STUDIES ON PROSTAGLANDIN CONTROL

OF

PRACTICAL BOVINE REPRODUCTION

by

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THESIS

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DEDICATION

For as long as I can remember I have taken pleasure from being with cattle. I have found satisfaction and enjoyment from working with them. I have been fortunate to earn my living from them. I hope that I may even have helped a few. As a mark of affection and gratitude, I dedicate these studies to the cows and calves, bulls, heifers and steers of the farms and fields of Britain - with nostalgic special mention for my first love, the Ayrshire.

DECLARATION

I declare that the work has been done and the thesis composed by me except in so far as a contribution was made by the undermentioned people. The extent of their contribution is indicated.

PART 1 Papers 1, 2 and 3.

In the studies reported in Part I, I worked under the guidance of D F Wishart. The papers in PART I were written by him and formed part of the subject matter of a Thesis entitled "Studies on the control of the ovarian cycle of heifers" presented by him for the degree of Doctor of Veterinary Medicine and approved by the University of Glasgow. I have his written permission to make use of the material in the three papers.

PART 2 Papers 4, 5, 6 and 7.

I designed the trials and protocols for all the studies, organised and supervised the field work in detail, with the assistance of D C Henderson who supervised the trial on one herd reported in Paper 7. I interpreted the results and wrote the trial reports and papers. I have received permission from Upjohn Ltd to reproduce the Technical Reports which constitute Papers 4, 5 and 6.

PART 3 Papers 8 and 9.

I designed the trials and protocols, organised and supervised the field work, interpreted the results and wrote the papers.

PART 4 Papers 10, 11 and 12.

I designed the trials and protocols, organised and supervised the field work, which was done by D B Anderson and R W J Plenderleith. I interpreted the results and wrote the papers.

The preparation of the thesis in its entirety, drafting, typing, composition and printing were done by me on an AMSTRAD PCW8256 Word Processor.

STATEMENT ON CONDUCT OF TRIALS

I was involved in the studies described in PART 1 as the main part of my duties as an employee of G D Searle Ltd. I worked under the guidance of D F Wishart, being involved in the practical work of the trials and gaining experience in the design, management and conduct of field trials on commercial farms.

The studies constituting the main body of the thesis in PARTS 2, 3, and 4, were carried out as part of my duties as an employee of Upjohn Limited since 1977. The object of the studies was to provide additional information to improve techniques involving the use of prostaglandin $F_{2\alpha}$ (dinoprost) as an aid to the management of bovine reproduction under practical agricultural conditions. The methods of investigation and techniques available to me were restricted by limited budgets. These precluded techniques involving frequent sampling and hormone assays. The Company does not own farm research facilities in the U K, so the practical work had to be done on commercial farms, which imposed the need for minimal interference with normal farm work routines.

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Finally but first, my wife Elspeth, for her encouragement, moral support, and forebearance of the disruption of domestic and social life that the preparation of this Thesis has entailed.

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PART 3

Strategic Use of Induced Oestrus

PART 4

Prostaglandin in the *post partum* period

SUMMARY

This thesis consists of a series of 12 Papers which are related as investigating exogenous hormonal control of bovine reproduction under practical agricultural conditions. In particular the objective was to produce additional information on the use of prostaglandin $F_{2\alpha}$. The methods and techniques which were used take account of the special requirements and restrictions of typical commercial husbandry conditions

In PART 1, the need for detection of bovine oestrus as a necessary preliminary to artificial insemination is discussed. This has been identified as a factor responsible for unsatisfactory conception rates. The physiological principles, practical problems, and economic consequences of these difficulties are examined in detail. Methods of improving the effectiveness of oestrus detection, and their limitations, are discussed.

The endogenous hormonal control mechanisms of the bovine oestrous cycle are examined in detail with particular regard to how they may be manipulated by administration of exogenous hormones. The development of techniques to control oestrus and ovulation by administration of different hormones is discussed. Detailed discussion of the principles involved, techniques required, and difficulties encountered are illustrated by reference to development trials for a progestagen-implant technique. The principles involved and techniques which were used, are related to bovine oestrous cycle control with prostaglandin $F_{2\alpha}$.

PART 1 describes the technical and practical stage of development which had been reached with techniques for the control of oestrus and ovulation in the bovine female when the studies were started. This sets the scene and gives the background for the studies which constitute the main body of the thesis.

In PART 2 the contribution made by controlled breeding techniques to practical cattle breeding is surveyed. Three main types of cattle are identified as, dairy cows, maiden heifers and beef suckler cows. Each has different physiological and pathological characteristics and different typical conditions of husbandry. The reproductive characteristics of each type are examined in detail. Trials are described which investigated the use of controlled breeding techniques in each type to identify the practical consequences and relevance of the differences.

Trials are described which demonstrate that dinoprost-synchronised ovulation with two fixed-time inseminations, results in conception rates equivalent to those achieved by inseminating at natural oestrus. An advantage was demonstrated for controlled breeding, because it allowed all treated cows an opportunity to conceive, not restricted by the need for oestrus to be detected. This advantage resulted in more pregnancies within the treatment period and significantly improved calving-to-conception intervals, compared with untreated controls.

It was further demonstrated with dairy cows, and also with heifers, that this advantage may also be achieved with dinoprost, using a single fixed-time insemination schedule. This results in more economic insemination practice. With suckler cows, a single-insemination schedule was associated with a lower conception rate.

Previously there was virtually no such information available in the literature specific to controlled breeding of dairy cows, which are the most economically important group of cattle and the type most frequently treated with prostaglandin $F_{2\alpha}$

The physiological bases are described and discussed for insemination at predetermined fixed times following synchronous ovulation induced by prostaglandin $F_{2\alpha}$. Published results of studies are cited which indicate that ovarian response to prostaglandin induction of ovulation varies according to the stage of the oestrous cycle when it is administerd. Evidence is cited which supports these findings and illustrates the mechanism involved. It is suggested that the evidence of these data might be applied to improve conception rates following fixed time insemination schedules by adjusting the relative timing of insemination.

In PART 3 criteria for selective use of dinoprost in herd fertility programmes were investigated. Induction of oestrus was demonstrated to be an effective therapy for cows presented clinically as not having been seen in oestrus. This agrees with cited evidence that the condition is largely a consequence of inadequate oestrus detection.

Two criteria were compared for selecting cows for induction of oestrus with dinoprost, to anticipate and minimise delayed breeding. Both schemes were equally effective in achieving a target calving-to-conception interval of 83 days, equivalent to a 365 day Calving Interval. One was more economical in operation because less prostaglandin was required.

An integrated dairy farm project over a twelve month period on a commercial farm is described. This incorporated techniques investigated in the thesis and demonstrated their economic effectiveness under practical farming conditions.

In PART 4, the *peri partum* and *post partum* periods of the dairy cow are examined. The anatomy, physiology, endocrinology and pathology of uterine and ovarian changes are discussed in detail, with particular reference to the length of the anoestrus period and return to fertile ovarian cyclicity. The most recent literature investigating the role of endogenous prostaglandin $F_{2\alpha}$ in the early *post partum* period of the cow was surveyed. This suggested an indication, which was investigated, for administration of prostaglandin $F_{2\alpha}$ in the early *post partum* period. Administration of exogenous prostaglandin was at a stage when blood progesterone concentrations were mostly basal, before resumption of oestrous cycles. The mechanism of an effect at this stage would therefore appear not to be due to the luteolytic property of prostaglandin $F_{2\alpha}$. First service conception rate of treated cows was significantly higher than that of controls. There was a significant advance of first oestrus in the treated cows compared with controls. The significance of, and possible mechanisms responsible for, these effects are investigated and discussed with reference to the relevant literature.

PART 1

PART I

BREEDING CONTROL

This is an introductory section. It sets out the agricultural background which had potential for the practical application of hormonal control of bovine reproduction. The physiological and hormonal principles involved are considered. Finally, a series of studies are described that developed a progestagen technique, starting with an identified progestagen with suitable characteristics for oestrus control, through to the stage when it was granted a Product Licence to allow it to be offered commercially for practical application. This establishes the 'state of the art' in the field of practical hormonal control of bovine reproduction, from which the following studies evolved to improve the effectiveness of the techniques under practical commercial conditions.

INTRODUCTION

From Noah's Ark until the middle of the 20th century, reproduction in the domesticated bovine species was controlled by management of mating. Female attractiveness to, and acceptance of the male were timed naturally. The first important interference with this independence was marked by the introduction of commercial artificial insemination services: by the English Milk Marketing Board in 1944, and by the Scottish Milk Marketing Board in 1949.

The role of the bull then became narrowed to the producer of semen, and his presence at the scene of action was no longer essential for fertilisation of a female. This marked the beginning of man's interference at a functional level.

At first artificial insemination involved collection and dilution of fresh semen, and insemination had to be done on the day of collection. The usefulness of the technique was improved by the development of successful freezing techniques which allowed semen to be stored for extensive periods, allowing the range and flexibility of the service to be increased. It also extended the value of exceptional sires, because the semen could be stored and used, even after the death of the bull.

The ability to dilute semen by factors of several hundreds, and to store diluted semen for years by freezing, has permitted widespread dissemination of the best genetic material available. The relatively small number of sires which are needed to service this extended use has allowed rigorous selection of only the most outstanding bulls to provide semen for artificial insemination. The effect of this greater selectivity of sires, and widespread use of their semen has increased enormously the milk production potential of dairy cows over the last forty years; and has improved beef production from superior genetic material, notably from exotic continental beef breeds, since the early nineteen seventies. The benefit to agriculture of the wide dissemination of superior genetic material to even the most modest commercial breeding enterprises has been immense. The availability of these advantages depends entirely on the use of artificial insemination, to permit distribution and use of the superior semen.

Although the function of the bull could be manipulated in this way, the female role was largely unaffected, except in the method of introducing semen into the uterus. The bovine female still retained control of her own cyclical oestrous pattern and determined the time of insemination. One severe limitation to the usefulness of artificial insemination in cattle has been that it relies on detection of oestrus in the female as an indicator of the approximate time of ovulation, so as to determine the optimum time to inseminate. The great variability between individual females in the expression of oestrus, and its random occurence within a herd, presents difficulties in its reliable detection. This markedly reduces the effectiveness which is possible with artificial insemination under farm conditions. If the timing of artificial insemination of the bovine female could be made more accurate, then the value of the technique could be further increased.

BOVINE OESTRUS

Overt oestrus, which describes a behavioural - not a physiological - state may be defined as the visible behavioural signs of sexual acceptance of the male, exhibited by the female. (Rowell, 1963) It depends on close quantitative and temporal relationships between progesterone and oestrogen from ovarian follicles near the time of oestrus. (Carrick and Shelton, 1969) The behavioural patterns and physiological changes make the female attractive to, and receptive of sexual attention from males (and in the bovine species, from other females). The consequence of these changes is to determine that copulation occurs close to ovulation, when it is most likely to produce conception. (Roberts, 1956)

The oestrous female shows restless activity, seeking the male and displaying signs of encouragement to him. Signs include - tail swishing, raised tail, swollen vulva, increased mucous secretion from the vulva, "cow-eyed" expression, bellowing, physical approaches to and contact with males in a provocative manner, nudging, receptive physical demonstration, mounting by the female and submissive reception by her of sexual approaches. In absence of a male, these characteristic behavioural patterns are directed to other females, not necessarily themselves in oestrus. The most obvious and characteristic sign of oestrus is attempted mounting, and solid submissive acceptance of riding by another. (Roberts,

1956) This latter is accepted as the diagnostic sign of true oestrus.

The female usually starts to show interest in the male in late pro-oestrus, when some of the signs described may be present but before she will stand willingly and solidly to be mounted; that is, when the female is not actually in oestrus. If more than one female in a group is in oestrus, and a number of others are showing sexual interest, it can be difficult to determine which is the one actually in oestrus. These complications explain part of the difficulty of accurate, reliable oestrous detection.

TIME ELEMENTS OF THE OESTROUS CYCLE

The length of oestrus has been recorded by many authors, who report average lengths mostly between 10 and 20 hours. (Hansel and Trimberger, 1952; Hough and others, 1955; Marion and others, 1950) Of considerable practical importance is not simply the mean, but the range of lengths of oestrus. These can vary from 4 to 24 hours, or more. (Wishart, 1972) The occurence of short periods of oestrus , and the fact that display of oestrus may be subdued, in response to many external factors, is of great importance, because it imposes the need for frequent periods of observation if the shorter oestrous events, and subdued expression, are to be detected. An interval of 12 hours between observation periods, which would be the case in twice-daily observation, would make it likely that many short oestrus periods would be missed. Even intervals of 8 hours which will occur between thrice-daily observations, may allow a number of short oestrus periods to pass undetected, which is a further possible source of oestrous detection error - omission. In farm conditions, it is usual simply to record oestrus : no attempt is made to estimate the stage, or identify the end of oestrus. The time of ovulation is measured from the end of oestrus. It would clearly be more precise if assessment of the time of ovulation could be estimated from a more precise point than "in oestrus".

OVULATION

Oestrus is comparatively short in the bovine female because a low oestrogen threshold causes an oestrogen block, so oestrus ends before the developing follicle has reached its maximum size. (Asdell and others, 1945) For this reason, ovulation in the bovine female, unlike the other farm animals, occurs at a variable period after the end of oestrus. (Roberts, 1956) The time interval from the end of oestrus to ovulation has been variously reported by many authors and the mean appears, by consensus, to be of the order of 11 to 15 hours ± a margin of some 5 hours. (Brewster and Cole, 1941; Hansel and Trimberger, 1952; Nalbandov and Casida, 1942) Much of this information was collected by repeated ovarian palpation, which may stimulate and accelerate ovarian activity. (Roberts and others, 1975) A range of 6 to 14 hours after the end of oestrus was reported in heifers when assesed by rectal palpation, and 12 to 24 hours when observed directly by endoscopy in a different series of heifers. (Wishart, 1972) The various means and ranges reported can be assumed to have been conscientiously recorded in each case; which indicates considerable variability in the average time interval from the end of oestrus to ovulation, and a much greater variability in individual cases when range is taken into consideration. The extent of this variability is further compounded when it has to be assessed from a starting point at an undefined time in 'oestrus', as discussed earlier.

INTER-OESTRUS INTERVALS

In line with the considerable variability of oestrous events, the length of oestrous cycles also exhibits considerable variations, and consecutive cycles may vary by as much as five days for animals showing an apparently normal cycle length of 18 to 24 days. (Wishart, 1972) It has been suggested, (Boyd and Reed, 1961) that 18 to 24 days may be considered 'normal' and cycle lengths outwith that range may be considered 'abnormal'. This is an interpretation that would not generally be considered controversial. The random occurence of oestrus within the herd and diversity of cycle lengths, make it impossible to predict oestrus with any degree of accuracy, or to impose regularity on the breeding schedule.

Therefore there is clearly much scope for inaccuracy in assessing the optimum time for artificial insemination in farm conditions. In addition, the logistics of operating a commercial artificial insemination service produces its own, additional complications. In the past, the practice was to inseminate heifers in the afternoon when they had been seen in oestrus in the morning. Those seen in oestrus in the afternoon were inseminated the following morning. Cows were inseminated the day following oestrus. In recent years, to minimise inseminator expense, it has become customary to inseminate both heifers and cows on the day following oestrus. The viable life of the ovum after ovulation is up to about 4 hours; that of spermatozoa is about 24 hours after insemination. Within these time constraints it is quite possible that commercial inseminations, may be done at the extreme limits of ovum/sperm viability - with the potential for poor conception rates.

Despite the difficulties and inaccuracies of its application artificial insemination has been very successful in improving the characteristics and potential of dairy and beef cattle. There is however still considerable scope for improving that performance. Better oestrous detection would allow more effective insemination, and developments in that field will be assessed. For artificial insemination, the only useful function of oestrus is to determine the time of ovulation. A better method for determining the time of ovulation could improve the performance of artificial insemination in cattle. A relatively recent increase in the understanding of endogenous control of the ovarian cycle has led to the development of some degree of control over the cycle, by administration of exogenous hormones. This can reduce the need to rely on oestrous detection for artificial insemination.

OESTRUS DETECTION

The reasons for inefficient oestrous detection are well recognised and their effect on presentation of females for insemination has been investigated. Successful oestrus detection by stockmen has been assessed to be of the order of 60 per cent by a number of cited authors, and detection rate was inversely related to calving to first service interval, calving to conception interval, and culling rate for failure to conceive. (Bailie, 1982) In a twenty-four day period, all animals displayed signs of oestrus when observed continuously by relays of observers. Over the same period, in conventional oestrus detection routines, stockmen only correctly identified 67 per cent of the same animals. (Esslemont, 1974)

Errors in oestrus detection have been identified as a major contributory factor to reproductive failure, on the evidence of returns-to-service being outwith normal range of 18 to 24 days. The incidence of returns outwith this range was significantly greater in cows with low conception rates compared with normal cows and this was considered by a number of authors to be largely an indication of inadequate oestrus detection. (Boyd and Reed 1961, Boyd 1973, O'Farrell 1979, O'Farrell and others 1983) In addition, cows described as anoestrous have been surveyed, with the conclusion that an important proportion of such cases are not anovulatory (Boyd, 1977) but reflect the difficulty of detecting oestrus.

Oestrous detection under practical farm conditions is not 100 per cent successful. There can be several explanations for this. (1) Poor detection can be due to lack of competence by the observer in interpreting the signs of oestrus correctly; especially if someone is delegated to the task who is not committed to the success of the herd breeding programme. (2) Oestrous activity is often sporadic, not continuous, and fairly long intervals of up to half an hour or so may lapse between episodes of oestrous activity. This may be partly in response to the presence of the observer, and the time needed for the interloper to cease to be a diversion. (3) Normal oestrus periods may be as short as four hours, so many may be missed because they happen between observation periods. For this reason, the more frequently it is possible to observe cattle, the better will be the detection rate. (4) In relatively adverse conditions or even without these, signs of oestrus may be more or less subdued, and escape the attention of the observer. It is also possible for physiological oestrus to occur with minimal signs, or without any outwardly detectable signs, the so-called 'silent oestrus'. (5) The actual time spent on this aspect of breeding is limited by the practicalities of farming. All of these factors make oestrus detection a source of error in the timing and administration of artificial insemination. Females undetected in oestrus may be capable of conceiving but are not given the opportunity.

Since full time observation for oestrus is impractical, a compromise must be accepted. A minimum of 30 minutes per session is essential if females that are actually in active oestrus are not to be missed. There need to be at least three, preferably four such observation periods per day, at times when the cattle are not being disturbed by external activity (feeding, gathering for milking, disturbance from tractors etcetera). The best times are early morning, the middle of the day, and late evening. Identification of individuals needs to be adequate for the conditions, as does lighting where appropriate. Detection will always be more successful when conditions, for cattle and observer alike, are comfortable and convenient

Progesterone assay has been used as a check on the stage of oestrus at which cows have been presented by farmers for artificial insemination. Reports from these investigations indicated that many cows were presented at an inappropriate time. Progesterone and oestradiol 17β were measured about the time of insemination followed by progesterone and rectal palpation of ovaries after insemination. On this basis, only 57 per cent of cows (n=46) were inseminated at a time when optimum conception rate could be expected. (Watson and MacDonald, 1984) Milk samples, with a progesterone concentration higher than the normal oestrus level of 0.3 ng/ml, taken from cows at the time of insemination; amounted to an important proportion of those studied ; 21.28 per cent (Appleyard and Cook, 1976); 22.4 per cent (Gunzler & others, 1979) ; 7.7 per cent (McCaughey and Cooper, 1980). A negative correlation was confirmed between milk progesterone concentration at insemination, and non-return rate, or conception rate. In a more detailed investigation on 1090 inseminations in 47 herds, (Henriksen and others, 1982) progesterone was assayed from milk samples taken at three times ; on the day of insemination and on days two and five later. The progesterone concentrations were then matched against the expected profile of concentrations at and following oestrus. Based on the assayed concentrations, cows were divided into five groups : (1) true oestrus (66 per cent), (2) pro-oestrus (4 per cent), (3) not in oestrus (5 per cent), (4) delayed ovulation or non-cyclical (20 per cent), and (5) acyclic (5 per cent). The pregnancy rate in the five groups were (1) 46 per cent , (2) 18 per cent , (3) 6 per cent , (4) 25 per cent , and (5) 2 per cent .The pregnancy rates confirm the definitions of the five groups.

There is thus ample evidence of the influence of inadequate oestrous detection on the outcome of artificial insemination. There is also evidence that illustrates one approach to reducing the problem. It may be assumed that provided the timing of insemination is correct relative to ovulation, fertilisation can be achieved whether or not oestrus is displayed, or detected. This has been illustrated in heifer studies on fixed-time insemination following induced ovulation. Oestrus was recorded, but fixed-time insemination was done on all induced females, whether detected in oestrus or not. (PAPER 1) Of 55 heifers not seen in oestrus but inseminated at fixed-times, 30 conceived to insemination (55 per cent).

Repeated failure to detect oestrus in individual females can be perceived as a fertility problem, and be presented by the stockman as a case for clinical examination. Cows presented clinically as not having been seen in oestrus were treated with prostaglandin $F_{2\alpha}$ and had first service conception rates of 61.1 per cent from artificial insemination following observed oestrus, and 73.7 per cent from artificial insemination on fixed-time basis. (PAPER 8) In a similar series of recorded clinical cases, 54.7 per cent conceived to first insemination (Eddy, 1977). Such conception rates indicate that the problem was not fertility but oestrus detection. It also demonstrates that removing the need for oestrous detection by hormonal induction of ovulation is one practical solution to the problem.

AIDS TO OESTROUS DETECTION

If the signs of oestrus are present but unseen, then detection may be improved by devising a suitable technique to record unseen oestrus. A number of approaches have made use of the characteristic homosexual behaviour of bovine females, and the fact that virtually all cyclical cows display oestrus to some extent. Mounting by other cows may be recorded by the use of a pressure sensitive capsule containing a dye marker (Kamar), which is fastened with adhesive to the sacral region of the pelvis. It is calibrated such that pressure from the brisket of a cow riding vigorously will express the colour dye from the opaque capsule so that it becomes visible.

These devices were used on several of the studies described in PAPERS 1, 2 and 3, as an additional aid to intensive oestrous detection. Under such intensive supervision they were found to be a useful aid to help identify oestrous cattle, but by no means infallible. Under the close scrutiny of these studies both false positives and false negatives were identified fairly often. False positives could be triggered by attempted mounting of a female not in oestrus, by rubbing on boughs of trees, stanchions of cubicles and other housing fixtures. False negatives were seen on cows ridden on numerous occasions as well as on some mounted infrequently. The viscous dye was more easily expressed in hot conditions than in cold conditions. The placing of the Kamar on the sacrum was critical, and varied, depending on the physical characteristics of cow's pelvis and sacrum. The efficacy of heat mount detectors was surveyed, with the conclusion that they were only a useful aid to regular oestrous detection. (Information Supplement, 1973) For these reasons, such a device cannot be a substitute for regular observation. Its main advantage was considered to be focusing attention on particular animals for closer observation.

Tail paint and tail paste have been used in a similar way. The paint is applied to the tail head, and is scuffed or removed by the brisket of riding cattle. This system appears to suffer from false positives and false negatives (Ducker and others, 1983) in a similar manner to that described for Kamar heat mount detectors and was considered to be useful only as an additional aid to regular observation.

Heat mount detectors and tail paint/paste are the only oestrous detection aids to have gained some commercial acceptance. Under commercial conditions they have proved to be useful aids. Under no circumstances can they be considered suitable as substitutes for regular oestrous detection by observation, and they have not been able to improve the level of detection to any great extent.

Teaser bulls which are incapable of fertilising the female may be used , and they identify oestrous females well. Methods of surgical preparation include; phallectomy, surgical displacement of the penis, vasectomy, epididymectomy. The major drawbacks are; the difficulty and danger of handling bulls; the risk of spreading sexually transmitted disease if penetration is permitted; the need for regular adjustment and replenishment of the marker apparatus ; the cost of surgery, and of buying, and feeding the bull. The practical difficulties have prevented their efficacy at oestrous detection being used to any important extent.

Several alternative approaches have been suggested, or attempted, to substitute for, or automate oestrous detection. All have suffered from operational complexity and inaccuracies.

Methods which have been proposed, but not as yet shown any real practical promise are : measuring walking activity with an electronic pedometer to recognise and signal the increased activity associated with oestrus ; measuring body temperature, to identify the rise associated with oestrus ; measurement of electrical resistance of vaginal mucus, which alters at oestrus ; employing 'sniffer' dogs to identify the pheromones in vaginal mucus, associated with oestrus - these dogs are apparently quite successful, and a project is currently attempting to use such dogs to try to identify the pheromones in the laboratory.

At present there are no indications that the difficulties of oestrous detection are likely to be solved by improved detection methods. The greatest promise in recent years has been the use of hormonal induction of oestrus and ovulation at a predetermined time, which can remove or reduce the need for identification of oestrus as a preliminary to artificial insemination.

The stringent requirements of oestrous detection had virtually excluded two categories of cattle from the benefits of artificial insemination : heifers and beef cows. Husbandry practice is usually to keep groups of such cattle on grazing, or in yards which are frequently remote from the farm buildings and inconvenient to visit regularly for oestrous detection. Both categories are often fed with a view to economy, which can result in poor expression of oestrus; and to poor conception rates to artificial insemination. In addition, beef cows are suckling calves at the time when they are to be inseminated and this also tends to subdue signs of oestrus . In deference to the difficulties that these husbandry practices present, it has been the practice to run a commercial quality stock bull with them for a period of time to mate naturally. The techniques of controlling and synchronising oestrus have now made artificial insemination more available to heifers and beef cows, but each category presents its own problems and special requirements.

The special requirements and problems of heifers, beef cows and dairy cows will be discussed and the application of controlled breeding techniques to each will be investigated.

ENDOGENOUS CONTROL OF THE BOVINE OESTROUS CYCLE

Any attempt to modify the time of oestrus and / or the time of ovulation must be related to an understanding of the natural oestrus cycle. Current understanding of endogenous hormonal control of the natural bovine oestrus cycle has been reviewed recently (Peters 1985; Peters and Lamming 1986; Lamming and others, 1975).

The stroma of the ovary contains large numbers of primordial follicles, and there is a continuing process of development and regression of these. The rate of development of follicles appears not to be constant, but to occur in waves, with growth phases between days 3 and 7, and again between days 7 and 13 of the cycle. (Ireland and Roche, 1983) The follicle which is destined to ovulate, the ovulatory follicle, developes late in the cycle, and may be identified only about 48 hours before oestrus. (Dufour and others, 1972) Following ovulation, the *corpus rubrum* developes in the ruptured follicle and is quickly invaded by cells from the granulosa and theca interna layers of the follicle. These are luteinised under the influence of luteinising hormone to form the *corpus luteum*. (Hoffman and others, 1974) The *corpus luteum* secretes progesterone, and the rate of secretion is positively related to its development and regression. Blood progesterone concentration rises by about day 4 after oestrus, reaches a maximum about day 7 and remains high until approximately 3 days before the next oestrous period when concentrations fall rapidly concomitant with the decline in size of the *corpus luteum*. (Hafs and Armstrong, 1968; Pope and others, 1969; Robertson, 1972) Progesterone is central to the endogenous control of the oestrus cycle.

PROGESTERONE

It is believed that luteal progesterone is responsible, by a negative feedback system, for inhibiting release from the anterior pituitary of the gonadotrophins necessary for ovarian follicular development, mainly follicle-stimulating hormone and luteinising hormone. (Convey and others, 1977; Roche and Ireland, 1981) The release of the pituitary hormones in a pulsatile manner is mediated directly from the hypothalamus via the hypophyseal portal system by the release of gonadotrophin-releasing hormone. This effect has been mimicked by administration of repeated injections of exogenous gonadotrophin-releasing hormone. (Riley and others, 1981) In the ewe, studies on hypophyseal portal blood have related the pulsatile release of gonadotrophin-releasing hormone with coincident pulsatile release of luteinising hormone. (Clarke and Cummins, 1982; Levine and others, 1982)

The onset of the events associated with the following oestrus are conditioned by the decline in luteal plasma progesterone activity. Simultaneously with the removal of its inhibitory effect, increased follicle-stimulating hormone causes an increase in follicular activity (Rahe and others, 1980) and a resulting rise in oestradiol 17β which reaches a peak at, or just before the onset of oestrus.

(Glencross and others, 1981) The raised plasma oestrogen, mainly oestradiol 17ß from follicles, is thought responsible, by positive feedback, for the pre-ovulatory surge of luteinising hormone which occurs near the onset of oestrus. (Kesner and others, 1981)

Luteinising hormone is believed to be the principal luteotrophic agent, probably the only one in the cow. (Hoffman and others, 1974) The ovulatory discharge of luteinising hormone initiates ovulation which occurs in 24 to 30 hours after the surge, (Swanson and Hafs, 1971) followed by luteinisation of the follicular granulosa and theca interna cells, which then contribute to the formation of the next *corpus luteum*. (Sprague and others, 1971)

These findings imply that when blood progesterone concentrations are high, ovarian follicular activity is suppressed; and when progesterone concentrations fall, the initiation of another oestrus is stimulated. This is confirmed in practice and is the basis for using progesterone or progestagens for oestrous control.

LUTEOLYSIS

The other hormonal effect that is relevant to oestrous control is luteolysis, which is the mechanism resulting in the lysis of the *corpus luteum* with a consequent decline and eventual cessation of progesterone production. The return to oestrus by the cyclical, non-pregnant animal is thought to be the result of hormones produced by the uterus causing lysis of the luteal tissue in the ovaries. Extensive studies in sheep in the sixties established the existence of such a substance. A number of teams, notably those led by McCracken and Goding produced extensive elegant research studies to elucidate the role of prostaglandin $F_{2\alpha}$ in the ovarian cycle of the sheep. It was demonstrated that a uterine luteolysin is produced about 3 days before the onset of oestrus, the substance, acting largely locally, to cause luteal regression with consequent decline of plasma progesterone concentrations. (Goding and others 1967a ; Goding and others 1967b ; Caldwell and others 1969).

Prostaglandin $F_{2\alpha}$ has been shown to occur in the venous outflow from the uterus of the sheep. There was some difficulty in explaining how it reached the ovary from that source in sufficient concentration to be luteolytic, because prostaglandin $F_{2\alpha}$ is rapidly metabolised, in the lungs, to the initial main plasma metabolite, 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$. To explain this anomaly, a theory suggested direct transfer of uterine prostaglandin $F_{2\alpha}$ to the ovarian artery. Studies demonstrated that, when tritiated prostaglandin $F_{2\alpha}$ was infused into the uterine vein, it appeared as such in the ovarian artery in concentrations far higher than those in the adjacent iliac artery. (McCracken and others, 1971 ; McCracken and others, 1972) Further evidence of counterflow transfer of prostaglandin $F_{2\alpha}$ from the uterine vein to the ovarian artery was provided (Baird and Land, 1973) by ligating the uterine vein proximal to the entry of the ovarian vein - to prevent access of prostaglandin $F_{2\alpha}$ to the segment of uterine vein which is in close apposition to the ovarian artery. This resulted in luteal maintenance in four of ten ewes so treated.

Detailed investigation of the relationships of the utero-ovarian vasculature of the ewe have revealed anatomical evidence supporting the concept. A portion of the ovarian artery is in an area where there is considerable surface contact between it and a branch of the uterine vein. The area of contact is greatly increased by the convolutions of the artery in this area, and in some specimens by the anastomoses among veins. (Ginther, 1976a) There is, therefore a considerable surface contact between vessels containing uterine venous effluent, and a vessel containing ovarian arterial affluent. This would facilitate transfer of hormones between the two vessels.

There is therefore a strong body of evidence supporting the counterflow theory; the local action of prostaglandin $F_{2\alpha}$ passing directly from the uterus, by direct transfer from uterine vein to ovarian artery, and thus to the ovary.

This prostaglandin transfer mechanism has not been demonstrated in the cow. However a utero-ovarian vascular shunt with anastomoses and anatomical relationships similar to that of the ewe has been demonstrated in the bovine female. (Ginther, 1976b) In addition, a threefold elevation in endometrial prostaglandin $F_{2\alpha}$, concomitant with a twentyfold elevation of venous prostaglandin $F_{2\alpha}$ has been demonstrated, as was the presence of prostaglandin $F_{2\alpha}$ in the ovarian artery during the 5 days before oestrus in the cow (Shemesh & Hansel, 1975).

In any event, prostaglandin $F_{2\alpha}$ has proved in practice to be an effective luteolysin in the cow. By causing luteolysis, blood concentrations of progesterone are rapidly reduced to basal levels in a way that mimics the natural decline and initiates the events of oestrus.

CONCLUSION

Initiation of oestrus can be achieved by artificially inducing progesterone decline, either by removing an artificial source of progesterone or a progestagen, or by inducing the same effect by luteolysis of the natural *corpus luteum* . These are the hormonal effects that have been investigated, and demonstrated to be capable of controlling the timing of the ovarian events that control oestrus and - more important for the successful timing of artificial insemination - ovulation. The investigations which form the body of this dissertation are concerned with the use of prostaglandin $F_{2\alpha}$. The earliest investigations in control of oestrus by administration of exogenous hormones involved the use of progesterone and progestagens. There is much common ground shared by oestrous control whether with progesterone or with prostaglandin $F_{2\alpha}$, and the development of both methods in cattle ran in parallel. Both will be discussed in detail.

EXOGENOUS CONTROL OF THE BOVINE OESTROUS CYCLE

In 1948, Christian and Casida reported that injection of progesterone inhibited oestrus and ovulation in cattle until 2 to 6 days after the end of treatment, but frequent injections were required. From that time, investigation of control of oestrus and ovulation have concentrated on progesterone and progestagens or prostaglandin $F_{2\alpha}$ and some of its analogues. The main object of the studies reported in this dissertation concern the development of techniques using prostaglandin $F_{2\alpha}$, which has evolved as the principal approach in practice. The role of prostaglandin $F_{2\alpha}$ in the artificial control of ovulation will be considered in detail later. However an understanding of the development of progesterone or progestagen techniques in the field is relevent to the overall development of ovulation control.

PROGESTERONE AND PROGESTAGENS

The first successful commercial application of a progestagen in the control of oestrus was in sheep. It used fluorogestone administered by impregnated intravaginal sponge left *in situ* for a period of 12 to 16 days. This acts as an artificial *corpus luteum* and its removal from the vagina produces the rapid fall in blood progestagen which induces oestrus after 36 to 48 hours. (Robinson, 1964). Medroxyprogesterone acetate has also been used in the same way for induction of synchronous oestrus, resulting in good conception. The method has become, and has remained firmly established as the method of choice in sheep to date (Gordon, 1970 ; Gordon, 1975), in which species it is extremely effective. Conversely, attempts to synchronise oestrus and ovulation in sheep using prostaglandin $F_{2\alpha}$ have had less success and have resulted in lower conception than with progestagens (Boland and others, 1978; Henderson and others, 1984). This is perhaps ironic, since the sheep was the experimental model used for the investigations leading to an understanding of the role of prostaglandin $F_{2\alpha}$ in the ovarian cycle.

In cattle, the use in this way of progestagen impregnated sponges has had considerably less success. It has been attempted using CAP (chlormadinone acetate), MGA (melengesterol acetate) and FGA (fluorogestone) (Carrick & Shelton, 1967; Shimizu and others, 1967; Hignett and others, 1970). The results have been variable and largely unsatisfactory. In sheep, the intra vaginal pessary has proved a relatively efficient means of administering a progestagen. In the cow however, inadequate retention of sponges, of the order of 80 per cent, has proved to be a persistent problem. (Hignett and others, 1970; Wishart and Hoskin, 1968)

One answer to this problem, a progesterone releasing intra-vaginal device has been developed. It consists of a stainless steel coil covered in silastic rubber which is impregnated with progesterone. Progesterone from this is absorbed into the systemic circulation. Concentrations in the milk of cows under treatment have been reported to be similar to those of the normal luteal phase (Bulman & Lamming, 1978). The device carries a gelatin capsule containing oestradiol benzoate, which is released rapidly after insertion, converting to oestradiol 17β, and is intended to assist premature regression of an existing corpus luteum. Removal of the progesterone releasing intravaginal device after an insertion period of 12 days causes a drop in the blood concentration of progesterone, which initiates the events of oestrus as previously discussed.

First service conception rates equivalent to those of contemporary untreated controls have been reported in dairy cows which were treated with progesterone releasing intravaginal devices and inseminated at predetermined fixed times (Drew and others 1978, Drew and others 1982). There is evidence that this method, using progesterone, is able to initiate oestrous cycles in a proportion of anoestrous/suboestrous cows (Lamming and Bulman 1976, Drew and others 1978). The value of this effect was demonstrated in beef suckler cows, when conception rates were significantly higher with progesterone releasing intravaginal device than with a prostaglandin $F_{2\alpha}$ analogue for oestrous induction. There was however, in the same study, no difference in conception rates between the two treatments when the experiment was done with Friesian dairy heifers (Roche, 1976). The difference in reproductive characteristics between different types of cattle, which is highlighted by this experiment is of considerable importance both in experimental design and in the practical application of the techniques, and will be considered in greater detail later.

The method of the progesterone releasing intravaginal device is a successful practical approach to the induction of oestrus and ovulation in cattle. It has retained a place in practice today and has some practical advantages. It has the potential to induce oestrus in some suboestrus cows, which can be a useful attribute particularly in beef cows, which as a class are anoestrous or suboestrous for longer after calving than are dairy cows. Prostaglandin $F_{2\alpha}$ on the other hand requires the presence of an active corpus luteum. In addition,the progesterone releasing intravaginal device may be administered by the stock owner without veterinary supervision; a freedom not shared by prostaglandin $F_{2\alpha}$. Despite these apparent advantages, the progesterone releasing intravaginal device has been less widely accepted than prostaglandin $F_{2\alpha}$ and some of its analogues.

Administration of a progestagen by means of an implant has also been established as a succesful route for oestrous control. Details of this particular approach are relevant to the development of practical methods of control of oestrus and ovulation, using prostaglandins in cattle, which occured at about the same time. Factors involved in the development of a practical progestagen implant technique for controlling bovine reproduction are reported and discussed in Part 1 of this thesis. It illustrates and explains the bases from which controlled breeding in cattle has evolved as a practical commercial method.

For the sake of completeness, passing reference should be made to oestrous control by the method of oral administration of progestagen. Progesterone itself is inactivated by the digestive process, but several synthetic analogues are active orally. The route of administration has some attractions because of its simplicity and suitability for mass medication. Progestagens which have been administered in feed in this way, in North America are, CAP (chlormadinone acetate), MGA (melengesterol acetate) and MAP (medroxyprogesterone acetate). Synchronisation has been achieved, but results have been variable. It seems probable that this is due to variations in dosage, dependant on individual food intake and rate of absorption from the gut. Relatively slow absorption from the digestive tract, complicated by variations in the mass of gut content in individuals inevitably make withdrawal of the hormonal influence slower and less precise following the end of treatment than is possible with the alternatives discussed. No orally administered progestagens are used in the U.K. The combination of imprecise action and current political attitudes to hormone medication makes it extremely unlikely that this situation will alter.

PROSTAGLANDINS

The term prostaglandin covers a large number of chemically similar, naturally occuring substances which are both ubiquitous and extremely potent, with a wide range of effects. Those of the E and F series are important for their roles in reproduction. They are known as primary prostaglandins because the other prostaglandins are derived from them. Chemically, the primary prostaglandins are unsaturated hydroxylated fatty acids with a five-membered ring in a twenty-carbon skeleton. All are derivatives of the parent compound prostanoic acid. Prostaglandins are classified according to their chemical structure, which is related to their pharmacological activity. The letters in the designation correspond to differences in the five-membered cyclopentane ring. The subscript number after the letter denotes the degree of unsaturation in the side chains of the prostaglandin molecule. Some 15 naturally occuring prostaglandins have been identified.

Unsaturated acids, derived from the essential fatty acid linoleic acid, are the precursors for the biosynthesis of natural prostaglandins under the control of a microsomal synthetase system (prostaglandin synthetase). Production of natural prostaglandins is by "*in situ* biosynthesis" immediately before release, rather than release from bound stores. They are rapidly metabolised, about 90 per cent during one circulation through the lungs and liver. The initial, main plasma metabolite is 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$. It is more stable than prostaglandin $F_{2\alpha}$, and has become widely used, for that reason, for assay to deduce plasma prostaglandin $F_{2\alpha}$ concentrations. Prostaglandins appear to be present in most animal tissues. Because of their instability and rapid metabolisation, it is considered that they function essentially as local hormones.

Prostaglandin $F_{2\alpha}$ is the prostaglandin most commonly associated with the reproductive process in domestic animals. The property of prostaglandin $F_{2\alpha}$ which is of principal veterinary interest is luteolysis, the morphological and functional regression of the *corpus luteum*. The mechanism by which this effect is produced is still unclear. It has been suggested that luteolysis is caused by a restriction of bloodflow to the *corpus luteum*, (Pharris and others, 1970) but this may be a consequence rather than a cause. There is evidence which appears to suggest that prostaglandin interferes specifically with the coupling of luteinising hormone and adenyl cyclase on the luteal cell membrane. (Henderson and McNatty, 1975; Wakeling and Green, 1981)

Although the mechanism of luteolysis is unclear, its effect is specific and predictable. The interval between administration of exogenous prostaglandin $F_{2\alpha}$ and the ensuing oestrus has been found to be fairly precise, in cows with an active *corpus luteum* . This suggested the possibility of artificial insemination without reference to signs of oestrus, (Rowson and others, 1972) but was effective only in females with an active *corpus luteum*.

Manual identification of *corpora lutea* per rectum has been shown to have limitations in assessment of the functional status, and to be only 85 per cent accurate (Watson and Munro, 1980) when confirmed by progesterone assay, or 67 per cent when confirmed by post mortem examination of ovaries. (Dawson, 1975). The practical difficulty of identifying the *corpus luteum*, would therefore reduce the reliability and effectiveness of inducing oestrus after identification of the *corpus luteum*.

In order to remove the need to identify active *corpora lutea*, a double injection regimen was devised, using two injections of prostaglandin $F_{2\alpha}$ with an interval of twelve days between (Cooper 1974, Hafs and others 1975). The first injection causes luteolysis in cows 5 to 18 days beyond oestrus. Twelve days later, they will again have an active *corpus luteum*, as will the cows which were in oestrus or had a refractory *corpus luteum* at the time of the first injection. In this way, the need to identify a *corpus luteum* is removed, and all cows in a group may have a synchronous oestrus/ovulation, and artificial insemination at a predetermined time. The conception rate of cows treated in this way was as high in cows inseminated 72 hours and 90 hours after the injection (56 per cent) as in cows inseminated in oestrus after the prostaglandin $F_{2\alpha}$ injection (52 per cent), and in cows inseminated in natural oestrus during a contemporary period (53 per cent) (Lauderdale and others, 1974)

The ability to induce oestrus and ovulation at a predictable time has considerable practical importance in the management of cattle breeding and the veterinary management of fertility. It has allowed insemination without the necessity for oestrus detection, batch insemination to improve batch management of groups of cattle, and selective techniques aimed at specific times, or groups of animals, or individuals. These aspects will be considered in detail in later sections.

COMMERCIALLY PRODUCED PROSTAGLANDIN $F_{2\alpha}$ AND RELATED ANALOGUES

The presently available commercially produced prostaglandins are manufactured by total synthesis. The parent compound, prostaglandin $F_{2\alpha}$, is available commercially as the synthesised tromethamine (THAM) salt, dinoprost (Lutalyse, Upjohn). In addition three synthesised analogues are available in the United Kingdom, registered for use in cattle - cloprostenol (Estrumate, Coopers), fenprostalene (Synchrocept B, Syntex), luprostiol (Prosolvin, Intervet). Tiaprost (Iliren, Hoechst) is no longer marketed in United Kingdom for use in cattle.

Prostaglandin $F_{2\alpha}$ and its analogues have the same basic chemical structure of a five-membered ring in a twenty-carbon skeleton. Small chemical substitutions or additions to the side chains have produced important differences to the pharmacological properties of the different analogues. Synthesised prostaglandin $F_{2\alpha}$ (dinoprost) is identical with the naturally occuring compound in its activity. The analogues have been selected for luteolytic potency, but with minimal side effects, in particular effects on smooth muscle.

The commercial prostaglandin analogues are more potent than natural prostaglandin $F_{2\alpha}$. This does not imply any difference in luteolytic potential, but does imply differences between the effective doses of compounds with different potencies. Dose titration studies select the optimum luteolytic dose for each analogue, which is what matters, not the actual potency. Pharmacological response indicates a threshold effect, as opposed to a weight related dose requirement. The optimum luteolytic effect at optimum dose for one analogue may not be assumed to be equally luteolytic with all the others. Indeed there is some indication that one analogue is less luteolytic than others.

Dose titration studies mainly use heifers, because they are convenient experimental animals for the purpose. In the field, prostaglandins are most often used on parous cows, which are considerably more variable in size and reproductive characteristics, and have the additional complicating influences of lactation.

In recognition of this difference, a detailed study investigated different doses of prostaglandin $F_{2\alpha}$ in lactating dairy cows; (Renegar and others, 1978) comparing doses of 15 mg, 25 mg and 35 mg for their luteolytic effect during the dioestrus period. The incidence of luteolysis was nine of eleven cows, eight of ten cows, and ten of ten cows respectively for the three dose rates. The differences were not statistically significant, but a similar non-significant positive effect was recorded in other hormonal concentration profiles measured. The authors concluded that 25 mg was an effective dose to induce luteolysis in lactating dairy cows. Whilst the scientific correctness of this conclusion cannot be denied, there can be no harm in making a mental note that only the 35 mg dose produced 100 per cent luteolysis in these cows.

A practical consequence of high potency is that a smaller total volume is needed for administration. This offers scope for operator error in formulations which in consequence of high potency, are dispensed in small volume doses. Any wastage occuring during loading of the syringe, careless administration, or the use of a relatively large needle will represent a larger proportion of the total dose, and could result in under-dosing with the possibility of failing to achieve a full luteolytic concentration in a proportion of animals. If the solution has a thick consistency, and a recommended 2 ml dose for cattle, as is the case with fenprostalene and loprostiol, then extra care is needed to ensure correct dosage.

ROUTE OF ADMINISTRATION

The usual route of administration for prostaglandin is intramuscular. Exceptions are tiaprost and fenprostalene, for which because of increased risk of tissue reaction, the recommended route of administration is subcutaneous. For simplicity, the rump muscles of the cow are mostly used. If a long needle, eg 1½ inch, is used, it is demonstrably possible for the needle to penetrate through into the pelvic cavity of dairy cows with thin rump muscles. In such a case absorption may be inadequate and luteolysis fail to be induced. The rump of dairy cows, particularly in some currently used housing systems, may be heavily contaminated with faeces, dust and dirt. In a small number of cases, contamination carried into the muscle by the needle, causes an inflammatory reaction, because the injection does not possess any antibacterial activity. A more hygienic site for injection, is the musculature of the neck, which carries less risk of such contamination.

Intra-uterine administration of prostaglandin can induce luteolysis at a considerably lower dose; of the order of half or less of the dose suitable for intramuscular administration. This effect has been demonstrated for dinoprost (Louis and others, 1974) and cloprostenol (Tervit and others, 1973). Despite the advantage of considerably reduced dose, and therefore cost, this route of administration has not been accepted in general practice. The reasons are presumably and understandably a combination of inconvenience, time required for the administration and the risk of introducing infection into the uterus. These disadvantages may be largely avoided, without losing the advantage of a smaller dose, when prostaglandin is injected by the intravulvosubmucous route (Horta and others, 1986). Using this route of administration, one quarter of the intramuscular dose of cloprostenol (125 μg compared with 500 μg) was stated not to reduce the luteolytic effect of the drug, or fertility, in heifers and cows. It was speculated that the cloprostenol reaches the ovaries without entering the systemic circulation, thus avoiding metabolisation in the lungs and liver. The studies were done on small groups, and results in adult cows were equivocal. This is an interesting and promising approach, but further dose titration studies using larger numbers of parous cows would be necessary before the technique could be considered for field use. High standards of hygiene would also be a prerequisite, because of the unacceptable consequences of introducing infection to this region.

SECONDARY PHARMACOLOGICAL PROPERTIES OF PROSTAGLANDINS

The prostaglandin analogues have been selected for luteolytic action combined with minimal smooth muscle activity. The point was made earlier, that small structural differences between analogues produce considerable differences in their characteristics. This is confirmed and illustrated by comparative investigations of some of these effects in cloprostenol, tiaprost and fenprostalene compared with prostaglandin $F_{2\alpha}$ (Jackson and Jessup, 1984).

Smooth muscle effects of all three analogues *in vitro* were less than prostaglandin $F_{2\alpha}$ in both prostaglandin-sensitive smooth muscle systems investigated; tiaprost and fenprostalene produced smooth muscle effects greater than cloprostenol but less than prostaglandin $F_{2\alpha}$. *In vivo,* cloprostenol is not significantly more or less potent than prostaglandin $F_{2\alpha}$ in raising rat blood pressure or inducing bronchospasm in the guineapig, but both tiaprost and fenprostalene are more potent in these respects. In two thromboxane-sensitive test systems both tiaprost and fenprostalene were markedly more potent than prostaglandin $F_{2\alpha}$ and both also induced irreversible aggregation of rat heparinised platelets. The significance of this is that both of these analogues have the potential to cause marked platelet aggregation which could lead to thrombus formation at the injection site, increasing the potential risk of site reaction and anaerobic infection. This implication is supported by field experience, and emphasises that prostaglandin $F_{2\alpha}$ and the analogues may not be assumed to be identical in their actions and characteristics.

It has been recognised for some time that prostaglandin $F_{2\alpha}$ can stimulate release of some anterior pituitary trophic hormones (Lauderdale, 1974) The effect of a number of analogues was investigated for their effect on secretion of pituitary hormones. (Schams and Karg, 1982) There was no effect on follicle stimulating hormone, and a raised luteinising hormone concentration appeared to be a response to falling progesterone concentration. Within 20 minutes of administration, an increase of prolactin, lasted two to four hours, and an increase of oxytocin, lasted one to two hours. The significance of this hormone release effect is not known.

The administration of prostaglandin $F_{2\alpha}$ has been associated with improved fertility in two different use schedules, which will be discussed in later sections. The mechanisms which produce the improvements are not understood. The possibility that a prostaglandin induced hormone release effect plays some part is worthy of consideration, and will be discussed in that context in PART 4.

The role of prostaglandin $F_{2\alpha}$ in management and control of breeding forms the subject matter of PARTS 2, 3 and 4. Specific properties and effects of prostaglandin $F_{2\alpha}$ will be discussed in detail in the context of the special demands of each situation. These will be ; synchronisation of ovulation for group insemination at predetermined fixed-times ; induction of oestrus and ovulation for strategic insemination schedules ; the role of prostaglandin $F_{2\alpha}$ in the *post partum* period.

PRACTICAL HORMONAL CONTROL OF CATTLE BREEDING

The value of artificial insemination has been well established, as has the limitation of the technique because of ineffective oestrous detection. A promising way of removing, or reducing reliance on oestrous detection was by controlling the time of oestrus. The management of oestrus and ovulation by hormonal means has been discussed, and two possible methods of doing this by administration of exogenous prostaglandin $F_{2\alpha}$ or progesterone have been outlined.

In the early nineteen seventies field trials were being run to establish effective techniques which would allow these methods to be offered commercially for the control of breeding in cattle. Development trials were being done more or less contemporaneously for prostaglandin $F_{2\alpha}$, dinoprost ; for a prostaglandin $F_{2\alpha}$ analogue, cloprostenol ; and for a progestin, norgestomet. The methods, standards established, and problems encountered were very similar, and were mostly not specific to one hormonal method of ovulation control.

The development of norgestomet is described in detail, because the author was intimately involved with that at a practical level. The standards established by the end of the trials represent the 'state of the art' when controlled breeding was made available commercially to agriculture. An understanding of the capabilities, potential, and limitations of the hormones, and techniques, are a prerequisite for the following studies which seek to improve understanding and application of these techniques. Farming conditions frequently impose limitations from economic or management system constraints. These may demand modification of theoretical standards to an acceptable practical compromise

DEVELOPMENT OF A PROGESTAGEN BASED BREEDING CONTROL SYSTEM

The development of a practical system of oestrus control is described, based on a progestagen. The studies are reported in detail in PAPERS 1, 2 and 3, which contain the references to literature cited. It starts with the potent progestagen, norgestomet (SC21009, 17α-acetoxy-11β-methyl-19-nor-preg-4-ene-20,dione) which is capable of suppressing oestrus and ovulation by daily intramuscular injection. This potent steroid had been incorporated in a small implant which provided a method of slow release depot administration.

The studies involved developing methods and 'recommendations for use', that would allow the system to be offered as a practical method of oestrus and ovulation control to be used under commercial agricultural conditions. The investigations and data obtained from the trials supported the efficacy of the norgestomet implant and resulted in the granting of a Product Licence by the Medicines Unit of M.A.F.F.

FERTILITY AND TIMING OF OESTRUS FOLLOWING

PROGESTAGEN TREATMENT

(PAPER 1)

Despite the demonstrated experimental effectiveness of the progestagen norgestomet, a system for using it had to be made workable and reliable, and some concern still remained concerning the known subfertility associated with the use of progesterone and progestagens. The purpose of the experiments reported in PAPER 1 was to determine the effectiveness of oestrus and ovulation control achieved by use of the norgestomet implant, to look for evidence of possible reduced fertility associated with its use, to establish the time relationships between the end of treatment, oestrus and ovulation, and finally, to determine the effectiveness of insemination at a predetermined fixed time following the end of treatment.

Prolonged administration of progesterone or progestagens, for twenty one days, has been associated with reduced fertility at the controlled oestrus. A shorter nine-day implantation period had been suggested to circumvent this effect.

Three equal groups of heifers were either; treated by daily injection with norgestomet for 21 days to suppress oestrus and to produce oestrus and ovulation a few days after the end of treatment; or norgestomet was administered by depot release from a polymer implant placed under the skin of the ear and removed after nine days, in conjunction with an injection of oestradiol valerate at the time of innplantation to induce early luteal regression. This treatment also produced oestrus and ovulation a few days after implant removal. Untreated control heifers were inseminated on detected oestrus during a contemporaneous period. The resulting ova from all heifers were removed surgically for microscopic examination. Of the embryos collected from 21-day treated heifers, a number exhibited delayed cleavage. Asynchrony between the embryo and the stage of oestrus of the maternal uterus is known from embryo transfer studies to result in considerable reduction in successful conceptions. (Newcomb, 1976 ; Wright, 1981) This supports indications that delayed cleavage was a factor in the reduced fertility associated with prolonged progesterone treatment. It was not found in embryos from untreated controls, or from norgestomet implant treated heifers, giving some experimental support for the improved fertility which had been associated with the shorter treatment.

A group of heifers was implanted, to establish the time relationship between the implant removal, oestrus, and ovulation. After implant removal oestrus observations were made every four hours for four days. Starting eight hours after the end of oestrus each heifer was examined by endoscopy every four hours until ovulation was observed directly. The mean interval from implant removal to start of oestrus was 36.0 hours (range 24 to 52 hours); mean duration of oestrus was 17.8 hours (range 4 to 28 hours) ; mean time from end of oestrus to ovulation was 14.6 hours (range

10 to 20 hours) ; mean time from end of treatment to ovulation was 68.5 hours (range 52 to 92 hours). Twenty-six heifers ((86.7 per cent) were in oestrus during the observation period. Of these, 96.1 per cent ovulated between 60 hours and 84 hours after implant removal. This gave a promising basis for investigating the possibility of inseminating one or two times at fixed intervals after implant removal, with reasonable expectation of conception rates close to those achieved by inseminating after oestrus detection.

Based on the ovulation times following implant removal which were established in the previous study, two fixed time insemination regimes were selected to be investigated on farm heifers. These were a single insemination given 48 hours after implant removal, and a double insemination given at 48 hours and 60 hours after implant removal without reference to oestrus. Control heifers were inseminated at about 9.30 on the morning following oestrus. On each of three herds of Friesian dairy heifers, three equal groups were selected on a random basis at the time of implant removal. Conditions and semen were standardised as far as possible. Observation for oestrus was done at four hour intervals from 8 a.m. until midnight for four days following implant removal. Pregnancy diagnosis was by rectal palpation 60 days and 90 days after implant removal. Of 150 heifers, 88.0 per cent were in oestrus following treatment, with 90.9 per cent being in oestrus 24 hours to 48 hours after implant removal. Conception rates were 51.0 per cent for control heifers, 41.2 per cent for single fixed time insemination , and 65.2 per cent for double fixed time insemination

These experiments provided evidence that norgestomet implant treatment for a nine day period can achieve good control of oestrus and ovulation, without the reduction of fertility associated with longer progestagen treatments. The time interval between implant removal and ovulation was sufficiently specific to permit successful insemination on a predetermined fixed-time basis without the necessity to detect oestrus and without reducing conception rate. At the times selected for artificial insemination in this study, the single insemination regime did not result in a satisfactory conception rate. The conception rate following the double insemination regime gave a good conception rate, but the timing of the two inseminations would mean that one of these would have to be done at a time outwith normal working hours. This is a drawback, as it would present some difficulties with farm labour and Artificial Insemination Service inseminators, such that it would limit the usefulness of the method. Fertility on two of the farms was poor in the controls and single insemination groups. This was judged to be due to inadequate nutrition, and an analysis of the assessed food intakes supported this opinion. This does not invalidate the conclusions of the comparisons, because all three groups were selected randomly, and received the same rations in the same conditions. However it was a matter of some importance that required investigation.

FERTILITY AFTER OESTRUS SYNCHRONISATION AND INSEMINATION AT DIFFERENT TIMES

(PAPERS 2 and 3)

The preceeding studies indicated that treatment with a norgestomet implant for nine days would induce oestrus and ovulation in a precise, predictable and synchronous pattern, with acceptable fertility at the induced oestrus. If the method of oestrus control was to achieve its full potential on farms, it would be necessary to identify an acceptable, convenient timing for insemination. It would also be necessary to demonstrate that poor fertility was not an effect of the hormone treatment, but could be avoided by providing adequate nutrition. The following four studies set out to investigate these factors on large numbers of heifers. (PAPER 2)

Using 257 animals in the first study, heifers were implanted with a norgestomet implant for nine days. Double inseminations were made at the previously most successful timing of 48 and 60 hours after implant removal and first service conception rates compared with insemination at alternative timings. Untreated controls were inseminated at observed oestrus over a contemporary 28 day period. Mean first service conception rates for the controls which were seen in oestrus and inseminated was 73.2 per cent ; for heifers inseminated at intervals after implant removal - 48 and 60 hours 74.6 per cent , 48 and 65 hours 54.6 per cent and 48 and 72 hours 59.1 per cent.

The fertility rate at the more convenient times was lower than might have been hoped for, but the number of heifers in the various groups was relatively small. Conception rate of control heifers was calculated as a percentage of heifers observed in oestrus and inseminated, in order to establish a baseline for fertility on the farm. When conception was calculated as a percentage of the total number of heifers allocated to the control group, it was less than for the fixed-time insemination groups. The reason for this is explained. Rigorous, oestrus detection was done at frequent intervals for all treatment groups, although it did not influence the insemination of fixed-time insemination groups. Of the heifers not seen in oestrus, but inseminated at predetermined times, about 50 per cent became pregnant. This is a clear demonstration of the inadequacy of oestrus detection, even when done with a thoroughness which would be impossible under commercial farming conditions.

In the second study using 1486 heifers, all were implanted and inseminated at either 42 and 60 hours, first service conception rate 59.6 per cent ; or 48 and 72 hours after implant removal, first service conception rate 55.7 per cent. The difference was not significant. A wide variation in fertility between farms was noted, as has become expected, and was a matter for concern.

In the third study involving 375 animals, the heifers were weighed and their condition scored at the beginning of the trial and allocated on a stratified random basis to two feeding schedules, 'control' and 'supplemented'. Control groups were fed a diet considered by the farmer to be adequate. The components of the diet were analysed, and predicted rates of liveweight gain calculated. For the controls, this averaged 0.3 kg per day. The supplemented groups were given additional cereal to provide an extra 20 MJ of metabolisable energy per day which was calculated to increase the liveweight gain to 0.7 kg per day. The supplement was fed for six weeks before until six weeks after insemination. All the heifers were treated with norgestomet implants and inseminated without reference to oestrus at 48 and 60 hours after implant removal.

The average first service conception rate of heifers on supplemented rations on all farms was significantly higher, average 68.9 per cent than that for the controls, average 50.0 per cent. This effect carried over to the pregnancy rates for first return-to-service. It should be noted that average conception rates still varied widely between the six farms involved in this study, but that on each farm there was an important advantage in favour of the supplemented heifers. The lowest calving rate of the supplemented group on any of the farms was 59.0 per cent , a figure exceeded on only one farm by the control group. This study confirms the importance of adequate nutrition for optimum fertility. It also makes clear that although this is so, there are other important factors of husbandry that demand careful management.

In the fourth study, all 512 heifers were fed a ration calculated to provide a daily liveweight gain of 0.7 kg per day for a twelve week period starting six weeks before insemination. They were treated with norgestomet implants and inseminated at 48 and 60 hours after implant removal, with a first service conception rate of 69.3 per cent ; or 48 and 72 hours after implant removal with a first service conception rate of 66.2 per cent. The difference is not significant, and mean values calculated for the total number of heifers on each schedule conceal wide variations between farms. Conception rates for heifers on the 48 and 60 hour schedule on the four farms where this was done had a range from 61 per cent to 86 per cent. For heifers on the 48 and 72 hour schedule where this was done, the range was 59 per cent to 72 per cent. All of these results on all farms could be considered at least perfectly acceptable for heifers, and scanning the individual results does not reveal any obvious advantage in favour of one schedule.

The regime for fixed time insemination 48 and 60 hours after implant removal consistently produced better conception rates in all the studies than the 48 and 72 hour regime. Under practical farm conditions, however, the relatively small difference would not be noticable because of wide variations in fertility within herds and between farms. These differences persisted even under conditions of adequate nutrition with apparently good standards of husbandry and management. The practical advantages of this timing would make it preferable in most circumstances and it seems improbable that the difference, which can be identified under carefully controlled conditions , could be perceived under normal farming conditions. The advantage of adequate nutrition is again underlined by the consistently high conception rates achieved on all groups of heifers in this study.

Although a double fixed-time insemination regime would probably be commercially acceptable if normal conception rates can be achieved, there can be no doubt that a single fixed-time insemination would be more attractive. It offers greater convenience, less handling of cattle, and economies in semen and inseminator costs. A single insemination 48 hours after implant removal which was reported in an early study resulted in reduced conception rate. Nutrition during that study was inadequate for optimum fertility. Mature consideration of the time selected, with reference to the time of ovulation relative to implant removal, (and taking account of the range of intervals) which was established in the endoscopy study, suggested that delaying the insemination by six hours might show an improvement in fertility.

In the final study in PART 1 (PAPER 3) involving 430 heifers on five farms, a single fixed-time insemination 54 hours after implant removal was evaluated. All heifers were fed a ration to provide 0.7 kg liveweight gain for a twelve week period starting 6 weeks before insemination. The standard norgestomet implant treatment was used, and a single insemination, 54 hours after implant removal, was compared with double insemination regimes at 48 and

60 hours, and 48 and 72 hours. The resulting conception rates were ; single insemination (54 hours) 65.7 per cent, double insemination (48 and 60 hours) 66.2 per cent ; (48 and 72 hours) 62.1 per cent. The differences are not significant. Although the, by now expected, variation between farms was again observed (range 55 per cent to 76 per cent), the conception rates were acceptable for heifers in all groups on all five farms involved. Thus two fixed-time insemination regimes which would be convenient for use on commercial farms had been identified, both of which gave normal conception without the necessity for oestrus detection.

CONCLUSION

The studies reported development of a practical application of a potent progestagen, norgestomet, to induce oestrus and ovulation in heifers in a synchronous pattern. It used a polymer implant to provide a depot release of norgestomet. Predetermined timings for fixed-time insemination that did not depend on oestrus detection were established to achieve optimum conception rates, economy and convenience. These regimes took account of practical considerations of working conditions on commercial farms. The important role of nutrition around the time of insemination was identified, and nutritional standards appropriate for the achievement of acceptable conception rates were established. Data and information from the studies were presented to the Medicines Unit of M.A.F.F. for registration of the norgestomet implant, and a Product Licence was granted. In the event, for reasons of company management policy, the product was never marketed in the United Kingdom, although it has since been marketed in Europe.

The studies reported, took place in parallel with similar studies that were going on about the same time, to establish similar timed oestrus and ovulation induction, for controlled breeding in cattle. The other research teams were investigating the use of prostaglandin $F_{2\alpha}$ (dinoprost), or a prostaglandin analogue (cloprostenol). They had to contend with similar problems, make similar adjustments and take account of similar considerations.

SUMMARY OF PART 1

Very considerable improvements of the breed characteristics and genetic quality of dairy and beef cattle have been witnessed in the United Kingdom since the second world war. Constantly rising standards, and aspirations, of agriculture have been responsible, and artificial insemination has made a major contribution to the speed with which these changes have been possible.

The unique contribution of the artificial insemination sevices has been rigorous selection of bulls with superior genetic potential, coupled with rapid and widespread dissemination of semen from these bulls to every commercial breeder who wishes to take advantage of the service. The ability to freeze and store semen successfully for a number of years has increased its flexibility and usefulness.

One weak link in the artificial insemination success story has been oestrous detection, which was a prerequisite for artificial insemination, is inefficient, and appears not to be capable of significant improvement.

The ability to control the time of oestrus and ovulation by administration of exogenous hormones has offered the possibility to avoid, or minimise these oestrous detection difficulties. The methods by which this may be done have been described and discussed.

The principles of controlled breeding were, and are, broadly similar whatever hormonal compound is used to induce ovulation, differing in pharmacological details of drug administration and response, but not in general management principles. The development of one such method, a progestagen implant, has been described in detail because the author was intimately involved at a practical level. This is used to illustrate the nature of the difficulties encountered, the practical considerations involved, and the standards of success that could be expected when breeding control methods became generally available on the farm.

STUDIES TO BE REPORTED IN THESIS

That was the situation and those were the standards when the studies, which will be reported, were started. Development studies had been done in heifers mainly, but also in beef cows, because these are more readily available for such trials. Many times, careful distinctions were not made between the categories of cattle or specific analogues used. When general principles were being established this may have been acceptable. However results of controlled breeding are quite variable, and it is important to categorise details critically so that differences may be identified and accorded appropriate importance.

The purpose of the present studies was to increase knowledge of practical details and methods of using, specifically dinoprost, in techniques for controlled breeding and improving fertility management.

A single fixed-time insemination schedule is economical and convenient. It had been recommended for dinoprost following synchronised ovulation, without making any distinction between types of cattle. Heifers, dairy cows and beef cows are three distinct categories of cattle, each with specific characteristics and different typical methods of husbandry and breeding management. They will not necessarily each respond in the same way to treatment. Other ovulation control products warn of reduced conception rate when a single fixed-time insemination schedule is used. No published work specifically on dairy cows and single fixed-time insemination could be found in the literature. It was considered particularly important that information should be available for dairy cows, because they form the group most often treated with dinoprost, and they were not well represented in development studies. The first group of studies was to compare and evaluate single and double insemination schedules, following dinoprost synchronised ovulation , in the three categories of cattle ; heifers, beef cows and dairy cows.

An optimum calving interval is of the greatest importance for the economics of milk production, and beef production. Routine synchronised ovulation and fixed-time insemination has been shown to be a cost effective method of improving calving intervals, but it has not gained general aceptance. One disadvantage of such a routine approach is that it involves treating all cows, while a proportion can be expected to perform acceptably without any treatment. The second group of studies investigated selecting, at an early stage in the breeding period, cows that would be most likely to gain advantage from induction of oestrus to advance first insemination and shorten the calving-to-conception interval. Alternative criteria were investigated to attempt to identify groups that would contain mostly those cows that would gain advantage from early oestrus induction, whilst leaving untreated those that might be predicted not to require assistance.

Minimising delay in breeding has to be met at two levels; on a herd basis, and in individual problem cows. The section also includes studies to investigate the efficacy, and alternative methods, of using induction of oestrus and ovulation, on problem cows presented clinically as anoestrous. The objective was to achieve insemination and conception as soon as possible in cows that were already late for their intended breeding time.

Luteolysis has been the principal action of prostaglandin $F_{2\alpha}$ that has received attention for veterinary use : but it also has myometrial activity. Endogenous secretion of prostaglandin $F_{2\alpha}$ has been demonstrated to have a significnat relationship with rate of uterine involution *post partum*, which is often a limiting factor in return to fertile cyclicity. It has been speculated that delayed uterine involution may be caused by defective endogenous prostaglandin $F_{2\alpha}$ production. That speculation prompted the idea that endogenous deficiency might be compensated by administration of exogenous prostaglandin $F_{2\alpha}$. The last group of studies investigated the administration of exogenous prostaglandin $F_{2\alpha}$ early in the *post partum* period, to identify any effect on the time needed for uterine involution to be completed, and also to look for any effect on return to ovarian cyclicity , and fertility.

The methods available for the studies were limited by lack of laboratory facilities, or funds, for extensive hormone assays. In absence of experimental farm facilities, the work had to be done on commercial farms, which restricted the acceptable degree of disruption of herd routines by experimental procedures. The positive effect of these restrictions was that trials had to be designed in a strictly practical direction, which ensured relevance of the results for application in practical conditions.

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Artificial insemination of progestin (SC21009)-treated cattle at predetermined times

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Three experiments involving 265 heifers are described. In the first it was demonstrated that delayed embryo cleavage—a major factor responsible for subfertility following progestin treatment of 18 to 21 days duration—does not occur following a nine day treatment period during which heifers were implanted with a 6 mg SC21009* polymer implant in the ear. At the time of implantation an injection of 3 mg SC21009 and 5 mg oestradiol valerate was given intramuscularly. In the second experiment the time of ovulation following implant removal was studied using endoscopy. On the basis of the endoscopic findings, times for artificial insemination were chosen relating the time of insemination to the end of treatment. In the third experiment it was shown that insemination of treated cattle, whether or not observed in oestrus, 48 and 60 hours after implant removal, resulted in levels of fertility comparable to the non return to service rates of the bulls when used in AI service. The conception rate in this predetermined insemination time group was superior to the group inseminated on detection of oestrus whether the conception rate was calculated on the basis of the number of animals in the group or on the basis of the number of animals exhibiting oestrus. It is concluded that for the first time it is possible for a farmer to have all his heifers inseminated on a single day on a "by the clock" basis without the need for detection of oestrus and without any adverse effect on fertility associated with the progestin treatment.

Introduction

CONTROL of the oestrous cycle in cattle was first reported by Christian and Casida (1948) using progesterone by daily injection. Since then research workers throughout the world have used a variety of synthetic steroids of differing biological activity, by a variety of routes and at varying dose levels to sychronise oestrus. It is now generally accepted that most animals will exhibit oestrus during a four to six day period after progestin treatment, and that sub-fertility follows insemination at this time. Normal fertility levels return at the subsequent oestrus. The within-heifer and between-heifer variation in oestrous cycle length (Wishart 1972a) is such that to delay insemination until the second oestrus after treatment would result in a loss of precision and hence loss of the advantage gained by synchronisation. Oestrous detection is a time consuming task; it is not by chance that the majority of cattle inseminated are dairy cows since they are more easily observed for oestrous behaviour than other classes of cattle. It is the responsibility of the farmer to detect oestrus accurately and to call for artificial insemination at the appropriate time.

*Norgestomet, G. D. Searle & Co

Incorrect timing of insemination leads to reduced conception rates (Laing 1970, Deas 1970). Accurate oestrous detection becomes more difficult in a synchronised group due to the varying degrees of sexual activity being shown by the majority of animals. Non-return to service rates are known to fall when a number of cows are inseminated at one visit; Frappell (1969) attributed this to inaccurate oestrous detection. Relieving the farmer of the responsibility of oestrous detection would be advantageous under these circumstances. This could be achieved by a treatment which would result in a response so precise that artificial insemination could be made at a predetermined or fixed time after the end of treatment rather than in relation to oestrus in individual animals. Such a method, using progestinated intravaginal sponges, is available for sheep and has been successfully used in cyclic (McClelland and Quirke 1971) and in anoestrous (Cooper and others 1971) ewes. Attempts to increase the precision of response in cattle to permit artificial insemination at a predetermined time after steroid treatment have produced disappointing results. Oestrogens (Ulberg and Lindley 1960, Lantz and others 1968, Smith and Zimbleman 1968, Hansel and others 1961) and human chorionic gonadotrophin (HCG) (Lantz and others 1968, Spahr and others 1970, Graves and Dzuik 1968, Roche and Crowley 1972 and 1973, and Boyd and Tasker 1971) have both been used in association with progestin treatment. Some modified treatments have interfered with normal expression of oestrus and have resulted in very low levels of fertility (Roche and Crowley 1973, Boyd and Tasker 1971).

A potent progestin, SC21009, 17α-acetoxy-11β-methyl-19-nor-preg-4-ene-20, dione has been shown to suppress oestrus and ovulation at a daily intramuscular dose level of 0·14 mg (Wishart 1972a). In common with other progestin treatments of 18 to 21 days duration, SC21009 is associated with a reduction of fertility following artificial insemination at the controlled oestrus (Wishart 1973). A shorter nine-day treatment period in which SC21009 in subcutaneous polymer implants, in conjunction with oestradiol valerate at the time of implantation to induce early luteal regression, is not associated with the same reduced levels of fertility (Whitman and others 1972, Burrel and others 1972), nor when SC21009 implants are used for five days in combination with prostaglandin F_2a administered at the time of implant removal (Wishart 1974). Oestradiol valerate (Smith and Vincent 1973), in common with $PGF_2\alpha$ (Liehr and others 1972, Rowson and others 1972) is ineffective in shortening the oestrous cycle of cattle treated within five to seven days of oestrus. Wiltbank (personal communication 1973) first incorporated SC21009 with the oestradiol valerate injection given at the time of implant insertion. The antiluteotrophic properties of SC21009 inhibit the development of corpora lutea in cattle which have recently ovulated. Thus, the short nine-day progestin plus oestrogen treatment results in the majority of treated animals exhibiting oestrus with normal levels of fertility following artificial insemination after oestrous detection. The stage of the cycle at the start of treatment affects neither the numbers of animals in oestrus nor their fertility. The aims of the experiments reported in this paper have been to determine, in heifers treated with SC21009 and oestradiol valerate, the time relationship of oestrus and ovulation, the time of ovulation after treatment and the fertilisation rate of ova collected surgically; and finally, to determine the fertility of heifers inseminated at a predetermined time following removal of SC21009 implants.

Materials and methods

Two hundred and sixty-five heifers (Friesian and Friesian X Hereford), weighing 650 to 750 lbs and aged 15 to 18 months, were used. SC21009 was administered in one of two ways, by 21 daily intramuscular injections of 0·2 mg SC21009 dissolved in 2·0 ml sesame oil with 10 per cent benzyl alcohol added as a bacteriostat; or by subcutaneous polymer implants containing 6·0 mg SC21009, weighing approximately 0·125 gm and measuring 18 mm in length and 3 mm in diameter. Implants were placed subcutaneously in the ear superficial to the conchal cartilage using a simple narrow bore trocar and cannula. Implants were used in conjunction with an injection of 2·0 ml sesame oil with 10 per cent benzyl alcohol in which was dissolved 5·0 mg oestradiol valerate and 3·0 mg SC21009. The injection was given at the time of implantation. Implants were removed after nine days. The study was conducted in three parts. In parts 1 and 2 all the heifers were housed in an experimental farm unit and were fed 4½ lbs rolled barley, 4½ lbs concentrates with barley straw and water ad lib. They also had free access to a mineral mixture*. In part 3 heifers were run on commercial farms.

PART 1:
Effect of SC21009 on fertilisation and development of early embryos

The objective of this part of the study was to determine whether the implant treatment reported by Wiltbank (1973) was associated with retarded cleavage of early embryos as has been demonstrated following progestin treatments of 21 days duration (Wishart unpublished data 1973).

Materials and methods

Seventy-five heifers were included in this experiment. Twenty-five of these were in oestrus after treatment with SC21009 by daily injection and 25 were in oestrus following SC21009 implant treatment. Twenty-five untreated heifers, fed and maintained under the same conditions and exhibiting oestrus spontaneously, acted as controls. At approximately 9.30 am on the morning following the day on which oestrus had been observed, heifers were inseminated with semen from one ejaculate from a Hereford bull. The semen, stored in Kassou straws in liquid nitrogen, contained a total sperm count of 25×10^6 cells in 1 ml of diluent. One inseminator made all the inseminations. On the fourth day after oestrus (taking oestrus as Day 0), anaesthesia was induced using either thiopentone sodium† (5 gm by intravenous injection irrespective of bodyweight) or methohexitone sodium‡ (1 gm per 400 lbs bodyweight) and was maintained using halothane§ and oxygen by closed circuit. Once positioned in dorsal recumbency on an inclined operating table, an area 8 inches square anterior to the mammary glands was prepared for surgery. A 4 to 5 inch incision was made into the abdomen using electro cautery. The uterus was located and exteriorised. Using a fine glass tube, the oviduct ipsilateral to the developing corpus luteum was cannulated. Thirty to 50 ml of tissue culture medium 199 (TCM 199*) were injected into the horn of the uterus and massaged through the utero-tubal junction and via the fallopian tube and cannula to a collecting dish. Flushings were examined using a stereo-microscope to find the ovum. Once found it was examined at magnifications of up to ×80 to determine whether fertilisation had taken place, the number of blastomeres present and whether there were any gross abnormalities. In this study, assessment of fertilisation was based on criteria used by other authors (Hunter and others 1955, Quinlivan and Robinson 1967), namely, evidence of cleavage and the presence of spermatozoa in the zona pellucida. Failure of recovery was admitted after the fallopian tube had been flushed three times without success. The uterus was then returned to the abdomen and the incision closed in the appropriate manner.

*DPE Mineral Mixture, Tuco Chemical Co Ltd
†Intraval Sodium, May & Baker Ltd
‡Brietal Sodium, Eli Lilli & Co Ltd
§Fluothane, Imperial Chemical Industries Ltd

Results

Sixty-four ova (85·3 per cent were successfully recovered; of these 95·3 per cent were fertilised (Table 1). Neither the ovum recovery rate nor the fertilisation rate were significantly affected by either form of SC21009 treatment. However, treatment with daily injections of SC21009 was associated with early cleavage forms which were absent, not only in untreated animals but also in those animals treated with SC21009 implants. Abnormal embryos were recovered from animals in each group. These abnormalities made the classification of cleavage stage impossible as either one or more blastomeres were degenerating. All unfertilised ova appeared normal.

Discussion

The passage of the ovum from the fallopian tube is dependent on ciliary action and peristaltic contractions (Parker 1931) and the rate of transport varies in differing parts of the oviduct (Schilling 1958). Ciliary action and peristalsis are hormonally controlled and there is considerable evidence in laboratory animals that the rate of transport of ova from the fallopian tube can be varied by the administration of progesterone or oestrogen (Chang and Pincus 1951, Chang and Harper 1966). Abnormal progesterone (Lamond and Gaddy 1972) and oestrogen (Hill and others 1972) levels are found in superovulated cattle and may account for the accelerated ovum transport reported (Hafez and others 1963). The ovum recovery rate can therefore be a useful indirect index of hormonal status. From the data in Table 1 it is clear that the levels of oestrogen and progesterone present following SC21009 treatment are not such as to accelerate ovum transport. The ovum collection rates obtained in this study are comparable to those obtained in untreated cattle by other authors (Tanabe and Casida 1949, Boyd and others 1969).

In progestin-treated ewes subfertility at the controlled oestrus is due to fertilisation failure (Quinlivan and Robinson 1967). This is clearly not the case in SC21009 treated heifers. The fertilisation rate of 95·3 per cent obtained in this study agrees with the fertilisation rates reported by Kidder and others (1954) in heifers inseminated with semen from high fertility bulls. Indeed, the bull chosen for this work had a 30 to 60 day non-return to service rate of 78 to 80 per cent when used in commercial artificial insemination service. Judged by this index the bull is in the upper quartile of fertility for bulls of the same breed used for artificial insemination (Stables personal communication 1973).

*Medium 199, BDH Chemicals Ltd

TABLE 1: Ovum recovery, fertilisation rate and cell stage of early embryos recovered from SC21009-treated and untreated heifers

Treatment	Number							
	Heifers	Ova recovered	Ova fertilised	Embryos				
				1 cell	2 cell	4 cell	8 cell	Total
SC21009								
(a) 21 daily injections	25	21	20	—	1	4	14	19*
(b) 9 day implant	25	22	20	—	—	—	18	18*
Control	25	21	21	—	—	—	20	20*
Total	75	64	61	—	1	4	52	57

*Indicates that the cleavage stage could not be assessed in one or two of the fertilised ova recovered

Morphological assessment of functional normality or abnormality is difficult, for the early embryo and care must be exercised in assessing the biological significance of histological abnormalities. For instance, Hancock and Hovell (1961) reported that of 14 fertilised sheep ova classified as abnormal on morphological grounds, 13 developed on transfer to suitable recipients. Conversely, Alliston and Ulberg (1961) found that morphologically normal embryos collected from heat stressed ewes failed to develop on transfer to suitable recipients. The blastomere breakdown reported here has previously been reported in synchronised cattle (Thompson 1970) and in hamsters (Weakley 1967). There can be little doubt that complete blastomere degeneration will result in pregnancy failure, but there is strong evidence to suggest that the same is not necessarily true when partial degeneration occurs. Single blastomeres of the rat, mouse, rabbit and pig are capable of further development (Nicholas and Hall 1942, Seidal 1952, Tarkowski 1959, Daniel and Takahashi 1965 and Moore and others 1969); and in the case of the rabbit, single blastomeres have developed to term (Moore and others 1968). There is, therefore, a distinct possibility that a proportion of fertilised ova, showing partial blastomere degeneration, retain the ability to develop normally and continue to term.

Table 1 shows clearly the association between SC21009 treatment for 21 days by daily injection and the presence of early cleavage forms. Hamilton and Laing (1946) have reported recovering from the fallopian tubes, two-cell ova from 40·5 to 55·5 hours; four-cell ova from 44 to 65·8 hours, and eight-cell ova from 62·5 and 96 hours post-oestrus. The recovery of two and four cell ova on Day 4 (Oestrus=Day 0) is clearly abnormal. There is good evidence from ovum transfer experiments in cattle to indicate that delayed cleavage could result in reduced pregnancy rates even if the early embryos showing this phenomenon were normal in every other way. Most investigators using ovum transfer have matched closely the stage of the oestrous cycle of the donor and the recipient. Rowson and others (1969 and 1972) showed that a high degree of success could be achieved when recipients were in oestrus on the same day as the donor and that an asynchrony of 24 to 48 hours dramatically reduced transfer success rates. Division of the fertilised ovum proceeds at about the rate of one division every 24 hours (Winters and others 1952). The delayed cleavage noted in 26·3 per cent of the early embryos recovered from heifers treated with SC21009 for 21 days by daily injection, results in asynchrony of development between the embryo and the maternal endometrium, which if duplicated in an ovum transfer situation would lead to low transfer success rates. It is unlikely that any single factor is responsible for the depression of fertility associated with the use of SC21009 for 21 days. However, it is concluded that retarded cleavage of the early embryo is a contributory factor.

PART 2

Time relationship of oestrus and ovulation in heifers treated with SC21009 implants

The aim of this study was to determine, by observation of oestrus, the time of onset of oestrus after treatment, the duration of oestrus, and by endoscopy, the time of ovulation relative to oestrus and relative to implant removal.

Materials and methods

Thirty heifers were treated at random during their oestrous cycle with SC21009 implants. After implant removal, observations for oestrus behavoiur were made at four hourly intervals for four days. Starting eight hours after the end of oestrus, each heifer was examined by endoscopy every four hours until ovulation occurred using the method described by Wishart and Snowball (1973).

Results and discussion

Twenty-six heifers (86·7 per cent) were in oestrus during the observation period. The results (Table 2) show that the duration of oestrus of 17·8 hours (SD=6·4) is greater than that previously reported for heifers treated with SC21009 by daily injection for 21 days (13·5 hours; SD=6·19) and for untreated animals (14·0 hours; SD=4·69; Wishart 1974). None of the heifers treated in this study ovulated earlier than 10 hours after the end of oestrus. This agrees well with endoscopic findings in a larger study in which SC21009 was administered by daily intramuscular injection. In that case, no heifer ovulated earlier than eight hours after the end of oestrus (Wishart unpublished data 1974). The accumulated percentage of heifers ovulated of those in oestrus in this study, was 0, 23, 69·2, 96·1 and 100 at 48, 60, 72, 84 and 96 hours after implant removal, respectively. Of the four heifers not observed in oestrus within four days of treatment, three were found to have recently ovulated when examined by endoscopy on Day 5. These results relating the time of ovulation to the end of treatment indicate that it should be possible to inseminate treated heifers on one or more occasions at predetermined times after the end of treatment with the expectation of the same level of fertility as would result if insemination were made following oestrus detection in each animal.

TABLE 2: Time relationships of oestrus and ovulation in heifers treated with SC21009 implants

	Mean	SD	SE	Min	Max
Interval to oestrus	36·0	8·9	1·7	24	52
Duration of oestrus	17·8	6·4	1·2	4	28
End of oestrus to ovulation	14·6	2·6	0·5	10	20
End of treatment to ovulation	68·5	9·7	1·9	52	92

TABLE 3: Number of heifers treated with SC21009 implants

	Group I	Group II	Group III	Total
Herd B	12	13	11	36
Herd L	15	14	13	42
Herd P	26	24	22	72
Total	53	51	46	150

TABLE 4: Distribution of oestrus following removal of SC21009 implants

	Number of heifers						
	Treated	In oestrus (hours after implant removal)					Not in oestrus
		0—24	25—48	49—72	Total	Per cent	
Herd B	36	Nil	31	1	32	88·9	4
Herd L	42	1	33	5	39	92·9	3
Herd P	72	Nil	56	5	61	84·7	11
Total	150	1	120	11	132	88·0	18

PART 3

Oestrous response and fertility in inseminated heifers treated with SC21009 implants

The aim of this part of the study was to compare the fertility of heifers inseminated in the usual way after oestrous detection with those inseminated at predetermined times following SC21009 implant removal.

Materials and methods

One hundred and fifty heifers on three farms were treated at random in their oestrous cycle with SC21009 implants. At the time of implant removal they were sorted at random into three insemination groups of approximately equal size (Table 3). Those allocated to Group 1 were inseminated at approximately 9.30 am on the day after oestrus was first detected. Group 2 and Group 3 heifers were inseminated whether they had been observed in oestrus or not. The times for insemination were chosen on the basis of the results of Part 2 of this study. Heifers in Group 2 were inseminated 48 hours after implant removal and those in Group 3 were inseminated 48 hours after implant removal and again, 12 hours later. Inseminations were made by inseminators of the local artificial insemination centre using semen from bulls of the farmers' choice. The same Friesian bull was used to inseminate heifers in Herd B and Herd L and a Simmental bull was used for heifers in Herd P. Over a four-day period following implant removal, observations for oestrus were made at four hour intervals from 0800 hours to midnight. On the 20th and 23rd day after insemination a blood sample was taken to give an early indication of pregnancy (Robertson and Sarda 1971, Mauleon 1974, Wishart and others in press). From day 16 to 28 following implant removal, observations for returns to service were made at 9 am, 4 pm and midnight. The same bull was used for inseminations made at this time except for Herd P heifers, semen from a Hereford bull was used for returns to service in this herd. Animals inseminated between 16 to 28 days following implant removal were used as controls. Fertility was also compared with the 30 to 60 or 90 to 120 day non-return to service rate for the bulls when used in artificial insemination service. On Day 28, Devon bulls were run with heifers in Herd B and L. Pregnancy was diagnosed by rectal palpation 60 and 90 days after implant removal.

Results and discussion

Implants were removed from all treated heifers without difficulty. No tissue reaction was present at the implantation site. The oestrous response following treatment is shown in Table 4. The precision of response was striking with 90·9 per cent of heifers in oestrus being in that state between 24 to 48 hours following implant removal. One hundred and thirty-two (88·0 per cent) of 150 treated animals were in oestrus after treatment. All heifers allocated to Group 1 in Herd B and L were seen in oestrus.

The number of animals pregnant to first insemination at 90 days is shown in Table 5. The conception rates in Group 1, 2 and 3 were 51·0 per cent, 41·2 per cent and 65·2 per cent respectively. The additional insemination at 60 hours after implant removal resulted in a significantly higher level of fertility than the single insemination at 48 hours after implant removal ($P=0·025$). The effect was particularly striking in Herds B and L in which fertility in Groups 1 and 2 was low. The pregnancy rate of Group 3 heifers was considerably higher than in Group 1 and 2 of all herds when the calculation was made on the basis of the number of animals treated. This is due to the fact that although oestrus was not observed in 18 heifers, seven of the 14 allocated to fixed time artificial insemination groups conceived. This confirmed the endoscopic findings in Part 2 and indicated that in non-oestrous heifers ovulation was sufficiently controlled by treatment to allow conception to artificial insemination at the predetermined times chosen.

Forty-eight heifers were in oestrus 16 to 28 days after implant removal, seven of these (Herd L–1; Herd P–6) were in oestrus for the first time after treatment. The remainder were returned to service. Forty-three were inseminated and acted as controls. (Herd B–13; Herd L–18; Herd P–12.) Twenty-two (51·1 per cent) conceived (Herd B–9; Herd L–9; Herd P–4). Subsequently 20 heifers (Herd B–6; Herd L–14) were diagnosed pregnant to service by the bulls when examined 90 days after implant removal.

For optimum fertility the provision of adequate energy and protein levels in the diet is important. Diets recommended by ADAS for 15-month old Friesian heifers gaining 1·5 lbs per day, provide approximately 8·5 lbs SE and 1·0 to 1·1 lbs DCP daily (MAFF 1973). Most of the cattle in the herds were older than 15 months. In Herd B and L growth had virtually stopped. In Herd P heifers were continuing to grow well inspite of adverse weather conditions on a diet providing some 8·8 lbs SE and 0·96 lbs DCP daily (5 lbs barley, 10 lbs good hay and straw ad lib). Herds B and L were fed diets

TABLE 5: Inseminated heifers pregnant at 90 days

Herd	Group I			Group II			Group III		
	Inseminated	Pregnant	Per cent	Inseminated	Pregnant	Per cent	Inseminated	Pregnant	Per cent
B	12	4	33·3	13	5	38·5	11	8	72·7
L	15	5	33·3	14	2	14·3	13	7	53·8
P	22	16	72·7	24	14	58·3	22	15	68·2
Total	49	25	51·0*	51	21	41·2	46	30	65·2

*Number of heifers pregnant of those treated = 47·2 per cent containing levels of energy and protein considerably below those recommended. Herd B was provided with some 3·5 lbs SE and 0·54 lbs DCP (10 lbs good hay plus whatever grass was available on bare paddocks in December), and Herd L was provided with some 6·22 lbs SE and 0·84 DCP (20 lbs silage, 3 lbs barley and 4 lbs average hay). Heifers in Herds B and L were out wintered and Herd P was wintered in a semi-open yard. When the conception rates of Herd B and L were pooled for Groups 1 and 2 and compared with the conception rates for the same groups in Herd P, the difference was statistically significant (P=0·01). It was concluded that an inadequate diet was contributory to the low overall fertility obtained in Herds B and L.

Another factor influencing pregnancy rate to first insemination is the choice of bull. Kidder and others (1954) and Bearden and others (1956) demonstrated differences in fertilisation rates of ova collected from heifers inseminated with semen from bulls of high and low fertility. Kidder and others (1954) reported essentially similar embryonic mortality rates in heifer groups inseminated with high and low fertility bulls. Boyd (1965) deduced from these figures that failure to achieve pregnancy with bulls of high fertility depends mainly on embryonic mortality, while for bulls of low fertility pregnancy failure depends on both fertilisation failure and embryonic death rate. The December 90 to 120 day non-return to service rate for the Friesian bull used on Herd B heifers that month was 67 per cent for 1056 first inseminations, and the January figure was 64 per cent for 764 first inseminations from the same bull used that month on heifers in Herd L (Dawson personal communication 1974). The January 30 to 60 day non-return to service for the Simmental bull used that month on heifers in Herd P was 81·4 per cent for 521 first inseminations (Stables personal communication 1974). Assessing bull fertility on a non-return to service rate is both widely accepted and widely used by artificial insemination centres, since it is a figure which can be conveniently calculated from records routinely kept. However, it is not an accurate method of assessing conception rate since some females failing to conceive are not reinseminated for a number of reasons (failure to detect oestrus, embryonic mortality, anoestrus, culling and use of natural service). Estimates of the differences between the pregnancy rate at 60 to 90 days and the non-return to service rate at that time vary—2·7 per cent (Bonadonna 1950), 4·8 per cent (Zwart 1964) and 10 to 12 per cent (Lutke-Vestert 1964). The Group 3 conception rates in all the test herds was of the expected order based on the non-return to service rates for the bulls used. The conception rate in Group 3 was in all herds greater than the conception rate following a single insemination at 48 hours after removal of SC21009 implants; and was comparable to or greater than the fertility resulting from insemination on detection of oestrus. It is concluded that artificial insemination at 48 and 60 hours after removal of SC21009 implants is not associated with a reduction in fertility when the comparison is made with animals returning to service after treatment or with the non-return to service rates for the bulls used in artificial insemination service.

General discussion

SC21009 implant treatment for nine days as described, reduced the oestrous cycle length in heifers treated within five days of oestrus. In parts 1 and 2, nine heifers were treated on the day of oestrus or on the next two days. Eight were in oestrus within 48 hours of implant removal. All 10 heifers treated on Days 3, 4 or 5 were in oestrus over the same period following implant removal. These findings confirm those made by Wiltbank (1973) and subsequently by Mauleon, in a personal communication (1974).

Two explanations can be made for the superior fertility reported for Group 3 heifers. It could be argued that progestin treatment might interfere with normal cervical mucus production and viscosity. Altered patterns of cervical mucus production have been reported in progestin treated ewes (Smith and Allison 1971), and reduced sperm penetration in cervical mucus has been reported in progestin-treated women (Zanartu 1964) and in progestin (Melengesterol Acetate) treated cattle (Boyd and others 1972). Any such progestin effect could be expected to result in a reduced fallopian tube sperm population. Sperm numbers reach a maximum at the uterotubal junction and oviducts about eight hours after artificial insemination (Dobrowolski and Hafez 1970). The ovum fertilisation rates obtained in Part 1 of this study do not suggest an alteration in the viscosity of cervical mucus nor do they suggest a failure to establish an adequate cervical sperm reservoir. If the latter were the case one would expect Group 3 fertility levels to be significantly greater than those in heifers inseminated after oestrous detection. It is suggested that the effect of a double insemination is not simply that of reinforcing an inadequate cervical reservoir or of compensating for impaired sperm transport. A more likely explanation is that the response following SC21009 implant treatment is not sufficiently precise as to permit high fertility to a single insemination at a predetermined time following implant removal. The average sperm survival time is approximately thirty hours (Laing 1945), whilst that of the ovum is a matter of only a few hours. The mean interval from implant removal to ovulation of 68·5 hours (SD 9·7; Table 2) supports this explanation.

In this study heifers in Part 3 were inseminated in the morning and again in the evening. From a practical point of view this is inconvenient to artificial insemination centres since the additional insemination was made outside normal working hours. However, the inconvenience to the centres must be balanced against the convenience to the farmer of having all his animals inseminated on a single day, on a "by the clock" basis, without the necessity for oestrous observation and without an adverse effect on fertility due to treatment with SC21009 implants.

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RÉSUMÉ.—Dans cet article sont décrites trois expériences portant sur 265 génisses. Au cours de la première, il est démontré que la division embryonaire retardée—important facteur entrainant la sous-fertilité à la suite d'un traitement de progestérone d'une durée de 18 à 21 jours—n'a pas lieu à la suite d'une période de traitement de huit jours pendant lesquels les génisses subissaient une implantation de 6 mg SC21009 polymére dans l'oreille. Au moment de l'implantation, on effectuait une injection intramusculaire de 3 mg SC21009 et de 5 mg de oestradiol valerate. Au cours cours de la deuxième expérience, on étudie le moment d'ovulation après retrait des implants par l'endoscopie. A partir des résultats donnés par l'endoscopie, on peut choisir le moment favouable à l'insémination artificielle en fonction du temps d'insémination par rapport à la fin du traitement. Dans la troisième expérience, il est démontré que l'insémination des bovins traités, observé ou non pendant l'oestrus 48 et 60 heures après retrait de l'implant, a donné des taux de fertilité comparables à ceux des non-retours à la saillie des teureaux utilisés pour les centres d'insémination artificiels. Le taux de conception dans ce groupe de prédétermination du temps d'insémination était supérieur à ceux du groupe inséminé par détection de l'oestrus, que le taux de conception soit calculé sur la base du nombre d'animaux du groupe ou sur la base du nombre d'animaux chez lesquels se manifestait l'oestrus.

On en conclue que, pour la première fois, il est possible que l'éleveur fasse inséminer toutes ses génisses en un seul jour "sur rendez-vous" sans avoir besoin de déceler l'oestrus et sans que cela ne présente de risque pour la fertilité associée avec le traitement de progestérone.

ZUSAMMENFASSUNG.—Es werden drei Experimente mit 265 Jungkühen beschrieben. Im ersten wurde aufgezeigt, dass verzögerte Eiteilung—die Hauptursache der auf eine 18-21-tägige Progestinbehandlung folgenden verringerten Fertilität—nicht nach einer 9-tägigen Behandlung vorkommt, in deren Verlauf Jungkühe eine 6 mg SC21009 Polymer-Implantation ins Ohr erhielten. Zu gleicher Zeit wurde den Tieren eine intramuskuläre Injektion von 3 mg SC21009 und 5 mg Oestradiol Valerat vera-bfolgt. Im zweiten Experiment wurde mit Hilfe von Endoskopie der Ovulationszeitpunkt nach Entfernung der Implantation ermittelt. Anhand der endoskopischen Befunde wurde der Zeitpunkt für künstliche Besamung gewählt und mit dem Behandlungsabschluss in Bexiehung gebracht. Im dritten Experiment wurde aufgezeigt, dass bei Besamung von behandelten Rindern im K.B.-Dienst, gleichgültig ob 48 und 60 Stunden nach Entfernen der Implantation Brunstanzeichen festfestellt wurden oder nicht, eine Fertilität erzielt wurde, die den Befruchtingsziffern der vom Bullen gedeckten Rinder entspricht. Die Befruchtungsziffern bei dieser Gruppe mit vorherbestimmtem Besamungszeitpunkt waren höher als diejenigen der bei Feststellung der Brunst besamten Gruppe, unabhängig davon, ob sie aufgrund der in der Gruppe enthaltenen Tieranzahl oder aufgrund der Brunstanzeichen aufweisenden Tieranzahl kalkuliert wurden.

Daraus folgt, dass es zum ersten Mal für einen Tierzüchter möglich ist, seine sämtlichen Jungkühe an demselben Tag "nach der Uhr" basamen zu lassen, ohne die Notwendigkeit, den Brunsteintritt festzustellen und ohne durch die Progestinbehandlung verursachte nachteilige Auswirkungen auf die Fertilität.

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Papers and Articles

Fertility of norgestomet treated dairy heifers

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Four studies are described involving 2573 Friesian heifers treated with norgestomet and oestradiol valerate to control the ovarian cycle. All treated animals were inseminated at fixed times following treatment. In study 1 insemination 48 and/or 60 hours after treatment resulted in a non-significant greater proportion of heifers becoming pregnant than at 48 × 72 hours. Large variations in fertility among farms were recorded in field trials of norgestomet (Searle) and oestradiol valerate. In study III the effect on fertility of nutrition, weight and body condition was studied. In section IV a pregnancy rate to fixed time insemination of 66·8 per cent of 500 heifers inseminated at 48 and 60 or at 48 and 72 hours after norgestomet/oestradiol valerate treatment was obtained. Heifers in study IV were fed a balanced ration designed to provide a predicted daily liveweight gain of 0·7 kg for a 12-week period starting six weeks prior to the date of insemination. The range of fertility was from 59·0 per cent to 85·7 per cent.

THE high biological activity of norgestomet (Wishart 1972) (17a-acetoxy-IIB-methyl-19-nor-preg-4-ene 20, dione) enables its incorporation into a small polymer implant which has been shown to control the breeding cycle of cattle (Burrell and others 1972, Whitman and others 1972). A short (nine day) implantation treatment period given in conjunction with a combination of oestradiol valerate and norgestomet in an intra-muscular injection is not associated with reduced fertility which is commonly found after long (18-24 day) progestin treatments. (Burrell and others 1972, Whitman and others 1972, Mauleon 1974, Wishart and Young 1974).

The oestrous response following norgestomet (Searle SC 21009) treatment is precise. Wishart and Young (1974) reported that 87·3 per cent of 150 treated heifers were observed in oestrus during the period 25-72 hours after implant removal. In heifers the interval from implant removal to ovulation was 68·5 hours (SD 9·7) (Wishart and Young 1974, Wishart 1975). This degree of synchrony of oestrus and ovulation permitted artificial insemination at a fixed time after the end of treatment. This has two main advantages; the elimination of oestrous detection which is time consuming and more difficult in a group of synchronised animals; and it gives non-oestrous but synchronously ovulating animals the opportunity of becoming pregnant. Oestrus is not a prerequisite for pregnancy so long as insemination is made close to and preferably prior to ovulation. Wishart and Young (1974) reported that half of the non-oestrous heifers inseminated at fixed times after implant removal became pregnant. They also reported that insemination at 48 and 60 hours after implant removal resulted in a significantly greater proportion of pregnant heifers than did a single insemination at 48 hours. However, a 12 hour interval between insemination is usually inconvenient, a single insemination or two inseminations spaced 24 hours apart would be preferable.

The proportion of treated animals calving to fixed time insemination is one of the most important factors determining the economic benefit likely to accrue from a controlled breeding programme. In this country it is usual for heifers to be reared on a system which maximises growth from grass. In winter when most heifers are mated the amount of food is usually restricted and often poor in quality. The onset of puberty in heifers is influenced by age, breed, growth rate, liveweight, level of nutrition and heterosis (Christian 1957, Wiltbank and others 1959, Reynolds and others 1963, Wiltbank and others 1966, Wiltbank and others 1957, Donaldson and Taaken 1968 and Wiltbank and others 1969). Pregnancy rates in cattle are known to increase with increasing body weight and condition (Osborne 1960, Donaldson 1962, Donaldson and others 1967, Lamond 1969). The effect of nutrition on the fertility of cattle can be accurately measured when controlled breeding techniques such as norgestomet/oestradiol valerate are used to standardise other variables. A total of 2630 hefers were used in four controlled breeding studies. In study I alternative times of insemination were compared with the 48 and 60 hour timing used successfully by Wishart and Young (1974). In study II the two most successful insemination timings in the first study were used on heifer groups throughout the United Kingdom. In study III the effect of level of nutrition, weight and condition score on fertility of heifers treated with norgestomet/oestradiol valerate was measured and the findings were implemented in study IV.

Materials and methods

Using a special applicator a polymer implant was placed subcutaneously in the outer surface of the ear of all treated animals (plate 1). Each implant contained 6·0 mg norgestomet, measured 3 mm × 18 mm and weighed approximately 0·125 gm. At the time of implantation a 2·0 ml injection containing 5·0 mg oestradiol valerate and 3·0 mg norgestomet dissolved in sesame oil was given intramuscularly. After nine days in position the implant was removed by nicking the skin at the outer end of the implant and expressing it with the thumb.

In study I three trials were run on two farms. 257 Friesian and Hereford X Friesian heifers aged 15 to 18 months and of average body weight 330 kg were allocated to four groups on a stratified random basis. The heifers in group I were untreated controls which were observed for 28 days. They were inseminated when the farmer saw them in oestrus. Heifers in groups II, III and IV were treated with norgestomet/oestradiol valerate. They were inseminated at 48 and 60, 48 and 65 or 48 and 72 hours respectively after implant removal. Oestrus was detected in these groups by observation at four-hourly intervals for five days. In each trial heifers were inseminated with semen from the same bull. No repeat services were made. Pregnancy was diagnosed by rectal palpation 42 days after insemination.

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In study II, 1486 predominantly Friesian or Hereford X Friesian heifers in 48 groups on 25 farms were treated as described and were inseminated either at 48 and 60 or 48 and 72 hours after implant removal. The heifers were inseminated with semen from bulls of the farmers' choice. For groups of more than 35 animals at least two inseminators were used to avoid the possibility of fatigue. At the return to service period a standard remating procedure was used for each group. Farmers who chose to rebreed their heifers either ran bulls with them from day 16 after implant removal or observed for oestrus and had them reinseminated. Heifers were examined for pregnancy by palpation per rectum 42 days after the fixed time insemination and all those in remated groups were re-examined 70 days after implant removal. It was then possible to differentiate between animals pregnant to fixed time insemination and those pregnant to natural mating or artificial insemination at the return to service period 16 to 20 days after implant removal.

In study III a total of 375 Friesian heifers aged 15 to 18 months with a mean body weight of 348 kg (± 38) were involved in six trials on commercial farms in Hampshire. The heifers were weighed and scored for body condition on a subjective 0-5 scale at the start of the study. They were allocated to two feeding groups (control and supplemented) on a stratified random basis. The control groups were fed a ration considered by the farmer to be adequate. The components of the ration were analysed and using estimates of food consumption, predicted rates of liveweight gains were calculated. For the control groups this averaged approximately 0·3 kg per day. The supplemented groups were given extra cereal to provide an additional 20 MJ of metabolisable energy per day which was calculated to increase the rate of liveweight gain to 0·7 kg per day. The supplemented ration was fed for a 12 week period starting six weeks before insemination. All the heifers were treated with norgestomet/oestradiol valerate. Implants were removed from both groups on the same day. No observations for oestrus were made. Each man inseminated approximately equal numbers of heifers in control and supplemented groups 48 and 60 hours after treatment. Semen from the same bull was also equally used. Animals in oestrus were remated at the return to service period (16 to 28 days after implant removal) using the bulls which would colour mark their progeny. All animals were examined for pregnancy by palpation per rectum 42 and 70 days after implant removal. At the time of the 48 hour insemination and at the 42 days pregnancy examination the heifers were reweighed and were scored again for body condition. Calving dates were recorded.

In study IV, 512 Friesian heifers in 15 groups were fed a diet calculated to provide a daily liveweight gain of 0·7 kg for a period of 12 weeks starting six weeks before the time of insemination. All heifers were treated with norgestomet/oestradiol valerate and were inseminated 48 and 60 or 48 and 72 hours after implant removal. No observations for oestrus were made. Pregnancy was diagnosed by palpation per rectum 42 days after implant removal.

Results

STUDY I

In study I all implants were removed without difficulty. None was lost and there was no detectable tissue reaction at the implantation site. Of the 57 control heifers 41 (71·9 per cent) were observed in oestrus over a four week period. Of the 200 treated heifers 191 (95·5 per cent) were observed in oestrus during the five day observation period. 189 were in oestrus during the period 25 to 72 hours after implant removal. The pregnancy rates obtained are shown in Table 1.

In the group of 57 control animals 30 (52·6 per cent) became pregnant. The mean oestrus detection rate was 72 per cent, and therefore the pregnancy rate based upon those observed in oestrus was 73·2 per cent. The overall pregnancy rate of the 200 treated heifers was 63 per cent. Analysis of

TABLE 1: Percentage of treated and untreated heifers pregnant of those inseminated—Study I

	Untreated		Treated		
	Group I		Group II	Group III	Group IV
Trial	a	b	(48 – 60)	(48 + 65)	(48 + 72)
1	75(12)	60(15)	80(25)	48(25)	58(24)
2	75(16)	60(20)	82(22)	57(21)	61(23)
3	69(13)	41(32)	60(20)	60(20)	60(20)
Mean	73·2	52·6	74·6	54·6	59·1

a = per cent of those inseminated.
b = per cent of those in group.

the pooled data showed that insemination at 48 and 60 hours resulted in a significantly greater proportion of pregnancies than did insemination at 48 and 65 hours ($\chi^2=5·87$; df=1; P<0·025) or in the controls when the calculation was made on the basis of the total in the group ($\chi^2=6·51$; df=1; P<0·025). The proportion of heifers pregnant following insemination at 48 and 60 hours, at 48 and 72 hours and in the control group calculated on the basis of the numbers inseminated did not vary significantly. Of the 41 treated heifers not observed in oestrus after implant removal 23 (56·0 per cent) became pregnant to fixed time insemination.

STUDY II

In this study 56 (3·8 per cent) of the 1486 treated heifers were subsequently excluded. 3 (0·2 per cent) lost the implant during treatment. 17 (1·1 per cent) were pregnant at the start of treatment and 36 (2·4 per cent) were freemartins. The pregnancy rates following artificial insemination at 48 and 60 hours or 48 and 72 hours after implant removal are shown in Table 2. Of the 1010 heifers inseminated at 48 and 60 hours, 602 (59·6 per cent) were pregnant when examined at 42 days. Of the 420 heifers inseminated at 42 and 72 hours after implant removal, 234 (55·7 per cent) became pregnant. The effect of timing of insemination on the proportion of heifers becoming pregnant was not statistically significant. Of the 494 heifers failing to conceive to fixed time insemination 170 were not remated; 186 were observed for reinsemination and the remainder were run with bulls. The effect of the method of rebreeding at the return to service period had a statistically significant effect on the proportion of heifers pregnant within the 30 days of treatment ($\chi^2=15·7$; P<0·001). A greater proportion was pregnant in groups remated by natural service.

STUDY III

In study III 2·9 per cent of 375 treated heifers were found to be freemartins when examined for pregnancy. These animals were excluded from the data. The results of pregnancy diagnosis and calving data are shown in Table 3. There was a wide variation in fertility between farms, but on each farm a higher proportion of heifers calved to fixed time insemination. Statistical analysis of the pooled data showed that the proportion of heifers calving to fixed time insemination was significantly greater for those in the supplemented groups ($\chi^2=13·0$; df=1; P<0·001). The combined proportion of heifers calving to fixed time insemination and to

TABLE 2: Percentage of treated heifers pregnant to fixed insemination; and percentage pregnant within 30 days of implant removal (fixed time AI and remating at the first return)—Study II

		Per cent pregnant		
Time of insemination	Total treated	Fixed time insemination	Combined fixed time and remating	
			Nat service	AI
48 and 60 hours	1010	59·6	82·1	70·9
48 and 72 hours	420	55·7	80·7	65·7
Mean		58·5	81·8	69·6

TABLE 3: Pregnancy and calving rates of treated heifers on control and supplemented rations—Study III

Ration	No of heifers	Fixed time insem. Pregnant (per cent)	Calved (per cent)	Fixed time and remating	Insemination first return
Control	178	52·8	50·0	73·6	66·4
Supplemented	186	67·2	68·9	84·4	83·5

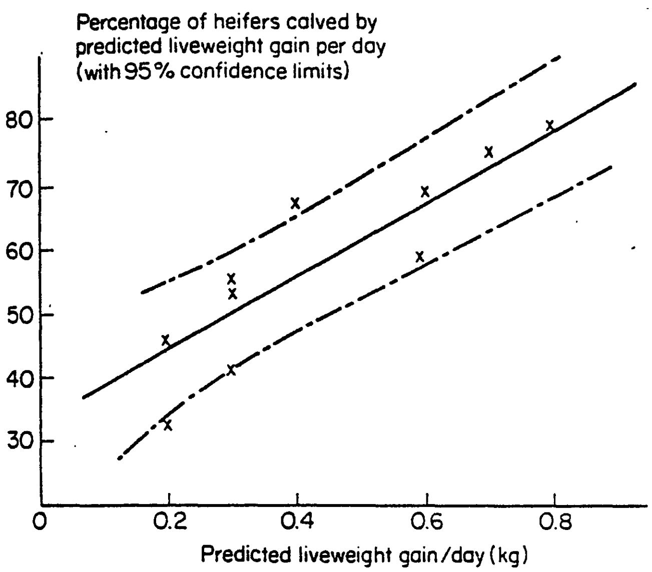

FIG 1:

breeding at the first return to service period was also significantly greater in the supplemented groups ($\chi^2=13{\cdot}1$; df=1; $P<0{\cdot}001$). No significant relationship was found between liveweight or condition score and fertility. Nor was there one between changes in liveweight or condition score and fertility. Fig 1 shows the calving rate of treated heifers in all groups plotted against predicted daily liveweight gain. There was a tendency for the proportion of heifers calving to increase with increasing rate of predicted liveweight gain.

STUDY IV

In study IV 12 heifers (2·3 per cent) of the 512 treated heifers were excluded from the pregnancy data because they were freemartins; 334 (66·8 per cent) of the remaining 500 heifers became pregnant to fixed time insemination. 264 (66·2 per cent) of the 399 heifers inseminated 48 and 72 hours after implant removal became pregnant, the corresponding figure for 101 heifers inseminated 48 and 60 hours after implant removal was 70 (69·3 per cent) (Table 4).

Discussion

The oestrous response in study I was similar to that reported by Wishart and Young (1974). The farmers ability to detect oestrus in untreated heifers varied from 59·0 per cent to 80·0 per cent. Farmers frequently calculate pregnancy rate on the basis of the numbers inseminated rather than on the total number of animals. In Table 1 the pregnancy rate of untreated controls is presented on the basis of the total number and on the number seen in oestrus. Only when the oestrous detection rate was high (trial 1 and 2) did the proportion of untreated heifers becoming pregnant approximate to the results achieved in the treated groups. A greater proportion of treated heifers became pregnant in groups inseminated 48 and 60 hours after implant removal. Under farm conditions, it is likely that differences in fertility among farms will mask the advantage of the 48 and 60 hours insemination over insemination 48 and 72 hours after implant removal. This was the case in study II. There was a wide

TABLE 4: Heifers pregnant to fixed time insemination of those treated with norgestomet (SC 21009) oestradiol valerate—Study IV

Timing of insemination

Farm	48 x 60 hours Treated	Repeat	Per cent	48 x 72 hours Treated	Repeat	Per cent
A	17	12	70·6	17	12	70·6
B	14	12	85·7	14	10	71·4
C	52	35	67·3	57	36	63·2]
	18	11	61·1	—	—	—
D	—	—	—	73	51	69·9
E	—	—	—	38	23	60·5
	—	—	—	39	23	59·0
F	—	—	—	25	17	68·0
	—	—	—	31	21	67·7
G	—	—	—	25	16	64·0
H	—	—	—	27	17	63·0
I	—	—	—	53	38	71·6
Total	101	70	69·3	399	264	66·2

range of fertility. For the groups inseminated 48 and 60 hours after implant removal the fertility ranged from five pregnant of 37 treated to 11 pregnant of 12 animals treated. For the 48 and 72 hour insemination, the comparable figures were from one pregnant of eight treated to 15 of 18 animals treated pregnant.

Farmers choosing to reinseminate heifers during the return to service period experienced some difficulty in detecting oestrus, some animals pregnant to fixed time insemination were thought to have been in oestrus and were reinseminated. Confusion may have arisen because during the return to service period of seven to 12 days there were many days during which more than one animal was in oestrus. It is known that inaccurate oestrous detection can occur under such circumstances (Frappell 1969). This coupled with an oestrus detection rate unlikely to exceed 60-70 per cent accounts for the greater proportion of heifers pregnant when fixed time insemination is followed by natural service at the return to service period. The widespread use of aids for detecting oestrus such as heat mount detectors (Baker 1965, Boyd and Hignett 1968, Williamson and others 1972) and surgically prepared bulls (Lang and others 1968), bears testimony to the difficulty experienced by some farmers in detecting oestrus and the length to which they will go to improve detection rate.

Farmers are accustomed to assessing fertility on the basis of non return to service rates. The results in these studies are based on established pregnancy rates. Most would consider acceptable a pregnancy rate to first insemination of 60·0 per cent. In study II half of the 48 hiefer groups had a pregnancy rate to fixed time insemination less than 60·0 per cent. A pregnancy rate to first insemination of less than 50 per cent would, in most cases, be considered unsatisfactory and 11 (22·9 per cent) of the groups fell into this category. At the other end of the fertility scale 10 (20·8 per cent) of the groups had a pregnancy rate to fixed time insemination greater than 70 per cent. It may well be that in these groups conditions for optimum fertility were operative at the time of insemination. Alternatively it may be that with comparatively small groups of animals calculation of pregnancy rate on a percentage basis gives an erroneous impression of super fertility.

Study I showed that norgestomet/oestradiol valerate treatment did not reduce fertility of treated heifers when compared with fertility of untreated animals inseminated with semen from the same bull. Nevertheless, under conditions of practical farm usage, unacceptable results occurred in a proportion of heifer groups. On these farms the level of nutrition provided during the immediate pre-service period was lower than recommended standards (MAFF 1973). In study III the results of the pregnancy diagnosis at 42 days and the calving data showed that a significantly greater proportion of heifers in the supplemented groups became pregnant. The supplemented ration was designed to support maintenance and a daily liveweight gain of 0·7 kg. The lowest calving rate in the six supplemented groups was 59·0 per cent of 56 animals. In only one control group was the calving rate greater than 59·0 per cent.

The tendency shown in Fig 1 for fertility to increase with increasing predicted liveweight gain should be interpreted with care because the farms were under different management systems and a proportion of the difference in fertility could have been a reflection of management factors other than nutrition.

In study III neither weight nor body condition significantly affected fertility. The average weight of the heifers was 348 kg. The mean group weight ranged from 264 kg (± 23 kg) to 423 kg (± 35 kg). The herd with the smallest heifers achieved pregnancy rates of 53·0 per cent of 34 animals in the control group and 67·0 per cent of 39 in the supplemented group. The comparable figures for the herd with the largest heifers were 32·0 per cent of 28 animals and 59·0 per cent of 28 respectively.

It is concluded from the results of study III that the traditional heifer ration provided in the six trials was insufficient to permit an acceptable level of fertility; also, that the provision of supplementary feeding to sustain a predicted daily liveweight gain of 0·7 kg resulted in a significantly greater proportion and an acceptable proportion of pregnant heifers.

The pregnancy results in study IV contrast in two ways to those obtained in study II. The overall pregnancy rate was higher (66·4 per cent) of 420 heifers v 58·5 per cent of 1430 heifers) and the range of fertility was narrower. In study IV the pregnancy rate of 101 heifers inseminated 48 and 60 hours after implant removal ranged from 65·7 per cent to 86·0 per cent and for 319 heifers inseminated at 48 and 72 hours after implant removal the range was 59·0 per cent to 74·0 per cent. In study II the comparable figures were 13·8 per cent to 91·7 per cent and 12·5 per cent to 83·3 per cent respectively. The results of this study confirm the beneficial effects on fertility of feeding recommended levels of nurtition to dairy heifers (MAFF 1973).

A pregnancy diagnosis by palpation *per rectum* at 42 days after fixed time insemination has been used as a measure of fertility in the studies reported in this paper. Early pregnancy diagnosis is not without hazard. Rowson and Dott (1963) reported that palpation of the amniotic sac could result in the rupture of the fetal organs. During examination the uterine horn was palpated carefully to detect the presence of fetal fluids. At no time were the fetal membranes rolled between the fingers in what has been referred to as the "pinch test". Hancock (1962) reported that of 388 positive diagnosis made by palpation at six weeks 97·9 per cent of the animals calved and of 72 negative diagnosis made 98·6 per cent were correct. The corresponding figures in study III were 97·2 per cent and 98·6 per cent.

The single most important factor determining the economic advantage of a controlled breeding programme is the proportion of animals becoming pregnant to a synchronised service. Genital tract abnormalities, fertilisation failure and embryonic mortality are the three main factors which reduce cattle fertility from the theoretical maximum. The most common genital tract abnormality in heifers is freemartinism. Drew (1975, unpublished) reported that of 434 dairy and beef heifers used in oestrous synchronisation trials, 2·1 per cent were freemartins. In the studies reported in this paper 2·2 per cent of 2630 heifers showed the same abnormality. It would seem probable that in a random population of heifers about 3·0 per cent are likely to have genital tract abnormalities which would make remote their chances of becoming pregnant.

Fertilisation failure has been shown to be affected by the fertility of the bull. Both Kidder and others (1954) and Bearden and others (1956) have reported that the difference in pregnancy rates of heifers inseminated with semen from high and low fertility bulls was due to the difference in ovum fertilisation rate. Fertilisation rates have also been shown to be affected by the level of nutrition on which cows are maintained. Hill and others (1970) reported that a diet sufficient to provide only 85·0 per cent of the maintenance requirements of heifers from day 5 of the cycle prior to insemination until day 3, 8 or 18 after insemination resulted in reduction of plasma progesterone concentrations, temporary reduction in the number of medium sized follicles, altered length of the oestrous cycle and reduction in the ovum fertilisation rate. Fertilisation failure may have been partially responsible for the differences in fertility between control an supplemented heifer groups in study III. Published work indicates that failure of fertilisation may occur in 6·0 per cent to 15·0 per cent of healthy cattle inseminated with semen from bulls of high fertility (Kidder and others 1954, Bearden and others 1956, Boyd and others 1969).

Embryonic mortality comprises the largest proportion of pre-natal loss in cattle. Boyd and others (1969) found that most embryonic mortality which was to take place before the 26th day of gestation would occur by the 12th day. They suggested from data from normal cows in good condition inseminated with semen from bulls of high fertility that an embryonic mortality rate of 7·5 per cent occurred prior to day 26 of gestation and 8·0 per cent thereafter. This latter figure agrees well with that of Fosgate and Smith (1954) who reported an embryonic mortality rate of 6·38 per cent in 690 dairy cows diagnosed pregnant 34 to 50 days after insemination. In study III it is apparent that the major proportion of embryonic deaths had occurred before day 42 of pregnancy because the discrepancy between then and calving was only 2·8 per cent. This difference also included errors in diagnosis. The embryonic death rate in healthy normal cattle will be in the order of 15·0 per cent to 20·0 per cent. David and others (1971) suggested that an expectation of 63 calves for every 100 inseminations represented a reasonable norm and suggested that this level of fertility would be difficult to improve upon. This estimate was similar to the survey results of Boyd and Reed in which an overall calving rate to first insemination of 61·2 per cent was reported for 146 Friesian herds. Both are similar estimates to those made by Kidder and others (1954).

Wide variations in fertility can occur in heifer groups. But this variation can be reduced when a balanced diet providing a predicted liveweight gain of 0·7 kg per day is fed to healthy heifers for a 12-week period starting six weeks before insemination. Pregnancy rates in heifers fed in this way and treated with norgestomet/oestradiol valerate and inseminated at 48 and 60 hours or 48 and 72 hours after implant removal can regularly exceed 60·0 per cent. The difference between the 42 day pregnancy rate and the calving rate will be small.

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copper levels remained similar. These results suggest that the copper deficiency was probably induced by the interaction of copper, molybdenum and sulphur in the rumen (Dick and others 1975).

The application of measures as described above to improve some hill pastures will result in a change in the balance of mineral uptake by plants and consequent intake by grazing animals. The occurrence of copper deficiency may reduce lamb performance and so limit production levels below those reasonably expected from such improvements.

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A comparison between the pregnancy rates of heifers inseminated once or twice after progestin treatment

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IN recent years progestins and prostaglandins have been widely used to control the ovarian cycle in cattle. It is now possible to dispense with oestrus detection and to inseminate at predetermined times following treatment. Fixed time insemination relieves the farmer from the need to observe oestrus; and has the additional benefit of giving non-oestrous but synchronously ovulating animals the opportunity of becoming pregnant. Normal levels of fertility appeared to depend upon a double fixed time insemination. This has been reported for prostaglandins (cloprostenol) by Roche (1976); and progestins (SC21009) by Wishart and Young (1974). It should be recognised that the success of a single insemination technique is likely to depend as much on the timing of the insemination as on the type of compound used to achieve ovarian control. Roche (1976 personal communication) has reported the successful use of a single insemination in a large scale series of field trials in Ireland. He reported a non-return to service rate of 71·0 per cent in 321 cows treated with progesterone impregnated intravaginal coils and inseminated 56 hours after treatment. The comparable figures for 522 untreated cows inseminated on detection of oestrus and for 653 treated cows inseminated 56 and 74 hours after coil removal was 62·8 per cent and 68·9 per cent respectively.

In the British Isles SC21009 (norgestomet; G. D. Searle & Co) has been extensively studied under laboratory conditions in heifers and under field conditions in heifers and suckler cows (Wishart 1972, Wishart and Young 1974, Wishart 1975, Wishart 1976, Sreenan 1976, personal communication, Wishart and others 1977). Wishart and Young (1974) reported that a single insemination 48 hours following SC21009 implant removal led to significantly fewer ($P<0.025$) heifers becoming pregnant than when two inseminations were made 48 and 60 hours after implant removal. Double insemination (at 48 and 60 hours or 48 and 72 hours) after implant removal has resulted in normal levels of fertility (Wishart and Young 1974, Wishart and others 1977). It was suggested that the response following SC21009 treatment was not sufficiently precise to permit high fertility to a single insemination after treatment. In an endoscopy study involving 30 heifers, the accumulated percentage of heifers ovulating, of those in oestrus, was 0, 23, 69·2, 96·1 and 100 at 48, 60, 72, 84 and 96 hours respectively following implant removal. The mean interval from implant removal to ovulation was 68·5 hr (range 52 to 92 hours; SD 9·7 hours). This suggests that the timing of the single insemination (48 hours) chosen by Wishart and Young (1974) was too early for optimum fertility. Also the heifers were on a ration subsequently shown to be inadequate.

This paper reports the use of a single fixed time insemination (54 hours) in SC21009 treated heifers fed a ration designed to provide maintenance and a daily gain of 0·7 kg and compares the proportion of heifers pregnant with heifers similarly treated but inseminated twice following treatment.

The study involved 430 heifers (75 Hereford-Friesian cross; 99 Shorthorn and 256 Friesian) on five farms (A to E) in England and Scotland. They were allocated on a stratified random basis according to body weight and condition score to three insemination groups. A diet calculated to provide for maintenance and 0·7 kg per day was fed for a period of 12 weeks starting six weeks before the date of insemination.

Using a special applicator, a polymer implant was placed subcutaneously in the outer surface of the ear. Each implant contained 6·0 mg SC21009, measured 3 by 18 mm and weighed about 0·125 g. At the time of implantation a 2·0 ml injection containing 5·0 mg oestradiol valerate and 3·0 mg SC21009 dissolved in sesame oil was given intramuscularly. After nine days the implant was removed by nicking the ear with a stylet and expressing the implant with the thumb. Heifers in group 1 were inseminated twice at 48 and 60 hours after implant removal. Heifers in group 2 were inseminated twice at 48 and 72 hours and heifers in group 3 were inseminated once only at 54 hours following implant removal. No observations for oestrus were made. Two inseminations, and semen from one bull was used at each farm. Pregnancy was diagnosed by palpation 42 days after treatment.

The results are shown in Table 1. Of the 430 treated heifers 278 (64·7 per cent) became pregnant. The proportion pregnant to insemination were for Group 1 (48 and 60 hours); Group 2 (48 and 72 hours) and Group 3 (54 hours) was respectively 66·2 percent of 148; 62·1 per cent of 145 and 65·7 per cent of 137. The effect of timing of insemination on the proportion of animals becoming pregnant was not statistically significant (Table 2). The pregnancy rate varied significantly among farms. The proportion of heifers pregnant on farms A, B, C, D, and E were respectively 66·7 per cent of 75; 55·6 per cent of 79;

TABLE 1: The effect of timing of insemination on the pregnancy rate of heifers treated with SC21009 implants

Farm	*Heifers pregnant of those treated (n)* Group 1 (48 & 60 Hr)	Group 2 (48 & 72 Hr)	Group 3 (54 Hr)	Total	Per Cent Pregnant
A	19 (25)	14 (26)	17 (24)	50 (75)	66·7
B	19 (35)	20 (34)	16 (30)	55 (99)	55·6
C	12 (17)	12 (17)	12 (17)	36 (51)	70·6
D	12 (14)	10 (14)	10 (14)	32 (42)	76·2
E	36 (57)	34 (54)	35 (52)	105 (163)	64·4
TOTAL	98 (148)	90 (145)	90 (137)	278 (430)	
Per Cent Pregnant	66·2	62·1	65·7	64·7	

AM2

TABLE 2: Analysis of variance

Source	Sum of squares	Degrees of freedom	Mean square	f Value	
AI	104·997	2	52·498	1·27	NS
Farm	703·152	4	175·788	4·27	P 0·05
Residual	329·461	8	41·183		
TOTAL	1137·61	14			

Norcestomet — G. D. Searle & Co

70·6 per cent of 51; 76·2 per cent of 42 and 64·4 per cent of 163.

These results indicate that a single insemination (54 hours) is likely to result in as great a proportion of heifers becoming pregnant following SC21009 treatment as with a double insemination (48 and 60 hours or 48 and 72 hours). Fertility can be expected to vary from farm to farm.

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The influence of cobalt-deficient diets on housed sheep

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EARLIER work (MacPherson and Moon 1974, MacPherson and others 1976) suggested that thiamine deficiency occurred as a secondary effect of maintaining sheep on a low cobalt diet. Biochemical tests (pyruvate, erythrocyte transketolase and pyruvate kinase) and a relatively high incidence of cerebrocortical necrosis among the cobalt depleted animals supported this conclusion. The experiment reported here was undertaken to follow the development of the thiamine deficiency and to determine its relationship to the progressive decline in cobalt status. Twenty Suffolk cross wethers, all derived from the same source, were housed and randomised into five groups of four animals and fed on timothy hay, flaked maize and maize gluten meal providing less than 0·05 mg cobalt/day. Group 1 was killed initially to give a base line for liver thiamine status; group 3 was given 0·7 mg cobalt (as the sulphate) orally per week while the other three groups were fed the basal ration until clinically but not severely cobalt-deficient, at which stage the sheep in group 2 were killed. The sheep in groups 4 and 5 continued on the deficient diet until death or until they were put down in the terminal stages of cobalt-deficiency at which time the sheep in group 3 (supplemented) were also slaughtered. Liver samples were collected post mortem and analysed for cobalt and thiamine concentrations and the whole brains were removed for histological examination. Faeces samples and samples of post mortem rumen contents were collected for thiaminase assay. One sheep died early in the course of the experiment from abomasal torsion and acidosis and has not been included in the examination of the results as it had not by that stage developed cobalt deficiency. The mean terminal liver cobalt and thiamine concentrations of the five groups are in Table 1.

The establishment of cobalt deficiency in groups 2, 4 and 5 was thus clearly demonstrated but liver thiamine levels were not correspondingly depleted, being normal in the sheep slaughtered in early cobalt deficiency, and still only borderline in the sheep slaughtered in advanced cobalt deficiency. However, four of the sheep in groups 4 and 5 did show deficient liver thiamine levels ranging from 0·62 to 1·17 µg/g.

TABLE 1: The mean terminal liver cobalt and thiamine concentrations of the 5 groups

Group		Days on experimental diet	Liver cobalt (µg/g dry matter)	Liver thiamine (µg/g fresh weight)
1	Slaughtered controls	48	0·05±0·002	a4·15 ± 0·35
2	Slaughtered in early deficiency	176	≤ 0·02	b2·79 ± 0·35
3	Cobalt supplemented	279	0·09 ± 0·008	c1·70 ± 0·35*
4/5	Slaughtered/died in late deficiency	235†	≤ 0·02	c1·29 ± 0·27*

*The same letter in superscript next to two means in the same column indicates that such means do not differ significantly from one another at the 5 per cent level of probability.
†Mean value. Individuals ranged from 215 to 279 days.

Reference to the table shows that irrespective of treatment there was a decline in liver thiamine with time, the correlation coefficient (−0·79) being highly significant. The reason for this decline is not certain but, except in the case of group 3, it does not seem to be associated with thiaminase activity as this enzyme was only detected in two out of 49 samples examined from the cobalt-deficient sheep.

An association between cobalt deficiency and a decline in liver thiamine could have been claimed if the liver thiamine levels of group 3 (cobalt supplemented) animals had been equal to or higher than those of the control animals in group 1.

An explanation for the anomalous results for group 3 may be provided by the thiaminase tests for this group; significant concentrations of thiaminase were found in eight out of 24 samples examined and in four out of six samples from the animal with the lowest liver thiamine. It has been reported elsewhere (Roberts and Boyd 1974) that the presence of specific cosubstrates is necessary for thiaminase activity.

Some of the typical clinical symptoms of cerebrocortical necrosis (CCN) were seen in the sheep of groups 4 and 5, six of them being ataxic when put down. However, the histological examination of the brains showed changes similar to but distinguishable from cerebrocortical necrosis. Thus, in contrast to previous work where a 25 per cent incidence of that disease among cobalt-deficient sheep was recorded this was the first of our experiments where no cases of cerebrocortical necrosis were confirmed. It is interesting to note that in a further experiment (to be reported in detail elsewhere) on the same dietary regime two cases of cerebrocortical necrosis have been confirmed out of seven fed a cobalt-deficient diet whereas no cases occurred among 20 supplemented sheep. It should also be noted that cobalt-supplemented sheep have been maintained on similar diets for much longer periods than the 280 days of the experiment described here. In other words they have had sufficiently long to develop low liver thiamine concentrations if the decline was maintained at even half the rate found here. Nevertheless, over a period of more than five years, no cases of cerebrocortical necrosis have ever been reported among such sheep.

Considerable evidence has therefore now accumulated to conclude that cobalt supplementation effectively protects against the development of cerebrocortical necrosis at least under the conditions described here. Also, low thiamine levels have been induced in sheep fed on a cobalt-deficient diet without the effective presence of thiaminase. Two possible explanations of the low thiamine levels suggest themselves. Firstly, the cobalt deficiency may affect the activity of the rumen micro-organisms responsible for thiamine synthesis and secondly there may be a liver involvement effected by the severe liver damage which is frequently a result of cobalt depletion.

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PART 2

PART 2

SYNCHRONISED OESTRUS AND OVULATION

UTILISATION OF CONTROLLED BREEDING

The widespread mass treatment of batches of cattle for synchronised insemination, the use which was anticipated when controlled breeding was introduced, did not really materialise. There was, at first, a surge of interest but it was not truly sustained or increased. The reason was quite simply that early results were mixed, and often disappointing. Although synchronised breeding now has an established role, greater numbers of cattle are treated on an individual basis. There has been a trend for prostaglandin to be used mostly in the treatment of individual animals or small groups, for problems related to oestrus detection and fertility. Often this is in the course of a regular herd fertility visit. Such routine veterinary servicing of the fertility needs of herds is increasingly being accepted by herd owners as a cost-effective approach. It owes much of its success to the ability of the veterinary surgeon to have positive control over reproduction by the use of prostaglandin $F_{2\alpha}$.

There are a number of reasons for the slow acceptance of synchronised insemination. It is almost inevitable with the introduction of a new and exciting technique, that initial expectations will be too great. This certainly was the case with controlled breeding - or 'synchronised oestrus' , as it was described at the time. The message, learned in development trials, of the need for high standards of management, and particularly nutrition, was not made sufficiently forcefully to the new users. The technique is undoubtedly more demanding than the less intensive methods that people were familiar with, and the difference not always clear to farmer or veterinary surgeon.

In addition there was, at the time, very considerable misunderstanding of the true rate of conception to artificial insemination. There was a belief that it was considerably higher than was in fact the case. At that time, accurate recording and analysis of herd fertility events was almost unknown, and without these, conception rate is not easily assessed or recognised. The best available standard was the 30/60 day 'non-return rate' used by Artificial Insemination Centres as a working standard to analyse and monitor the conception rates achieved by individual inseminators, and by bulls.

For that purpose, non-return rate is a useful enough working standard. It is not, however, the accurate measure of conception rate, which farmers believed it to be. There are a number of reasons why a repeat insemination may not be requested for a cow that did not conceive to artificial insemination. Not all of them imply the pregnancy that is assumed by the recording system. There may be a policy decision not to re-inseminate the cow, which may be sold or culled; the cow may suffer disease or injury, or die; she may not be detected in oestrus, and be presumed pregnant. Early pregnancy diagnosis by progesterone assay will identify such non-pregnant cows at the end of the first oestrous cycle but rectal palpation is not practical until about the seventh week of gestation. Thirty/sixty-day non-return rate was assessed to be about 20 per cent higher than calving rate by a survey done by Hampshire Cattle Breeders Society reported in 1979.

The consequence of this long established misunderstanding of realistic expectation for first service conception rate, was that farmers believed an acceptable standard to be about 75 per cent. This would be a typical order of 30/60 day non-return rate, but represented a true calving rate of about 55 per cent. With the advent of synchronised insemination - and 'synchronised' calving, it suddenly became possible in these circumstances to measure calving rate with ease and reasonable accuracy. In that situation, even respectable conception rates to synchronised breeding , of the order of 50 per cent, were thought to be failures. Since average conception rates in commercial dairy herds are frequently of the order of 45 per cent, 'synchronised oestrus' earned a poor reputation, not all of which was justified.

DIFFERENCES BETWEEN TYPES OF CATTLE

Development studies on techniques for the hormonal control of breeding used mainly maiden heifers. These are convenient for research studies, being more readily available, cheaper, less variable physiologically, and less likely to have chronic pathological conditions of the reproductive tract than parous cows. They are not, however, representative of a cross section of the national cattle population. This population may be roughly divided into three distinct categories of cattle, differing in physical characteristics, and in typical conditions of husbandry, management and production methods. These are maiden heifers, beef cows and dairy cows. Controlled breeding under commercial farming conditions must take account of these differences.

The group of trials to be discussed in PART 2 investigated the use of prostaglandin $F_{2\alpha}$ tromethamine (dinoprost), to control and synchronise oestrus and ovulation in groups of cattle, so that they might be inseminated at predetermined fixed-times. At the time of the studies, dinoprost already had been issued a Product Licence. The regulatory data sheet recommendations for its use in controlled breeding, consisted of two intramuscular injections, each of 25mg dinoprost, with an eleven day interval, followed by insemination at observed oestrus, or at fixed-times 72 and 90 hours after the second dinoprost injection, or a single insemination 78 hours after the second injection.

The trials were conducted on each of three categories of cattle; heifers, beef cows and dairy cows. At the time of the studies, literature searches had not revealed any published work dealing specifically in dairy cows, with the comparison between double and single insemination regimes following hormonally induced ovulation. This was an important omission, because dairy cows represent by far the largest proportion of cattle to be submitted to hormonal control of reproduction. The conclusions from the dairy cow studies differed from current conventional wisdom, which was based mainly on results from beef cow studies and from clinical impressions. Beef cows had tended to be used for clinical trials, in preference to dairy cows because they were more readily available for treatment in batches. Reasonably sized contemporary groups were necessary to achieve the numbers needed for statistical results. Beef cows are not however directly comparable with dairy cows in reproductive characteristics.

Additionally, some studies reported results from heifers and suckled cows, using dinoprost and cloprostenol, (eg. Hafs and others, 1978) drawing conclusions on mean values, and making no distinction between the different factors. There appeared to be a need to take account of real practical differences, which it seemed were not always given due weight, and not necessarily to expect unlikes to conform to a common pattern. Sufficient difference can be demonstrated between reported results, between properties of prostaglandin $F_{2\alpha}$ and its analogues, and between types of cattle, for there to be a need to investigate each component specifically

It was with this background that the trials described in PART 2 were arranged. A need was felt for greater precision in assessing techniques that continued to produce variable results; to provide additional data specific to the type of cattle and specific to dinoprost. The intention was to provide more precise information about its use for synchronised batch insemination.

The reproductive characteristics of the different types of cattle, and relevant features of typical husbandry systems that have a bearing on controlled breeding, will be discussed in detail. The significance and importance of the time elements of fixed-time insemination schedules will be surveyed and discussed. Finally the trials will be described and results discussed in light of the objectives of the studies.

REPRODUCTIVE CHARACTERISTICS OF THREE CATEGORIES OF CATTLE

Attention has been directed to the difference in characteristics and typical methods of husbandry of heifers including heifers of beef breeds, dairy cows, and beef suckler cows. These will be considered in relation to the effects of the differences on hormonal control of reproduction.

HEIFER REPRODUCTION

The maiden heifer has a relatively uncomplicated reproductive system. It has not been exposed to damage and infection from the hazards of parturition, and so is unlikely to be subject to the effects of chronic pathological conditions of the uterus. Cystic ovarian disease is not common in heifers. Two important reproductive conditions, however, are frequently met in heifers under normal farming conditions.

SUBFUNCTIONAL OVARIES

It is not uncommon to encounter immature ovaries in heifers, a condition that can reduce fertility or prevent conception. This may be due to uncomplicated immaturity of the animal, ie. those simply too young to have reached puberty and developed functional ovaries. A similar condition is also commonly seen in heifers which are of sufficient age, but have had puberty delayed by inadequate nutrition. The end result is the same, a small infantile uterus and very small hard featureless ovaries which are incapable of developing follicles and ovulating until they have developed to maturity. The incidence of prepuberal heifers presented for oestrus synchronisation studies has been reported to be, 12 per cent, (Dailey and others, 1983) and 17.9 per cent (Britt and others, 1978) The puberal status of heifers was found to vary widely among herds and was dependent mostly on bodyweight. Heifers as young as 12 months were puberal in some herds, and heifers as old as 22 months were prepuberal in other herds.

In the field, investigation of unsatisfactory conception rates in heifers that have had oestrus synchronised for batch insemination, not infrequently reveals that a proportion are suffering from a combination of puberal immaturity and suboptimal nutrition, with consequent ovarian inadequacy. This can result from methods of rearing heifers, for example outwintering on sparse pasture with minimal nutritional supplementation. Inadequate rations may also be offered to yarded heifers which are little better off.

The consequences of reduced and delayed fertility from such husbandry may be less obvious when heifers are bred by natural mating. Traditionally a stock bull would be turned out with them for a prolonged period. In this situation, the result is a prolonged calving period. If, on the other hand, the group of heifers has oestrus synchronised and fixed-time-insemination, then the poor conception rate will be all too obvious when they calve or are diagnosed for pregnancy.

A less extreme example of the same effect is illustrated by the reduced conception rates observed in PAPERS 1 and 2 when heifers were fed a ration considered by the farmers involved, to be adequate, but demonstrated to be well below optimum when compared with heifers fed supplementary grain. Such suboptimal nutrition is commonplace, particularly in heifers of the beef breeds, which may be overwintered on barely more than maintenance rations, as 'store cattle'.

These effects of suboptimal nutrition are characteristic hazards of traditional systems of heifer rearing which did not seek optimal weight gain. Unfortunately attempts are frequently made to combine such traditional feeding practice with the modern practice of mating heifers at 15 months of age or less, so as to have them calving at two years old. To be effective, calving at two years old demands adequate nutrition to provide near optimum liveweight gain and ensure proper physical maturity at mating. If these conditions are not met, then controlled synchronised oestrus for group insemination is contra-indicated, and likely to result in unsatisfactory conception rates caused by low fertility.

HERMAPHRODISM

The second reproductive problem characteristic of heifers is freemartinism. This is a distinctive form of intersexuality in cattle which results from the development of vascular anastomoses between heterosexual foetuses in multiple pregnancies. It results in the male hormones of one interfering with the development of the female reproductive tract of the other. The external genitalia of affected heifers are female in appearance but the internal genitalia commonly show masculinisation and some physical abnormality of the reproductive tract. Such heifers are infertile. In the trials reported in PAPER 2 the incidence of freemartins in farm-bred heifers was; 2.4 per cent of 1486 heifers; 2.9 per cent of 375 heifers and 2.3 per cent of 512 heifers. Heifers for the trials reported in Parts 1 and 2 of PAPER 1 were purchased from a cattle dealer and kept on an experimental farm. It was usual for the preliminary clinical examination to reveal a small proportion, of about the same order, to be freemartins. A similar incidence, 2.2 per cent, has been reported in oestrus-synchronisation studies on heifers. (Dailey and others, 1983) It has been recorded that of a group of 46, week-old heifer calves bought at market as breeding heifers, 19 proved to be freemartins.(Wilkes and others, 1981). A high incidence in female dairy calves bought from markets in this way may be expected because dairy farmers have long been aware that females twinned with males are liable to be freemartins. Hence such local traditional descriptive terms as, Willjills, or wijels, and the practice of selling them early rather than risk the uneconomic consequences.

MISALLIANCE PREGNANCY

It is common to find a variable proportion of 'maiden' heifers to be already pregnant. Although not strictly speaking a reproductive problem, it is nevertheless a barrier to the intended breeding schedule. This usually results from having run groups of mixed sex calves together long enough for precociously developed males to mate the more sexually mature females. It can also happen when males are improperly castrated, and are left with some potency. Careless husbandry may permit bulls intended to sire the cows, to have access to puberal heifer calves, or inadequate fencing may produce the same result. When purchasing 'bulling heifers' intended for breeding, there is always a risk that a proportion will already be pregnant.

For the trials reported in Parts 1 and 2 of PAPER 1, maiden heifers were purchased from a cattle dealer and yarded on an experimental farm. They were acquired in batches of 80 and given a clinical examination on arrival. It was usual for a small proportion, of the order of 5 per cent, to be diagnosed pregnant on arrival. An incidence of 9.2 per cent pregnant to unknown matings has been reported in heifers presented for trial studies. (Dailey and others, 1983)

When the condition is recognised early enough pregnancy may be terminated by inducing abortion with prostaglandin $F_{2\alpha}$. This should be done after five days from service, to ensure the presence of a responsive *corpus luteum*. After about 70 days, the response becomes gradually less reliable, until about 150 days, when pregnancy is no longer dependant on luteal progesterone and termination with prostaglandin $F_{2\alpha}$ is unreliable. In areas where the farming tradition is to sell batches of heifers for slaughter or for breeding, it is a common practice to inject the whole group with prostaglandin $F_{2\alpha}$ as an abortifacient, as an insurance against penalties invoked by the slaughterer or breeder for unwanted pregnant animals.

NUTRITION AND FERTILITY IN HEIFERS

The practice of breeding heifers to calve at two years of age requires optimum growth so that they will achieve a sufficient size, and so that they will be puberal by 15 months for breeding. Adequate feeding of heifers presents no fundamental difficulties. The growth targets are daily weight gain of 0.7 kg per day, to achieve a body weight by 15 months of at least 300 kg. The energy component of the ration is the critical element, and can be easily and economically provided by grain to supplement inadequate forage. In the studies reported in PAPER 2 it was found necessary to supplement the diet considered adequate by competent farmers by 20 MJ metabolisable energy per day to achieve the required growth.

This was provided by 6 to 8 pounds of barley per head per day, and produced a significant increase in calving rate compared with heifers fed the farmer's ration. The value of adequate nutrition cannot be over emphasised.

These common reproductive difficulties met in heifers are characteristic of, and derive from the husbandry practices typical in the breeding management of this category of cattle. In fact, when proper precautions are taken, they need not present any great problem, and the fertility of maiden heifers should be and usually is higher than that of cows.

BEEF COW REPRODUCTION

Fertility in commercial beef cattle is mostly lower than in dairy cattle. This is probably more to do with husbandry and breeding practices than with any basic physiological difference between the two types. However, an apparent difference in fertility was recorded between two French beef breeds, Salers and Charolais. (Chupin, 1977) with first service conception rates of 59 per cent and 44 per cent respectively. This represented a significant difference ($P < 0.01$) in fertility, which may represent a true breed difference, and indicate that at least some beef breeds have relatively low fertility. Certainly there are well recognised differences in the breeding characteristics of milk and beef types of cattle which have important consequences.

The feeding of beef cattle is usually a minimum-cost exercise, so requirements for optimum reproduction and minimum feed costs, are in conflict. The importance of adequate dietary energy for optimum conception has been recognised in the previous section on heifer reproduction. The same principle holds good for beef cattle and its significance will be illustrated later in this section. Controlled breeding systems to be practically effective have to take account of the potential effects of suboptimal nutrition.

Beef production to be economically viable, has to be organised for minimum labour input and supervision. (Meat and Livestock

Commission, 1980-81) Advantages are available from batch breeding by the use of controlled breeding techniques, which allow economical and convenient batch management of feeding, housing and husbandry tasks.

The usual practice is for beef calves to be suckled by their dams until they are weaned at an age of several months. This suckling has an inhibitory effect on ovarian cyclicity, with important reproductive consequences which will be considered in detail.

EFFECT OF SUCKLING ON OVARIAN ACTIVITY IN THE COW

For optimum productivity, the beef cow needs to produce and rear one calf per year. This means that the cow must conceive within three months of parturition; at a time when she is usually still suckling her last calf. Suckling has a demonstrably negative effect on resumption of regular ovarian cyclicity, such that the *post partum* anoestrous period may be prolonged to a variable extent, from less than 50 days to more than 90 days. (Peters and Riley, 1982; Chupin, 1977 ; Morrow and others, 1969; Oxenreider, 1968; Wiltbank and Cook, 1958)

A study investigated the length of the *post partum* anoestrous period and the incidence of short oestrous cycles in beef cows. (Graves and others, 1968) In this study, the mean interval from parturition to first oestrus was 85.8 days in suckled *versus* 36.5

days in non-suckled beef cows ($P < 0.005$). The effect of suckling frequency on *post partum* ovarian cyclicity was investigated. (LaVoie and others, 1981) Beef cows were suckled twice daily, or *ad libitum*, and compared with non-suckled cows. The non-suckled control cows had their calves removed 3 days *post partum* and were not suckled or milked thereafter. The mean interval from parturition to first oestrus was shorter ($P < 0.05$) for non-suckled cows, 20 days, than for twice suckled, 34 days, or suckled *ad libitum*, 38 days.

Although there is an apparent trend to suggest that the frequency of suckling did prolong the anoestrous period, this was not a significant effect. Nutrition in this study was carefully controlled, and the difference was independant of nutritional effects. Another report failed to observe any difference in length of acyclic period between cows suckling one or two calves. (Peters and Riley, 1982) It appears that, in beef cows, the difference between suckling and non-suckling is more important than the difference between degrees of suckling. This appears to differ from the situation in dairy cows. In a study cited later under the heading of 'Dairy Cow Reproduction', no difference was observed in the time to onset of *post partum* ovarian cyclicity in dairy cows, when suckled were compared with milked cows.

The phenomenon of short oestrous cycles is particularly common at the first *post partum* cycle and has been reported in dairy cows. (Macmillan and Watson, 1971) and in beef cows. (Odde and others, 1980; LaVoie and others, 1981 ; Graves and others, 1968). The shorter cycle is due to a shortened luteal phase, and it has been suggested that cows with a short cycle at breeding are less likely to sustain conception. This is because the embryo has no apparent effect on the lifespan of the corpus luteum until Day 15 to 17 post oestrus. (Odde and others, 1980). In order to maintain a yearly calving pattern there is pressure to attempt breeding as early as possible. In beef cows, because of their delayed return to ovarian cyclicity, this is liable to coincide with short cycles, which will further delay conception.

A seasonal effect on fertility has been noted. (Chupin, 1977) The fertility of induced oestrus fell steadily, between early March and the end of April, then increased in May. The management system involved turning the cows out to grass about 1st April, so there was about a month at grass before fertility improved. It is difficult to differentiate the relative effects on the timing of these events, which could be attributed to winter underfeeding, or to the stress of turning out to grass.

A seasonal effect has also been reported on the length of the *post partum* anoestrous period. (Peters and Riley, 1982) Beef cows calving between February and April were acyclic significantly longer, 83 days, than those calving between August and December, 44 days ($P < 0.001$). There was a significant negative correlation ($P < 0.001$) between the length of the acyclic period and days from calving to the next longest day of the year. This would seem to point to a true effect, probably associated with photoperiodism. Recently the hormone melatonin, which is released from the pineal gland and influences the return of seasonal ovarian cyclicity in sheep, has been administered to ewes. (Arendt and others, 1983) This was successful in shortening the period of seasonal anoestrous which is known to be influenced by photoperiodism in sheep. Perhaps melatonin might also be administered to cattle to minimise the seasonal effect on fertility and *post partum* anoestrous.

HORMONAL ELEMENTS OF POST PARTUM ANOESTRUS IN SUCKLED COWS

Follicular development is depressed in the *post partum* cow, and recovery is slowest in the suckling cow. (Wagner and Oxenreider, 1971) *Post partum* anoestrous is not caused by persistence of the corpus luteum of pregnancy. (Labhsetwar and others, 1964) It appears that uterine involution occurs rapidly after parturition in the suckled cow. (Wagner and Hansel, 1969) Low conception at the first *post partum* oestrus was attributed to factors other than incomplete involution of the uterus, which had occurred by 28 days in autumn-calving beef cows. (MacFarlane and others, 1977)

The endocrinology of the *post partum* period is not well understood. A study is reported, that investigated and correlated the recovery of ovarian activity, the occurence of first oestrus and the temporal patterns of plasma concentrations of luteinising hormone, oestradiol-17β and progesterone during the *post partum* period of the suckling beef cow. (Rawlings and others, 1980) Seven Hereford cows, suckling single calves were bled daily from parturition, for 79 days. At ten-day intervals blood samples were taken every 15 minutes for 8 hours.

The average *post partum* interval to first oestrus was 59.8 ± 3.7 days for 5 of the cows. In cows returning to oestrus , plasma concentrations of progesterone were low until 55.5 ± 3.0 days *post partum*, rose to exceed 0.5 ng/ml plasma for 4.0 ± 0.4 days, declined for 5.0 ± 0.5 days and then rose again to normal luteal phase concentrations. First oestrus followed the initial rise in progesterone in 4 cows, and preceeded it in one. Ovarian palpation revealed considerable follicular development before the initial rise in progesterone, but no clearly discernable corpus luteum until normal luteal phase progesterone concentrations were detected. Plasma concentrations of oestradiol-17β fell after parturition and, although very variable, showed no apparent trend thereafter. Plasma concentrations of luteinising hormone varied in an episodic manner, with an apparent increase in frequency and magnitude of peaks up to 10 to 33 days before the first elevation in plasma progesterone. Subsequently there was little change, except for a decline in peak luteinising hormone concentrations after the initial elevation in plasma progesterone.

Progesterone is thought to have an organising effect on gonadotrophin secretion. The source of the early elevation of plasma concentrations of progesterone is unclear, as is the mechanism that controls the duration of its secretion. In the dairy cow, the length of the *post partum* anoestrous period is dictated largely by the time needed for uterine involution to be completed. In beef cows, return to ovarian cyclicity when uterine involution has been completed may still be delayed by the suckling effect.

The mechanisms by which suckling delays the resumption of oestrous cyclicity have been investigated. Experimental evidence has been produced and supporting literature cited to indicate that there is a neural inhibition of release of gonadotrophin-releasing hormone from the hypothalamus. (Schallenberger and others, 1982) The authors cite further studies showing that suckling stimuli are able to enhance corticoid levels in cows, and these have been implied to suppress *post partum* luteinising hormone release other than via hypothalamic inhibition. The end result is to delay the characteristic pre-oestrous build-up of luteinising hormone pulses from the pituitary.

Cows in a high nutrition group had shorter ($P < 0.10$) intervals from parturition to first oestrus than cows on low nutrition group, 44.6 days *versus* 52.0 days. (Hinshelwood and others, 1982) It is well recognised among cattle breeders that fertility improves at spring turnout to pasture, and this effect has been observed. First service conception rate in beef cows inseminated after progestagen induction of oestrus was 25 per cent (n=40) during the period 23 to 29 April, about the time of turnout; compared with

55.8 per cent (n=43) during the period 6 to 12 May, after turnout. (Chupin, 1977) The low fertility immediately after turnout was thought to be associated with environmental and dietary stress associated with turnout.

SUMMARY

Delay of *post partum* return to oestrous cyclicity caused by suckling is well recognised. It is believed to have its effect by inhibition of the release of luteinising hormone, and a neural mechanism is thought to be involved. Indications of a true seasonal effect suggest that hypophyseal release of gonadotrophin-releasing hormone may be suppressed. It may be that the effect of suckling, and of photoperiodism, are separate and can therefore be additive. Adequacy of nutritional energy contributes to return of cyclicity, and may superimpose an additional influence on the outcome. It is fortunate that adequate nutrition makes a major contribution to fertility, because it is the one factor which can readily be manipulated.

In the beef cow, the economic importance of a short calving-to-conception interval is fraught with physiological and environmental difficulties. These influences go a long way to explaining why good reproductive performance in beef cattle requires a high standard of husbandry, breeding management and stockmanship. Controlled breeding with prostaglandin $F_{2\alpha}$ has a

useful role in reducing the inherent delay and improving batch breeding if used with proper regard to the factors that can limit its effectiveness.

NUTRITION AND FERTILITY IN BEEF COWS

In heifers, appropriate nutritional management is relatively straightforward: an average daily liveweight gain of the order of 0.7 kg per day was demonstrated in studies reported previously, to be an appropriate target, and presented no real difficulties in attainment. In the dairy cow, rumen capacity and the demands of milk production complicate the issue. In beef cattle, the marginal profitability of suckler cow enterprises requires minimising feed costs (Meat and Livestock Commission, 1983), which places economic constraints on justifiable expenditure. These limitations must be recognised and accomodated if practically acceptable recommendations are to be made.

Suckler herds are traditionally bred seasonally to calve either in the spring or the autumn. Suitable nutritional requirements are more readily met in spring-calving herds, when adequate dietary needs are provided by good quality grass at the time when mating is taking place. Autumn calving cows on the other hand face their greatest nutritional demands when grass quality and quantity is at its lowest, and expensive supplementary feeding has to be provided, grudgingly if at all.

It is generally recognised that fertility improves at spring turnout to pasture, and this effect has been reported. (Chupin, 1977) The conception rate of beef cows was monitored in relation to the time of treatment with norgestomet implants First service conception rate fell steadily from early March until late April, then increased in May. There was a delay in positive response to turnout in early April, which was attributed to nutritional and environmental stress associated with the changes at turnout.

The delay of *post partum* return to ovarian cyclicity already noted in beef cows can be modified by feeding. The effect of nutrition, on length of *post partum* anoestrous period and ovulation rate, at various stages *post partum*, was studied in beef cows fed individually either; to maintain body weight (M); or gain 1.0 kg per day (G). (Henricks and others, 1986) Oestrus and ovulation had started by day 28 *post partum* in group (G), compared with day 70 *post partum* in group (M). In a similar study, cows in a high nutrition group had shorter ($P < 0.10$) intervals from parturition to first oestrus than cows in a low nutrition group, 44.6 days *versus* 52.0 days. (Hinshelwood and others, 1982)

Beef and dairy cows behave differently in metabolising available energy. An additional 10 MJ per day fed to autumn calving beef cows produced an increase in 150 day milk yield of only 37.5 kg, compared with the 300 kg response predicted from a similar increment in feed intake for dairy cows. (Lowman, 1985) The implication is that the beef cow tends to increase body reserves rather than convert surplus rapidly to milk as does the dairy animal.

Investigating these effects in a controlled study, beef cows were fed a 2 kg rolled barley supplement, equivalent to 21 MJ metabolisable energy for a period from 2 weeks before to 4 weeks after mating. (Lowman, 1985) Enhanced feeding produced a significant reduction ($P < 0.10$) in pregnancies. This was attributed to the dietary stress caused by removing the supplementary diet before embryonic implantation was established. Repeating the study with dietary supplementation from two weeks before until eight weeks after mating resulted in an improved pregnancy ratio in the supplemented cows compared with the controls. The cows had been scored for degree of ovarian activity before starting the supplementary feeding, and the positive response was greatest in cows that were anoestrous or had a low score.

A similar effect was noted in heifer trials during the norgestomet studies reported in PART 1. In these, feeding a supplement from 3 weeks before until 3 weeks after insemination, resulted in reduced conception in the supplemented heifers. (unpublished data) This effect was reversed by feeding a supplement from 6 weeks before until 6 weeks after insemination, as reported. At that time the negative effect was also attributed to increased early embryonic mortality resulting from dietary stress. Being mindful of the need for economical feeding of beef cows it seems probable that a combination of these two observations would suggest feeding a supplement from 2 weeks before until 6 weeks after insemination.

Despite these difficulties in identifying a minimum effective schedule for supplementary feeding, improved fertility from improved nutrition has been clearly demonstrated. Nutritional studies similar to those described on heifers in PART 1 were also done on 161 beef cows. (Drew, Wishart and Young, 1979) Control cows were fed a diet considered by the farmer to be adequate. 'Supplemented' cows were fed a supplement calculated to provide maintenance plus 9.0 kg milk per day. This required a extra 20 MJ metabolisable energy per day, fed as rolled barley. The pregnancy rates were for farm ration, 37.9 per cent and for supplemented 59.7 per cent.

A series of experiments examined the response of autumn-calving beef cows to three planes of nutrition during lactation in 191 cows. (Somerville and others, 1979) The cows were offered individually, either 175 (high), 125 (medium) or 90 (low) per cent of their estimated energy requirement for maintenance, based on their mean liveweight 12 hours *post partum*. At 100 days *post partum* the cows were approximately 8, 16 and 21 per cent lighter than at calving on the high, medium and low planes of nutrition respectively. The reproductive performance of cows on the high and medium planes of nutrition was satisfactory, but 18 per cent of the cows on the low plane failed to conceive during a 100 day mating period.

SUMMARY

The importance of adequate nutrition for optimum fertility is established beyond doubt. However, under extensive breeding conditions, the consequences of poor nutrition are not obvious. Even if the stockman does recognise the value of nutrition, his idea of a suitable ration has frequently been shown to be inadequate. Subsequent discussion about controlled breeding in the beef cow, is set against this background. The first step in any controlled breeding programme is to ensure adequate nutrition. Supplementation need only apply to energy, and can be economically provided by grain fed over a short period, from two weeks before until six weeks after insemination. The difference, in calves born, is very cost effective, and controlled breeding in beef cows without this precaution can result in poor results.

Supression of ovarian cyclicity by suckling as seen in beef cows is not a problem in the dairy cow, because most dairy cows do not suckle calves. However when a group of four milked Friesian cows was compared with a group of Friesian cows each suckling three or four calves, no difference was found in either duration of the *post partum* acyclic period or in the pattern of luteinising hormone and oestradiol 17β secretion. (Fisher and others, 1986)

Inadequacy of energy in the ration, as encountered with heifers and beef cows, is not a problem in the dairy cow. The dairy cow is usually fed for optimum milk production, and while rumenal capacity and appetite may prevent optimum feed intake for that purpose, usually it is sufficient for reproductive needs.

In the dairy cow, the main problems of breeding tend to be sequelae to the trauma and infection associated with parturition under the intensive or semi intensive conditions typical of dairy cow husbandry. These include ; dystocia, retained foetal membranes, endometritis, metritis and pyometra. Delayed uterine involution, which may be associated with the other sequelae of parturition, can also delay the onset of fertile oestrous cycles.

Uterine infection after calving is virtually the norm in dairy cows, and mostly will resolve without treatment. The rate of self cure and the time required is difficult to estimate, but is an important source of delay for uterine involution. The time needed for uterine involution to be completed determines the time when conception is again possible, so it is a limiting factor in attaining an optimum calving-to-conception interval. It is certainly a critical factor in return to reproductive competence in the dairy cow.

Malfunction of the endogenous hormonal control of ovarian function as seen in cystic ovarian disease can also interfere with reproduction. The condition is more common in dairy cows than in heifers or beef cows. It has a heritable tendency which has been shown to be influenced by the female line. A sire effect has also been demonstrated.

These factors which influence the *post partum* reproductive performance of the dairy cow are the subject of PART 4, where they are examined and discussed in detail. The endocrinology of the early bovine *post partum* period, and in particular, the latest understanding of the role of prostaglandin $F_{2\alpha}$ will also be discussed in detail.

Effective controlled breeding of dairy cows is thus influenced by physiological and pathological factors which are different and distinct from those affecting heifers and suckler cows.

TIMING OF ARTIFICIAL INSEMINATION FOR INDUCED OESTRUS

The time interval between an injection of prostaglandin $F_{2\alpha}$ and the induced ovulation which follows, is important for the timing of successful fixed-time insemination. That interval is fairly predictable, but exact synchrony is not achieved within a group of randomly cyclical females. It is probable that precise synchrony is not achievable, because of natural individual variation and seasonal variations.

An investigation into the length of component events of the natural oestrus cycle in yarded Friesian heifers, (Wishart, 1972) recorded average cycle length (n=211) as 22.02 days ± 3.5 days. During the Spring-Summer period the average (n=147) was 20.02 ± 4.6 days (range 17 to 26 days) and in the Autumn-Winter (n= 64) it was 26.17 ± 0.8 days. (range 17 to 65 days) Although variation in cycle length in individual animals was mainly ± one day, the overall range was ± 5 days. The mean duration of oestrus (n=68) was 14.7 ± 1.6 hours (range 4 to 24 hours), and the mean duration from end of oestrus to ovulation as determined by rectal palpation (n=68) was 9.16 ± 1.3 hours (range 6 to 14 hours). Direct observation by endoscopy suggested that ovulation occurred over a range of 15 hours, starting 9 hours after the end of oestrus. This latter is probably a more accurate assessment because repeated palpation may stimulate ovarian activity and shorten the interval to ovulation.

A difference in follicular development was noted when prostaglandin $F_{2\alpha}$ was injected on different days of the oestrus cycle. (Edqvist and others, 1975) Injection on Day 8 or Day 14 of the cycle in dairy heifers resulted in reduction of the size of the corpus luteum within 24 hours. When the injection was made on Day 8 of the cycle, the dominant follicle at mid-cycle appeared to continue to be the ovulatory follicle; when the injection was made on Day 14 of the cycle, a new follicle formed within 48 hours of the prostaglandin $F_{2\alpha}$ injection and remained to be the ovulatory follicle. This does not entirely concur with data from direct endoscopic observation of the ovaries. (Wishart and Snowball, 1973) The observations from these studies appeared to show that the ovulatory follicle was identifiable only after the end of oestrus. (Wishart, 1976) Further supporting evidence indicates that the follicle which eventually ovulates is only identifiable approximately 48 hours before oestrus. (Dufour and others, 1972)

A study investigated follicular development in the ovaries of heifers after injection of cloprostenol on Day 8 or 9 of the oestrus cycle. (Ireland and Roche, 1982) The data suggested that recruitment of antral follicles to ovulate is very rapid. The large follicle, present at the time of the cloprostenol injection, appeared to regress and a new follicle was recruited within 12 hours. The poor synchrony of ovulation which is sometimes observed after prostaglandin $F_{2\alpha}$ induction of oestrus, was not considered to be attributable to variability of luteolysis. It was considered probable that the timing of the luteinising hormone preovulatory surge, which is variable, may be influenced by the number and size of follicles present in the ovary at the time of prostaglandin injection. The discussion of the mechanism of such an effect was equivocal.

A reliably short lifespan of the ovulatory follicle would seem to be an important condition for accurate synchronous induction of oestrus and ovulation by prostaglandin injection. If that factor is variable between individuals, or between different stages of the oestrus cycle, then the interval from prostaglandin injection to ovulation will also be variable

Oestrus cycle length may of course be materially controlled by prostaglandin $F_{2\alpha}$. The ensuing cycle however is subject to the normal individual variability noted above. So by the mid to late phase of dioestrus a degree of asynchrony can have developed between females injected at the same time for induction of ovulation. When a second injection of prostaglandin $F_{2\alpha}$ is then administered to induce synchronous oestrus, not all females will be at precisely the same stage of dioestrus. Asynchrony of the interval from induction with prostaglandin $F_{2\alpha}$ to ovulation is considered to be a contributory factor in failure to achieve maximum conception rate by this method. This interval may be made more precise by supplementary administration of progesterone, or GnRH, but such improved precision has not yet been reliably associated with improved conception rate.

Investigating oestrus synchronisation in heifers with two injections of cloprostenol, Roche (1977) noted that only 31 per cent had ovulated by 78 hours after the second injection. In an attempt to produce more synchronous ovulation cloprostenol treated heifers were injected with 100 μg GnRH 48 hours after the second cloprostenol injection. This resulted in 93 per cent having ovulated by 78 hours after the second cloprostenol injection. Following cloprostenol synchronisation with or without supplementary GnRH treatment heifers were inseminated at a fixed-time, either once (n=34) or twice (n=147) after the second cloprostenol injection. GnRH treatment had no effect on conception rate. In a similar study synchronising oestrus in heifers (n=130) with dinoprost, either with or without an injection of 100 μg GnRH given 72 hours after the first dinoprost injection. (Graves and others, 1985) treatment groups did not differ in conception rates or in pregnancy rates. In these circumstances, improved synchrony of ovulation was not associated with the expected improvement in conception rate.

It was noted from surveyed literature, (Higgins and others, 1986) that combined treatments involving progestagens for 5 to 9 days followed by a single injection of prostaglandin $F_{2\alpha}$, before, at, or after withdrawal of progestagen, resulted in increased proportions of animals exhibiting oestrus , improved synchronisation, and reduced intervals to onset of oestrus, when compared with a single injection of prostaglandin $F_{2\alpha}$. These workers attempted to emulate this improvement in synchronisation by giving a single supplementary injection of progesterone 5 days, or 24 days before treatment with prostaglandin $F_{2\alpha}$. Progesterone pretreatment combined with prostaglandin $F_{2\alpha}$ appeared to enhance oestrus synchronisation but again did not influence either pregnancy or calving rates.

It is surprising that improved precision of ovulation, allowing improved precision of insemination, which is considered to be critical for optimum conception, has not necessarily resulted in improved conception. The explanation for this is not clear. The time of insemination relative to ovulation is presumed to be critical. In the bovine female the ovum remains viable for only 4 to 6 hours after ovulation but spermatozoa remain viable for about 24 hours after insemination. These 'safety margins' could be expected to allow some flexibility in the interval from insemination to ovulation for succssful fertilisation, of the order 24 hours before to 6 hours after the optimum time. If the optimum time for insemination is 12 hours before ovulation, ie. roughly the end of oestrus, then that would suggest that successful insemination might be achieved from 24 hours before the end of oestrus until 6 hours after. In practice, it would appear that the period suitable for successful insemination may be somewhat longer.

The effect of the time of oestrus, relative to a fixed-time insemination 48 hours after implant removal, was investigated in norgestomet treated dairy heifers. (Anderson and others, 1982) In these studies, the time of oestrus was recorded but the fixed-time insemination was not altered to take account of any variations noted. Oestrus was first detected from 24 hours before to 24 hours after artificial insemination. The resulting conception rate averaged 63 per cent. In a similar study, an average conception rate of 71.2 per cent was recorded in heifers first detected in oestrus from 32 hours before to 24 hours after insemination. (Graves and others, 1985) These are high conception rates, and support the indications that precise timing of insemination relative to ovulation may be less critical than has been supposed.

Current wisdom accepts that timing of insemination relative to time of ovulation is critical for optimum conception rate. The observations above show that improved precision of insemination relative to the time of ovulation does not necessarily produce improved conception. The observations also show that conception is not necessarily reduced when there is considerable divergence from optimum insemination timing relative to oestrus. There is no immediately apparent explanation for these observations. It may be that some current assumptions do not have the validity attributed to them. Factors other than precision of timing may make an essential contribution, or may be more important

When all animals in a group are at precisely the same stage of the ovarian cycle when a prostaglandin $F_{2\alpha}$ injection is given, good synchrony of oestrus within the group is possible: but the stage of the cycle at which the injection is made influences the length of the interval from injection to oestrus and ovulation.

(Watts and Fuquay, 1985) Within a random group of animals the stage of the cycle of individuals varies. The effect of this difference is to widen the range of intervals from injection to ovulation, such that a given fixed-time insemination is not equally appropriate for all animals in the group.

A lower oestrus response rate and lower fertility was observed in dairy heifers injected with prostaglandin $F_{2\alpha}$ during early dioestrus as compared with those injected after Day 10 of their cycle. (Watts and Fuquay, 1982 ; Watts and Fuquay, 1983)

In a two year study, conception rates were recorded for dairy heifers (n=250) which were injected with prostaglandin $F_{2\alpha}$ on different days of the oestrus cycle. (Watts and Fuquay, 1985) After an observed oestrus , heifers were injected once with prostaglandin $F_{2\alpha}$ either on cycle Days 5 to 7 (Group E, n=86), 8 to 11 (Group M, n=104), or 12 to 15 (Group L, n=60) ; oestrus was Day 0. In this study, for 5 days after the prostaglandin $F_{2\alpha}$ injection, heifers were inseminated about 12 hours after oestrus was first observed. Observed rates of successful oestrous response were, 43.0 per cent, 83.6 per cent, and 100 per cent respectively for E, M, and L. Average time from prostaglandin $F_{2\alpha}$ injection to observation of oestrus for E, M, and L was 59, 70 and 72 hours.

Conception rates for heifers responding to prostaglandin $F_{2\alpha}$ were 56.8 per cent, 62.1 per cent, and 78.3 per cent for Groups E, M, and L respectively. Group E had a significantly lower response rate and conception rate compared with Groups M and L, as well as a shorter period between injection of prostaglandin $F_{2\alpha}$ and observation of oestrus. Peak conception rates were achieved by heifers that had been injected with dinoprost on Days 11 to 14 of their oestrous cycle. The conception rates were : Day 11 (n=16) 81.2 per cent ; Day 12 (n=22) 77.3 per cent ; Day 13 (n=16) 100 per cent ; Day 14 (n=10) 70.0 per cent.

Data from a study examining ovaries from dairy cows slaughtered at different stages of the oestrus cycle provide support for this finding and illustrate the probable mechanism involved. (Kruip, 1982) Two active phases of follicular growth were identified. Follicles which reach a large size within the first eight days of the cycle cannot maintain their dominant position and degenerate. This degeneration gives a new chance for small follicles to grow (Day 8-13) leading to the second group of dominant follicles present from Day 13 until the next oestrus. The follicles of this second ovarian phase would be reaching maturity about the time when dinoprost induction in fact produced highest conception rates. Further supporting evidence (Ireland and Roche, 1983) also suggests that there are two phases of follicle growth and atresia during the oestrous cycle, one between Days 3 and 7 and the other between Days 7 and 13.

The investigation by Watts and Fuquay (1985) was a carefully executed, well documented practical study, and the reported results warrant further investigation. The data suggest that the currently used interval between prostaglandin $F_{2\alpha}$ injections in fixed-time insemination scheduling might be improved. An 11-day interval between injections means that females with responsive *corpora lutea* at the first injection, will be about Day 8 of the new cycle for the second injection; those which had immature corpora lutea will be at Days 8 to 11. By extending the interval between prostaglandin $F_{2\alpha}$ injections to 14 days, the second injection would be made on Day 12, instead of Day 8; and on Days 12 to 15 instead of Days 8 to 11. This would bring all into the range of days after oestrus which have produced optimum conception rates. The longer interval between injections would also accomodate the small proportion of females that have delayed oestrus after the first injection, and the longer oestrous cycle observed in heifers during the autumn/winter months.

The timings of hormonal and physiological events around oestrus and ovulation have been well documented. The underlying message is that while the sequences and broad time relationships are well recognised, they are subject to quite considerable natural and individual variation. Exogenous hormonal control of these events has met with mixed success. The ability to initiate oestrus with prostaglandin $F_{2\alpha}$ or progestagen has had considerable advantages for the management of breeding. However the induced oestrus still retains all the variability of time relationships characteristic of natural oestrus. It has been possible to induce ovulation with greater precision by administering exogenous hormones.

Unfortunately, this improved precision has not been accompanied by the anticipated improvement in conception rate.

Schedules for artificial insemination at predetermined fixed-times, following group induction of synchronised oestrus, have to allow for the range of time responses within a group of treated animals. Although suitable timings may be predicted from a knowledge of the physiological events, the imponderability of variables has meant that there has been no substitute for experimental trials to establish optimum timings. The following studies will describe assessment of the relative effectiveness of established timings for fixed-time insemination of oestrous synchronised cattle in commercial conditions.

FIXED-TIME INSEMINATION SCHEDULES

The ability to control the time of oestrus and ovulation has provided the possibility of inseminating a group of bovine females at a predetermined time. This offers advantages both in the management of insemination and in the management of the cattle during and after the ensuing pregnancy. The technique of fixed-time artificial insemination can produce conception rates equivalent to those of untreated contemporaries inseminated at natural oestrus. In addition, when synchronous oestrus is induced in a group of females, every one is inseminated and given the opportunity to conceive. If the same group is inseminated at natural oestrus, only those detectd in oestrus can be inseminated, and oestrus detection is at best an inefficient exercise, for reasons detailed in PART 1. The end result is that synchronised oestrus with fixed-time insemination results in more pregnancies than can be achieved by insemination on detected oestrus, other things being equal.

The timing of the events of oestrus and ovulation have been well documented, both in naturally occuring oestrus and in artificially induced oestrus. Whilst these are largely predictable, the ranges of natural variations introduce a degree of imprecision. Whilst schedules for prostaglandin injections and insemination have been based on research data in the first place, it has been necessary to establish optimum timings by trials conducted under practical agricultural conditions. For reasons of availability and convenience, such work has mainly been done with heifers and beef cows, without drawing any very clear lines of distinction between them.

In practice, oestrous synchronisation techniques are used on three types of cattle which have different reproductive characteristics and different conditions of husbandry and management. It is likely that such underlying differences will have some influence on the outcome of oestrous synchronisation. Consideration of each class specifically, might suggest alternative recommendations for treatment, or different expectations following treatment.

Techniques for synchronisation of oestrus with prostaglandin $F_{2\alpha}$ have evolved to a single treatment schedule and a small number of insemination schedules. The recommended schedule for oestrous and ovulation synchronisation with all current commercially available prostaglandin $F_{2\alpha}$ products is two injections of prostaglandin $F_{2\alpha}$ with an interval of eleven days between. The recommended schedule for insemination following induction with the prostaglandin analogues is 72 and 96 hours after the second injection. Single fixed-time insemination is not recommended, but if used should be done at 72 hours after prostaglandin $F_{2\alpha}$ injection - with a warning that this is likely to result in reduced conception.

The insemination schedule recommended for dinoprost is two inseminations, 72 and 90 hours after the second injection. A single insemination schedule is also recommended, with insemination 78 hours after the second injection

For the prostaglandin $F_{2\alpha}$ products commercially available for cattle in Britain, no differentiation is made between the three types of cattle, either for recommended treatment schedule, or for expected results. The physiological and hormonal data discussed earlier, and the differences in reproductive characteristics and typical husbandry methods, suggest that differences may be expected. There is a special need for this information in dairy cows because these account for the greatest usage of prostaglandin $F_{2\alpha}$ in practice. No complete study investigating a single insemination schedule, specifically in dairy cows, could be found in a search of the published literature. The trials which will be reported and discussed, were to investigate two practical alternative insemination schedules; in heifers, beef cows and dairy cows. The schedules were for a single insemination 78 hours after, or for two inseminations 72 and 96 hours after the second dinoprost injection.

CONTROLLED BREEDING IN HEIFERS

(PAPER 4)

The trial in PAPER 4 was designed to investigate the relative effectiveness of a single fixed-time insemination, compared with two fixed-time inseminations, following recommended treatment for oestrus synchronisation with dinoprost in heifers.

The trial involved 137 Friesian dairy heifers, all given two intramuscular injections of 25 mg dinoprost with an interval of eleven days between. They were randomly allotted to two groups, one given a single insemination 75 to 80 hours after the second dinoprost injection, the other given a double insemination 72 and 96 hours after the second injection. Semen from two bulls was shared equally between the two groups, and heifers on double inseminations received semen from the same bull on both occasions. Three inseminators did an equal number of inseminations in each group, and heifers on the double insemination regime were inseminated by the same inseminator on both occasions. The first service conception rates were; single insemination 38 per cent, double insemination 34 per cent. The difference is not significant.

The overall conception rate is low for dairy heifers, so the data were analysed in detail to seek an explanation. It had not been possible to have controls, either inseminated at natural oestrus, or inseminated at observed oestrus after dinoprost induction.

Investigation revealed that a major constituent of the ration had been changed abruptly three weeks after insemination, because supplies had run out. The initial diet was maize silage which is low in β-carotene. Deficiency of β-carotene has been associated with reduced fertility. (Jackson, 1981) Such a drastic change of diet at precisely this stage of gestation was found to cause a dramatic reduction in conception rate in previous studies on controlled breeding in heifers with norgestomet implants. On that occasion, a supplement of grain was withdrawn from the trial heifers three weeks after insemination. (unpublished data) The same effect has been recorded in beef cows. (Lowman, 1985) About 10 to 15 per cent of early embryonic mortality occurs about the time of implantation. (Sreenan anf Diskin, 1983) The change of ration about three weeks after insemination would approximate to the time of implantation and it is likely that the resulting nutritional stress contributed to the poor conception rate.

Investigation also revealed that 112 of the 137 heifers on trial were under the stipulated age of 15 months by one or two months. The combination of nutritional stress at a critical stage and puberal immaturity are probably sufficient to account for the disappointingly low conception rates.

A number of additional factors appear to have made a contribution to low conception.

1. Conception rates for the semen from the bulls used were, KD: 48 per cent, SS : 34 per cent. The overall low fertility can be attributed to the female component. Bulls are known to vary in the fertility of their semen, however, and bull SS appears to have been less fertile than bull KD.

2. Conception rates analysed by inseminator were 36 per cent, 38 per cent and 56 per cent. Operator efficiency varies between inseminators and this component may also have made a contribution to overall results.

3. Heifers from six heifer rearing farms were brought together and mixed about a week before the start of the trial. The heifers contributed by individual farms did not all do equally badly. Conception rates by contributing farm were : 59 per cent, 50 per cent, 48 per cent, 36 per cent, 19 per cent, and 0 per cent. It is also likely that the stress of mixing strange heifers would make a contribution.

It is unfortunate that the first study had just about everything go wrong that could, particularly as heifers generally make good experimental models. However the trial design distributed these effects evenly between the study groups and the comparison between the groups may be accepted. The difference in this study between single fixed-time insemination, 38 per cent and double fixed-time insemination 34 per cent, is not significant. From the results of this study it may be concluded that after synchronised ovulation with dinoprost, one fixed-time insemination is as effective as two fixed-time inseminations under adverse conditions.

DISCUSSION

In a Survey of insemination results confirmed by calving data for the year 1983, (D Smith, personal communication) the Aberdeen and North of Scotland Milk Marketing Board recorded the following data for dairy heifers synchronised with dinoprost and batch inseminated : Single fixed-time insemination (n=221), average conception rate 63.8 per cent, Double fixed-time insemination (n=260), 58.8 per cent. For dinoprost synchronised, single inseminated beef heifers (n=159) the average conception rate was 57.9 per cent. The number of double inseminated beef heifers was too small (n=29) for comparison.

In a study in dairy heifers (Graves and others, 1985) reported conception rates in untreated control heifers inseminated at observed oestrus (n=42) 64.3 per cent compared with heifers synchronised by two injections of dinoprost and inseminated 80 hours after the second injection (n=44) 65.9 per cent. They also compared in heifers synchronised by two injections of dinoprost (a) inseminated on observed oestrus following synchronisation (n= 78) 56.4 per cent with (b) heifers inseminated 80 hours after the second injection (n=81) 53.1 per cent, and (c) control heifers, untreated and inseminated on observed oestrus (n=81) 70.4 per cent.

This body of evidence suggests that no practical advantage is achieved by inseminating dinoprost synchronised heifers more than once in fixed-time insemination schedules.

Documented evidence of conception rates in heifers to single fixed-time insemination schedules using cloprostenol is sparse and involves numbers too small for realistic appraisal. In a study on Friesian heifers, (Roche,1977) conception rates were compared between (a) untreated control heifers inseminated on observed oestrus (n=24) 46 per cent, and heifers synchronised by two injections of 500 μg cloprostenol eleven days apart (b) inseminated at observed oestrus (n=73) 66 per cent, or (c) one insemination 60 hours after the second injection (n=35) 29 per cent, or (d) inseminated twice 60 and 74 hours after the second injection (n=36) 53 per cent. Heifers in a second study were synchronised in the same way. Conception rates were : untreated controls inseminated at observed oestrus (n=10) 60 per cent, heifers synchronised by two injections of cloprostenol eleven days apart and (a) inseminated 48 hours after the second injection (n=20) 10 per cent, (b) inseminated 72 hours after the second cloprostenol injection (n=12) 25 per cent, (c) inseminated twice 48 and 72 hours after the second injection (n= 12) 50 per cent. The selection of non-standard insemination timings, combined with variable but small numbers renders the results equivocal and difficult to interpret.

The data sheet recommendation for fixed-time insemination following synchronisation with two injections of cloprostenol with an eleven day interval is : for double insemination, 72 and 96 hours after the second cloprostenol injection : for single fixed-time insemination, 72 hours after the second injection. A warning is given that reduced conception may result from the second schedule. Such évidence as is available would support the warning.

In view of the known differences between prostaglandin $F_{2\alpha}$ analogues, and between types of cattle, it is important that trial design should be specific in both parameters. The differences are not large and cannot be clarified in absence of such careful distinction. Conversely, failure to make such distinctions may conceal identifiable differences, so interpretation of trial results must be alert to inconsistencies.

A trial was reported on oestrus/ovulation synchronised cattle with either dinoprost or cloprostenol, without making any distinction between the two, and included both heifers and suckler cows, again without distinction. (Hafs and others, 1978) The study consisted of standard oestrus synchronisation by two injections of unspecified 'prostaglandin' with an eleven day interval, followed by one insemination 80 hours after, or two inseminations 70 and 88 hours after the second prostaglandin injection. In view of the differences between prostaglandin $F_{2\alpha}$ products, and the differences between heifers and beef cows illustrated in PAPERS 4, 5 and 6, the conclusions of this study must be of limited value.

CONTROLLED BREEDING IN BEEF COWS

(PAPERS 5 AND 6)

The trials reported in PAPERS 5 and 6 were designed to investigate the relative effectiveness, in beef suckler cows, of a single insemination regime, compared with a double insemination regime, after recommended oestrous and ovulation synchronisation with dinoprost. The results of this study were to be compared with those from similar studies in maiden heifers and dairy cows, to look for differences which might be attributable to the different reproductive characteristics of the different types of cattle.

The first study (PAPER 5) was done on 120 parous, single-suckled Hereford X Friesian cows. All were at least 50 days *post partum*. The herd was run as a single group until calving, when it was divided according to the sex of the calves into two separate herds, each with calves of one sex. This division was accepted for allocation of treatment, all members of each herd being on the same insemination schedule. There were 60 cows in each herd.

Synchronisation of ovulation was by two intramuscular injections of 25 mg dinoprost with an interval of 11 days. Single insemination was 78 hours after, and double insemination 72 and 96 hours after the second dinoprost injection. Semen was from two bulls, FS and SP and was used on the same proportion of cows in each group. Insemination was by two inseminators from the Scottish Milk Marketing Board. Cows were identified . Those on the two-insemination schedule were inseminated with semen from the same bull and by the same inseminator on each occasion.

Results were calculated from calving data. The conception rate of single inseminated cows was 53 per cent, and of twice inseminated cows was 67 per cent. There was no difference between results analysed by bull (57 per cent *versus* 63 per cent), or by inseminator (59 per cent *versus* 61 per cent).

The second study (PAPER 6) was done on 50 parous Hereford X Beef Shorthorn single suckled cows which were randomly allocated to the two treatment groups. Semen from one Charolais bull was used and insemination was by two inseminators from the Scottish Milk Marketing Board. Results were calculated from calving data.

The conception rate for single inseminated cows was 52 per cent, and for twice inseminated cows was 68 per cent.

DISCUSSION

The conception rates in both treatment groups would be considered high for suckler cows in both studies. The difference between treatments was remarkably similar, of the order of 15 per cent in favour of double insemination in each study.

In a Calving Survey of oestrous synchronised batch inseminations carried out by the Aberdeen and North of Scotland Milk Marketing

Board in 1983 (D Smith, personal communication) data was collected for suckler beef cows. Only identifiable calving results were included. The conception rate for beef cows synchronised with dinoprost and after the second injection ; inseminated at 72 and 96 hours (n=93) was 62 per cent ; inseminated once at, 75 to 80 hours (n=727) was 59 per cent).

In a study on 162 single suckled beef cows where oestrous synchronisation was by norgestomet implant (Drew, Wishart and Young, 1979), a single insemination 48 hours after implant removal was compard with two inseminations, 48 and 72 hours after implant removal. The conception rates were 44 per cent and 61 per cent respectively

In combined results from comparative trials with cloprostenol on lactating beef cows (n=2676) conception rate for cows inseminated 72 and 96 hours after the second cloprostenol injection (n=954) was 41.8 per cent ; for cows inseminated once 72 hours after the second cloprostenol injection (n=824) it was 35.3 per cent. (Estrumate Handbook for planned breeding of Beef and Dairy Cattle)

A report of trials comparing single and double fixed-time insemination schedules in suckler cows following oestrous synchronisation with prostaglandin (Hafs and others, 1978) does not help to resolve the situation because it fails to differentiate between dinoprost and cloprostenol , or between dairy heifers and suckler cows.

The rather sparse data from controlled experimental evidence indicates that a single insemination schedule for oestrous synchronised suckler cows is likely to give lower conception rates than a double insemination schedule, by some 5 to 10 per cent. The recorded practical experience of the Aberdeen and North of Scotland Milk Marketing Board does not, however, confirm the inevitability of this. In that survey, conception rates for both schedules were high for suckler cows. It may be that in the beef cow, active ovarian cyclicity and sound breeding management are more significant than the insemination schedule.

CONTROLLED BREEDING IN DAIRY COWS

(PAPER 7)

The purpose of this study was to compare the effectiveness of a single fixed-time, with a double fixed-time artificial insemination regime after oestrous synchronisation with dinoprost. This information could not be found in a search of the published literature, which mainly relies on data from heifers and beef cows. The three types of cattle may not be assumed to respond in identical fashion because each has different reproductive characteristics and dissimilar conditions of husbandry and management. The dairy cow is the most frequent, and most important subject of prostaglandin treatment.

This trial (PAPER 7) was conducted on a total of 535 lactating Friesian dairy cows, on three commercial dairy farms. On each farm cows were grouped into weekly batches on the basis of calving dates and allocated on a statistically random basis to one of three groups. Inseminations were started in accordance with farm policy, after 42 days *post partum* on two farms, and after 49 days *post partum* on the third. Cows in treatment groups were injected with 25 mg dinoprost twice with an 11-day interval. Those on the single insemination regime were inseminated once, 75 to 80 hours after the second dinoprost injection; those on the double insemination regime, were inseminated 72 and 96 hours after the second dinoprost injection. Control cows were observed over a contemporaneous three-week period and inseminated at detected oestrus. Insemination was by the Milk Markting Board Inseminator Service, and choice of bull was at the discretion of the farmer. Data were calculated from calving results.

The First service conception rate for cows on the single insemination schedule (n=183) was 46 per cent; for those on the double insemination schedule (n=176) was 47 per cent ; and for contemporary controls (n=107) was 50 per cent. There was no statistically significant difference between conception rates of cows which were inseminated.

DISCUSSION

The relative effectiveness of a single insemination schedule in the dairy cow here demonstrated, compares with the reduced conception following the same protocol in beef cows demonstrated in the previous study. Only one published account of a similar study, specific to dairy cows, has been found in the literature. Using two injections of cloprostenol to synchronise ovulation dairy cows were inseminated on a fixed-time schedule 80 hours, or 72 and 96 hours after the second injection. The conception rates were (n=88) 43.5 per cent and (n=102) 37.7 per cent respectively. (Chupin, 1977)

The benefit of fixed-time insemination in the dairy cow is twofold: reducing reliance on inefficient oestrus detection, and shortening the interval from calving to service. Both were illustrated in the study.

Cows allocated to the control group could be inseminated only if detected in oestrus. The oestrous detection rate was 61 per cent. Of the total number of control cows (n=176), 53 were pregnant to insemination during the 21 day service period of the trial. The comparable figures for treated cows was; single insemination (n=183) 84 pregnant and double insemination (n=176) 83 pregnant. This represents an increase of first service pregnancies in excess of 50 per cent resulting from removal of reliance on oestrous detection.

Induction of ovulation, by definition, shortens the time to next oestrus by an average of half of one oestrous cycle. Cows which do not conceive to first insemination will continue to cycle on average half a cycle in advance of their natural cyclical pattern. So all treated cows have a continuing half cycle advantage over untreated cows. This was illustrated in the study. Treated cows compared with controls, had statistically significant advantages in calving-to-first service interval, 10 days and calving-to-conception interval, 12 days.

The presumed reason for lack of published information from dairy cows on this aspect of controlled breeding is the dificulty of assembling large groups of cows at a stage suitable for treatment at the same time. This complicates the organisation and design of trials. Under farming conditions, it also complicates the application of a useful technique in a regular way. Alternative methods of making use of the principles will be investigated in PART 3.

CONCLUSION - PART 2

Synchronised oestrus and ovulation followed by fixed-time insemination can produce conception rates equivalent to those obtained by insemination at detected oestrus. Ovulation is synchronised by two injections of 25 mg dinoprost with an 11-day interval. Insemination may then be once, 78 hours after, or twice, 72 and 96 hours after the second dinoprost injection.

Results vary and depend on the type of cattle being treated and on conditions of husbandry and management.

Fertility in heifers is generally good, provided that they are sufficiently mature and that adequate energy is fed. A supplement of energy to the ration will usually increase conception rates, but must not be abruptly withdrawn before foetal implantation. A single insemination schedule is as effective as two inseminations, and may be slightly better.

In beef cows, the suckling effect tends to delay return to ovarian cyclicity and this effect shows a seasonal influence, being greater during the winter months. This effect may be reduced, and fertility improved by feeding a ration adequate in energy, again taking care not to increase early embryonic mortality by withdrawing it too soon. Some beef cattle may have inherently low fertility, additional to the more common effect of suboptimal nutrition. Single fixed-time insemination is likely to result in conception rates lower than double fixed-time insemination by a factor of the order of 10 per cent.

The principal benefit of batch breeding in heifers and beef cows is the ability to use semen from superior sires by artificial insemination. There are also advantages from batching of calving and management routines.

In dairy cows, a single fixed-time insemination regime can result in conception rates equivalent to those from double fixed-time insemination. Delayed uterine involution resulting from *post partum* uterine infection is the most common factor delaying return to ovarian cyclicity. Adequate nutrition is not usually a problem in the dairy cow. The advantages for the dairy farmer are reduced reliance on inefficient oestrus detection and a reduced calving-to-conception interval.

There are indications that conception rates from fixed-time insemination might be increased by extending the interval between the two prostaglandin $F_{2\alpha}$ injections used to induce synchronised ovulation.

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PAPER 4

REPORT CODE NO. ________

TECHNICAL REPORT

TRIAL CODE NO. CWS/80/LUT/CAT/12

DATE 9th December, 1981

TITLE: DAIRY HEIFER CONTROLLED BREEDING : SINGLE ARTIFICIAL INSEMINATION VERSUS DOUBLE ARTIFICIAL INSEMINATION

AUTHOR: I.M. YOUNG

ABSTRACT: One hundred and forty heifers were presented for treatment with 2 x 25 mg dinoprost injections followed by either single (75-80 hours) or double (72 and 96 hours) fixed time artificial insemination (A.I.). Conception rates for the two groups were 38% and 34% respectively. Pregnancy results were also recorded by bull and by A.I. Technician. Differences of up to 20% were recorded between Technicians. The low overall conception rates were due to immaturity of the heifers, with 17.5% only 13 months old at treatment and 64.2% only 14 months old.

70-1704 12/77

CWS/80/LUT/CAT/12
9th December, 1981

INTRODUCTION

The object of the trial was to compare in Friesian diary heifers, single and double insemination regimes after treatment with dinoprost.

Estates operate a number of dairy farms. The heifers used on the trial were on the heifer rearing unit which receives calves from the various farms. They are reared and mated on the unit and returned to the farm of origin in late pregnancy so that they can be introduced into the milking herd at calving.

METHOD

It was stipulated that heifers for the trial should be at least 15 months old at mating, that they should weigh 300 kilo's or greater and that the ration should not be changed for at least 6 weeks after insemination.

The heifers, which had been on grass, were housed in covered yards prior to the start of treatment to allow time to settle and adjust to the change of diet.

Heifers from each farm were allocated randomly to treatment groups. Untreated controls were not feasible as natural mating with bulls was the alternative to synchronised inseminations.

Two intramuscular injections of 25 mg dinoprost were given to all heifers with an interval of 11 days between injections.

Heifers on the single insemination regime were inseminat d 75 to 80 hours after the second dinoprost injection.

Heifers on the double insemination regime were inseminated 72 and 96 hours after the second dinoprost injection.

Semen from two M.M.B. bulls (KD and SS) was used in similar proportions in both treatment groups and the insemination was done by inseminators of the M.M.B. A.I. Service. The identity of the inseminator performing each insemination was recorded, and in the case of double inseminations, the same inseminator performed both. Heifers on double insemination regime were inseminated with semen from the same bull on both occasions.

Pregnancy diagnosis by rectal palpation was carried out at 7 weeks after A.I. Calving dates were recorded and the results arre calculated from these.

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RESULTS

Heifers presented for trial	140	
Removed from trial	3	(1 pregnant, 1 free-martin, 1 escaped treatment)
Total treated and inseminated	.137	
Pregnancy diagnosis (7 weeks post A.I.)	49	pregnant

DISCUSSION

This is a disappointing result. A number of factors have been identified which would explain the poor conception rate to fixed time inseminations.

Age of Heifers on Trial

Calculating from the birth dates of the heifers revealed that at the time of inseminations, only 25 of the 137 had achieved the age of 1 year 3 months or more; 88 were 1 year 2 months old and 24 were 1 year 1 month old (see Table 4).

This is very disappointing as it was specifically agreed before starting the trial that only heifers at least 15 months old would be used. Sexual maturity and ovarian activity is related to age. It is not sufficient to rely on target weights. While optimum liveweight gain will result in optimum sexual growth, there are physiological changes which require time, and there is a minimum time which is needed for these to occur. For an acceptable majority of a group of Friesian heifers to have achieved fully functional sexual maturity it is necessary that they should be more than 15 months old.

Change of Ration During Trial Period

At was found necessary to change the major constituent of the ration molassed beet pulp for maize silage - during the critical first 6 weeks of pregnancy. Diet stress at this stage, (before the embryo is implanted) can result in an increase of early embryonic deaths. The importance of this was explained before the start of the trial in the expectation that the need for such a change could be anticipated and avoided.

Other Factors

The results have been analysed by farm of origin of the heifers which were randomly distributed between treatments. It appears that heifers from different farms did not all perform equally well. Heifers bearing code HW, PV and FV achieved notably lower conception rates than the others (see Table 5).

Analysis of results by bulls and by inseminators is included (Tables 2 and 3). The trial was not designed to compare these performances so it would be wrong to attempt to draw firm conclusions from the differences.

CONCLUSION

The conclusion must be that the low conception rate overall was due principally to the immaturity of the majority of the participating heifers, probably compounded by the change of a major constituent of the diet in the immediate post insemination period and by factors relating to some of the farms which contributed heifers.

Despite the poor overall conception rate to fixed-time insemination, the better result to single A.I. frequently seen in heifers is still apparent.

CWS/80/LUT/CAT/12
9th December, 1981

Table 1

Conception Rates

	Treated	Pregnant	Percent
Conception rate to Single A.I.	69	26	38%
Conception rate to Double A.I.	68	23	34%
Overall conception rate	137	49	36%

CWS/80/LUT/CAT/12
9th December, 1981

Table 2

Bull Results
(Pregnant/Inseminated)

Bull	Double A.I.	Single A.I.	Total	Percent
KD	25/50	18/40	43/90	48%
SS	1/8	15/39	16/47	34%

Table 3

Inseminator Results
(Pregnant/Inseminated)

Inseminator	Double A.I.	Single A.I.	Total	Percent
X	12/30	6/20	18/50	36%
Y	8/18	8/24	16/42	38%
Z	11/20	14/25	25/45	56%

Table 4

Age of Heifers

Days Over 1 Year Old at A.I.

Pen No.	30-39	40-49	50-59	60-69	70-79	80-89	90+	Total
1	-	2	2	-	5	11	9	29
2	-	-	-	3	6	7	-	16
3	3	-	1	4	13	4	3	28
4	-	5	1	3	10	4	10	33
6	1	3	6	3	7	8	3	31
Total	4	10	10	13	41	34	35	137

24
2 months premature

88
1 month premature

Table 5

Pregnancy Results by Herd of Origin

Herd	Pregnant	Treated	Percent
XW	13	22	59%
NW	16	32	50%
CW	15	31	48%
HW	12	33	36%
PV	3	16	19%
FV	0	4	0%

PAPER 5

AGRICULTURAL RESEARCH AND
DEVELOPMENT LABORATORIES,
THE UPJOHN COMPANY

REPORT CODE NO. ______

TRIAL CODE NO. 81/LUT/CAT/13

DATE 2nd December, 1981

TECHNICAL REPORT

TITLE: FERTILITY OF BEEF COWS FOLLOWING OESTRUS SYNCHRONISATION WITH DINOPROST

AUTHOR: I.M. YOUNG

ABSTRACT: One hundred and twenty mature beef cows were treated with dinoprost to synchronise oestrus. Artificial insemination of animals was either with Aberdeen Angus or Simmental semen at either 78 (single) or 72 and 96 (double) hours after second dinoprost treatment. The conception rate to single A.I. was 53% and to double A.I. 67%. Results were also recorded by bull, 57% Aberdeen Angus, 63% Simmental; and by inseminator, 59% Inseminator 1, 61% Inseminator 2.

70-1704 12/77

81/LUT/CAT/13
2nd December, 1981

METHOD

The cows used on the trial were Hereford X Friesian single suckled beef cows aged 3 to 6 years. All cows included in the trial were at least 50 days post-partum. It is the practice of the farm to run the cows in two groups according to the sex of the calf, and this was accepted as the basis for allocation to the two treatment groups. The whole herd was eligible for the trial and fortunately the division between the sexes of calves was exactly equal.

Dinoprost injections for the synchronisation of ovulation, two injections at an interval of 11 days, were administered by a veterinary surgeon from the local practice in accordance with the protocol. The double A.I. group were inseminated 72 hours and 96 hours after the second dinoprost injection; the single A.I. group 78 hours after the injection.

Semen from two bulls was used - Aberdeen Angus (Fraser of Stern) and Simmental (Scottish Plato). Thirty cows in each group were inseminated with Aberdeen Angus semen and thirty with Simmental semen. Cows in the double A.I. group were identified and marked so that they were inseminated with semen from the same bull both times.

Two inseminators from the Scottish Milk Marketing Board performed the inseminations. Each inseminated 6 cows, then rested by preparing insemination rods for the other. Cows on the double A.I. groups were identified and inseminated by the same inseminator on both occasions.

Stock Hereford bulls were turned out with the cows one week after A.I. to serve those cows which returned to service. Results are calculated on calving data.

RESULTS AND DISCUSSION

The conception rate (Table 1) to single A.I. of 53% is a satisfactory result. The conception rate to double A.I. pf 67% is exceptionally high for beef cows. This is a well managed farm and the conditions of husbandry and of the cows are high, which accounts for such satisfactory results.

The result is not in agreement with the conclusions of Trial LUT/CAT/10 in 535 dairy cows where the small difference between insemination regimes (single and double A.I.) was not significant. It would be valuable to know if the difference is a real difference between the response of dairy cows and of beef cows, or whether it is exaggerated by the between-herd differences which are well recognised.

The fertility of the semen from both bulls was of a high standard (Table 2). The inseminators performed equally well overall, although it is interesting to speculate if the difference in performance of inseminator 2 between the two regimes is more than a chance effect - probably not.

TABLE 1

Conception Rates

Calculated on calving results

	Cows Treated	Pregnant	Percent
Single A.I.	60	32	53%
Double A.I.	60	40	67%
TOTAL	120	72	60%

TABLE 2

Conception Rates by Bull

	Single A.I.			Double A.I.			Total		
	Insemi-nated	Preg-nant	(%)	Insemi-nated	Preg-nant	(%)	Insemi-nated	Preg-nant	(%)
Aberdeen Angus	30	15	(50%)	30	19	(63%)	60	34	(57%)
Simmental	30	17	(57%)	30	21	(70%)	60	38	(63%)

81/LUT/CAT/13
2nd December, 1981

TABLE 3

Conception Rates by Inseminator

Inseminator	Single A.I.			Double A.I.			Total		
	Insemi-nated	Preg-nant	(%)	Insemi-nated	Preg-nant	(%)	Insemi-nated	Preg-nant	(%)
1	30	18	(60%)	29	17	(59%)	59	35	(59%)
2	30	14	(47%)	31	23	(74%)	61	37	(61%)

PAPER 6

AGRICULTURAL RESEARCH AND
DEVELOPMENT LABORATORIES,
THE UPJOHN COMPANY

TECHNICAL REPORT

REPORT CODE NO. ______________

TRIAL CODE NO. ______________

DATE 6th May, 1982

TITLE: BEEF COW CONTROLLED BREEDING: COMPARISON OF SINGLE ARTIFICIAL INSEMINATION AND DOUBLE INSEMINATION REGIMES AFTER SYNCHRONISATION OF OVULATION WITH DINOPROST

AUTHOR: I.M. YOUNG

ABSTRACT:

Fifty mature beef cows were treated with dinoprost to synchronise ovulation. Artificial insemination (A.I.) of the cows was at either 78 (single) or 72 and 96 (double) hours after the second dinoprost treatment.

The conception rate to single insemination was 52%, and to double insemination was 68%.

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METHOD

Fifty beef cows, Hereford X Beef Shorthorn, aged 5 to 7 years were used for the trial, divided randomly into two equal groups. For the synchronisation of ovulation, two intramuscular injections of 25 mg dinoprost were given with an eleven day interval. The double insemination group was inseminated 72 and 96 hours after the second dinoprost injection; the single insemination group, 78 hours after the injection. Insemination was by inseminators from the Aberdeen and District Milk Marketing Board A.I. Service. Semen from a Charolais bull was used for inseminations. A stock Aberdeen Angus bull was turned out with the cows to serve those which returned-to-service.

RESULTS AND DISCUSSION

The conception rate (Table 1) to single A.I. of 52% is a satisfactory result. The conception rate of 68% to double A.I. is better than would usually be expected for beef cows. Overall 60% of the cows were pregnant to fixed-time inseminations, and the remaining 40% were pregnant to the stock bull.

It is interesting that these results agree very closely with a previous trial (LUTCAT13) in beef cows, also in Scotland, where the conception rates in a trial using 120 mature beef cows were for single and double inseminations, 53% and 67% respectively.

These results in beef cows contrast with the results of Trial LUTCAT10 in 535 dairy cows where the conception rates did not differ significantly between single A.I., double A.I. and controls at 46%, 47% and 50% respectively.

This suggests that there may be a difference between beef cows and high producing dairy cows in terms of 'normal' fertility. The two classes of cows differ in physical characteristics, also in the conditions of feeding, housing and management under which they are kept. At present it is possible only to speculate as to whether the difference is real and what the underlying mechanism might be.

TABLE 1

CONCEPTION RATES

Calculated on Calving Results

	Cows Treated	Pregnant	Percent
Single A.I.	25	13	52
Double A.I.	25	17	68
TOTAL	50	30	60

LUTALYSE TRIAL - BEEF COWS - A.E. WALL

Farmer	Name:	W. Everitt
	Address:	Evelix Farm, Dornoch, Sutherland.
Cows	Breed:	H X SH
	Age/Age Range:	5-7 years
	Total Number Treated:	50
Lutalyse	Date of first injection:	31-10-80 Time: 9 a.m.
	Date of second injection:	11-11-80 Time: 9 a.m.
Single AI:	Number of cows:	25
	Time of AI:	14-11-80 3 p.m.
	Number calved to induced oestrus:	13 — 52%
Double AI:	Number of cows:	25
	Times of AI:	First AI time: 14-11-80 9 a.m. Second AI time: 15-11-80 9 a.m.
	Number calved to induced oestrus:	17 — 68%
Bulls:	Breed of AI Sire:	Charolais
	Breed of Herd Bull:	Aberdeen Angus
Calving:	Total number of cows calved:	30 to AI on induced oestrus (Char) 20 to AA bull running with cows afterwards
	60%	

Evaluation of single and double artificial insemination regimes as methods of shortening calving intervals in dairy cows treated with dinoprost

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Conception rates in 535 commercial lactating Friesian dairy cows on two farms were compared between treated animals after two injections of dinoprost at an 11-day interval, and untreated contemporary controls bred by conventional artificial insemination. The conception rate for a single insemination 75 to 80 hours after the second dinoprost injection was 46 per cent, for two inseminations 72 and 96 hours after the second injection was 47 per cent, and for untreated controls was 50 per cent. The differences are not statistically significant. Both dinoprost treated groups had a mean calving interval of 366 days compared with 378 for controls. The time advantage of 12 days in calving interval was principally due to the shorter calving-to-first-service interval of treated cows. This 10-day advantage in calving-to-first-service was increased to a 12-day advantage in calving-to-conception in treated cows, and applied also to cows which failed to conceive to the induced oestrus. The accuracy of pregnancy diagnosis was confirmed by calving data; pregnancy diagnosis by rectal palpation was 94 per cent and by milk progesterone assay 81 per cent accurate, overall. Oestrus occurred in 3·8 per cent of pregnant cows, on the basis of stockmen's observations. The relevance of the information to herd fertility control is discussed.

THE economics of calving intervals are of major concern to the dairy farmer and his veterinary adviser. Under most circumstances a calving interval of 320 to 360 days is recommended for optimal profitability from milk production (James and Esslemont 1979). The average calving interval or calving index for most dairy herds in the UK is well above this target. A significant component of the role of the veterinarian in fertility work in the dairy herd is to give advice and treatment which will help the client to achieve shorter calving intervals. Absence, delay or failure of recognition of oestrus is at the heart of the problem and the reasons for anoestrus in its variety of manifestations are many (Boyd 1977). Inadequate oestrus detection is a fundamental problem, characterised by a low detection rate, 58 per cent being the average for dairy herds in England and Wales (Wood 1976), and by presentation of a substantial proportion of cows (about 7·7 per cent in Northern Ireland) at an incorrect time for insemination (McCaughey and Cooper 1980).

One approach to handling such undesirable delays in achieving successful insemination of cows with functional ovaries is to induce oestrus and inseminate at a predetermined time. This may be done in the course of routine fertility programmes when target service dates have not been achieved or in dealing with individual cases which are presented when delay has been identified (Young 1979) or on a planned basis to anticipate and prevent delay (Esslemont and others 1977).

The effectiveness of prostaglandin in causing luteolysis and inducing oestrus and ovulation in cattle is well established. In the presence of a responsive corpus luteum, oestrus can be induced by a single administration of prostaglandin. However, the resulting oestrus and ovulation is not precise and is unsuitable for insemination at a predetermined time (Eddy 1977). In order that cows with cyclical ovaries can be treated regardless of the stage of cycle, prostaglandin may be administered on two occasions with an 11- to 13-day interval. This produces a synchronised oestrus and permits insemination at two predetermined times, with resulting fertility similar to that of untreated controls (Hafs and others 1975).

Comparison of fertility rates between a single and a double insemination regime and contemporary controls bred by conventional use of artificial insemination on detection of spontaneous oestrus has been reported in heifers and beef cows. There was no significant difference between the three methods (Hafs and others 1978). A study on French Friesian dairy cows compared a number of different methods and combinations of oestrus synchronisation and insemination regimes. Within that study, oestrus was synchronised in two groups of cows by two injections of prostaglandin at an 11-day interval and fertility compared between those inseminated at a single predetermined time and those inseminated at two predetermined times. Fertility was determined by progesterone levels 21 days after insemination and non-return by five to six months after insemination and it was found that fertility to a single insemination was not less than to two inseminations (Chupin and others 1977). These results in dairy cows disagree with earlier work on beef cows which reported a significant decrease in calving rates after a single insemination compared with double insemination and untreated controls (Cooper and others 1976).

The purpose of this study was to investigate, in lactating dairy cows on commercial farms, the relative effectiveness, as determined by calving rates, of insemination at a single predetermined time with insemination at two predetermined times after inducing ovulation with dinoprost and to compare these with untreated contemporary controls. The effects of the methods of treatment on subsequent events and intervals of importance to the management of herd fertility were compared and details of information relevant to herd fertility control were recorded.

Materials and methods

The trial was conducted on a total of 535 Friesian cows on two commercial dairy farms. Farm L in Lincolnshire, with an autumn calving policy, is run as two herds. The two herds are under identical management and are fed the same ration, but herd L(1) consists of cows in their first or second lactation while herd L(2) consists of older cows mainly in their third and fourth lactations. When the two herds are combined they produce an age profile typical of a single herd. Farm S in Somerset has an all-year calving policy.

Cows were allocated to weekly groups by calving dates and were then subdivided on a statistical random basis into three equal groups, two treatment groups and one control group. In order to accommodate farm policy on the timings of first service, no cows were inseminated before day 42 post partum on farm L and day 49 post partum on farm S. All cows were examined per rectum for reproductive normality before inclusion in the trial.

Cows in the two treatment groups were given an intramuscular injection of 25 mg $F_{2\alpha}$ dinoprost (Lutalyse; Upjohn) followed 11 days later by a second injection of dinoprost. Cows in the single insemination group (A) were inseminated once only at 75 to 80 hours after the second injection. Cows in the double insemination group (B) were inseminated at 72 and 96 hours after injection. Cows in the control group (C) were observed for spontaneous oestrus and inseminated according to conventional artificial insemination (AI) usage over a 21 day period. This period was timed to include the week before and the week after that in which the contemporary treatment groups were inseminated.

The inseminations were performed by the local AI services. Veterinary work was performed by the farm's veterinary surgeon.

Breeding management of treatment groups A and B after the fixed-time insemination and of control group C after the 21st day of the service period was at the discretion of the owner, in line with his normal practice and taking no account of the group to which the cows had been allocated.

All return-to-service and repeat insemination dates were recorded. Rectal pregnancy diagnosis was carried out about 49 days after insemination and calving dates were recorded for all cows which calved, whether to an insemination within the trial period or to a repeat insemination. The data are calculated on calving information.

Calving-to-first-service interval, calving-to-conception interval, calving interval and gestation lengths were measured for all cows which proceeded to calve at full term.

Cows which aborted were included in the conception rate data if they had been inseminated only on a fixed time schedule, or only during the allocated service period for controls.

The accuracy of rectal pregnancy diagnosis about seven weeks after service was measured against calving results. In a number of cows (88) on farm L, samples were sent for the Milk Marketing Board's milk progesterone test and the accuracy of these was measured against calving results.

Oestrus observation periods were not changed from the normal procedures on the farms. These were at least twice per day but were not strictly defined on either of the farms, being fitted in at times convenient to the stockmen.

Statistical method

Conception frequency data were compared using three-way chi-squared tests of independence (Kendal and Stuart 1961) (comparison over farms, treatments and conception categories).

Non-significant categories were combined for subsequent analyses. Confidence intervals on mean frequencies were calculated using normal approximations to the binomial distribution (Armitage 1971).

The calving interval data were subjected to analysis of variance using a general linear model of the form (Searle 1971).

$$Y_{ijk} = \mu + F_i + Tj + \alpha ij + \varepsilon ijk$$

Where Y_{ijk} is the observed value
μ is the overall mean
F_i is the effect of the ith farm
T_j is the effect of the jth treatment
αij is the interaction term
ε_{ijk} is the error term ε_{ijk} $N(0,\sigma^2)$

Treatment effects were corrected for the between farms effects in the analysis of variance tables.

Multiple comparison tests were carried out for significant factors using the Student-Newmann-Keuls' multiple range test. Note that when the numbers in each group are not equal this test is not exact.

Results

The mean conception rate to a single fixed-time insemination was 46 per cent, to double fixed-time insemination was 47 per cent (Table 1) and of controls inseminated during the contemporary service period was 50 per cent (Table 2). The difference between treatments is not significant, nor is the difference between both treatments and controls, at the level $P = 0{\cdot}05$.

The conception rate of control cows was recorded as a contemporary monitor of fertility on the trial farms and demonstrated no significant reduction of fertility in treated animals. For this purpose conception rate was calculated as cows pregnant expressed as a percentage of those inseminated.

Of the cows in the control groups, 61 per cent were detected in oestrus, so 39 per cent were not given an opportunity to breed within the comparative period. Expressed as a percentage of the allocated group, the pregnancy rate of controls was 30 per cent and of treated groups was 46 per cent and 47 per cent. This difference between controls and treatment groups is significant at the level $P = 0{\cdot}05$.

TABLE 1: Cows pregnant to fixed-time insemination, expressed as a percentage of those treated, calculated on calving data. Cows which aborted after receiving only a fixed-time insemination are included

Farm	Single AI Treated	Single AI Pregnant	%	Double AI Treated	Double AI Pregnant	%
L herd (1)	54	27	50	49	19	39
L herd (2)	34	12	35	33	17	52
S herd	95	45	47	94	47	50
Combined	183	84	46*	176	83	47†

* 95 per cent confidence interval ± 0·072
† 95 per cent confidence interval ± 0·073

TABLE 2: Control cows pregnant, expressed as a percentage of cows detected in oestrus and inseminated during the contemporary service period, calculated on calving data. Cows which aborted after being inseminated only during the allocated period are included

Farm	Allocated to control	Observed oestrus + AI	Pregnant	Pregnant %	Oestrus detection rate %
L herd (1)	50	24	11	46	48
L herd (2)	31	26	16	62	84
S herd	95	57	26	46	60
Combined	176	107	53	50*	61

* 95 per cent confidence interval ± 0·095

TABLE 3: Mean calving-to-first-service (C-S) and calving-to-conception (C-C) interval (days)

Interval	Farm	Single AI (range)	Double AI (range)	Control (range)
C-S	L	54·3 (50-61)	53·7 (50-60)	70·1 (45-125)
	S	58·7 (55-62)	59·7 (55-62)	65·9 (48-105)
	Combined	56·6	57.2	67.8
C-C	L	86·0 (50-213)	81·5 (50-182)	94·7 (45-209)
	S	83·8 (55-305)	86.6 (55-212)	97.2 (48-302)
	Combined	84·8	84·4	96·1

Difference between treatments not significant
Difference between treatments and control significant at level P = 0·05

The ability to select an optimal service date for treated cows gave both treatment groups an advantage of approximately 10 days shorter calving-to-first-service interval over the untreated controls (Table 3).

This advantage persisted after the induced oestrus and cows which returned to service in treatment groups maintained the advantage over their counterparts in control groups in calving-to-conception interval (Table 3), and in calving interval. Both treatment groups achieved a mean calving interval of 366 days compared with 378 days for controls, which is significant at the P = 0·05 level.

Seven cows from farm L and four cows from farm S were excluded from the trial on veterinary advice at the initial rectal examination. Of the 535 cows allocated to the trial, 56 (10 per cent) were removed before completion of the trial (Table 4).

Gestation period lengths were recorded for all cows and revealed no important differences between groups or between farms. Pregnancy diagnosis by rectal palpation was carried out routinely approximately seven weeks after the first insemination. The diagnosis made at this time was compared with calving data and showed an overall accuracy of 94 per cent, with diagnosis of non-pregnancy being slightly more accurate at 97 per cent (Table 5). Milk samples for pregnancy diagnosis were collected from 88 cows on farm L and submitted in accordance with MMB recommendations. When compared with calving data, negative results proved to be 93 per cent accurate and positive results 75 per cent accurate, with an overall accuracy of 81 per cent.

Three cows which were returned as 'doubtful' on the milk progesterone test were not resampled at six weeks. All three were correctly diagnosed by rectal palpation seven weeks after insemination.

Oestrus detection was carried out routinely for all cows on the trial by stockmen and recorded in the trial data. Oestrus as judged by the stockmen was detected in 18 cows which subsequently proved to have been pregnant at the time, an incidence of 3·8 per cent. Four of these cows exhibited oestrus on more than one occasion (Table 6).

Discussion

The principal reason for induction of ovulation in the dairy cow is to shorten the interval from calving to conception,

TABLE 4: Cows removed during the period of the trial

	Farm	Single AI	Double AI	Control
Aborted	L	—	—	2
	S	3	4	—
Sold	L	5	4	5
	S	8	6	12
Died	L	1	2	1
	S	1	2	—
Total		18	18	20

TABLE 5: Pregnancy diagnosis

Diagnosis	Rectal palpation* Number examined	Correct diagnosis	%	Milk progesterone† Number tested	Correct diagnosis	%
Positive	220	202	92	57	43	75
Negative	262	253	97	28	26	93
Combined	482	455	94	85‡	69	81

* Performed approximately seven weeks after insemination
† According to MMB recommendations — farm L only
‡ Not included in this data were three cows diagnosed as 'doubtful'

TABLE 6: Oestrus displayed during pregnancy based on oestrus detection by stockmen

Farm	Pregnant cows	Pregnant cows observed in oestrus	%
L	212	9	4·2
S	259	9	3·5
Combined	471	18*	3.8

* Four cows were observed in oestrus on more than one occasion

whether as part of a planned fertility programme, or on an individual basis, or as a method of dealing with the clinical syndrome of no visible oestrus and the problems of oestrus detection. It is usual at present to use a double fixed-time insemination regime in preference to single fixed-time insemination, in the belief that this results in a better conception rate. Published results comparing conception rates to single or double insemination regimes are somewhat contradictory and are mostly derived from beef cattle or dairy heifers, neither of which are directly comparable with the dairy cow. This is unfortunate because induction of ovulation has its major practical application in the dairy herd. The technique is principally used on small groups of cattle or on individual cows in circumstances where objective comparisons are impossible. For this reason it was felt necessary to make the comparison in a sufficiently large number of dairy cows under normal commercial management, in conditions which would permit valid statistical conclusions to be drawn. The single insemination regime has obvious economic advantages in terms of reduced semen cost, inseminator time and cattle handling time. This study demonstrates that when dinoprost is used to induce ovulation, there need be no significant reduction in conception rate from using a single fixed-time inseminination scheme in lactation cows, and that between herd variation is likely to be greater than any difference between the two insemination regimes (Tables 1 and 2).

The conception rates in treated cows were not significantly different from those in control cows bred by the conventional application of AI at a comparable period post partum. The overall conception rates achieved in the trial are in line with those obtaining in large herds in the UK as demonstrated by recorded conception rates of 46·1 per cent from 12 large commercial herds (Eddy 1977).

For the purpose of comparing conception rates between treated cows and controls, only those controls which were inseminated during a 21 day period corresponding to the first service of treated contemporaries were considered. While all cows allocated to treatment groups were inseminated on schedule, only those controls which were observed in oestrus could be inseminated during the corresponding period. Oestrus detection rate is a factor which limits the proportion of cows which can be inseminated in time to achieve a respectable calving-to-first-service interval. The oestrus detection rate for control cows of 61 per cent in this trial compares with the national average of 58 per cent for dairy cows in England and Wales (Wood 1976).

The advantage to treated cows compared with controls in days saved in calving-to-first-service interval was reflected in a shorter calving-to-conception interval and subsequently in a shorter calving interval. This advantage was consequent on treated cows in both single and double insemination groups being given the opportunity to conceive earlier than the controls.

The reduction in calving-to-conception interval of 12 days recorded here for both single and double insemination regimes agrees with a reduction of 12·5 days reported in similar conditions using cloprostenol and a double insemination regime (Esslemont and others 1977).

It is usually assumed that animals which have not become pregnant to the controlled oestrus have failed to derive any benefit from the treatment. In fact the absolute measure of benefit is the interval between successive calves. The results from this study suggest that the conception rate to the controlled oestrus is not quite so critical as might be assumed, and that treated cows which did not become pregnant to the induced ovulation nevertheless did maintain an advantage over their untreated contemporaries. In both single and double insemination regimes a useful reduction in calving intervals was achieved. The explanation would appear to be that treated but non-pregnant cows have their following oestrus advanced by an average half oestrous cycle compared with their untreated contemporaries. This is demonstrated by the advantage gained in calving-to-first-service interval by both treatment groups being improved in calving intervals.

The farm policy decision on the optimum interval from calving to first service is of paramount importance in achieving respectable calving-to-conception and calving intervals. It is also important to identify non-pregnant cows as early as possible so that appropriate action can be taken to minimise delay. This trial demonstrates that the MMB milk progesterone test at 24 days after insemination is accurate at an early stage for the detection of non-pregnancy. Rectal palpation at seven weeks after insemination gave a high degree of accuracy in detecting both pregnant and non-pregnant animals. In deciding on the treatment of non-pregnant animals consideration has to be given to the 7 per cent of false negative to progesterone assay, the 3 per cent false negative to rectal palpation and the 4 per cent of pregnant cows which displayed oestrus.

To conclude, in dairy cows, following dinoprost induced ovulation, a single insemination at a predetermined time is a practical alternative to double insemination at predetermined times and offers economies in operation. Inducing ovulation and inseminating at a predetermined time allows more cows to be served at a selected time than does reliance on oestrus detection. The time advantage gained by induction of ovulation applies both to cows pregnant at the induced oestrus and to the returns-to-service which are an average half oestrous cycle earlier. Early detection of non-pregnancy is necessary to achieve low calving intervals.

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Abstracts

Brown adipose tissue in pigs

FIFTEEN young (eight to 26 weeks) pigs were examined post mortem and tissue which was fatty but not typical white adipose tissue was examined by light and electron microscopy. Based on accepted criteria used to identify brown adipose tissue, the authors concluded that brown fat occurred in pigs aged two to three months, and it was embedded in connective tissue or white adipose tissue. This finding may lead to a better understanding of the control of non-shivering thermogenesis.

DAUNCEY, M. J., WOODING, F. B. P. & INGRAM, D. L. (1981) *Research in Veterinary Science* **31**, 76

Pancreatic polypeptide and gastrin in sows

CANNULAE were implanted in the circumflex iliac arteries and veins of three sows. Plasma gastrin and pancreatic polypeptide levels, and pulse rates were measured before and after the sows ate. All three measurements increased within about 10 minutes of the pigs eating after fasting overnight. Atropine given intravenously reduced pancreatic polypeptide to below resting levels but gastrin and pulse rates rose. The function of pancreatic polypeptide is not clear but it seems that pigs have an atropine-sensitive mechanism stimulating post-prandial increases in pancreatic polypeptide and gastrin.

COMLINE, R. S., HANSKY, J., REYNOLDS, G. W., STIFFE, G. & TITCHEN, D. A. (1981) *Research in Veterinary Science* **31**, 140

Infection and the bursa of Fabricius

THE bursae of Fabricius in 10 one-day-old chickens were infected via the cloaca and duct, with the virus of infectious laryngotracheitis. At five weeks, the infected birds, together with six uninfected controls, received intramuscular injections of *Brucella abortus*. At seven weeks, blood serum was collected from each bird just before it was killed. The infectious laryngotracheitis infection had severely reduced the bursa weight/bodyweight ratio and bursal regeneration had not progressed past the mucogenic crypt stage. The antibody responsiveness was reduced in some birds. It appears that infectious laryngotracheitis may have destroyed the B-lymphocyte precursor cells.

ROBERTSON, G. M. (1981) *Research in Veterinary Science* **31**, 136

Absorption of colostral IgG_1 by lambs

EIGHTEEN lambs were bottle-fed 2·5 per cent of their birthweight of pooled bovine colostrum, containing 40 mg IgG_1/ml, at four, eight, 12, 16, 20, 24, 28 and 32 hours after birth. Blood samples were taken at birth and at two and four hours after birth, then at five, seven, nine, 11, 15, 20 and 30 days after birth. All the lambs were born spontaneously between 135 and 145 days of gestation. Results of plasma examinations showed that level of IgG was inversely correlated with length of gestation, birthweight, thyreostimulin levels, time at which maximum IgG_1 levels occurred, length of gestation, and thyroxin levels, and that the half-life of bovine IgG_1 was related to the thyroxin level at birth. It seems that the lightweight immature lamb may be more efficient than its physiologically more mature mates at absorbing colostral immune bodies.

CABELLO, G. & LEVIEUX, D. (1981) *Research in Veterinary Science*, **31**, 190

PART 3

PART 3

STRATEGIC USE OF INDUCED OESTRUS

HERD FERTILITY

Until comparatively recent years, the fertility management of a dairy herd consisted of treatment for fertility of individual cows, as and when that became necessary. Of recent years, awareness has increasingly grown that the herd should have an overall policy, with targets set for performance at strategic stages. As a corollary, regular monitoring was necessary to confirm success in achieving targets, or to reveal lack of progress at an early stage. Although computerised herd records have facilitated this approach and encouraged its growth, they are by no means essential. The influence of this style of management has encouraged many dairy farmers to accept the philosophy, and adapt the principles and practices in a form individually, often loosely, modified to suit their own conditions and inclinations.

These improvements have been instigated or encouraged by progressive veterinary surgeons, and have made an impact on the contribution of all veterinary surgeons in cattle practice. Prostaglandin has made a large contribution in the move towards better management of control of breeding, because uniquely, it has given the veterinarian a tool with positive effect on the bovine reproductive system in a variety of conditions and circumstances. Continuing economic pressure on the milk producer has increased the need for improving efficient management of the dairy herd. Not least is the need to improve breeding efficiency.

ECONOMICS OF FERTILITY IN THE DAIRY HERD

Profit from the dairy cow is from lactational milk, and from calves. The aim is to have one live calf per year, and a lactation of about 305 days. This accepts a dry period of about 60 days, when no milk is produced. Any extension of the dry period represents a loss of potential income from the cow which is not accompanied by any reduction in feeding and overhead costs. The target must therefore be to have the cow calve every 365 days. The Calving Interval of a cow is the length of the period between successive calvings, measured in days. The Calving Index of a herd is the mean of the Calving Intervals of all the cows in the herd. The breeding component of prime importance for the economics of the dairy herd is the Calving Index, and the target is 365 days.

This fundamental standard has been well substantiated by commercial dairy usage. It has also been verified by analysis of relevant practical data. A computerised mathematical model was used to test the economic effect of changing calving intervals under typical high-yielding herd conditions. (James and Esslemont, 1979) It concluded that optimum profitability was obtained with a Calving

Interval between 320 and 360 days. The effect on annual milk yield resulting from increasing the Calving Interval of a cow from 380 days by one day, varied according to the month of the year, but was a reduction, of the order of 10 litres per extra non-productive day per cow.

Analysis of lactational data from 48,669 cows showed that cows in high-yielding herds achieved highest productivity with 41 to 90 days-dry, while cows in moderately-yielding herds were most productive when mated as early as possible. (Bar-Anan and Soller, 1979)

'Days-dry', the period between the end of one lactation and the start of the next at calving, is mostly determined by the interval from calving until the next pregnancy is conceived. This is known as the calving-to-conception interval, and must average 85 days to maintain a 365 day calving interval. Time is needed after calving for *post partum* uterine involution, and for vascular, glandular and mucosal changes of the endometrium to be completed. These changes are necessary to provide a fertile uterine environment capable of sustaining conception. It is usual to allow a minimum of 42 days for this process to be completed, before attempting to breed a cow. This leaves only 43 days, or roughly two oestrus cycles, in which to achieve conception on schedule for a 365 day calving interval. It is this very tight breeding schedule that presents the dairy farmer with most of his breeding problems and prevents most from achieving the optimum Calving Index.

Three components which contribute to the calving-to-conception interval are; calving-to-first-service interval; conception rate, and ; oestrus detection rate. The effects of the three are inter-related. A tabular correlation of the three has been assembled, and confirmed by field data from the computer based Melbread fertility recording scheme. (Esslemont and Ellis, 1974) An example from this data illustrates the principles involved. If a herd's average interval to first service is 80 days and average conception rate is 50 per cent, then the calving-to-conception interval will range from 106 days with an 80 per cent oestrus detection rate to 122 days with a 50 per cent rate.

The interval to first service is determined by a combination of : herd policy as to the interval after calving until breeding should be started; completion of uterine involution, and: oestrus detection rate. Oestrus detection rate is a critical factor in attaining optimal Calving Index, but conception cannot be achieved until uterine involution is complete. If the process of involution is delayed into the proposed breeding period, then that will delay the onset of potentially fertile oestrus. This topic will be dealt with in detail in PART 4.

Differences in calving-to-conception interval of the magnitude indicated by the example cited above, which relate entirely to oestrus detection rate, can have a critical effect on the profitability of a dairy enterprise. Data presented in PART 1 indicate that practical oestrus detection rates in general are mostly of the order of 60 per cent. The effect of this weak link in the process of artificial insemination may be reduced by eliminating the need for oestrus detection, by prostaglandin induced ovulation combined with fixed-time insemination. The results reported in PAPER 7 demonstrated that in herds with an average oestrus detection rate of 61 per cent, inducing ovulation and fixed-time insemination could provide an average advantage of 10 days in calving-to-first-service, and 12 days in calving-to-conception. This advantage was in comparison with untreated contemporary control cows, and took account of all returns-to-service.

A computerised decision model has been constructed to evaluate the economic outcome when (a) using or not using prostaglandin. After prostaglandin induction (b) insemination on detected oestrus or fixed time insemination. Each possible outcome was evaluated at each stage, and given a weighting according to the probability of it occuring. Returns-to-service were bred up to, but not after 175 days post partum. (Fetrow and Blanchard, 1987)

The model was based on the premise that a cow not seen in oestrus by day 50 post partum should be examined. If the cow has a *corpus luteum* the veterinary surgeon may 1) have the cow inseminated at observed oestrus, 2) inject with prostaglandin and inseminate at observed oestrus 3) inject prostaglandin and inseminate 84 hours after injection without oestrus detection.

The following variables were evaluated at a realistic level for inclusion in the calculations: Oestrus detection rate (60 per cent); oestrus detection after prostaglandin induction (70 per cent); ovulation rate at natural oestrus (100 per cent), or after prostaglandin treatment (90 per cent); conception rate from artificial insemination; untreated, at observed oestrus (50 per cent); prostaglandin treated, at observed oestrus (50 per cent); prostaglandin treated with fixed time insemination (40 per cent). It was assumed that the cow will be rebred at returns-to-service until 175 days post partum (allowing 5 return cycles) but not thereafter. A range of specific options, different from the assumed values, were fed into the model to see what effect they would have uniquely on the outcome. These included; a full range of oestrus detection rates and a wide range of semen costs.

Costings have been converted, at an exchange rate £1 = $1.50, to the sterling equivalent of the original dollar values which were assumed for the computer model. These are; cost per day not pregnant £1.33 ; cost of semen £10. ; prostaglandin injection £4. An added cost applies to those cows that need a second insemination. If the cow is in oestrus after 24 hours from the fixed-time insemination, this is given a weighting of 15 per cent.

The model expresses the outcome relative to a baseline which was the decision not to give prostaglandin. It does this in two ways; saved days not-pregnant, and profit, which is the value of days not-pregnant which hve been saved by prostaglandin treatment.

The following fundamentals were demonstrated by the model. Treated cows are in oestrus sooner than if they had not been treated. If they do not hold they will return to service sooner than if they had not been treated. The more future cycles available to detect oestrus within the 175 day limit, the more likely the cow is to become pregnant.

The results were : prostaglandin $F_{2\alpha}$, artificial insemination if in oestrus - days saved 6.06 (£4.08) : prostaglandin $F_{2\alpha}$, fixed-time artificial insemination - days saved 6.56 (£2.40) Cost of prostaglandin is included in the calculation, so investment of £4 for a prostaglandin injection would give a return of 100 per cent , and 60 per cent, respectively for the two methods. With prostaglandin treatment, oestrus detection saves slightly less time but is more cost effective because it uses less semen. When semen costs less than £3.33 fixed-time insemination was more profitable, as it was when oestrus detection rate is less than 40 per cent.

As oestrus detection improves beyond 40 per cent, insemination of prostaglandin treated cows on detected oestrus becomes more profitable and becomes progressively more profitable as detection improves. No matter how good oestrus detection is, it is more profitable in conjunction with prostaglandin treatment. An increased pregnancy rate during the cycle in which the decision was made is not sufficient to make prostaglandin profitable, without the opportunity to continue to inseminate cows that fail to conceive. The more future cycles that are potentially available for breeding, the more profitable prostaglandin becomes.

Thus prostaglandin is more profitable if used early rather than late; postponing the use of prostaglandin until all else has failed is an economic mistake. The use of prostaglandin to induce oestrus in dairy cows is profitable over nearly the complete range of of values for all variables. The results of this computer model provide logical support for the trial results reported in PAPER 7.

There is thus ample evidence to validate the cost effectiveness of using prostaglandin to reduce the limitations imposed by inefficient oestrus detection, on artificial insemination practice, and to shorten the interval to oestrus.

In addition to causing loss from reduced milk production, infertility is a principal cause for premature culling of milk cows. Failure to conceive was the commonest reason given by farmers for culling milk cows in a survey of 80 herds over four years. (Young and others, 1983) As a percentage of total culls it was given as the reason for 31.0 per cent of cows culled in lactations one to three; 28.4 per cent in lactations four to six and ; 22.4 per cent in lactations seven or more. In an analysis of data from the Melbread Dairy Herd Health Recording Scheme, done over a three year period, (Gartner, 1983) infertility was the most common reason for culling on all three years, ranging from 32.3 per cent to 43.2 per cent of cows culled. In view of the generally poor standards of oestrus detection, it is likely that not all of that large proportion of culled cows were truly infertile. Results of an abattoir survey of cattle genitalia tend to support this speculation. Of a total of 8071 genital tracts examined, 9.96 per cent had macroscopic genital abnormalities, and 23.36 per cent were pregnant. (Al-Dahash and David, 1977). It is probable that most of the pregnant cows were slaughtered because they were believed to be infertile.

BOVINE ANOESTRUUM

Anoestrus has been proposed as the most critical reproductive disorder of the *post partum* period in the dairy cow, because of its effect in delaying conception. (Boyd, 1977) Its effect is similar to that of inefficient oestrus detection, and the two can be complementary components of one practical problem. (Martinez and Thibier, 1984) Attention has been focused on the importance of the condition by the use of progesterone assay, (Bulman and Lamming, 1978; Bulman and Wood, 1980) and by herd reproductive health surveys. (Esslemont, 1976)

Anoestrus in cows is simply failure to exhibit the normal oestrous cycle. Any factor which seriously impedes the physiological mechanisms involved in oestrus expression is liable to cause anoestrus. The complex control mechanisms of the oestrous cycle which were described and discussed in PART 1 indicate that there are innumerable opportunities for its disruption. Some causes have already been discussed in PART 2 : puberal immaturity in heifers, either from age or from inadequate nutrition; inhibition of *post partum* ovarian cyclicity in beef cows caused by a neurally transmitted effect of suckling. In the early *post partum* period of the dairy cow, the process of uterine involution has an inhibitory influence on oestrus which will be discussed in PART 4.

The inhibitory effect of inadequate nutrition on fertility has already been discussed in relation to heifers and beef cows. If there is a sufficient degree of nutritional inadequacy, it can produce anoestrus by the effect of low blood glucose concentration on the hypothalamic centres. (Boyd, 1977)

Physical and pathological conditions may also result in anoestrus. Hermaphrodism in heifers was discussed in PART 2. Many forms of this congenital abnormality result in anoestrus. In the dairy cow, *post partum* uterine infection, which will be examined in detail in PART 4, can result in the condition of pyometra, which is usually associated with a persistant *corpus luteum* and resulting anoestrus. This pathological influence also applies to the less commonly encountered foetal mummification and foetal maceration, conditions which can be considered as mimicking pregnancy. The condition of bovine cystic ovarian disease, also to be examined in PART 4, may take the form of luteinised ovarian cysts, which are commonly associated with anoestrus.

A classic reason for anoestrus is, of course, pregnancy. Although this may appear self evident, it is a possibility that must always be considered in farm conditions. It is not unusual for cows which are presented to the veterinary surgeon as anoestrus to prove pregnant on rectal examination. It was recorded in PART 2 that a proportion, roughly between two and ten per cent, of maiden heifers purchased for breeding, were found to be already pregnant. In an abattoir survey of genital tracts cited previously, (Al-Dahash and David, 1977) 23.36 per cent were pregnant. It is likely that many of the cows were culled on grounds of infertility, identified as persistant anoestrus. It is therefore imperative in practice to confirm absence of pregnancy in such cases, particularly if there is a possibility of considering administration of prostaglandin.

Anoestrus has been recognised on a functional basis as either *post partum* anoestrus, which was defined as non-observance of oestrus during the first 60 days after calving, or as post service anoestrus, defined as non-observance of oestrus during the 25 days following artificial insemination. (Martinez and Thibier, 1984) Both require confirmation by rectal palpation, and preferably progesterone assay. Although there might be some slight difference of opinion as to the specific intervals selected, such definitions would be generally accepted.

The difficulties of oestrus detection, and the consequent poor average performance of stockmen under commercial farming conditions has been detailed in PART 1. The demonstrable inefficiency of oestrus detection has given rise to common usage of the expressions 'silent oestrus' and 'suboestrus'. These terms are used to indicate that ovulation has taken place but no external signs of oestrus have been exhibited, or at least *have not been observed*.

In most dairy cows which are thought by the stockman to be anoestrus, ovarian activity is occuring. Only 5 to 15 per cent of these animals exhibit true anoestrus. (Bulman and Wood, 1980;

Lamming, 1980; Humblot and Thibier, 1980) So administration of a luteolytic agent during the luteal phase of the oestrous cycle is indicated as a means of minimising delay of insemination and reducing unprofitability. This was the reason for the studies reported in PAPER 8.

TREATMENT OF 'NO VISIBLE OESTRUS'

(PAPER 8)

The cow which is presented as failing to display oestrus but which on examination has no detectable abnormality of the reproductive tract and ovaries, has in the past presented the veterinary surgeon with considerable difficulty. While he might be reasonably confident that the fault was one of inadequate oestrus detection, what best to do about it was a real problem. Advising better oestrus detection is an unsatisfactory solution which is not likely to be well received by the stockman.

Before more effective treatments were available, an injection of stilboestrol had the advantage of inducing signs of oestrus , which might satisfy the owner but did not induce ovulation. Indeed it carries the risk of stimulating the formation of ovarian cysts. (Roberts, 1956; Palsson, 1961) Ovulation may be induced when a Graffian follicle is present in an ovary by injection of a preparation such as Human Chorionic Gonadotrophin which contains luteinising hormone. Or release of luteinising hormone from the anterior pituitary may be induced by an injection of a gonadotrophin-releasing hormone analogue. The exogenous, or endogenous luteinising hormone will stimulate ovulation followed by oestrus.

There is a *corpus luteum* present in the ovary for about 65 per cent of the oestrous cycle. So that proportion of randomly examined cyclical cows are likely to have an ovary with a *corpus luteum*. In the past, manual enucleation of the *corpus luteum* was the only method available to induce oestrus in such cows. The practice is now contraindicated because of the unacceptable risk of causing ovarian haemorrhage or adhesions, and because an effective alternative is available. Oestrus and ovulation may now be induced more or less predictably by luteolysis with prostaglandin $F_{2\alpha}$.

PAPER 8 describes two series of clinical cases of cows either presented as 'no visible oestrus', or disclosed as such at negative pregnancy diagnosis. The first series of 18 cows were injected with 25 mg dinoprost after periods of anoestrus ranging from 48 to 137 days. All but two exhibited oestrus within 2 to 8 days of injection, and 61.1 per cent of the total group were pregnant to first insemination.

It is suggested that if poor oestrus detection played an important part in the incidence of no visible oestrus, then requiring oestrus detection for the remedy might be inappropriate.

The second series of 19 cows, after periods of anoestrus ranging from 60 to 137 days were therefore inseminated at fixed-times after oestrus induction. Fifteen were injected twice with 25 mg dinoprost with an eleven day interval, and inseminated twice, 72 and 96 hours after the second injection. Four were injected once with dinoprost and inseminated 72 and 96 hours later: all four were pregnant, Of the series, 73.1 per cent were pregnant to fixed-time insemination. The numbers involved were too small to warrant statistical analysis or firm conclusions, but in this study the advantage sometimes associated with insemination at observed oestrus following prostaglandin induction (Macmillan and Day, 1982) was not evident. It does suggest that if poor oestrus detection is considered to be an important element of the syndrome in a given situation, then dispensing with it entirely from the treatment is logical and may be an advantage.

A similar series of cases was reported, of clinical 'no visible oestrus' cows injected with cloprostenol and inseminated at detected oestrus. (Eddy, 1977) Of the 253 cows injected, 159 (62.8 per cent) were seen in oestrus and inseminated, with a resulting pregnancy rate of 54.7 per cent of inseminated cows. The oestrus detection rate was close to that considered to be typical in farm conditions. The overall conception rate for the series of cloprostenol treated cows was 34.3 per cent. It is tempting to speculate that the conception rate might have been increased by fixed-time insemination, removing the need for oestrus detection. The author of that study considered that the interval from injection to induced oestrus was not sufficiently specific to warrant fixed-time insemination. Against that is the consideration that cows, which may be presumed to have ovulated, were not given the opportunity to conceive.

The ability to induce oestrus and ovulation in so-called 'no visible oestrus' cows is a valuable tool in the armoury of the veterinary surgeon for bovine fertility control. There is a case for fixed-time insemination to avoid the effects of inefficient oestrus detection following such treatment

OESTRUS INDUCTION IN THE DAIRY HERD

The inefficiency of extended calving intervals, which result from deficiencies in oestrus detection as a means of timing artificial insemination, may be reduced by inducing synchronised ovulation with prostaglandin, and inseminating at a predetermined fixed-time, or times. This method was shown in PAPER 7 to produce significantly more pregnancies, earlier than was achieved in the same herd by oestrus detection. The use of prostaglandin in conjunction with oestrus detection, where that is done efficiently, can also reduce calving-to-conception intervals.

Despite the demonstrable effectiveness of prostaglandin, and the indisputable need for improved early conception rates, the method has not been accepted generally by dairy farmers as a routine procedure. There are a number of probable explanations for this reluctance.

(1) Conception rates following fixed-time insemination, in cows, as opposed to heifers, still tend to be perceived by the farmer as low. In fact, although that is not necessarily so, results do tend to be variable, and may be capable of some improvement.

(2) The effective, constructive use of prostaglandin requires good breeding records, which are frequently not available, or are inadequate. Records are needed of individual calving, oestrus, service, and return-to-service dates, treatment and early

pregnancy diagnosis results. They are required to identify when cows are due to be served, dates for possible return-to-service, and for early pregnancy diagnosis. They are also needed to identify, at an early stage, cows that are not meeting necessary target breeding intervals, so that appropriate examination and action may be taken at the correct time. Above all, they are the only method by which progress may be monitored accurately within the complex conditions of a dairy herd, problems identified early, and corrective measures taken promptly. Without adequate records problems are not recognised until a late and serious stage, and the information needed to identify and correct problem areas is not available.

(3) It is not possible to inseminate dairy cows in large batches as can be done with heifers. Even in herds with a seasonal breeding pattern, calving spreads over a period of months. To maintain her 365 day calving interval, each cow must be served as soon as possible after calving. So individual cows, or small groups need to be inseminated at the earliest time suitable for them. It has been observed (Simmons and others, 1979) that synchronising oestrus in large groups of dairy cows is probably less desirable than inducing oestrus in a few females at a time. This would entail frequent visits by the veterinary surgeon to administer prostaglandin, which is not economical.

(4) The response to this situation, which is becoming increasingly adopted, is organisation of regular routine herd fertility visits by the veterinary surgeon, often at two-weekly intervals. Within this framework, fixed-time insemination is easier to organise. The interval of eleven days between prostaglandin injections, however involves an extra visit by the veterinary surgeon to administer the second prostaglandin injection - or leaving a second dose with the farmer. The first solution is unpopular with the farmer, the second with the veterinary surgeon. If a fourteen day interval between injections of prostaglandin, to synchronise ovulation, should prove effective as discussed in PART 2, this may increase the practicality of regular synchronised ovulation and fixed-time insemination as a routine practice.

PROSTAGLANDIN BREEDING SCHEDULES

Prostaglandin has not been used widely, in a regular routine way, in the management of breeding in the dairy herd, with the occasional exception of heifers. For the reasons given above, the farmer has mostly shown little recognition of, or enthusiasm for its potential to improve Calving Indices. Its use has been limited to treatment, on the initiative of the veterinary surgeon, of individual cows presented as having a fertility problem, or not having been seen in oestrus, or having been diagnosed as not-pregnant on rectal examination. A number of approaches have been adopted in those circumstances.

When a palpable *corpus luteum* is present, the practical limitations of rectal palpation do not permit accurate assessment of its maturity, but most will be responsive to administration of prostaglandin $F_{2\alpha}$ which will usually cause luteolysis. In those circumstances, administration of prostaglandin $F_{2\alpha}$ is indicated, and will usually be followed in two to seven days by oestrus. (Macmillan and others, 1977; Macmillan and others, 1980) In fact, there is evidence to suggest that the oestrus induced in this way is more fertile, by a factor of almost 10 per cent, than natural oestrus. (Macmillan and Day, 1982) In specific comparisons involving only those cows observed in oestrus during the 24 hours before they were inseminated, the average pregnancy rate to first insemination for over 2,000 prostaglandin treated dairy cows was 69 per cent, compared to 60 per cent in a comparable number of untreated herdmates.

HERD FERTILITY PROGRAMMES

Although there are differences in detail, the structure and aims of routine herd fertility schemes are broadly similar. They are typified by one such that has been reported. (Whitaker, 1980) Animals which fall into various categories are selected by reference to the herd records before the visit and are presented to the veterinary surgeon for examination and treatment as necessary. These categories are :

(1) Pregnancy diagnosis: 49 to 77 days after the last service without a subsequent oestrus period.

(2) No visible oestrus: 49 days after calving, or an oestrous period without a service.

(3) Failure to conceive: two or three services without conceiving, or 6 months after calving without conceiving.

(4) Post natal: after assisted calvings, retained placentae, prolapsed uterus, vaginal discharge reported by the farmer.

(5) Abnormal oestrous cycles, after positive pregnancy diagnosis.

(6) Re-checks requested after previous visits, or by the farmer.

In conjunction with such a scheme, a number of criteria from the herd records are monitored regularly, and corrective action taken when achievement drops to an 'interference level'. Eddy (1980) suggested that appropriate levels for interference are :

(1) Calving to conception (days)	95
(2) Mean calving to first service (days)	70
(3) Mean first service to conception (days)	25
(4) Oestrus detection rate	70 per cent
(5) Conception to first service	50 per cent
(6) Conception rate to all services	50 per cent
(7) Per cent conceived of all served	90 per cent

Within this framework advice, instruction and treatment are given as appropriate to improve performance. The single most effective pharmacological aid available to the veterinary surgeon is prostaglandin, for induction of oestrus (discussed in PART 1), for treatment of endometritis (discussed in PART 4), for termination of pregnancy or induction of parturition. For induction of oestrus, it will usually be administered to cows with a palpable *corpus luteum*, with instructions to inseminate on detected oestrus. The effectiveness of prostaglandin for such use has been discussed, and shown to be superior to methods not using prostaglandin under almost all circumstances. (Fetrow and Blanchard, 1987) If oestrus is not detected following the first injection, a second may be given, followed by insemination at a fixed-time.

This approach to herd fertility, monitors both individual and herd performance. The aim is to confirm acceptable standards regularly, and identify falling standards at an early stage so that corrective action may be taken. An alternative approach, which need not be exclusive of the first, would be to instigate a routine procedure designed to anticipate breeding delay. That was the thinking behind the methods to be reported in PAPER 9. The method chosen, was to define standards which are identifiable early in the breeding programme and which would select cows with an increased likelihood of delay. Those cows can then be treated with prostaglandin to ensure early insemination.

SELECTION OF COWS FOR OESTRUS INDUCTION

(PAPER 9)

In PAPER 7 it was demonstrated that by using routine induction of oestrus with dinoprost, followed by fixed-time insemination, it is possible to get more cows pregnant early in the breeding season, with a consequent reduction in calving-to-conception interval. The resulting improvement in herd Calving Index has important economic advantages for the milk producer which were discussed earlier in this section.

Despite these demonstrable advantages, there has been virtually no acceptance in commercial dairy herds of the technique of routine induction of ovulation followed by fixed-time insemination. One reason for this reluctance to make use of an effective aid for production is that it is perceived as being wasteful. The farmer knows that a proportion of his cows can be bred, on time, without the need for prostaglandin treatment. He therefore perceives a prostaglandin injection for those cows as money wasted.

This is not necessarily the case, because it is not possible to know in advance which cows will breed on schedule. If there is not a strict management policy to identify at an early stage these cows which will not do so, then inevitably many of them will slip into delay. To prevent this, it is necessary to adopt corrective measures on a strict schedule. PAPER 9 investigates methods of attempting selection, at an early stage, of cows with a potential for delay and using prostaglandin to ensure that they will be inseminated as soon as possible. In this way it should be possible to obtain most of the advantage of early induction of ovulation, while selecting-out from treatment cows that probably will not require assistance to breed on schedule.

Two schemes were tested which sought to use criteria that could be applied, at an early stage in the breeding cycle, to select groups of cows that would be likely to include a large proportion of cows with a tendency to delay.

Cows that have not been seen in oestrus by Day 42 after calving will include cows that are 'anoestrus' at that stage and could persist. This was the basis for selecting Group 1. Likewise, cows that have not been inseminated by Day 66 after calving, that is after having been allowed one cycle to exhibit oestrus, will contain cows which could later prove to be 'anoestrus'. These were chosen for Group 2. The study, on a total of 120 Friesian cows in a commercial dairy herd, was planned to compare the relative advantages of acting earlier with Group 1, with the likelihood that some cows would be treated unnecessarily, compared with losing 24 days initiative in Group 2 , but not wasting treatment on cows that would breed early without assistance. Cows were grouped for treatment at regular weekly visits.

Cows in Group 1 which had been seen in oestrus before Day 42 were inseminated at the first natural oestrus thereafter: those which had not been seen in oestrus were injected immediately after Day 42 with dinoprost, inseminated at induced oestrus , or if not seen in oestrus, were given a second injection eleven days later and inseminated 72 and 96 hours later.

Cows in Group 2 were inseminated at natural oestrus between Days 42 and 66 *post partum* : those not inseminated by Day 66 were then injected with dinoprost , inseminated at induced oestrus , or if not seen in oestrus by eleven days later, were given a second injection and inseminated 72 and 96 hours later. All returns to service from both groups were inseminated at detected oestrus . Calving-to-conception intervals were recorded for all cows in both groups.

Calving-to-conception intervals for treated and untreated cows were combined within each allocated Group. The average calving-to-conception interval for both Groups was 83 days. The economic advantage was with Group 2 because 50 per cent fewer dinoprost injections were needed to achieve the same calving-to-conception interval. A direct comparison cannot be made, but the average calving interval for all cows on the trial compared very favourably with the Herd Calving Index for the previous year. The report of a controlled study on dairy cows (Korenic, 1981) is cited which compared two groups selected on very similar criteria. The same conclusion was reached, and the treatment groups had significantly shorter calving-to-conception intervals compared with untreated controls.

Non-detection of oestrus by Day 42 *post partum* appears not to be a reliable criterion for selecting cows which are likely to experience delay before first service. Induction of oestrus with dinoprost in the manner described is a useful method of achieving benefits in shortened calving-to-conception interval similar to those demonstrated in PAPER 7, without the disadvantage of treating every cow in the herd routinely.

INTEGRATED HERD FERTILITY PROJECT

The purpose of the studies which are the subject of this dissertation was to provide information which is of value in the practical conditions of stock breeding on commercial farms. Of necessity each study was investigated in isolation so that scientifically valid conclusions might be reached. In order to relate the usefulness of the findings to more normal farm conditions a project was devised to investigate the practicality of the techniques when they become a regular component of daily working practices on an average commercial farm. Those are the circumstances under which such techniques must operate, without special attention or priority, and taking their place alongside numerous other demands on the attention of the stockman.

In such circumstances, recording, evaluation and relevant standards for comparison, presented considerable difficulties, complicated as they must be by interactions with associated and unassociated factors in the normal daily working of a dairy farm. To provide useful results, the investigation required the contribution of specialists in the recording and analysis of data in the fields of reproductive performance and of agricultural economics.

That was the background for an integrated dairy farm project. (Johnson, Young, Scott, Turner and Esslemont, unpublished data) A typical small West Country family dairy farm of 183 acres was selected for the project. It had a milking herd of 83 Friesian cows. Fertility, breeding and mastitis records were available, but were not in an organised usable form.

Data for the 12 month period before the start of the project were extracted from the farm records and analysed to provide a control baseline. Fertility data were analysed by the DAISY herd fertility computer programme of Reading University. The DAISY bureau service was used to analyse and monitor the herd fertility programme weekly during the term of the project. Farm accounts and economics were analysed by the Exeter University Agricultural Economics Unit who provided quarterly analyses during the term of the project.

The co-operation of the farm's veterinary practice was enlisted and regular two-weekly veterinary herd health visits were initiated, concentrating on reproduction and mastitis.

The following protocol was determined for reproduction control :

1. Specific oestrus detection time allocated by farmer. Three times per day, 30 minutes per session. One session late evening.

2. Routine herd visit by veterinary surgeon every 14 days.

3. Insemination at observed oestrus from 42 days *post partum*.

4. Cows not inseminated by 55 days after calving were examined and if appropriate, injected with 25 mg dinoprost. Insemination at observed oestrus. Cows not seen in oestrus injected with dinoprost 14 days later and single fixed-time insemination.

5. All cows were presented for pregnancy diagnosis by rectal palpation 8 weeks after insemination. If negative, inject dinoprost and continue as (4).

6. All cows returned-to-service three times were examined and treated or culled as appropriate.

RESULTS

Data for 1985 were existing pre-trial standards; for 1986, results over the first full year of operating the protocol :

	1985	1986
Calving-first service interval (days)	83	60
Calving-conception interval (days)	107	81
Pregnancy, first service	55%	44 %
Oestrus detection, first service	50%	84%
Oestrus detection, repeats	30%	50%
Calving Interval (days)	389	361
Cows culled for infertility	11	8
Gross margin per cow (£)	404	536
Veterinary fees, per cow (£)	10.15	21.24

Herd reproduction improved on every parameter measured except one. Presumably as a result of serving cows relatively soon after calving, the first service conception rate dropped from a satisfactory 55 per cent to 44 per cent. In view of the marked improvement of all other economically important fertility parameters, this was acceptable. It is anticipated that as fertility control improves from the effects of the programme, conception rate can be improved. Milk production also improved on every parameter measured as a result of a similarly strict mastitis control protocol.

Veterinary costs were increased by approximately 100 per cent in 1986 compared with 1985, but nett income per cow increased by 33 per cent in the same year. It is not possible to identify accurately the contribution made by improved reproduction, but the data show that it was considerable.

When specific techniques for improving reproduction are developed, even under farm conditions, they become the focus of attention and may receive special detailed care. Once adopted into farm daily routine, that element of extra input is lost in the multiplicity of demands on the stockman's time and attention. This integrated dairy farm project has demonstrated that individually developed techniques, developed and assessed in isolation, can be successfully absorbed into practical systems of management and make a positive contribution.

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Treatment of the clinical syndrome of no visible oestrus: Alternative schemes using prostaglandin $F_{2\alpha}$

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THE significance and importance of anoestrus in dairy cattle is well recognised. It has been reviewed by Boyd (1977) in the full width of the clinical syndrome, from pathological conditions of the genitalia at one end of the spectrum, to simple failure to observe oestrus at the other.

Esselmont and Ellis (1974) have demonstrated the economic significance of the high rate of failure to detect oestrus in cyclic cows. Esselmont (1977) has evaluated in precise financial terms the economic consequences of delay.

Prostaglandin $F_{2\alpha}$ and some of its analogues have been valuable to the practitioner in treating the "no visible oestrus" (NVO) syndrome. Eddy (1977) has reported a well documented series of 253 NVO dairy cows treated with a prostaglandin analogue (cloprostenol). Of these, 159 were observed in oestrus and inseminated. Eighty-seven (54·7 per cent) conceived. From the study, he concluded that in this class of animal, fixed time insemination was unlikely to be successful because of the lack of predictability of the induced oestrus following one injection.

Two clinical field investigations which look at this question of the suitability of fixed time insemination in the NVO cow treated with prostaglandin $F_{2\alpha}$ (dinoprost [Lutalyse; Upjohn]) are reported. It is suggested that a double injection regimen, followed by double insemination may have advantages in this condition.

In series 1 (Table 1) cows presented as NVO or non pregnant at pregnancy diagnosis were given a single injection of 25 mg of dinoprost and inseminated the day following observed oestrus. Of 18 cows presented, 16 exhibited oestrus and were inseminated an average of 4·8 days (range two to eight days) after injection. Eleven of the 18 cows presented were pregnant to the induced oestrus (61·1 per cent). Two of the seven which did not conceive, became pregnant to a second treatment, and three to natural return to service. Thus of the 18 presented, 16 achieved pregnancy.

This level of fertility supports the opinion that frequently the NVO syndrome is a reflection of difficulty or inefficiency of management and is not truly a fertility problem. Therefore, if the problem is one of detecting oestrus, is the best solution one which requires the visual detection of oestrus?

Series 1 demonstrates that the method can be successful, and there are two probable reasons for this. Firstly, the timing of oestrus can be anticipated fairly precisely, and is therefore more likely to be noticed. Secondly, specific animals are being watched carefully and not just as part of a large group. However, that does not radically alter the underlying difficulty. Even under these conditions some animals were not seen in oestrus. It is probable that, away from the special effort that goes into supporting a special project and when the procedure becomes an unexceptional routine, the success rate will fall off.

Series 2 (Table 2) consisted of 19 cows presented as NVO or negative to pregnancy diagnosis.

Fifteen received two injections of 25 mg of dinoprost 11 days apart and were inseminated at 72 hours and 96 hours after the seond injection.

Four received a single injection of 25 mg of dinoprost and were inseminated at 72 and 96 hours after the injection. Of the 19 cows treated, 14 (73·7 per cent) became pregnant to fixed time insemination. Of the non pregnant cows, one became pregnant to natural return to service, making 15 pregnant (78·9 per cent) of the original group.

As in series 1, this does not suggest any real fertility problem with the presented cattle and a satisfactory result has been achieved in both cases. This must be a more logical and satisfactory solution on farms where NVO is a problem. The principal objection to the double injection and double insemination will be that of additional cost. On the farm with difficulties over NVO, profits are already being lost. The best solution in this situation is the one which is most precise and relies least on third parties whose reliability is uncertain. The additional cost is unlikely to be as much as the potential loss of milk for even one further cycle.

There is a place for the clinical treatment of the "NVO" syndrome in cows with two injections of dinoprost followed by double fixed time insemination. By using fixed time insemination, the underlying management problem, failure to detect oestrus, has been completely bypassed.

TABLE 1: Series 1: Single injection of 25 mg prostaglandin $F_{2\alpha}$. Insemination on observed oestrus

Cow No	Length of anoestrus period (days)	Induced oestrus observed	Interval injection to AI (days)	Pregnant at induced oestrus	Pregnant to subsequent oestrus
3	79	+	4	+	
5	64	+	6	—	—
6	77	+	6	—	+
8	77	+	4	+	
10	66	+	3	—	+
12	100	+	6	—	+
13	87	+	5	+	
15	80	—	—	—	+
16	125	+	8	+	
18	86	+	4	+	
24	63	+	3	+	
25	81	+	5	+	
26	74	+	4	—	—
27	137	—	—	—	+
28	56	+	2	+	
33	65	+	4	+	
35	48	+	5	+	
36	61	+	8	+	
	Average 79·2 Range 48–137	16/18 (88·9%)	Average 4·8 Range 2–8	11/18 (61·1%)	Total 16/18 (88·9%)

TABLE 2: Series 2

Cow No	Length of anoestrus period (days)	Pregnant to induced oestrus	Pregnant to subsequent oestrus	Double injection	Single injection
MFS 161	83	—	—	++	
MFS 94	86	—	—	++	
MFS 441	103	+		++	
MFS 443	132	+		++	
MFS 360	96	+		++	
MFS 361	68	+		++	
MFS 362	86	+		++	
MFS 453	136	+		++	
MFS 470	127	+		++	
MFS 475	137	—	—	++	
MFS 440	129	+		++	
MFS 268	89	+		++	
MFS 100	98	—	+	++	
MFS 327	107	—	—	++	
MFS 348	72	+		++	
MFS 472	60	+			+
MFS 109	109	+			+
MFS 74	N/K	+			+
MFS 102	73	+			+
	Average 99·5 Range 60–137	14/19 (73·7%)	Total 15/19 (78·9%)		

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TABLE 2: Details of the morphological features of the fringed virus-like particle

Case number	Mean (±sd) size (range) (nm) Particle	Particle + fringe	Fringe
1 (Dog)	28·6±0·8(27·6–29·5)	44·8±1·0(43·3–45·3)	16·2±1·3(14·8–17·7)
2 (Dog)	28·6±1·2(26·6–29·5)	43·7±0·8(42·4–44·3)	15·1±1·3(12·8–16·7)
3 (Calf)	29·7±1·5(27·6–31·5)	45·1±1·3(43·3–46·3)	15·4±2·1(12·8–18·7)
Mean size of cases 1 to 3	29·0±1·3(26·6–31·5)	44·5±1·2(42·4–46·3)	15·5±1·6(12·8–18·7)

morphology, size and superficial surface structure (Figs 1 and 2). They were spherical in shape and had an overall size ranging from 42 to 46 nm with a mean diameter of 44 nm (Table 2). While the fringed virus-like particle detected in case 3, a calf with enteritis, tended to be larger than those observed in either of the other two cases, it should be stated that the magnification of the electron microscope had not been calibrated before the examination of each specimen.

A conspicuous feature was the presence of a fringe of surface projections arranged in a halo around each particle. The projections were relatively broad, regularly spaced and appeared to have a sessile mode of attachment to the virus-like particle. The fringe varied from 13 to 19 nm in width (mean 15 nm). Difficulties were experienced in measuring the precise width of the particles because of poor definition of the distal extremity of the projections.

All efforts at cultivation of the particle either in cell or organ culture were unsuccessful, based on negative electron microscopic results with culture supernatants, cells or tissue harvests. Similarly, attempted transmission of the particle to specific pathogen free pups could not be demonstrated. No virus-like particles were observed on electron microscopy of faecal specimens, nor were any agents isolated in cell or organ cultures.

The morphological features of the fringed virus-like particle observed in this study differentiate it from any of the previously described viruses associated with the gastrointestinal tract of man or animals. Whereas the size of the virus-like particles without the surrounding fringe approximates to that of astroviruses, the five or six-pointed star surface configuration characteristic of both astroviruses and caliciviruses was not evident (Madeley 1979).

There are some similarities in the morphological appearance of this particle and members of the family Togaviridae. In purified preparations of certain viruses belonging to this group, a halo of fine surface projections resembling post-like structures or massed filamentous material has been demonstrated surrounding the nucleocapsid (Walton 1981). This closely resembles the fringe of projections associated with the virus-like particles described in the present report. However, the diameter of the fringed virus-like particle is less than that recorded for eastern, western and Venezuelan encephalomyelitis viruses (Walton 1981).

The particle was detected very infrequently, with only 0·25 per cent of canine samples positive (Hammond and Timoney 1983), indicating that the agent was not widespread in the bovine or canine populations sampled during the period under study. Whether this virus-like particle is a normal inhabitant of the gastrointestinal tract of dogs or calves or whether it occurs in other domestic or wildlife species, remains to be determined. Despite association of the virus-like particle with acute enteritis in the dog and calf, the available evidence would not suggest a direct causal relationship, even though no other virus was demonstrated by electron microscopy in two of the three positive cases. Only a single small aggregate of the particle was observed in each case, despite exhaustive examination of the respective grids.

Failure to isolate the virus-like particle in the selected cell or organ culture systems may reflect their unsuitability for cultivation of this agent or insufficient viable virus in the inoculum. Inability to transmit the fringed virus-like particle to specific pathogen free beagle pups may similarly have been caused by an inadequate virus challenge. On the other hand, it could also indicate previous exposure to this or another closely related agent. Unfortunately it was not possible to determine the immune status of the pups to the fringed virus-like particle before or after challenge. It remains to be seen whether the particle described in this report is yet another virus to be added to the growing list of agents associated with the gastrointestinal tract of man and animals.

FIG 2: Fringed virus-like particles observed in a faecal sample from a two-week-old calf with diarrhoea (case 3). Bar = 100 nm

Addendum

Since this manuscript was submitted for publication, an unidentified virus-like particle of similar morphology has been reported in a limited number of diarrhoeic faecal specimens from dogs, calves and piglets examined at the State veterinary diagnostic laboratory, Nashville, Tennessee (J. Black, personal communication). In each instance, the particles were very few in number and though varying somewhat in size, measured approximately 44 nm in width.

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Selection of specific categories of dairy cows for oestrus induction with dinoprost

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ONE of the main concerns in dairy herd fertility work is to reduce to an economic minimum the interval between calving and the subsequent conception. It is possible, by routine use of dinoprost, to reduce the calving-to-conception interval of treated cows as compared with comparable cows subjected to conventional oestrus detection and artificial insemination (Young and Henderson 1981).

An objection to a routine approach is that a proportion of cows treated could be expected to be seen in oestrus at a suitably early time without induction. Rather than treat every cow in the herd, it would be preferable to select categories of cows that are more likely to benefit from early oestrus induction, while leaving alone those that would probably make suitable progress without interference.

A comparison was made, under practical conditions, to assess the relative values of two such methods. Cows selected as being more likely to benefit from induction of oestrus at an early stage were (a) those that had not been seen in oestrus during the first 42 days after calving (group 1) and (b) those that had not been inseminated 66 days after calving (group 2).

A group of cows that have not been seen in oestrus by 42 days after calving will include any that are anoestrous. This term, in cattle, covers a wide range of conditions (Boyd 1977). However, this is probably not a major practical problem. Cows that had been presented for veterinary attention because they had not been seen in oestrus were found to have normal conception rates when they were inseminated at fixed times following induction of oestrus with dinoprost (Young 1979). This suggested that the main problem in these was one of oestrus detection.

A commercial herd of Friesian dairy cows was used for the study. All cows that calved between September 10, 1980 and March 14, 1981 were allocated to the trial, a total of 137 cows. Seventeen of these were excluded, categorised as: not to be rebred; culled; sick or reproductive abnormality. All cows were examined per rectum for reproductive normality 42 days after calving. One hundred and twenty cows were assigned alternately to two treatment regimes in sequence of calving dates.

Cows that had been observed in oestrus by day 42 after calving were not treated, and were inseminated normally at the first detected oestrus thereafter (group 1). Cows that had not been seen in oestrus by 42 days after calving (group 1) were injected with 25 mg dinoprost (prostaglandin $F_{2\alpha}$, Lutalyse; Upjohn) and inseminated at observed oestrus. Cows not seen to be in oestrus were given a second injection of dinoprost 11 days after the first and inseminated 72 and 96 hours after the second injection, without reference to oestrus.

Cows observed in oestrus between 42 and 66 days after calving were inseminated (group 2). Those not inseminated by day 66 after calving (group 2) were injected with 25 mg dinoprost and inseminated at observed oestrus; those not observed in oestrus were given another injection of dinoprost 11 days after the first and inseminated 72 and 96 hours later.

Cows that returned to service in both groups were inseminated following detected oestrus. Calving-to-conception intervals were recorded for all cows in the two groups.

Artificial insemination was carried out by the farm's usual AI service. All cows were examined rectally for pregnancy about seven weeks after insemination. Results were calculated from pregnancy diagnosis data and mean calving-to-conception intervals are used as criteria for comparison (Table 1).

Group 1 cows, which had ovulation induced 42 days after calving, had a 12 day advantage in calving-to-conception interval over those induced at 66 days after calving (group 2), which was significant (P = 0·001). When the treated and untreated cows were combined, each group had the same calving-to-conception interval of 83 days, which is equivalent to a calving interval of 365 days.

Cows observed to be in oestrus after the first dinoprost injection were inseminated. About half of the treated cows in each group were in this category, which gave them an advantage in the calving-to-first-service interval over cows which needed a second dinoprost injection and fixed time insemination.

TABLE 1: Mean calving-to-conception (C-C) intervals calculated from results of rectal pregnancy diagnoses seven weeks after insemination

Treatment	Group 1 Number of cows (sd)	Group 2 Number of cows (sd)	Total
	63	57	120
Insemination at spontaneous oestrus	14	30	44
C-C interval (days)	79 (42·6)	73 (27·8)	
Insemination after first dinoprost injection	20	10	30
C-C interval (days)	78 (24·7)	96 (32·2)	
Insemination after second dinoprost injection	22	11	33
C-C interval (days)	91 (39·1)	99 (27·9)	
All oestrus induced	42	21	63
C-C interval (days)	85 (33·3)	97*(29·3)	
Totals	56	51	107
C-C interval (days)	83 (35·5)	83 (30·6)	
Not pregnant	7	6	13

* Significant at level P = 0·001

Approximately 75 per cent of cows in group 1 were treated, compared with 50 per cent of cows in group 2. Since the mean calving-to-conception intervals of both groups were the same this indicates that postponing intervention until 66 days after calving is likely to be more economical.

There was a significant advantage of 12 days in favour of treated cows in group 1 compared with treated cows in group 2. The failure of the groups as a whole to demonstrate this difference indicates that failure to detect oestrus by 42 days after calving is not a good criterion for allocating the treatment.

Korenic (1981), in a similar controlled study, compared prostaglandin induction of oestrus in all cows after 42 days post partum, with prostaglandin induction of cows not inseminated by 60 days post partum. The calving-to-conception interval of 95·5 days for controls was significantly longer than 84·9 days in the former group and 82·5 days in the latter. This broadly agrees with the relative merits of the criteria for selection and the calving-to-conception intervals achieved in the present study.

Useful savings were made by inseminating cows at a detected oestrus after the first dinoprost injection, so that only about half needed a second injection followed by fixed time insemination. Two inseminations, 72 and 96 hours after the second dinoprost injection, were used on this occasion. However, a further economy might be made by using only one insemination 75 to 80 hours after the second injection because it has been shown that, when dinoprost is used, a single fixed time insemination can be equally effective in dairy cows (Young and Henderson 1981).

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PART 4

PART 4

PROSTAGLANDIN IN THE POST PARTUM PERIOD

THE POST PARTUM DAIRY COW

The early return of the dairy cow to fertile ovarian cyclicity after calving is of prime economic importance for the dairy farmer, for reasons outlined in PART 3. A Calving Index of 365 days has been established as a cornerstone for a profitable dairy enterprise, and a strict breeding schedule is needed to meet that target. Although oestrus can occur before uterine involution has been completed, (Buch and others, 1955; Menge and others, 1962) conception in the postgravid horn cannot. (Gier and Marion, 1968) In PART 3, the importance of calving-to-first-service interval was stressed, and delayed uterine involution was given as a critical factor which can, and does, delay the start of breeding.

The return to ovarian cyclicity after parturition involves a complex series of physical, physiological and hormonal interactions involving the uterus and ovaries , the full mechanisms of which are not entirely understood. The rate at which uterine involution is completed is somewhat variable, and may be extended by uterine infection varying in degree from slight to gross contamination, possibly complicated by retained placenta. Although these uterine complications are largely self-curing, the rate of cure depends on the severity of the original infection. The considerable range of severities, which will be examined in detail in this section, implies an equivalent range of times needed for uterine involution to be completed.

ANATOMY AND PHYSIOLOGY OF POST PARTUM UTERINE INVOLUTION

The process of uterine involution was studied in the uteri of 57 clinically normal *post partum* dairy cows. (Gier and Marion, 1968) This was to determine the anatomical changes that occur in the normal *post partum* uterus and to determine at what stage the uterus can be considered to have returned to 'normal'. The information in this section is based mainly on the report of this detailed, meticulous and exhaustive study

During the first few days after parturition there was a rapid decrease in the dimensions of the postgravid horn, due to vasoconstriction and muscular contraction. This early reduction in size may be partially explained as resulting from peristaltic contractions at 3 to 4 minute intervals for one day, and at extending intervals for two to three days, (Jordan, 1952; Venable and McDonald, 1958) but loss of fluids appeared to be an even more important factor. The length of the gravid horn was reduced to half of the parturition size of approximately a metre, by 15 days, and to a third by 30 days. The gravid diameter, about 40 centimetres, contracted by half in about 5 days. Generally the non-gravid horn was expanded relatively little, and the gravid horn was much larger and continued to be larger, even into the next pregnancy. The weight of the average uterus decreased from 9.0 kg at parturition to 1.0 kg at 30 days, and 0.75 kg at 50 days.

The changes in the cervix were mainly reduction in size, by elimination of tissue fluid, and some reduction in muscle tissue. At 2 days after parturition the diameter of the cervix was about 15 centimetres; 9 to 11 centimetres at 10 days; 7 to 8 centimetres by 30 days, and; 5 to 6 centimetres by 60 days. There seemed to be early constriction between 5 and 10 days and secondary relaxation at 10 days - at the time of final sloughing of the caruncular masses.

The caruncles in the gravid horn at parturition averaged 70 mm long, 35 mm wide, and 25 mm high. Each caruncle consisted of a crumpled mass of septa and blood vessels capping an endometrial base. By 5 days after parturition, necrosis due to vasoconstriction, and evidenced by infiltration of leucocytes, resulted in complete septal disorganisation. By 12 days, most of the caruncular septa had sloughed, leaving a raw surface with protruding remnants of blood vessels. Sloughing was complete to the *stratum compactum* by 15 days.

The uterus contained a considerable quantity of decidual detritus and blood from the time of parturition, becoming mixed with sloughed caruncular material after Day 4. The luminal contents changed by 12 days to a more lymph-like fluid which was found in decreasing quantities until Day 23.

Regeneration of of the uterine epithelium began almost immediately after parturition in the areas which were not seriously damaged during parturition, and re-covered the entire inter-caruncular luminal surface by 8 days after parturition. If bacterial infections occurred during the period of tissue loss, the epithelium was again partially or completely destroyed. It was found that, even under the most favourable conditions, the processes involved in tissue loss, and the centripetal growth of new epithelium from the surrounding uterine glands to cover the raw surfaces of the caruncles, was not complete before 25 days after parturition, 10 days after obvious sloughing had ceased. Caruncles in the postgravid horn did not return to normal with uterine glands restored to cycling condition until 40 to 60 days after parturition. It is suggested that early pregnancies are probably dependant on development in the previously non-gravid horn.

Shrinkage of the vascular system, and muscular contractions continued to reduce the size of the postgravid uterus until it reached a near pregravid size by 40 to 50 days after parturition. Data obtained on uterine involution, by rectal palpation, indicated that involution was nearly complete by 40 days after parturition. (Buch and others, 1955; Foote and Hunter, 1964) In a review of *post partum* ovarian activity and uterine involution, (Morrow and others, 1969) six studies on dairy cows are cited which showed the interval to be 26.2 to 52.0 days.

Uterine involution in the suckled cow has been supposed to be accelerated by the 'suckling reflex'. This supposition was not supported by three studies on beef cows, cited in the survey, which showed involution times of 37.7 to 56.0 days. Furthermore, one study, (Wiltbank and Cook, 1958) comparing milked and suckled beef cows showed involution times to be 44.0 and 56.0 days, respectively. There is considerable evidence of this nature which indicates that differences in reproductive characteristics between beef and dairy cows would mostly be more accurately described as differences between suckled and milked cows.

It is doubtful that the slight reductions in size which occur between 40 and 60 days after parturition are dectectable by rectal palpation. The subjectivity of rectal palpation for the purpose of accurate assessment of uterine dimensions has recently been improved upon by the use of an ultrasonic linear scanner. Observation by ultrasonic scanner, of the progress of uterine involution was started on Day 8 and continued to Day 43 *post partum*. (Okano and Tomizuka, 1987) Involution was completed by approximately Day 40 *post partum*, which agrees closely with results obtained by rectal palpation cited above.

The changes involved in uterine involution may be considered as three overlapping processes; reduction in size; loss of tissue; and repair. The drastic reduction in size and reorganisation of tissues is necessary before another pregnancy may be maintained. This process is delayed by uterine infection because of its effect on loss of tissue and repair. As the incidence of such infection is usual, but variable, it may be presumed that the degree of delay will be similarly variable in response to the degree of tissue damage.

All phases of uterine involution were noticably delayed by secondary uterine infections, retained placenta, or poor physical condition of the dam. It was considered probable that the delay was due to leucocytic infiltration of the endometrium which would require a longer interval for the tissues to return to normal. Several studies cited in a review (Morrow and others, 1969) reported involution to be retarded by 5 to 10 days following abnormal parturitions. A seasonal effect was also recorded in the time from normal parturition to completion of involution in winter, spring, summer and autumn as 51, 47, 42 and 44 days respectively. Following abnormal parturition the respective intervals were 56, 54, 44 and 51 days. (Buch and others, 1955)

PERIPARTURIENT ENDOCRINOLOGY OF THE COW

After parturition there follows a period of anoestrus before return to normal ovarian cyclicity and optimum fertility. The return of fertility is linked with uterine involution. Although much is known of the events of the *post partum* period, the physical and hormonal inter-relationships are complex, and the precise mechanism of the link is not clear.

A balanced, co-ordinated endocrine system is essential for normal reproductive function. (Leslie, 1983) This involves homeostasis between gonadotrophin-releasing hormone from the hypothalamus, follicle-stimulating hormone and luteinising hormone from the anterior pituitary, prostaglandin $F_{2\alpha}$ from the uterus, and the ovarian steroids.

PREPARTUM AND PERIPARTUM ENDOCRINOLOGY

During pregnancy one of the ovaries contains a well developed *corpus luteum*, which produces the progesterone which is necessary to maintain pregnancy. From about Day 150 of gestation to Day 250, pregnancy is not entirely reliant on progesterone from this *corpus luteum*. During that period, additional progesterone is produced from extra-ovarian sources to support pregnancy. There is evidence that this extra-ovarian progesterone is produced mainly by the foeto-placental unit, (Pimentel and others, 1986) and although that may not be the only source, it is likely to be the most important one. Evidence has been provided to support the view that placental progesterone production is controlled by the foetal pituitary-adrenal axis, (Challis and others, 1977) and that maturation of this control system is responsible for the terminal reduction of progesterone and increase of oestrone which stimulates the terminal surge of prostaglandin $F_{2\alpha}$ production that initiates parturition.

Maternal ovarian follicular activity continues at a decreasing level during pregnancy, and the diameter of the largest follicles are progressively smaller throughout pregnancy until at parturition the diameter of follicles is minimal. (Schirar and Martinet, 1982) Several authors are cited as reporting that active ovarian follicular activity is resumed by Day 10 *post partum*, and that by Day 15, there are present large follicles which are capable of ovulating.

Throughout most of gestation an adequate blood concentration of progesterone is essential for the maintenance of pregnancy. Towards the end of pregnancy at term, preparation for parturition is organised. The endocrinology of this phase has been investigated and recorded.

Measurement of 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$, the main prostaglandin $F_{2\alpha}$ metabolite, has provided an alternative method of estimating the release of prostaglandin $F_{2\alpha}$. The metabolite is not metabolised as quickly as prostaglandin $F_{2\alpha}$ and allows the collection of physiological data by frequent blood sampling from peripheral veins. This avoids the necessity for surgical intervention to cannulate vessels close to the organ being investigated. (Kindahl and others, 1976a; Kindahl and others, 1976b)

Concentrations in the circulating blood of progesterone, oestrone and 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$, were determined during the peripartal period in 12 cows. (Edqvist and others, 1978) Plasma concentrations of progesterone showed a gradual and continuous decrease during the last 60 days before parturition. This gradual decrease was followed by an abrupt decline in the progesterone concentration occurring 24 to 48 hours before delivery. The plasma concentrations of oestrone started to increase about 30 days before parturition, reaching high concentrations during the last days of pregnancy. After delivery the oestrone content had decreased to base-line levels within 48 hours. It is postulated, with support from cited authors, that the concomitant reduction of progesterone and increase of oestrone reflects the utilisation of progesterone for oestrone production by the foeto-placental unit. Increased concentrations of prostaglandin $F_{2\alpha}$ metabolite occurred before, or in conjunction with prepartum luteolysis. Prostaglandin $F_{2\alpha}$ metabolite concentrations remained high during parturition and did not return to base-line until 10 to 20 days after delivery.

A similar sequence was found when prostaglandin $F_{2\alpha}$ metabolite and progesterone concentrations were investigated in peripheral blood of cows at premature parturition induced in late pregnancy with dexamethasone. (Lindell and others, 1977)

The endocrinology associated with abortion at earlier stages of gestation has also been studied. Abortion was induced in 12 heifers at pregnancy stages from 39 to 146 days with cloprostenol, a prostaglandin $F_{2\alpha}$ analogue. (Lindell and others, 1981) The analogue was used so that it would not interfere with assay of prostaglandin $F_{2\alpha}$ metabolite. The peripheral plasma concentrations of progesterone decreased rapidly following the injection of cloprostenol. All heifers had short-lasting peaks of prostaglandin $F_{2\alpha}$ metabolite in connection with luteal regression. In animals pregnant for less than 80 days this release ceased at the time of delivery of the foetuses, which were expelled with unruptured foetal membranes. Standing oestrus was observed in connection with the expulsion of the foetuses, and two heifers which were mated at this oestrus became pregnant. In contrast, animals pregnant for more than 100 days released massive amounts of prostaglandin $F_{2\alpha}$ metabolite during the two to five days after calving, and retained foetal membranes. No oestrus was observed in connection with these abortions.

The difference in response between the early and late abortions appears to be a reflection of the degree of placental attachment at the two stages. Before 80 days of pregnancy there was little attachment and minimal trauma at delivery. The absence of uterine trauma was reflected by cessation of prostaglandin $F_{2\alpha}$ release at delivery and a simultaneously induced oestrus, which was fertile. Conversely, at the later stage of pregnancy, placental attachment had been firmly established, causing trauma to cotyledons at delivery and stimulating release of prostaglandin $F_{2\alpha}$ for several days. The 'later' abortions were between Days 102 and 146 of gestation, so it is unlikely that the foetal-placental unit had any influence on the hormone profiles at that time because it does not begin to operate until about Day 150 of gestation. (Pimentel and others, 1986)

Parturition at term, or abortion after the first 100 days of gestation is followed by an anoestrous period before regular oestrus cycles resume. The endocrinology of the anoestrous period is of importance because it dictates the time when re-breeding may be commenced.

POST PARTUM OVARIAN CYCLICITY

A hypothetical endocrine model has recently been proposed for the *post partum* regulation of ovarian function in the cow, based on the results of endocrine assay studies. (Peters and Lamming, 1986) This section is largely based on these studies.

Ovarian function is closely controlled by the hypothalamus-pituitary axis, through secretion of gonadotrophins, and the hypothalamus and pituitary functions are in turn partly controlled by the secretion of steroid hormones from the ovaries. It is postulated that the following sequence of events occurs. At first, gonadotrophin-releasing hormone is secreted infrequently from the hypothalamus in small quantities. Concentrations of follicle-stimulating hormone are low, about 20 ng/ml, during the last 10 days of gestation but increase within five days of parturition to around 40 ng/ml. There then appears to be a tendency to some reduction of responsiveness of follicle-stimulating hormone to gonadotrophin-releasing hormone up to Day 30. So, early after parturition the development of follicles is stimulated by follicle-stimulating hormone. This results in production of oestradiol and inhibin by the follicles, and there is a gradual restoration of the positive feedback mechanism by which preovulatory gonadotrophin release occurs. After parturition, there is a gradual increase in responsiveness of luteinising hormone, to gonadotrophin-releasing hormone causing increasing frequency of pulsed release of luteinising hormone. This latter is the most consistent endocrine change which has been reported to precede ovulation in the *post partum* period. (Schallenberger, 1982)

It has been found that the greater the frequency of luteinising hormone pulses between Days 10 and 17 *post partum*, the shorter the time to the onset of ovarian cycles. (Peters and others, 1981) There is evidence that the positive feedback mechanism of oestradiol 17β on follicle-stimulating hormone release is inhibited until Day 5 *post partum*, and on luteinising hormone release is inhibited until about Day 10 *post partum*. (Schallenberger, 1982) Also that the neural components of this regulatory system can be inhibited by neural stimuli evoked by calf suckling and can be modulated by environmental, hormonal and nutritional influences. This observation provides some explanation of the well recognised inhibitory effect on fertility of suckling, or of adverse nutritional or environmental conditions; a response which protects the cow from the additional stress of pregnancy under unfavourable conditions.

BOVINE CYSTIC OVARIAN DISEASE

A pathogenic condition which may interfere with reproductive performance is cystic ovarian degeneration, a disease principally of the dairy cow. Cystic ovaries, defined as containing a cyst of diameter 2.5cm or greater, were the most commonly encountered abnormality (3.80 per cent) in an abattoir survey of bovine genital abnormalities. (Al-Dahash and David, 1977a) This incidence was in close agreement with findings of a survey by one of the authors in the same abattoir six years previously. The authors cite numerous references confirming that the incidence of the condition is much lower in beef than in dairy cattle.

The condition is strongly associated with milk production, having its greatest incidence in high producing dairy cows. (Johnson and others, 1966) It typically occurs following the second to fifth parturition, and usually appears about the second or third month of lactation. The incidence is greatest in housed cattle during the winter months. (Roberts, 1956) The incidence reported in four different categories of cows was 3.4 per cent, 6.8 per cent, 8.5 per cent and 10.6 per cent. (Casida and Chapman, 1951) The higher incidences were attributed to higher milk production, increased feeding, more frequent daily milking, or all three factors.

It is a disease complex of an endocrine nature with primary pathological changes in the hypophysis, adrenals and ovaries, from which arise the secondary general symptoms, and the clinically demonstrable pathological changes in the internal and external genital organs and the pelvic ligaments. (Palsson, 1961) Although it is believed to be caused by a derangement of the anterior pituitary, the precise mechanism is not known. The evidence points to inadequacy of luteinising hormone release from the pituitary, because a specific curative effect followed the administration of exogenous luteinising hormone in the form on an unfractioned extract of pituitary gland. (Casida and others, 1944) Normal development of the *corpus luteum* is prevented by either failure of the release mechanism, or an actual deficiency of luteinising hormone, (Roberts, 1956) A number of causes which would produce the same end result, of inadequate luteinising hormone release, were suggested. These include, a lack of progesterone in the mature follicle to stimulate the release of luteinising hormone, an imbalance between follicle-stimulating hormone and luteinising hormone, increased production of prolactin causing high milk production levels, inhibition of the release mechanism of gonadotrophin-releasing hormone to stimulate release of luteinising hormone, or possibly other effects on the pituitary by associated endocrine glands.

There is strong evidence indicating a heritable predisposition to the condition. Studies of cows which had been in a herd for four service periods or more showed that daughters of dams with cystic ovaries had an incidence of 48.1 per cent cystic ovaries, compared with an incidence of 21.7 per cent in daughters of non-cystic dams. (Casida and Chapman, 1951) A sire effect has also been demonstrated. Analysis of data gathered by a Swedish artificial insemination society was used to investigate the incidence of cystic degeneration of the ovaries. (Palsson, 1961) The daughters of one bull of five studied showed a significantly higher incidence of the condition, compared with the daughters of the other bulls.

The disease is characterised by the development of pathologically enlarged cystic ovarian follicles varying in size from 2.5 to 7.5 cm in diameter. In many cases of bovine cystic ovaries, gross or histological examination of excised follicular cysts reveals varying amounts of lutein tissue in the cyst wall. (Roberts, 1956) Clinically, two syndromes are recognised within the disease; thin walled follicular cysts, which are usually associated with frequent, irregular, or continuous oestrus, and thick walled luteal cysts often associated with anoestrus. These occur in roughly the proportions 70 per cent and 30 per cent respectively. (Roberts, 1955) Of the 486 cysts examined in an abattoir survey, 22.84 per cent had luteinisation, the others had none. (Al-Dahash and David, 1977b)

A similar proportional incidence was reported in a clinical study, (Booth, 1981) when rectal palpation of 91 affected cows revealed 70 per cent follicular cysts and 30 per cent luteal cysts. Differential diagnosis by rectal palpation did not produce accurate differentiation between the two types of cysts which is better confirmed by progesterone assay. In the study, manual diagnosis of follicular and luteal cysts, when confirmed by progesterone assay, was correct in 80 per cent and 52 per cent of cases respectively.

The current treatment of choice for follicular cysts is administration of gonadotrophin-releasing hormone analogue, to stimulate release of luteinising hormone ; and for luteal cysts, administration of prostaglandin $F_{2\alpha}$ to induce luteolysis and oestrus. In a clinical trial treating field cases of bovine cystic ovarian disease, the response rate to the above treatments was recorded : the response to treatment of follicular cysts (n=46) was 78 per cent, and of luteal cysts (n=13) was 85 per cent. (Booth, 1982) The high success rate makes treatment an attractive option, however, the strong heritability of ovarian cystic disease calls to question the wisdom of breeding herd replacements from affected cows.

UTERINE INFECTION POST PARTUM

Retained placenta and endometritis are the most frequently occuring abnormalities in the cow covering the first 6 weeks of the puerperal period. (de Bois, 1982) These abnormalities cause considerable financial loss not only due to the expense of treatment, and in a few cases to the death of the animals, but also to the fact that fertility is temporarily or even permanantly altered in a number of animals.

RETAINED PLACENTA

The definition of abnormal retention of the placenta is generally accepted as retention for more than 24 hours. The duo of retained placenta and endometritis have frequently been linked as a single syndrome. However evidence from the literature indicates that, although strong correlations exist, the two require separate consideration. A significant increase in the incidence of retained placenta with increasing parity has been reported; from 5.6 per cent after the first calving to 38.4 per cent for 4 or more calvings. (Markusfield, 1984) The converse was the case for the incidence of endometritis; 52.2 per cent after the first calving to 23.0 per cent after 4 or more calvings ($P < 0.01$)

Factors positively associated with increased incidence of retained placenta include; short gestations, dystocia, incidence of twinning, induction of parturition, stillbirth and abortion, metabolic disease, and a seasonal influence of the winter months. (Markusfield, 1984; Vandeplassche and Bouters, 1982) In a review of the subject, the impact of retained placenta on bovine fertility is discussed, on the evidence of a number of cited investigators. (Paisley and others, 1986) It is considered that uncomplicated cases have minor adverse effects on future fertility. However complications, especially in the metritis complex, occur in more than half of the cows with retained placenta.

Retention of foetal membranes for longer than is usual following uncomplicated parturition, is of itself evidence of some disruption of the normal process. Part of the aetiology of the condition begins to act before the start of parturition. Delayed maturation of the placenta prior to parturition was identified mainly as immaturity of foeto-maternal placental contact, which was characterised by an intact epithelial layer of the endometrial crypts. (Vandeplassche and Bouters, 1982)

Successful expulsion after parturition is by uterine mechanical pressure, and depends on anaemia of the foetal villi, and reduction of the size of the caruncles. These processes are capable of disruption by numerous physiological abnormalities, immune reactions or infectious agents, most of which are not responsive to specific therapy. (Paisley and others, 1986) If the normal loosening process has not occured by the time of parturition, retained placenta and its sequelae are further complicated by the decrease in myometrial activity after 24 to 48 hours *post partum*. (Jordan, 1952; Venable and McDonald, 1958) As the underlying cause, failure of separation, is well established by the time diagnosis is possible, it is not surprising that treatment has at best, had limited effect.

Retained placenta is a common sequel to induced parturition. This may be assumed to be a result of inadequate time for the maturation processes discussed above to be effective. A high incidence has been reported for all methods of induction using short-acting steroids and / or prostaglandin and has mostly been of the order of 60 per cent retention. (Plenderleith, 1978; Murray and others, 1982; Johnson and Jackson, 1982; Claydon, 1984)

Evidence has been reported which shows that this is not an inevitable sequel. (Gross and others, 1986) Dairy cows (n=66) were induced to calve with dexamethasone treatment 5 days before expected calving date. Half of the cows, allocated randomly, were injected with 10 mg prostaglandin $F_{2\alpha}$ within one hour of calving. The other cows served as controls, and were injected with 2 ml sterile saline within one hour of calving. There was 90.5 per cent placental retention in the saline injected control cows, and 8.8 per cent in prostaglandin treated cows. Prostaglandin administered more than one hour, but less than three, or two hours after calving, was not effective. This suggests that the conditions for placental retention are not finally determined until parturition, but within one hour become irreversible.

The traditional method of dealing with retained placenta was manual removal. There is still considerable pressure on the veterinary surgeon, from the farmer, to remove retained placentae manually, for reasons of cleanliness, hygiene and aesthetics. There has, however, long been support for the view that manual removal is contra-indicated. "If the placenta is allowed to drop away of its own accord, or if it is gently withdrawn from the uterus even 10 to 12 days after parturition, involution of the uterus occurs sooner, and uterine discharge ceases more quickly than when the placenta is removed in a rough manner and portions are left in the uterus." (Roberts, 1971) There is physical evidence which provides support for this opinion. (Paisley and others, 1986) After manual removal of retained placentae was attempted, the uteri of the cows were examined at necropsy. Haemorrhages, haematomata and vascular thrombi were found in the uteri even when removal was accomplished with no external evidence of trauma. In many cases, when removal was thought complete, many of the caruncles had portions of the foetal cotyledons attached.

In a review of the subject (de Bois, 1982) the balance of evidence supports non-intervention in cows with uncomplicated retained placenta. Manual intervention carries a strong risk of uterine injury, as illustrated above. In addition it was reported that phagocytosis by uterine leucocytes was completely inhibited for several days following attempts at manual removal. (Vandeplassche and Bouters, 1982) There is evidence that intra-uterine chemotherapy or antibiotic therapy is likely to have minimal effect because of the nature and quantitiy of intra-uterine contents which tend to inhibit and dilute medicaments. (Vandeplassche and Bouters, 1982) All types of intra-uterine antiseptics had a similar inhibiting effect on uterine phagocytosis. If antibiotic support treatment is indicated, it is more effective when administered systemically. The balance of evidence appears to support non-intervention, with systemic antibiotic support if necessary.

There is little about the treatment and sequelae of bovine retained placenta that may be stated concisely and confidently. A borrowed summary illustrates the best information currently available (de Bois, 1982) :

- it is generally concluded that broad spectrum antibiotics are quite capable of controlling putrefaction,
- freedom from infection is quite often not achieved despite repeated intra-uterine treatments with antibiotics,
- it is likely that the fertility rate of cows which calved normally, retained their placenta and were treated with intra-uterine antibiotics, is less than of cows which calved normally and dropped the foetal membranes promptly.

ENDOMETRITIS

From the the evidence of the last section, it appears that retention of foetal membranes has some influence on subsequent endometrial infection. In any event, post parturient infection of the uterus is demonstrated to be almost the usual sequel to calving. Studies indicate that 85 to 93 per cent of cows have uterine infections 2 weeks *post partum*, but only 5 to 9 per cent are infected by 46 to 60 days. (Elliot and others, 1968; Johanns and others, 1967) Comprehensive investigations showed that bacteria invade the uterus of up to 90 per cent of cows during the first 10 days *post partum*. (Griffin and others, 1974a) In fertile cows this flora is eliminated and puerperal endometritis is resolved before presentation for first service. A total of 93 dairy cows from nine herds had uterine samples taken aseptically for bacteriology, and biopsy samples for histopathology, at weekly intervals for the first seven weeks after calving. The cows were selected randomly, and only cows which had calved normally and expelled the placental membranes promptly were used. In all the cows the composition of the uterine flora fluctuated constantly throughout the seven-week period as a result of spontaneous contamination, clearance and re-contamination.

The range of bacteria isolated included *Staphylocci, Streptococci, Enterobacteria, Diphtheroids, Proteus, Bacillus*, and *Corynebacterium pyogenes*. The prevalence of uterine infections for *post partum* Weeks One to Seven were ; 92 per cent, 96 per cent, 77 per cent, 64 per cent, 30 per cent, 30 per cent, and 25 per cent respectively. The incidence of samples with endometrial lesions for Weeks One to Seven were 62 per cent, 91 per cent, 93 per cent, 89 per cent, 54 per cent, 46 per cent, and 34 per cent respectively. The incidence of moderate/severe degrees of endometritis was greatest during Weeks Two, Three and Four, when approximately 70 per cent of cows were in this category.

A direct correlation was found between *Corynebacterium pyogenes* infection and endometritis. During the early stages of *Corynebacterium pyogenes* infection the endometritis was usually mild or moderate, but if the infection persisted for more than one week, severe endometritis developed, which frequently required up to a month after the clearance of the pyogenic infection for resolution to be complete. When *Corynebacterium pyogenes* was present in the uterus at any time during the fourth to the seventh weeks *post partum*, the probability of conception to first service was markedly reduced. The endometritis induced by *Corynebacterium pyogenes* usually persisted for some weeks after clearance of the organism, and during that period the uterus was frequently invaded by other non-specific bacteria. These did not appear to influence the course of resolution of the existing endometritis.

The incidence and nature of endometritis during the *post partum* period is important because of its effect on the rate of uterine involution, but the bacteriology and health status of the endometrium is of immediate importance at the time of service, for its effect on conception. The previous study was continued into the early breeding period, on cows in which the involutionary patterns of infection had been studied. (Griffin and others, 1974b) In order to assess the immediate effect of the uterine flora and uterine histopathology on fertility, microbiological and histopathological specimens were obtained from the uteri of fertile and infertile cows before and after service. All the cows were artificially inseminated at the first oestrus after Day 50 *post partum*. Uterine samples were obtained within the 14 days immediately preceding and the 10 days after first service.

Forty eight per cent of the cows harboured a uterine microflora in the two weeks preceding the first service. Non-pathogenic organisms were predominant and there was no evidence from this study that conception rate at first service was influenced by either the presence or the composition of the uterine flora during that period. It was observed however that cows which conceived to first service had a significantly lower incidence of metritis during the 14 days preceding first service than those which conceived to second or third inseminations. The majority of this pre-service endometritis was the result of lesions persisting from *Corynebacterium pyogenes* and severe endometritis during the latter part of the involutionary perioid.

During the 10 days after service, the cows which conceived to first service had no uterine bacteria or endometrial lesions, compared with those which required two or more services and of which approximately 15 per cent had bacteria isolated and approximately 25 per cent had endometrial lesions. Although the difference was not significant, it seems that degree and severity of uterine infection during the *post partum* period has a carry-over effect on conception rate at first service.

NATURAL ELIMINATION OF ENDOMETRIAL INFECTION

The economic impact of endometritis may be overestimated because insufficient attention is paid to the normal involutional process and uterine defence mechanisms. (Paisley and others, 1986) Bacteria are eliminated mechanically when contraction of the myometrium forces the lochia out through the cervix. They are also eliminated by the phagocytic activity of leucocytes in the uterine fluids and endometrium, and by antibacterial substances produced by the uterine glands. (Morrow, 1969; Griffin and others, 1974a)

The stage of the oestrous cycle has a major influence on the resistance of the uterus to bacterial infection. Bacteria introduced to the uterus at oestrus are rapidly eliminated. (Rowson and others, 1953) however during the luteal phase the bacteria are likely to persist and cause endometritis. A study investigated the opsinising capacity of uterine secretions during both follicular and luteal phases of the oestrous cycle. (Watson, 1985) Killing of bacteria *in vitro* was significantly higher ($P < 0.001$) when blood neutrophils were opsinised with uterine flushings from cows during the follicular phase (39 per cent), than during the luteal phase (22 per cent) There was also a significant difference between individual cows.

Uterine infections, which delay involution, are more readily eliminated in cows with regular oestrus cycles. (Steffan and others, 1984) But re-establishment of normal cyclical oestrous activity is dependant on uterine involution. In dairy cows, follicular growth begins as early as four to ten days after calving, but the interval to the first *post partum* ovulation is influenced by many factors, including periparturient disease. (Morrow, 1969)

The uterus therefore has substantial defence mechanisms which are capable of eliminating infection and permitting return to a normal healthy intra-uterine environment and fertile ovarian cyclicity after parturition. As will be seen below, intra-uterine administraion of chemotherapeutic and antibiotic substances can interfere with this process by inhibiting leucocytic phagocytosis. The economic losses resulting from unecessary therapy and the requirement to discard milk during and after after antibiotic treatment must be weighed against any gains from such therapy. (Paisley and others, 1986)

TREATMENT OF ENDOMETRITIS

The treatment of endometritis appears to follow the same broad outlines as that of retained placenta, perhaps not surprisingly as the two are so closely linked. Chemotherapy and antibiotic therapy have had mixed success, and results are equivocal, both in resolution of the disease, and in subsequent fertility.

In a review of intra-uterine antibiotic therapy, (Paisley and others, 1986) the reasons for diminished efficacy are catalogued. They include; inhibitory effect of pus and organic debris; the anaerobic environment rendering aminoglycosides ineffective; inactivating enzymes produced by many organisms; diminished absorption of drugs when organisms are not confined to the uterine cavity and surface of the endometrium; and an inhibitory effect on leucocytic phagocytosis. For these reasons, systemic administration of antibiotics is considered more appropriate.

A study in 357 Jersey cows investigated preventive therapy for endometritis. (Dowlen and others, 1983) Two different antibiotic formulations in the form of boluses, were administered within 24 hours of calving. Neither treatment resulted in any significant improvement over control animals in any of the reproductive efficiency parameters measured.

With the intention of stimulating natural uterine defence mechanisms, prostaglandin $F_{2\alpha}$ has been used in the treatment of endometritis when a *corpus luteum* is present. This induces oestrus and stimulates 'natural' defence mechanisms of myometrial expulsion and the protective effect of the mechanisms described above.

A series of cases of *post partum* pyometra (n=26) were treated with prostaglandin $F_{2\alpha}$ by intravenous or intramuscular routes, and at various dose levels. (Gustafsson and others, 1976) Twenty two of the cows (85 per cent) started to empty the uterus within 24 hours of treatment, and evacuation of uterine contents was completed at the oestrus which occurred three to four days after treatment. The authors considered prostaglandin $F_{2\alpha}$ to be the treatment of choice for bovine pyometra.

A series of clinical field cases of bovine metritis (n=55) were reported. (Coulson, 1978) These were treated by injection of 25 mg dinoprost, and a recovery rate of 76.3 per cent was recorded. A comparison was made in 209 cows, between cloprostenol and a proprietary intra-uterine antibacterial formulation. (Vujosevic and others, 1984) Although no difference was found in rate of uterine involution, or restoration of normal vaginal discharge, prostaglandin treated cows had better conception rates at first service and better calving-to-conception intervals. In a study on 83 dairy farms in France, the overall incidence of metritis was 32.9 per cent. (Vallet and others, 1987) Treatments involving the use of prostaglandin $F_{2\alpha}$, with or without antibiotics was found to give better results than treatments not using prostaglandin $F_{2\alpha}$. Pregnancy rates were 78.9 per cent *versus* 62.8 per cent, and conception rates at first service were 46.9 per cent *versus* 38.5 per cent respectively.

In a clinical study on the occurence and treatment of chronic endometritis, (Pepper and Dobson, 1987) it was observed that treatment was more successful if it was initiated before 40 days *post partum* than after that time. This was considered to be due, in part at least, to the tendency for self cure. Treatments used were either intra-uterine antibiotic irrigation, oestradiol benzoate injection, or parenteral dinoprost treatment. Of the cases treated more than 40 days *post partum*, only 25 were treated with oestradiol or irrigation compared with 46 treated with dinoprost. Also the dinoprost treated cases had a higher average diagnosis-score for severity before treatment, of 2.2 compared with 1.4 for irrigation and 1.9 for oestradiol. Conception rate at first service was 39 per cent for dinoprost treatment compared with 24 per cent for non-dinoprost treatment.

There seems to be little doubt, from the evidence, that stimulation of the natural uterine defence mechanisms with prostaglandin $F_{2\alpha}$ is the treatment of choice for bovine endometritis. When antibiotic cover is necessary, it should be administered by a systemic route to achieve adequate submucosal protection and avoid inhibition of uterine leucocytic phagocytosis.

EFFECT OF UTERINE DISEASE ON INVOLUTION

The size, and degreee of involution of the uterus, particularly of the post gravid horn, is frequently used as a clinical guide to the cow's progress towards return of *post partum* fertile ovarian cyclicity. In the hands of the experienced clinician this is a useful indicator. It suffers however, from the inaccuracies of subjective assessment of what is a large, soft and relatively formless organ. For many cows, the diameter of the uterus cannot be palpated easily during the first three weeks *post partum*, so the size of the cervix may be a more useful indicator of the involution process.

Because the condition of the reproductive tract in the early *post partum period* appears to be a biological factor closely related to subsequent reproductive performance of a cow, Oltenacu and others (1983) in a study on 462 calvings, attempted: 1) to quantify effects of parity, type of parturition, and type of discharge from the genital tract early *post partum*, on rate of involution of the cervix; and 2) to evaluate effects of type of parturition, type of discharge, and degree of involution of the cervix on subsequent reproductive performance of Holstein cows. A total of 14 per cent of all calvings were abnormal. In the first six weeks after calving 38 per cent of cows had abnormal discharges. There was a positive relationship between both abnormal calving and abnormal uterine discharge, and delayed cervical contraction. Of the factors considered in the studies, diameter of the cervix estimated by rectal palpation 12 to 26 days *post partum* was the best indicator of the cow's subsequent reproductive performance.

The ability to predict future reproductive performance, combined with the ease of estimating cervical diameter during routine *post partum* examination, would make cervical diameter potentially a good management tool for identifying cows likely to have deficient reproductive performance. Regression analyses indicated that the optimum time to determine cervical dimensions would be about three weeks *post partum*. At that time the difference between normal cows, and cows with uterine problems is largest. When the size of the cervix is used as a management tool, parity should be taken into account. For Holstein cows in their first lactation, the critical cervical diameter is 55 mm, and cows with a greater diameter should be considered 'problem cows'. For cows in a second or later lactation, the critical diameter is 60 mm, and cows with a greater cervical diameter should be considered 'problem cows'.

This information on uterine involution about three weeks *post partum* has a bearing on the role of endogenous prostaglandin $F_{2\alpha}$ in the mechanisms of involution which will be discussed later.

Immediately preceding parturition, regression of the *corpus luteum* of pregnancy is demonstrated by an abrupt decrease in the concentration of progesterone in the maternal peripheral blood plasma. (Edqvist and others, 1973) Release of prostaglandin $F_{2\alpha}$ is the key hormonal event responsible for terminating luteal function in the pregnant cow. This has been demonstrated by measuring the main metabolite of prostaglandin $F_{2\alpha}$, 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$ at the time of *pre partum* luteolysis. (Edqvist and others, 1976) Furthermore, administration of exogenous prostaglandin $F_{2\alpha}$ will terminate function of the *corpus luteum* in a pregnant cow. (Lauderdale, 1972)

The uterus has been identified as the source of *post partum* prostaglandin $F_{2\alpha}$ release in a controlled study in naturally calving cows (n=12) which were allocated to three groups in which treatment was carried out within 12 hours of calving. (Thatcher and others, 1982) Five were hysterectomised after ligation of the major uterine blood vessels; three had a sham operation in which the uterus was manually prolapsed and returned after 20 minutes; four were untreated controls. The characteristic prostaglandin $F_{2\alpha}$ metabolite release profile over approximately 15 days *post partum* was demonstrated in the control cows, and in slightly modified form in the sham operated cows. There was no release of prostaglandin $F_{2\alpha}$ metabolite in the hysterectomised cows. In addition, the uterine vein, jugular vein and uterine artery were cannulated in three cows. Blood samples were taken at frequent intervals on *post partum* Days Zero to Five. Concentrations of prostaglandin $F_{2\alpha}$ metabolite were consistently higher in the uterine vein than in the uterine artery or jugular vein.

Collectively, the results indicate that the uterus is the primary source of *post partum* prostaglandin $F_{2\alpha}$ release. Laboratory tissue studies by the same authors indicate that maternal caruncular tissue is probably the main source of uterine prostaglandin $F_{2\alpha}$ production.

In the sheep, two uterine sources of prostaglandin $F_{2\alpha}$ have been associated with different stages of parturition. (Liggins and others, 1976) Blood was collected simultaneously from a maternal vein draining a cotyledon (mainly a maternal component of the placenta) and from a uterine vein (blood from both placenta and myometrium) in sheep at 120 to 130 days of gestation. Prostaglandin $F_{2\alpha}$ was measured by radioimmunoassay. The concentration of prostaglandin $F_{2\alpha}$ from both was determined 18 hours after a maternal injection of oestradiol 17β. Levels were elevated in both veins, but particularly in the cotyledonary vein. The uterus was then stimulated to contract by giving a bolus injection of oxytocin. A sharp rise in concentration of prostaglandin $F_{2\alpha}$ occurred which was more marked in the uterine vein. This data is consistent with prostaglandin $F_{2\alpha}$ production from two sources, each responding to a different stimulus. The authors suggest that prostaglandin $F_{2\alpha}$ of placental origin is likely to be the more important for initiation of parturition, in that it may activate the myometrium whereas prostaglandin $F_{2\alpha}$ from the myometrium may be concerned with maintenance rather than initiation of contractility. This work has not been done in the bovine female.

The significance of prolonged *post partum* release of prostaglandin $F_{2\alpha}$ in the bovine female is not known, and it was suggested that it might be released by the myometrium in proportional response to the degree of endometrial damage or repair. (Edqvist and others, 1978) The duration and possibly the magnitude of the release appeared to be directly related to the time for completion of uterine involution.

Data supporting this theory was provided by inducing abortion in pregnant heifers at different stages of gestation, with cloprostenol, (Lindell and others, 1980/1981) as discussed earlier. The heifers which were aborted before 80 days *post partum* delivered foetuses within their placental membranes, showed no sign of uterine damage, and 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$ release terminated after abortion. Conversely, in heifers aborted after Day 100, when there is greater placental attachment, prostaglandin $F_{2\alpha}$ metabolite release remained raised for two to five days after delivery with placental retention. It was suggested that the degree of endometrial damage determined the length of prostaglandin $F_{2\alpha}$ release, and the time required for uterine repair and involution

Further support for the theory was provided by a study in normally calving cows (n=59). In this study, (Lindell and others, 1982), levels of prostaglandin $F_{2\alpha}$ metabolite were high at parturition and remained elevated for periods of 7 to 23 days. Uterine involution was completed during periods ranging from 16 to 53 days. When compared with cows which had an uncomplicated puerperal period, cows with uterine discharge during the first 30 days had significantly longer periods of prostaglandin $F_{2\alpha}$ metabolite release, and also required longer periods for completion of uterine involution.

However the same was not true of cows with an uncomplicated puerperium. The study revealed in these cows a significant inverse relationship between the duration of elevated *post partum* prostaglandin $F_{2\alpha}$ release and the time required for completion of uterine involution. When the puerperium was uncomplicated, longer durations of *post partum* prostaglandin $F_{2\alpha}$ release were associated with shorter involution times.

The authors concluded that completion of uterine involution seemed to be conditioned by maintenance of high concentrations of prostaglandin $F_{2\alpha}$. They noted that elevated levels of prostaglandin $F_{2\alpha}$ were maintained during almost the whole involutionary period in cows which completed involution before about 24 days *post partum.* In cases with 'wavering' of prostaglandin $F_{2\alpha}$ synthesis at an earlier stage, the involutionary period became prolonged, reaching 40 to 50 days in some cases. They speculated that uterine synthesis of prostaglandin $F_{2\alpha}$ increases uterine tone and thus promotes involution, and that long involution times seem to be caused by deficient synthesis of prostaglandin $F_{2\alpha}$. A similar study on periparturient and *post partum* endocrinology in the dairy cow supports these findings, and reached similar conclusions. (Eley and others, 1981)

It was this concept, of delayed uterine involution being attributable to deficiency of endogenous prostaglandin $F_{2\alpha}$ synthesis, that was the stimulus for the studies reported in PAPERS 10, 11 and 12, which investigated administration of exogenous prostaglandin $F_{2\alpha}$ during the period of potential deficiency.

SPONTANEOUS MYOMETRIAL ACTIVITY

Spontaneous motility of the bovine uterus, and intra-uterine pressure changes have been studied during the oestrous cycle in non-lactating dairy cattle. (Rodriguez-Martinez and others, 1987a) A pair of miniature pressure transducers were used, mounted 15 centimetres apart at the distal end of a catheter placed in one uterine horn via the cervix. This technique has advantages over previously reported methods cited by the authors: implanted multiple electrodes or strain guage systems do not permit recording of intra-uterine pressure; intra-uterine rubber baloons or an open ended catheter require a slight increase of intraluminal volume, which may induce uterine response and not reflect the natural state precisely. Further, they cannot record directional myometrial motility.

Three non-lactating cows and a mature heifer were used in the studies. Stages of oestrus were identified by rectal palpation, oestrus detection and assay of regular blood samples for oestradiol 17β and progesterone concentrations. The intra-uterine catheter was placed such that the anterior transducer was located near the middle of the uterine horn, and the posterior transducer was located near the body of the uterus.

Measurements were recorded during proestrus, oestrus, metoestrus and dioestrus. Frequency of contractions, about 0.6 per minute, did not vary appreciably between the various stages of the oestrous cycle. Amplitude and length of tonic contractions was greatest at oestrus and minimal, but still registering some activity, during early dioestrus. The degree of active intrauterine pressure also followed this pattern. About the time of ovulation, regular antiperistaltic waves of contraction moved towards the uterine tubes. During early metoestrus and late proestrus, waves of contractions were irregular in their directions. The most striking feature of the uterine activity was the progressive development of regular patterns culminating with the highest synchronised activity during oestrus. High concentrations of progesterone were associated with minimal uterine activity, and high concentrations of oestradiol 17β with peak activity. There was however a delay of approximately two days between peak levels of oestradiol 17β and maximum uterine activity. The reason for the time lag is not clear; however the effect of the delay is to produce greatest organised myometrial activity near the time of ovulation.

EFFECT OF EXOGENOUS PROSTAGLANDIN ON UTERINE MOTILITY

The effect of prostaglandins on uterine motility was investigated in four non-lactating dairy cows, using intraluminal pressure microtransducers as described above. (Rodriguez-Martinez and others, 1987b) Spontaneous activity was recorded for the first 30 minutes. A single dose, 10 per cent of the luteolytic dose, of prostaglandin $F_{2\alpha}$, prostaglandin E_2, or cloprostenol was injected intravenously during dioestrus, proestrus, oestrus and metoestrus, and their effects recorded. The prostaglandin administrations did not affect the duration of oestrous cycles. Single doses of prostaglandin $F_{2\alpha}$ and prostaglandin E_2 significantly increased intra-uterine pressure, frequency of contractions and amplitude of contractions at all stages of the oestrous cycle. The spontaneous pattern of contractions resumed within 20 minutes after injections. There appeared to be partial uterine refractoriness following treatment with either of the natural prostaglandins, reducing mean responses by approximately 20 per cent. Cloprostenol had no effect on intrauterine pressure or myometrial activity.

A third study investigated the effect of intravenous injection of oxytocin on uterine myometrial activity. (Rodriguez-Martinez and others, 1987c) There was a significant increase in intra-uterine pressure and myometrial activity at all four stages of the oestrous cycle. There is evidence to suggest that the mechanism of action of oxytocin and of prostaglandin $F_{2\alpha}$ and prostaglandin E_2 are interrelated. The authors, however, cite evidence which suggests that the uterotonic and prostaglandin releasing actions of oxytocin are separate functions, each acting on a different receptor site and / or mechanism.

Myometrial activity has also been demonstrated *in vitro*. Myometrial strips from non pregnant uteri spontaneously contracted rhythmically and vigorously. (Singh and others, 1979) The frequency of contractions was not affected by addition of prostaglandin $F_{2\alpha}$ but the amplitude immediately increased significantly and persisted for 8 to 10 minutes. A similar response was reported from similar investigations on bovine myometrial strips *in vitro*. (Patil and others, 1980) The increase of tonus and amplitude of contractions persisted for 15 to 18 minutes after administration of prostaglandin $F_{2\alpha}$.

Strips of follicular tissue have also been shown to display spontaneous irregular contractions *in vitro*. (Singh and others, 1979) The effect of administering prostaglandin $F_{2\alpha}$ was to produce slower and stronger contractions persisting for 20 to 30 minutes after stimulation. This effect would also appear to relate to a regulated response to raised concentrations of endogenous prostaglandin $F_{2\alpha}$ about the time of ovulation.

POST PARTUM PROPHYLACTIC PROSTAGLANDIN ADMINISTRATION

(PAPERS 10, 11 and 12)

It is accepted wisdom that economic production from the bovine female demands calving intervals of 365 days. Equally it is recognised that the short calving-to-conception interval needed to achieve this target presents difficulties to the breeder. The physiological, endocrinological and pathological foundations for these dificulties have been discussed in the previous sections.

Common ground for delay in conception is encompassed in the early *post partum* period, and rate of uterine involution has been regarded as an indicator of progress towards resumption of fertile ovarian cyclicity. Many of the causes of delay are expressed in delayed uterine involution.

Links have been demonstrated between blood concentrations of endogenous prostaglandin $F_{2\alpha}$, uterine involution and resumption of ovarian cyclicity. Experimental evidence has led to speculation that one cause of delayed uterine involution may be deficiency of endogenous prostaglandin $F_{2\alpha}$ at a critical stage of the *post partum* period. If there is an endogenous deficiency, then a possible therapeutic response would be to compensate by administration of exogenous prostaglandin $F_{2\alpha}$. This was the concept that led to the investigations described in PAPERS 10, 11 and 12.

The conditions under which the trials had to be organised, placed restrictions and conditions on the methods and trial designs that were possible. The studies had to be capable of being done on commercial dairy farms, with minimal interference to farm routine, without the need for frequent examinations of cows, or frequent sampling and assay techniques. A protocol was devised that could be done at 14 day intervals, during a routine herd fertility visit.

TRIAL DESIGN

Because of the very short half life of prostaglandin $F_{2\alpha}$, replacement therapy would appear best served by frequent administrations over a period of time. This is not an economically feasable approach under practical agricultural conditions, but a single injection of prostaglandin $F_{2\alpha}$ at a strategic time *post partum* would be acceptable. The measurable effects of exogenous administration of prostaglandin $F_{2\alpha}$ have been shown to last for periods of the order of 10 to 30 minutes in studies cited earlier, so there could be no expectation of a single injection providing realistic replacement therapy. However endogenous prostaglandin $F_{2\alpha}$, in common with many other hormones controlling reproductive function, appears to be released in a pulsatile fashion. It is possible that a significant exogenous pulse might stimulate a lagging endogenous system into increased activity. The appropriate time to administer such a stimulatory pulse, might be at a time when the endogenous supply is near the end of its active phase. The *post partum* release of prostaglandin $F_{2\alpha}$ has been shown to last some 15 to 20 days. Twenty one days *post partum* was selected as an appropriate time for prostaglandin $F_{2\alpha}$ injection, and this was bracketed plus or minus seven days, to accomodate a visiting schedule once every 14 days.

The intention of the study was to investigate whether acceleration of *post partum* uterine involution might be induced, and if it was, might advance return to fertile ovarian cyclicity. Two of the most urgent breeding parameters are first-service conception rate, and calving-to-conceptiion interval. These may be measured accurately and objectively, and were chosen as the main parameters for comparison. The perceived target of the treatment, uterine involution, is important mainly for its effect on these two parameters. As its measurement is extremely subjective and imprecise it was rejected as a comparative parameter for the study. Blood concentration of progesterone at the time of treatment would supply useful information, and its assay was included in the protocol.

The mechanism by which prolonged high *post partum* concentrations of endogenous prostaglandin $F_{2\alpha}$ accelerate uterine involution is not known. Influences during the periparturient and early *post partum* period which can affect uterine involution are numerous, diverse and difficult to identify. Prostaglandin $F_{2\alpha}$ has been shown to be effective in treating endomitritis, and subclinical infection may well be common about three weeks after calving. On the other hand, early *post partum* administration has been shown to accelerate uterine involution in apparently normal cows. In absence of an obvious basis for selecting cows for inclusion in the study, all cows that calved within a defined trial period were included, regardless of periparturient history.

TRIALS

PAPER 10. Three commercial dairy herds were selected with middle ranking reproductive performance, and which were already participating in two-weekly routine herd fertility visits. Fertility data were recorded on a computerised fertility programme. All cows that calved between 1 January and 30 April 1983 were allocated alternately to treatment or control. On the day of the herd visit, all cows 14 to 28 days *post partum* had blood samples taken for progesterone assay. 'Treatment' cows were given a single intramuscular injection of 25 mg dinoprost, control cows were not injected. Data recorded were; blood sampling / dinoprost injection date, blood progesterone concentration, service date, return to service dates, conception date, calving date.

The 64 dinoprost injected cows had a a significantly higher (P = 0.007) first-service conception rate, (68 per cent) compared with 64 untreated controls (43 per cent). The advantage was only seen in cows with basal blood progesterone concentrations, of less than 0.5 ng/ml. Dinoprost treated cows had a non-significant six day advantage in calving-to-conception interval.

PAPER 11. The repeatability of the effect of early *post partum* administration of prostaglandin $F_{2\alpha}$ on first-service conception rate was reported. The protocol was the same as for PAPER 10, First-service conception rate for prostaglandin $F_{2\alpha}$ injected cows (n=74) was 56 per cent , and for untreated controls (n=74) was 47 per cent. The difference is not statistically significant. As in the previous study, the advantage was restricted to cows with blood progesterone concentrations under 0.5 ng/ml. Statistical analysis of interaction and year effects were not different, so results of the two studies were combined. The conception rate for 138 prostaglandin $F_{2\alpha}$ injected cows was 62 per cent , and for 138 control cows was 45 per cent. The difference is highly significant (P=0.004). A seven day advantage in calving-to-conception rate in favour of treated cows was not significant but was in close agreement with the first study.

PAPER 12. The previous paper was accepted for publication by the Veterinary Record only if presented as a Short Communication. As the second study contained new information, a full report of the two studies, including the new information, was published in Theriogenology.

The first study had omitted the recording of first oestrus. The protocol for the second study was identical with the first, with the single addition of recording every oestrus in all cows, including oestrus too early for breeding. The average interval from calving to first oestrus was 57 days for 74 prostaglandin $F_{2\alpha}$ injected cows, and 70 days for 74 untreated control cows. The 13 day difference in calving-to-first-oestrus interval was significant (P=0.17).

First oestrus was recorded within seven or fewer days of the treatment date in a number of cows in the study. Of these, 16 were in the prostaglandin $F_{2\alpha}$ injected group compared with five in the control group. Of the 16 prostaglandin $F_{2\alpha}$ injected cows to show oestrus within seven days, eight had blood progesterone concentrations of less than 0.4 ng/ml.

This new information suggests that prostaglandin $F_{2\alpha}$, administered in the early *post partum* period, may be able to reduce the interval to first oestrus. It also may be able to induce oestrus in absence of an active *corpus luteum*, at least in the early *post partum* period. The mechanism by which oestrous cyclicity is stimulated at an early stage is not known. It may be associated with accelerated uterine involution. It may be associated with a direct effect of prostaglandin $F_{2\alpha}$, or a hormone release effect on the ovaries. Such an effect may be associated with, or independent of, uterine involution. The reason for the increased first-service conception rate is not known. It may be a result of oestrous cyclicity starting earlier, so that first or second *post partum* oestrus, which tend to be less fertile, are not used for service. It is also possible that prostaglandin $F_{2\alpha}$ has a therapeutic effect on subclinical endometritis, creating a uterine environment more suitable for conception.

The precise significance of prolonged release of endogenous prostaglandin $F_{2\alpha}$ in the early *post partum* period of the bovine female is unclear, and evidence is somewhat equivocal. It has been demonstrated that endometrial trauma associated with detachment and expulsion of the foetal membranes, stimulates production and release of myometrial prostaglandin $F_{2\alpha}$. (Lindell and others, 1980/1981; Thatcher and others, 1982) This may be accounted for by response to the extensive tissue damage caused by disruption of cotyledons at parturition. (Gier and Marion, 1968) A similar explanation would apply to tissue damage associated with placental retention, and related, or independent uterine infection. Release of prostaglandin $F_{2\alpha}$ has been demonstrated in response to bacterial endotoxins. (Fredriksson, 1984) In these circumstances, prostaglandin release is prolonged, but uterine involution and return to fertile cyclicity are delayed. (Kindahl and others, 1982; Lindell and others, 1982) The prostaglandin $F_{2\alpha}$ release in such conditions appears to be a response to tissue damage.

The situation is different when the puerperal period has been untroubled. In that circumstance, prolonged *post partum* release of prostaglandin $F_{2\alpha}$ is associated with accelerated uterine involution and return of ovarian cyclicity. (Lindell and others, 1982) The stimulus for its prolonged release in these conditions has not been identified, but the effect suggests a positive mechanism accelerating involution, rather than a response to damage.

The presence of non specific bacteria in the uterine lumen after parturition is regarded as virtually inevitable. In absence of certain specific pathogenic bacteria, this does not appear to have an adverse effect on fertility. (Griffin and others, 1974a,b.) It may be that the presence of bacteria, or their metabolites, or breakdown products, have an irritant effect on the endometrium, which might stimulate release of myometrial prostaglandin $F_{2\alpha}$.

The positive relationship between uterine involution and resumption of ovulation is well recognised, but the mechanism has not been elucidated. Return of ovarian cyclicity is not dependent on uterine involution, only ovulation is. (Paisley and others,1986) Moreover the delaying effect applies only to the ovary adjacent to the recently pregnant horn, (Thatcher and others, 1982) and may therefore be deduced to depend on a mechanism acting locally.

Exposure to prolonged heat-stress during pregnancy induced very high blood concentrations of endogenous prostaglandin $F_{2\alpha}$ metabolite, which persisted until approximately 20 days after parturition. (Thatcher and others, 1982) In the heat-stressed cows with abnormally high prostaglandin $F_{2\alpha}$ levels, the expected inhibition of folliculogenesis in the ovary adjacent to the recently pregnant horn did not occur. Folliculogenesis started simultaneously in both ovaries. This did not happen in unstressed controls, so an inhibitory factor would appear to have been removed.

The inhibitory action of pregnancy on follicular development continues after parturition, and it has been suggested that the mechanism involves the previously gravid horn and/or the persisting *corpus luteum* of pregnancy. (Rexroad and Casida, 1975) The follicular contents of ovaries were analysed from dairy cows, up to 35 days after calving. (Dufour and Roy, 1985) It was found that the number of atretic follicles varied significantly according to the *post partum* interval and to whether they were in the ovary containing the *corpus luteum* of pregnancy. It was concluded that the *corpus luteum* of pregnancy and/or the conceptus have a carryover effect on the rate of growth of antral follicles after parturition. There appears therefore to be a link, which acts locally in the ovary, between the persisting *corpus luteum* of pregnancy and inhibition of folliculogenesis. The evidence from the heat stressed cows suggests that exceptionally high concentrations of endogenous prostaglandin $F_{2\alpha}$ can suppress such a local inhibitory influence.

These observations may hold a clue as to the mechanism by which administration of exogenous prostaglandin $F_{2\alpha}$ in the early *post partum* period resulted in advance of *post partum* onset of oestrus cyclicity. The observation in PAPERS 10, 11 and 12, that the effect was observed at a time of basal blood progesterone concentrations would not be in conflict with the observations cited above, which would also be operating at a time of basal blood progesterone. The apparent anomaly may be explained by the presumed inhibitory effect being local to the specific ovary.

Accelerated uterine involution following administration of multiple injections of exogenous prostaglandin $F_{2\alpha}$ on days 3 to 13 *post partum* has been demonstrated, (Lindell and Kindahl, 1983) but the effect on fertility was not investigated. It would be expected that earlier uterine involution would be asociated with earlier return to fertile cyclicity as has been recorded in normal cows. (Madej and others, 1984) The mechanism by which uterine involution influences ovarian cyclitity has not been elucidated.

The short half life of prostaglandin $F_{2\alpha}$ has been invoked to discount the probability of a single injection producing a prolonged effect such as might be thought necessary to influence the rather protracted duration of the uterine involutionary process. Myometrial activity stimulated by a single administration of prostaglandin $F_{2\alpha}$ has been reported to last for up to 30 minutes. (Patil and others, 1980) This is longer than can be accounted for by the presence of the exogenous prostaglandin $F_{2\alpha}$, which would have been metabolised. To account for such an extended period of activity following administration, a "cascade effect" may be proposed and explained as follows. An injection of prostaglandin $F_{2\alpha}$ causes myometrial contraction; myometrial contraction stimulates release of endogenous prostaglandin $F_{2\alpha}$, which stimulates myometrial contraction, which stimulates release of endogenous prostaglandin $F_{2\alpha}$. In this way a prolonged succession of gradually decreasing waves of myometrial activity may be induced by administration of a single pulse to initiate the activity.

In addition, it may be that high peripheral blood concentrations of prostaglandin $F_{2\alpha}$ metabolite are not a necessary concomitant of myometrial prostaglandin $F_{2\alpha}$ activity. A recent tissue cage study has demonstrated the presence of raised concentrations of prostaglandin $F_{2\alpha}$ metabolite in a tissue cage for longer than six hours following a a single intramuscular injection of prostaglandin $F_{2\alpha}$. (Kindahl and others, 1985) In the same animal, prostaglandin $F_{2\alpha}$ metabolite in peripheral blood had returned to pretreatment levels after two hours. Prostaglandins are recognised to function as local mediators, being produced and having their action locally, and being rapidly metabolised. That being the case, it may be that local activity need not necessarily be accompanied by measurable concentrations in peripheral blood.

The *post partum* rate of uterine and cervical reduction in size has been used as a functional indicator of return to fertile ovarian cyclicity. (Oltenacu and others, 1983) However the physical condition of the uterus, whilst it may be a necessary prerequisite, must mediate its effect on the ovaries by a hormonal mechanism. The appropriate mechanism has not been identified, and it is possible that the *post partum* release of prostaglandin $F_{2\alpha}$

may have a role in mediating ovarian function in addition to its presumed myometrial role. This could be a direct effect, or might be indirectly modulated through the action of associated release factors.

Prostaglandins, and in particular prostaglandin $F_{2\alpha}$ have been demonstrated to fulfil such hormone release functions. Infusion of prostaglandin $F_{2\alpha}$ at the rate of 0.2 mg per minute into the jugular vein of bulls increased plasma concentrations of luteinising hormone, glucocorticoid and testosterone, or prolonged the periods when hormone concentrations were elevated when compared with controls. (Haynes and others, 1977) Furthermore, a dose of prostaglandin $F_{2\alpha}$ which was ineffective by the jugular route, was effective when administered by the carotid artery, indicating that the effect is probably mediated via the hypothalamus-pituitary axis. A similar, but slower response has been recorded in cows. (Hafs and others, 1975) A further similar study in bulls demonstrated that the effect was not a general response to prostaglandins. (Haynes and others, 1978) Prostaglandin $F_{2\alpha}$ produced a significant increase in serum luteinising hormone concentrations within 40 minutes, which had not returned to baseline by 6 hours. Conversely, prostaglandin E_2 depressed luteinising hormone relative to controls. The authors observe that both prostaglandin $F_{2\alpha}$ and prostaglandin E_2 are present in hypothalamic tissue , and speculate that if endogenous prostaglandins are indeed involved in the normal modulation of luteinising hormone secretion, the combination of an activating and a suppressive effect of different prostaglandins may offer a sensitive system for modulating luteinising hormone release.

The most consistent endocrine change which has been reported to precede ovulation in the *post partum* period is the onset and increase in frequency of pulsatile release of luteinising hormone. (Lamming and others, 1981; Peters and Lamming, 1986; Schallenberger and others, 1982) Furthermore, the earlier and more frequent these pulses are, the earlier is ovarian cyclicity resumed. (Peters and others, 1981; Peters and Riley, 1982). If, as is indicated above, endogenous prostaglandin $F_{2\alpha}$ has a positive role in the release of luteinising hormone from the anterior pituitary, then administration of exogenous prostaglandin $F_{2\alpha}$ at an appropriate time during the *post partum* anoestrus period may overcome the suggested inhibitory effect of prostaglandin E_2 and stimulate pulsatile release of luteinising hormone sufficiently to initiate ovarian cyclicity.

A controlled study on 305 cows in a commercial Friesian herd investigated injection with gonadotrophin-releasing hormone on Day 15 *post partum* and / or cloprostenol on Day 24 *post partum*. (Etherington and others, 1984). It was concluded that treatment with gonadotrophin-releasing hormone was generally detrimental. Treatment with cloprostenol significantly decreased calving-to-first-observed-oestrus interval by 15 days (P=0.003), and calving-to-conception interval by 15 days (P=0.046).

In a similar controlled trial, Holstein cows (n=234) were injected with gonadotrophin-releasing hormone between Days 10 and 14 *post partum* and / or prostaglandin $F_{2\alpha}$ (dinoprost) 10 days later. (Benmrad and Stevenson, 1986) Treatment with gonadotrophin-releasing hormone reduced intervals to first ovulation and first detected oestrus as well as increasing the proportion of cows with three or more ovulations before first service to 83 per cent, compared with 57 per cent for saline treated controls. Treatment with prostaglandin $F_{2\alpha}$ reduced intervals to second and third ovulation and shortened the first oestrous cycle. The interval from calving to conception was reduced by 43 days to 48 days in cows with an abnormal puerperium, treated with either gonadotrophin-releasing hormone or prostaglandin $F_{2\alpha}$ compared with saline injected controls. When cows with untroubled puerpera were included in these two groups, calving to conception interval was reduced by 27 days to 29 days. Cows treated with prostaglandin $F_{2\alpha}$ or with gonadotrophin-releasing hormone required 26 to 41 per cent fewer services per conception than did control cows.

The limited evidence available suggests that either prostaglandin $F_{2\alpha}$ or gonadotrophin-releasing hormone administered in the early *post partum* period has a stimulatory effect on resumption of ovarian cyclicity, with somewhat better results following the prostaglandin $F_{2\alpha}$ treatment. The responses are not identical, and this may indicate a difference in the mechanism, however the administration of gonadotrophin-releasing hormone earlier in the *post partum* period than was prostaglandin $F_{2\alpha}$, may also be important. In both trials cited above, the treatments which combined gonadotrophin-releasing hormone injected 15 days *post partum* followed by prostaglandin $F_{2\alpha}$ 10 days later, did not result in an improvement of the reproductive parameters studied. A further study comparing the same combined gonadotrophin-releasing hormone and prostaglandin $F_{2\alpha}$ treatment with saline injected controls also failed to record an advantage. (Richardson and others, 1983)

The studies reported in PAPERS 10, 11 and 12 have demonstrated advantageous effects on reproductive parameters from a single injection of prostaglandin $F_{2\alpha}$ administered during the period from 14 to 28 days after calving. First service conception rate was increased, and the onset of fertile ovarian cyclicity was advanced. The time for injection was selected because concentrations of endogenous prostaglandin $F_{2\alpha}$ appear to have a critical influence on reproductive events about this stage of the *post partum* period. Studies cited above agree with the effect of prostaglandin $F_{2\alpha}$ administered in the early *post partum* period, and suggest that gonadotrophin-releasing hormone may have a similar, or associated role in the mechanism. It also appears possible that luteolysis of the *corpus luteum* of pregnancy may be a factor. A luteinising hormone release effect of prostaglandin $F_{2\alpha}$ appears to be another possible mechanism for the observed response.

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Addendum

One hundred and ninety four of the 200 ewe lambs in flock 1 that had been seronegative in October 1983 were resampled in June 1984 at the age of about 15 months. Two (1·0 per cent) were found to have developed positive titres for maedi-visna virus. The lambs had been managed separately from the rest of the flock from their arrival in September 1983 and there had been no direct contact between the two groups of sheep at any time. However, there had been opportunity for indirect contact via the pasture, communal farm tracks and sheep collection points.

Although the possibility that the lambs had acquired infection from the older ewes cannot be completely excluded, it seems much more probable that they were infected before arrival in East Anglia although at the age of seven months they had not seroconverted. These latest serological findings therefore appear to indicate an origin for the maedi infection in this flock.

Increased conception rate in dairy cows after early post partum administration of prostaglandin $F_{2\alpha}$ THAM

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Veterinary Record (1984) **115**, 429-431

Commercial dairy cows were given a routine injection of dinoprost tromethamine (prostaglandin $F_{2\alpha}$ THAM) in the early post partum period. The first service conception rate of 64 cows given a single 25 mg injection of dinoprost during the period 14 to 28 days after calving was 68 per cent, that of 64 untreated controls was 43 per cent. The difference was highly significant at the level P = 0·007. In cows with no blood progesterone and with basal progesterone concentrations at the time of treatment, indicating absence of an active corpus luteum, the mean conception rates for 30 treated and 38 control cows were 70 and 44 per cent, respectively, demonstrating that this is not a luteolytic effect. Although that implies a positive myometrial effect, the interval from calving to first service was not shortened in treated cows.

THE complex endocrine changes involved in the post partum return to cyclic activity in cattle have been reviewed (Leslie 1983).

Although the precise mechanism is not clearly understood there is an apparent relationship between completion of uterine involution and return to ovarian cyclicity. The diameter of the previously pregnant horn is commonly regarded as the main indicator of involution of the genital tract, however, reduction in the diameter of the cervix has also been used as an indicator of the involution process. In cows with medium or large cervices first service conception rate was lower and days from parturition to conception were higher than in cows with small cervices (Oltenacu and others 1983).

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Blood concentrations of prostaglandin $F_{2\alpha}$ were investigated during the periparturient period by measurement of its main plasma metabolite 15-keto-13,14-dihydro-$PGF_{2\alpha}$, (PGFM) (Lindell and others 1982).

High concentrations of prostaglandin $F_{2\alpha}$ metabolite at parturition remained elevated for periods of between seven and 23 days and uterine involution was completed in periods of 16 to 53 days. Progesterone concentrations remained low until the post parturient prostaglandin release had ceased.

In cows with no periparturient complications, a significant correlation was found to exist between the time needed for complete uterine involution and the duration of elevated levels of prostaglandin $F_{2\alpha}$. Cows with a long duration had relatively shorter involution times.

Lindell and others (1982) speculated from their data that uterine synthesis of prostaglandin $F_{2\alpha}$ increased uterine tone which promoted involution and they associated long involution times with insufficient synthesis of prostaglandin $F_{2\alpha}$.

Prolonged uterine contractions have been induced after a single intramuscular injection of prostaglandin $F_{2\alpha}$ in non-pregnant women (Karim and others 1971) and sheep (Edqvist and others 1975). Similar effects have been demonstrated in in vitro studies on bovine myometrium (Patil and others 1980).

The possibility that exogenous prostaglandin $F_{2\alpha}$, administered during the early post partum period, might influence uterine involution and shorten the time for return to optimum fertility after calving is reported here.

Materials and methods

Three commercial dairy farms were selected for the study. The herds had been participating in routine herd fertility schemes run by the University of Glasgow veterinary practice. They were visited at two-weekly intervals.

Data were recorded on the practice microcomputer, a 64K Tandy TRS 80 microprocessor with expansion disk drive and

TABLE 1: Calving to first service interval, calving to conception interval and first service conception rate for prostaglandin treated and untreated control cows

	Prostaglandin injected	Control
Average calving to first service interval (days ± sd)	73±26·1	69±22·1
Range	21–136	20–121
Average calving to conception interval (days±sd)	87±35·8	93±39·9
Range	21–227	20–230
First service conception rate (%)	68*	43

*Difference significant at level P = 0·007

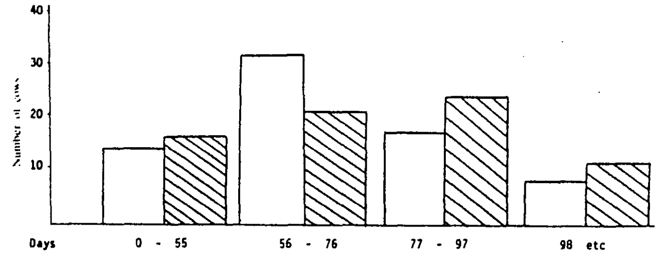

FIG 1: Number of days from calving to first service. Shading – prostaglandin injection; no shading – control

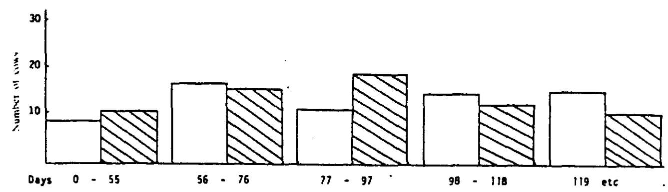

FIG 2: Number of days from calving to conception. Shading – prostaglandin injection; no shading – control

printer. The fertility programme used by the practice produces analyses and histograms of criteria of importance for monitoring herd breeding performance.

A total of 139 Friesian cows participated in the study. Eleven cows were culled during the study period, three from the treatment groups and three from the control groups for infertility, the remainder for mastitis and management policy reasons, leaving 64 each in treatment and control groups.

All cows which calved between January 1 and April 30, 1983, inclusive, were allocated, before examination, on each farm to treatment or control alternately on the basis of chronological order of calving dates. All cows had a blood sample taken during the period 14 to 28 days after calving for progesterone assay.

Treated cows were given a 25 mg intramuscular injection of dinoprost (Lutalyse; Upjohn) immediately after blood sampling; control cows received no injection.

Blood samples were centrifuged on the day of collection and the serum stored at −18°C until dispatched to the Milk Marketing Board veterinary laboratory at Worcester for serum progesterone determination by radioimmunoassay.

Fertilisation was by artificial insemination and should have been started after 56 days from calving. A small number of cows in both groups and on all three farms were served before day 56. Pregnancy diagnosis by rectal palpation was carried out about seven to nine weeks after insemination. The criteria used for comparison were calving to first service interval, calving to conception interval and conception rate to first service.

Statistical comparisons for calving to first service interval and calving to conception interval were carried out using *t* tests. Comparisons for first service conception rate were carried out using Fisher's exact test on the 2 × 2 contingency table. Comparisons for the effect of blood progesterone concentration at the time of treatment on first service conception rates were carried out using a linear model for categorical responses.

Results

The first service conception rate for 64 prostaglandin treated cows was 68 per cent and for 64 untreated controls was 43 per cent; this difference was significant at the level P = 0·007. The average calving to first service interval was four days longer for treated cows than for control cows, but the average calving to conception interval was six days shorter (Table 1).

This trend does not achieve statistical significance. These opposing trends are reproduced graphically for calving to first service intervals (Fig 1) and for calving to conception intervals (Fig 2). The degree of luteal activity at the time of treatment had no significant influence on the outcome even when zero levels of progesterone indicated absence of active luteal tissue (Table 2).

The protocol did not require the recording of non-service oestrus, however, this was recorded for a number of cows on all three farms, 13 treated and 13 controls. The average calving to first oestrus interval was 45 days for treated cows and 43 days for controls, which agrees with the trend seen in calving to first service intervals.

TABLE 2: Effect of blood progesterone concentration at time of treatment on conception rate of prostaglandin treated and control cows

Blood progesterone (ng/ml)	Prostaglandin injected		Controls	
	Number of cows	Number pregnant to first service	Number of cows	Number pregnant to first service
0·0	16	12	25	11
0·1 – 0·4	14	9	13	6
0·5 – 3·0	22	13	12	7
3·1 and over	4	3	6	2
Not sampled	8	7	8	2
Total (%)	64	44 (68)	64	28 (43)

Discussion

The evidence of this study suggests that prostaglandin $F_{2\alpha}$ has a role in increasing fertility in bovine reproduction. The effect is not mediated through luteolysis because the influence can be seen in cows with no corpus luteum, as indicated by no blood progesterone, or inactive corpora lutea, as indicated by basal levels (less than 0·5 ng/ml). The trend is seen also with moderate luteal activity (0·5 to 3·0 ng/ml) and active corpora lutea (3·1 ng/ml plus), which suggests that it is independent of luteal phase.

The first service conception rate of the control cows may appear low; however, it lies in the middle of the range (27 to 52 per cent) which was recorded for the conception rates for untreated control cows in a recent computer recorded fertility study of six commercial dairy herds (Jackson and others 1983).

The relationship between concentrations of endogenous prostaglandin $F_{2\alpha}$ and rate of uterine involution observed by several authors (Kindahl and others 1982, Lindell and others 1982, Thatcher and others 1982) suggests a myometrial role for prostaglandin $F_{2\alpha}$ in this context, which prompted this study. For this application it was considered that prostaglandin $F_{2\alpha}$ THAM would have a greater myometrial effect than prostaglandin analogues which have been shown to have less smooth muscle activity (Jackson and Jessup 1984).

Administration of prostaglandin $F_{2\alpha}$ THAM in the early post partum period has been reported (Kindahl and others 1982) but the fertility criterion examined was time to first ovulation, which was not shortened; the effect on the conception rate was not reported.

That observation is in agreement with the present study where, in fact, a comparable parameter, calving to first service interval, is slightly longer in the treated cows compared with controls. Similarly in a number of cows where the interval from calving to first oestrus was recorded, that too was slightly longer for treated than for control cows.

This observation, however, does not necessarily preclude the importance of an initial myometrial effect, because the mechanisms by which uterine involution influences resumption of reproductive function and increase in fertility during the post partum period are complex and have not been elucidated. Certainly the influence of prostaglandin $F_{2\alpha}$ in this context appears to extend beyond luteolysis, or a quicker return to ovarian cyclicity.

Prostaglandin has been shown to have a role in the treatment of uterine infection (Ott and Gustafsson 1981) and it might be speculated that its administration in the early post partum period would reduce the incidence of subclinical uterine infection and hasten the return to a suitable uterine environment for fertilisation and pregnancy.

In this context it is the luteolytic function of prostaglandin which has been supposed to operate. Its effectiveness, however, may not depend necessarily or exclusively on luteolysis.

These results suggest a potentially valuable prophylactic role for postaglandin $F_{2\alpha}$ THAM to increase fertility in the cow.

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Ectopia cordis thoracoabdominalis in a piglet

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A congenital anomaly characterised by displacement of the heart through a ventral body wall fissure involving the thoracic and cranial abdominal regions was recorded in a female Yorkshire-cross piglet. Dissection to assess the morphology of the developmental defect and a summary review of the literature on ectopia cordis were made. This case appears to be one of only three of ectopia cordis thoracoabdominalis reported in swine.

A NEWBORN female crossbred Yorkshire piglet from the Virginia Polytechnic Institute and State University swine herd was received and examined by the authors following its sacrifice as a source of liver tissue for experimental purposes. Although able to suck, the piglet had been selected because of doubts concerning its viability caused by the presence of a defect in the ventral body wall through which the heart protruded.

Post mortem examination

The piglet was of normal size (26 cm crown to rump length) and conformation except for the body wall and heart anomaly. The body wall defect (Fig 1) consisted of a ventral midline fissure which involved all of the sternum except the manubrium and which extended caudally as far as the umbilicus. The defect was bridged by a thin, transparent membrane which covered the partially exteriorised heart, the junction between the membrane and normal skin being clearly demarcated. The membrane joined the cranial aspect of the umbilical cord approximately 11 cm distal to the union of the umbilical cord with the body wall.

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Dissection

Dissection of the body wall revealed that the lateral abdominal wall musculature (external abdominal oblique, internal abdominal oblique and transverse abdominal muscles) was complete.

The right and left rectus abdominis muscles extended from the pubis to the level of the umbilicus and were separated by a typical linea alba. However, cranial to the umbilicus, the rectus abdominis muscles lay immediately dorsolateral to the lateral margins of the body wall defect.

At the level of the sixth costal cartilage, the rectus muscle system was continued cranially by the rectus aponeurosis and by the rectus thoracis muscle overlying the first three ribs. Intercostal musculature was present.

Right and left sternal elements were present, but were separated by the fissure and only met cranially at the non-fissured manubrium. Although 14 or 15 pairs of ribs are considered typical for swine (Getty 1975), 16 pairs were observed in this piglet.

Deeper dissection revealed that the ventral part of the diaphragm had been removed when the liver was removed. The dorsal portion of the diaphragm was present and was attached to the body wall dorsolaterally at the level of the 13th ribs. The partially exteriorised heart was contained within a pericardial cavity, the dorsal extent of which occupied the floor of the thoracic cavity and extended as far cranially as the thoracic inlet.

The parietal layer of the intrathoracic portion of the pericardium was continuous with the membrane that bridged the ventral body wall defect. The left pleural cavity, as defined by its pleural membrane, was complete although

Disease associated with *Mycoplasma mycoides*, subspecies *mycoides* in sheep in Nigeria

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IT has generally been thought that the occurrence of *Mycoplasma mycoides* subspecies *mycoides* is limited to cattle under natural conditions. However, in recent years it has been isolated from disease conditions of sheep and goats in different parts of the world (Erno and others 1972, Cottew 1979, Perreau and Breard 1979, Rosendale and others 1979, Bar-Moshe and Rapapport 1981, Perreau and Bind 1981, Jones 1983). In Nigeria natural infection of goats with *M mycoides* has been reported (Ojo 1973, 1976). However a *M mycoides* disease outbreak, per se, in sheep has never been confirmed in Nigeria.

A confirmed natural outbreak of the disease in 500 indigenous Balami-Yankassa crossbred sheep is reported here. The sheep were housed and given subsistence feed but derived most of their roughage from the open grazing land. On June 2 1985, depression, dyspnoea, fever, anorexia, reluctance to move, polyarthritis and diarrhoea were seen in the flock. About 45 per cent of sheep had clinical signs. Ensuing mortality averaged four to six sheep daily from June 7 but increased to 10 to 12 on June 20. Deaths in adult sheep (including pregnant ewes) and lambs were high. This resulted in about 60 per cent total mortality in the flock despite administration of lincomycin and spectinomycin (Linco-Spectin; Upjohn) late in the course of infection in drinking water for about a week by the sheep owner.

The most common gross lesions were pleurisy and bronchopneumonia with soft gelatinous adhesions of lungs and heart to the thoracic wall.

All sheep carcases necropsied from June 7 to July 10 consistently showed consolidation of the lungs with apical and cardiac lobes of either or both lungs hepatised. Other gross lesions seen were hydropericarditis (hydropericardium) and hydrothorax, haemorrhagic enteritis and acutely congested brains.

Histopathological examination of lung tissue showed a severe, acute lobular purulent alveolitis with marked congestion and oedema associated with fibrinous pleurisy and pericarditis.

Gel precipitation tests carried out on lung tissues and thoracic exudates of necropsied animals produced characteristic precipitation lines. Inoculation of such lung tissues and thoracic fluid in Newing's tryptose broth (Brown and others 1965) and tryptose serum agar (Davies and Read 1968) produced typical *M mycoides* subspecies *mycoides* colonies and the organism was confirmed from characteristic cultural, biochemical and growth inhibition tests as described by Davies and Read (1968) and Ojo (1976). No significant bacteria were cultured from the lungs, thoracic fluid or heart blood of the sheep.

Mortality in the outbreak was quite high and it is thought likely that other concurrently diagnosed infections, namely heartwater and type B *Clostridium welchii* enterotoxaemia, might have played some part. Epidemiologically *M mycoides* of sheep may be important in countries such as Nigeria where the disease caused by the organism in cattle is being controlled. In contagious bovine pleuropneumonial-endemic countries like Nigeria, cattle, sheep and goats are herded together and the epidemiology of *M mycoides* in sheep and goats could therefore be important.

It has not been conclusively shown that *M mycoides* isolated from sheep can infect cattle and goats and this subject is being investigated as it may be of great practical importance in Nigeria and other countries in West Africa where tremendous efforts are being made to control contagious bovine pleuropneumonia.

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First service conception rate in dairy cows treated with dinoprost tromethamine early post partum

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A SINGLE injection of dinoprost tromethamine administered routinely to apparently normal dairy cows between 14 and 28 days after calving significantly improved conception to first service and shortened the interval from calving to conception (Young and others 1984). This response was thought to be associated with the smooth muscle effect of dinoprost tromethamine on the myometrium because it was demonstrated mostly when blood concentrations of progesterone were zero or less than 0·5 ng/ml.

Abnormal delay in uterine involution after calving has been associated with inadequate production of endogenous prostaglandin during the period shortly after calving (Lindell and others 1982). Repeated administration of dinoprost twice daily from days 3 to 13 after calving shortened the time

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TABLE 1: Calving to first service and conception intervals and first service conception rate for dinoprost treated and untreated control cows

	Dinoprost tromethamine injected	Control
Average calving to first service interval (days ± sd)	75 ± 22·9	78 ± 28·2
Average calving to conception interval (days ± sd)	96 ± 34·7	103 ± 37.3
First service conception rate (%)	56	47

TABLE 2: Effect of dinoprost treatment on first service conception

	Dinoprost injected		Controls	
Year	Number of cows	Pregnant to first service	Number of cows	Pregnant to first service
1983*	64	44	64	28
1984	74	42	74	35
Total†	138	86 (62%)	138	63 (45%)

* Difference significant at level P = 0·007
† Difference significant at level P = 0·004

TABLE 3: Effect of blood progesterone concentration on first service conception

	Dinoprost injected			Controls		
Progesterone	Number of cows	Pregnant	(%)	Number of cows	Pregnant	(%)
Less than 0·5 ng/ml	51	31	(60)	41	18	(43)
0·5 ng/ml and more	22	11	(50)	29	17	(58)
Not blood sampled	1	0		4	0	

needed for the recently pregnant uterus to involute (Lindell and Kindahl 1983).

In veterinary practice, multiple administrations over a period of 10 days are quite impractical, so a single injection of dinoprost tromethamine was chosen (Young and others 1984). The improved reproductive performance warranted further investigation. The repeatability of improved first service conception rate in treated cows is reported here.

Three commercial dairy farms were used for the study. They were visited at two weekly intervals. Data were recorded on the fertility programme of the microcomputer of the University of Glasgow veterinary practice.

A total of 163 Friesian cows were available for the study. Fifteen cows were culled during the trial and were not included in the data. There were 74 cows each in the treatment and control groups.

Cows were allocated to treatment or control groups alternately on chronological order of calving dates on each farm. All cows calving during the first three months of 1984 in all three herds were included in the study. All cows had a blood sample taken for progesterone assay at the appropriate visit between 14 and 28 days after calving. At the same time cows in the treatment group were injected with 25 mg of dinoprost tromethamine (Lutalyse; Upjohn) intramuscularly. Control cows were not injected. Blood samples were centrifuged as soon as possible on the day of collection and were stored at −18°C until dispatch to the Milk Marketing Board veterinary laboratory at Worcester for progesterone assay.

Insemination was started 56 days after calving on all three farms.

The data used for comparison were the mean intervals in days from calving to first service, conception and the calving rate to first service.

The first service conception rate for 74 dinoprost injected cows was 56 per cent and for 74 untreated control cows was 47 per cent (Table 1). This difference does not achieve statistical significance. Statistical analysis of interaction and year effects were not significantly different so the 1983 and 1984 data may be combined. The combined conception rate for 138 dinoprost injected cows was 62 per cent and for 138 control cows was 45 per cent. The difference is highly significant at the level P = 0·004 (Table 2).

The average calving to conception interval was 96 days for dinoprost injected cows and 103 days for controls. This seven day advantage does not achieve statistical significance but it is consistent with the six day advantage recorded previously (Young and others 1984).

There was no statistical difference between the average calving to first service intervals of treated and control cows at 75 and 78 days respectively.

The recent ability to deduce blood concentrations of prostaglandin $F_{2\alpha}$ from assay of its less transient metabolite 15-keto-13,14-dihydroprostaglandin $F_{2\alpha}$ (PGFM) has produced new information.

The studies by authors cited in this paper, and others, have drawn attention to a considerable variability in the time needed for involution of the uterus of the cow after calving, before it is again capable of a successful pregnancy. There is also a hormonal dimension involved in restarting ovarian activity and regular fertile oestrous cycles. Longer high concentrations of PGFM have been associated with both a faster rate of uterine involution and a stimulatory effect on follicular and luteal components of the ovary (Thatcher and others 1982). The occurrence of the first ovulation followed by a normal luteal phase length after calving in 29 cows was positively correlated with the time needed for completion of uterine involution (Madej and others 1984).

Involution of the uterus is an integral part of the process of return to ovarian cyclicity, but subjective manual assessment of its physical form may not be necessary to measure its progress in this context. It was notable in the present study that improved conception was mostly in dinoprost treated cows with a blood progesterone concentration of less than 0·5 ng/ml at the time of treatment. Progesterone assay of blood or milk is now readily available to the practitioner and might be used to identify cows at an early stage after calving with a low progesterone concentration, as suitable candidates for this treatment.

A clinical study assessed the improved rate of uterine involution by rectal palpation and calving intervals after early post partum prostaglandin treatment. While lacking controls, a convincing performance was recorded, measured against accepted practical fertility standards (Tindall 1984).

The results of these studies suggest that resumption of ovarian cyclicity after calving may be advanced by the administration of dinoprost tromethamine, soon after calving. It may be possible to identify the cows most likely to respond to such treatment by milk progesterone assay about two to four weeks after calving. Such cows amounted to some two thirds of those in the studies. The technique might be used to improve conception rate to first service at an earlier time than usual in a herd's breeding period.

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THERIOGENOLOGY

IMPROVED REPRODUCTIVE PERFORMANCE FROM DAIRY COWS TREATED WITH DINOPROST TROMETHAMINE SOON AFTER CALVING

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ABSTRACT

The first-service conception rate for 74 commercial dairy cows that were given a single injection of dinoprost tromethamine (prostaglandin $F_{2\alpha}$ THAM) between 14 and 28 d after calving was 56%. For 74 untreated control herdmates the rate was 47%. The average interval from calving to first oestrus was 57 d for treated cows and 70 d for the control group. The difference is significant at the P = 0.017 level. The advantage in the conception rate of treated over control group cows occurred mostly in cows with a blood progesterone concentration of less than 0.5 ng/ml at the time of treatment; this numbered about two-thirds of the cows in the trial. The results support the findings of a previous study.

Key words: dinoprost, post-partum, oestrus, conception rate

INTRODUCTION

Evidence has been produced to show that a single injection of dinoprost tromethamine ('Lutalyse', Trademark, Upjohn Ltd. Crawley, U.K.) administered routinely to apparently normal dairy cows between 14 and 28 d after calving can significantly improve conception from the first service and can shorten the interval from calving to conception. The response was thought to be associated with the smooth muscle effect of dinoprost tromethamine on the myometrium, because the improvement was demonstrated mostly when blood concentrations of progesterone were less than 0.5 ng/ml. This was assumed to infer that ovarian cyclicity had not been resumed after calving at the time of treatment and that luteolysis did not play a part in the response (1). Delay in the time taken for the uterus to involute after calving and return to a non-pregnant condition has been related to delay in the time needed to achieve the next pregnancy (2). In addition, and related to that, abnormal delay in uterine involution after calving has been associated with inadequate production of endogenous prostaglandin during the period shortly after calving (3). It was found that repeated administration of dinoprost twice daily from Day 3 to 13 after calving did in fact shorten the time needed for the recently pregnant uterus to involute (4).

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The authors thank C. Fenton for statistical advice and analyses.

Repeated administration of prostaglandin over a period of time to compensate for suspected endogenous deficiency is logical because of the short half-life of prostaglandin. In the economics of veterinary practice, however, multiple administrations over a period of 10 d are quite impractical. It was for this reason, and in the knowledge of prolonged myometrial effect following a single administration of prostaglandin $F_{2\alpha}$ (5, 6, 7), that a single injection of dinoprost tromethamine was chosen in a previous study (1). In that study, the effect of dinoprost treatment on the occurrence of the first oestrus after calving was not recorded. The study reported here recorded an advance of oestrus in treated cows. The repeatability of increased first-service conception rate was confirmed in treated cows, and also the importance of low blood progesterone concentration at the time of treatment. The data suggest the possibility that dinoprost may be able to induce oestrus at a time when basal blood progesterone concentration indicates the absence of an active corpus luteum.

MATERIALS AND METHODS

In order to achieve comparable results, the conditions and protocol were deliberately kept the same as in our previous 1983 study(1), with the single addition of recording pre-service oestrus in all cows.

The three commercial dairy farms used in the 1983 study were again used. They were visited once every 14 d. Data were recorded on the microcomputer fertility programme at the University of Glasgow Veterinary Practice.

A total of 163 Friesian cows were available for the study. Fifteen cows were culled during the trial and were excluded from the data. One cow from the treatment group and four from the control group were culled for infertility; the remainder were culled for mastitis, lameness, hypomagnesaemia, and for management policy reasons. Of the remaining 148 cows, 74 cows each were placed into the treatment and control groups.

The cows, from each of the 3 farms, were allocated alternately to either the treatment or control group according to the chronological order of their calving dates. All cows that had calved during the first 3 months of 1984 in all 3 herds were included in the study. All cows had a blood sample taken for progesterone assay at the appropriate visit between 14 and 28 d after calving. At the same time, cows in the treatment group were injected with 25 mg of dinoprost tromethamine intramuscularly. Control cows were not injected.

Blood samples were centrifuged as soon as was possible, within about two hours of collection. The plasma samples were stored at -18°C until dispatch to the Milk Marketing Board Veterinary Laboratory, Worcester, for progesterone assay in one batch. The assay is a direct radio-immunoassay method described by Holdsworth (11), giving results closely correlated to, but somewhat higher than, the conventional extraction method. An identical standard internal control serum was used for the assay, so that results from different years are comparable.

Insemination of cows was started 56 d after calving on all three farms. The commercial inseminator service of the Scottish Milk Marketing Board, using frozen semen, was used for all cows, and the choice of semen was not specified. Oestrus detection was done by stockmen at least three times per day. Pregnancy diagnosis was made by rectal palpation about 7 to 8 weeks after insemination and confirmed by the calving date.

The data used for comparison were the mean intervals in days from calving to first oestrus, calving to first service, calving to conception, and the calving rate resulting from the first service. These were the same parameters as those in the 1983 study, with the single addition that all prebreeding oestrus was also recorded. The influence of the concentration of the blood progesterone at the time of blood sampling on the conception rate to the first service was compared between dinoprost-treated cows and untreated controls.

For comparisons between calving to first oestrus, calving to first service and calving to conception intervals, the Wilcoxon Rank Sum Test, a non-paramatic equivalent of 't' tests, was used because the data was found to deviate from the normal distribution. Where statistical analysis of interaction and year effects were not significantly different, results of the 1983 and 1984 studies were also combined for comparison. Fisher's Exact Test was used for 1984 first-service conception rate data, and a linear model was used for the combined data for 1983 and 1984. We used a linear model for categorical responses in comparing the effect of blood progesterone concentrations at the time of treatment on the conception rate.

RESULTS

The average interval from calving to first observed oestrus was 57 d for dinoprost-injected cows and 70 d for the control group. The difference is significant at the level P = 0.017 (Table 1). The average calving-to-conception interval was 96 d for dinoprost-injected cows and 103 d for the control group. This 7-day advantage does not achieve statistical significance, but it is consistent with the 6-day advantage recorded in the 1983 study data.

Table 1. Calving to first oestrus interval, calving to first service interval, calving to conception interval and first service conception rate for dinoprost-treated and untreated control group cows, 1984

	Dinoprost injected	Control
Average calving to first oestrus interval (days ± SD)	57 ± 31.2[a]	[illegible] ± 33.5
Average calving to first service interval (days ± SD)	75 ± 22.9	78 ± 28.2
Average calving to conception interval (days ± SD)	96 ± 34.7	103 ± 37.3
First service conception rate (%)	56%	47%

[a] Difference significant at level P = 0.017.

The first-service conception rate for 74 dinoprost-injected cows was 56%; for 74 untreated control cows it was 47% (Table 1). This difference is not statistically significant. However, when the first-service conception rate data for 1983 and 1984 are combined, the conception rate is 62% for 138 dinoprost-injected cows and 45% for 138 control cows. This difference is highly significant at the P = 0.004 level (Table 2).

Table 2. Effect of dinoprost treatment on first-service conception. Combined results 1983 and 1984.

Year	Dinoprost injected: Number of cows in study	Dinoprost injected: Pregnant to first service	Control: Number of cows in study	Control: Pregnant to first service
1983	64	44 (68%)[a]	64	28 (43%)
1984	74	42 (56%)	74	35 (47%)
Total	138	86 (62%)[b]	138	63 (45%)

[a] Difference significant at level $P = 0.007$.
[b] Difference significant at level $P = 0.004$.

A plasma progesterone concentration of less than 0.5 ng/ml at the time of treatment was selected as an arbitrary measure of inactive ovaries in the context of this study. In this range, the first-service conception rate for 81 dinoprost-injected cows was 64% and for 79 control group cows was 44%. The difference is significant at the $P = 0.010$ level. Cows with a blood progesterone concentration of 0.5 ng/ml or greater at the time of treatment were considered as a group. The first-service conception rate of 48 dinoprost-injected cows was 56%, and it was 55% for 47 control cows. This difference is not statistically significant (Table 3).

Table 3. Effect of blood progesterone concentration at the time of treatment on first-service conception. Combined results 1983 and 1984.

		Dinoprost injected			Control		
Progesterone (ng/ml)	Study year	Number of cows	Pregnant	(%)	Number of cows	Pregnant	(%)
Less than 0.5 ng/ml	1983	30	21	(70)	38	17	(44)
	1984	51	31	(60)	41	18	(43)
	[a]Total	81	52	(64)[a]	79	35	(44)
0.5 ng/ml and more	1983	26	16	(61)	18	9	(50)
	1984	22	11	(50)	29	17	(58)
	Total	48	27	(56)	47	26	(55)
Not blood sampled	1983	8	7		8	2	
	1984	1	0		4	0	

[a] Difference significant at level P = 0.010.

Sixteen treated cows were seen in oestrus by 7 d after blood sampling and dinoprost injection, while only 5 of the control group cows were in oestrus by 7 d after blood sampling. Comparable numbers of both groups had basal progesterone concentrations at the time of treatment (Table 4).

Table 4. Days from dinoprost injection/blood sampling, to oestrus, and progesterone concentration in all cows exhibiting behavioural oestrus in less than 8 d

Dinoprost injected (n = 16)				Control (n = 5)	
Days	Progesterone ng/ml	Days	Progesterone ng/ml	Days	Progesterone ng/ml
1	0.0	4	0.0	2	0.6
3	0.0	4	0.3	4	0.0
3	0.0	4	1.3	5	1.0
3	0.1	4	4.3	5	1.1
3	0.9	5	0.8	6	1.6
3	0.9	5	2.3		
3	1.1	7	0.0		
3	2.1	7	0.0		

DISCUSSION

The ability to deduce blood concentrations of prostaglandin $F_{2\alpha}$ from assay of its less transient metabolite 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$ (PGFM) has produced new information. It has been shown that high concentrations which arise early in the process of parturition do not quickly subside but are sustained for long periods of 1 to 4 wk (3, 8). Moreover, the longer that high concentrations of PGFM persist, the shorter is the time needed for uterine involution to be completed in cows with no detectable uterine or ovarian abnormality or pathology (3).

The studies cited here as well as others have drawn attention to the considerable variability in the time required for involution of the uterus of a cow after calving before it is again capable of a successful pregnancy. Physical uterine dimensions and characteristics have been used as a working measure of return to function, but there is also a hormonal dimension involved in restarting ovarian activity and regular fertile oestrus cycles. Longer periods of high concentrations of PGFM have been associated with both a faster rate of uterine involution and a stimulatory effect on follicular and luteal components of the ovary (9). The occurrence of the first ovulation followed by a normal luteal phase length after calving in 29 cows was positively correlated with the time needed for completion of uterine involution (8).

In addition to a significant shortening of the interval to first oestrus in treated cows in our study, more treated cows (n = 16) than control cows (n = 5) were in oestrus within 7 d of treatment/blood sampling (Table 4). If, as appears possible, oestrus was induced by dinoprost, then the basal concentrations of progesterone in many cows at the time of injection suggest that a mechanism other than luteolysis is involved.

Although involution of the uterus is an integral part of the process of return to ovarian cyclicity, subjective manual assessment may not be necessary to measure its progress in the context of these studies. It was notable in the present study that improved conception occurred mostly in dinoprost-treated cows with a blood progesterone concentration of less than 0.5 ng/ml at the time of treatment. Progesterone assay is now readily available and as an alternative to assessment of uterine involution by rectal palpation, it might be used to identify cows with a low progesterone concentration at an early stage after calving.

In deciding on a suitable time to administer exogenous prostaglandin, it was thought that a stimulus to natural synthesis would be most likely to be effective when endogenous concentrations were declining. The frequency of visits was planned to be compatible with routine herd fertility visits at two-week intervals, hence the choice of the two-week period 14 to 28 d after calving. About this time, PGFM concentrations can be expected to decline and progesterone concentrations start to rise, so basal concentrations of progesterone are compatible with the required hormone profile for effective treatment. The first-service conception rates in the study tend to support this concept that cows with low progesterone concentrations had a poor conception rate of 44%, which was improved to 64% by dinoprost treatment. Cows with higher progesterone concentrations, indicative of ovarian activity, had an acceptable conception rate of about 55% with or without dinoprost treatment; this is a further indication that luteolysis may not be important in this context.

It seems that ovarian cyclicity was achieved sooner in treated cows with low progesterone concentrations at the time of treatment, thus allowing them to reach optimum fertility sooner. The prevailing level of fertility in the herd then applied equally to treated and untreated cows. Increased fertility per se in treated cows would infer a hormone release function which has not as yet been attributed to dinoprost.

Trends similar to those reported here are recorded in a multifactorial trial that included a comparison between control cows and cows injected with a single dose of prostaglandin on Day 24 after calving. Prostaglandin treatment in that study reduced the interval from calving to first observed oestrus and from calving to conception. In the entire study, plasma progesterone concentrations on Day 24 after calving were less than 1 ng/ml in about two-thirds of the cows (10).

The results of these studies suggest that resumption of ovarian cyclicity after calving may be advanced by the administration of dinoprost tromethamine soon after calving. It may be possible to identify the cows most likely to respond to such treatment by milk progesterone assay about 2 to 4 wk after calving.

Such results occurred in about two-thirds of the cows in the two studies. The technique might be used to improve conception rate to first service earlier in a herd's breeding period and would be suitable to employ in routine herd fertility care systems.

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