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SOME CONSIDERATIONS ON THE VALUE OF HORMONAL ASSAYS AND A KNOWLEDGE OF HORMONAL PROFILES TO PRODUCTION OF RED MEAT ANIMALS*

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Despite the immense effort expended on research in animal productive physiology, relatively little appears to have been achieved in terms of practical application. The major advance has been the widespread application of artificial insemination of dairy cattle, commonly associated with the use of frozen semen. *T.J. Robinson, 1974.*

Except for the rapidly increasing use of embryo transfer in cattle, and the widespread availability of prostaglandin $F_2\alpha$ ($PGF_2\alpha$) and gonadotropin releasing hormone (GnRH) for use in that species, one might conclude that the situation described by Robinson (1974) has not changed very much. If anything, the glowing example he cited, artificial insemination (AI) in dairy cattle, is in a state of stagnation or decline. In the U.S.A., there have been several recent reports of lower and lower average conception rates and declining use of AI. Reasons for this decline will be examined later in this paper, in relation to problems in management detected by assays for progesterone in milk.

Have basic and applied research efforts, using the highly sensitive hormonal assays, yielded answers to some of the questions regarding factors which limit both reproductive performance and the application of our knowledge of reproductive physiology to effective management of animals? We believe the answer is yes; studies utilizing hormonal assays or determining hormonal profiles have made important contributions to animal agriculture. Like other tools, hormonal assays have not provided all the answers. Assays may not be effective tools or even the methods of choice for solving many of the problems that remain. The contributions made using these assays have had both positive and negative impacts. Assays have been used both in identification of problems and in finding solutions to them; some of these solutions have been realized and some are still to be realized, some have been applied in practice and others have been, so far, ignored. To assess all of these contributions in detail would require an entire book; the literature that must be evaluated and assimilated to sort out real contributions from redundancies and correct uses and interpretations of assays and of hormonal data from the improper and incorrect is monumental in scope. Furthermore, the publication of such literature is occurring at an ever increasing rate. Probably no one scientist or group of scientists is capable of such a sorting process. So this paper is, by necessity, only illustrative in nature and is not a comprehensive review.

In evaluating the contributions to animal agriculture achieved by use of hormonal assays, it is appropriate to consider those contributions which have occurred through use of assays as tools in (1) basic research, (2) applied research and product development and (3) regulatory activities. Each of these classifications will receive some consideration among the examples we have chosen to illustrate the values of hormonal assays, but none will receive full coverage. Rather we will restrict most of our examples to areas in which we have some experience in the use of validated radioimmunoassays in basic and applied research in reproductive physiology of the female.

Valid uses of assays

Hormonal assays are tools and as such must be utilized properly on samples from well designed experiments. Both the assay and the experiment must be evaluated by appropriate statistical analyses if results are to be meaningful. Midgley, Niswender & Rebar (1969) have defined the appropriate methods for assessing linearity, parallelism with standard, precision, sensitivity (limits of detection), specificity, accuracy, and repeatability of an assay. Parallelism with standard is too often assessed only by recovery of added hormone in a single volume of sample. Parallelism should be assessed with different volumes of sample (Pexton, Ford, Wilson, Butcher & Inskeep, 1975a; Lewis, Jenkins, Fogwell & Inskeep, 1978). The need for repeatability over volume is comparable to the need for absence of a dose by treatment interaction in a valid bioassay (Bliss, 1952).

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In regard to specificity, a valid radioimmunoassay should measure a single (usually biologically-active) form of the hormone in question. Failure to establish this fact is still a limiting factor in interpretation of many assays or values that are reported. In some cases, a treatment or change in the physiological state of the animal may modify the hormonal molecule being secreted (from the same or a different source). For steroids, a change in ratio of progesterone to 20α– or 20β–OH–pregn–4–en–3–one, a less biologically-active steroid, is a well known example in rabbits and cattle, respectively. Since specific assays allow each steroid to be quantified separately, this is not normally a problem. For peptide hormones, areas of disagreement in immunological and biological potency are less well understood. But the most widely utilized antiserum to ovine LH detects some LH in the serum of hypophysectomized monkeys, whereas the rat interstitial cell testosterone secretion assay does not (Neill, Dailey, Tsou & Reichert, 1977). When this antiserum was used for assay of LH in serum of intact monkeys, it overestimated LH during the follicular phase and underestimated LH during the preovulatory surge when compared to the bioassay. Abnormal cycles had higher LH in both follicular and luteal phases by radioimmunoassay than by the bioassay (Dailey & Neill, 1981). So both the user and the reader must be aware of the fact that radioimmunoassays measure immunological activity and that biological activity may differ.

Experiments must be designed rigorously to allow clear tests of hypotheses and must be based upon current knowledge of the patterns of secretion of the hormone in question. For example, nothing can be learned about the roles of gonadotropins in the puberal process by taking monthly samples of jugular blood; the half-lives of these hormones are measured in minutes and they are secreted in pulses as frequent as once per hour; clearly sampling on a monthly basis will miss changes in amplitude or frequency of release of hormones. In addition, infrequent sampling may inflate variability and make detection of real differences very difficult. Similarly, nothing is learned by measuring the steroids in a single pool of follicular fluid from 101 follicles which are 2 mm in diameter and comparing the value obtained with the value for a pool of 9 follicles which are 4 mm in diameter. No matter how good the assay is, there must be opportunity for expression of biological variation in the material being assayed; every treatment or classification must be replicated; in the example given above, there are no degrees of freedom for the appropriate error term to test for differences. Yet papers with these kinds of errors in planning both experiments and assays continue to appear in the literature, even in highly respected, refereed journals. In short, assays are only as good as the people who use them; these people must have hypotheses to test and they must design experiments to test those hypotheses, whether basic or applied.

Analyses of assay data must be stringent and appropriate. Gill & Hafs (1971) pointed out that repeated measurements within an animal are related one to another and several authors have developed methods to apply least squares techniques to comparisons of profiles of hormones and other substances over time (Chenault, Thatcher, Kalra, Abrams & Wilcox, 1975; Webb, Head & Wilcox, 1969). We have utilized the approach of comparing hormonal profiles in several studies, using time as a continuous, independent variable in the subplot of a split-plot design (Fogwell, Weems, Lewis, Butcher & Inskeep, 1978; Lishman, Allison, Fogwell, Butcher & Inskeep, 1979; Lewis, Lishman, Butcher, Dailey & Inskeep, 1981). In other studies it has been useful to determine the natural logarithm of the variance of a hormone within a time period, as a means to assess whether there are changes in amplitudes of frequent oscillations, for example with luteinizing hormone (LH) (Keisler, Dailey & Inskeep, 1980; Keisler & Dailey, 1981) or prostaglandins (Silvia, Ottobre & Inskeep, 1981). Others have developed a variety of methods for identifying secretory spikes in hormonal patterns (e.g. Christian, Everson & Davies, 1978) or studying rhythmicity of secretory episodes and differentiating real endocrine changes from noise in the assay (Yates, 1981).

The value of hormonal assays is enhanced by the fact that concentrations of hormones in plasma and serum reflect secretion rates. This was shown clearly for follicle stimulating hormone (FSH), LH and prolactin by Akbar, Nett & Niswender (1974) in sheep. They determined that metabolic clearance rates for these hormones did not differ with stage of the oestrous cycle. Absence of variation in metabolic clearance rate has been demonstrated for progesterone in sheep by Bolt & Rollins (1976). In the case of prostaglandins, rapid degradation by the lungs may make peripheral measurements of the native hormone inappropriate. Metabolites of prostaglandins do reflect secretion patterns, but some cautions must be raised concerning the use of peripheral concentrations of metabolites (Lewis, Wilson, Wilks, Pexton, Fogwell, Ford, Butcher, Thayne & Inskeep, 1977; Inskeep & Murdoch, 1980). In some species, sampling is further complicated by the fact that the blood platelets release prostaglandins. It is fortunate that this is not the case for $PGF_2\alpha$ in the sheep, the agricultural animal which has received the most attention with respect to prostaglandins (Ellinwood, Nett & Niswender, 1979). Even concentrations of hormones in venous plasma collected locally may be affected by changes in blood flow through the organ in question, associated with changes in hormonal secretion rate (Pexton, Weems & Inskeep, 1975b).

Development of prostaglandin $F_2\alpha$ and its analogues for use to synchronize oestrus in cattle

At present AI is used in less than 5% of beef cattle in the U.S.A. Limiting factors include a high cost of labour for detection of oestrus and for insemination. Hafs (1979) has predicted the use of prostaglandins as a means for synchronizing oestrus could bring about the use of AI in

20 to 40% of the beef cattle population of the U.S.A. by 1990. This could provide a means for rapid genetic improvement. The average increase in calf weaning weights achieved in beef herds using performance-tested bulls, some AI at synchronized oestrus and some cross-breeding to exotic breeds, was 3,3 kilograms per year of participation in West Virginia University's Allegheny Highlands Project (Baker, Lewis, Colyer, Woodson, Inskeep & Maxwell, 1981). If this kind of annual increase in weaning weights were to occur for 5 years in 40% of the beef herds of any country, it should bring about some combination of the following: (1) a decrease in brood cows required, (2) a decrease in feedlot costs or feed requirements, (3) a decrease in beef prices to the consumer, or (4) an increase in beef available to the consumer. In the Republic of South Africa, for example, it could mean an annual increase of 7,968,000 kilograms of calf weaned from the present cow herd of 6,000,000. Selection of replacement heifers from among the daughters of dams mated by AI to superior sires, and breeding them by AI to superior sires in turn, could bring about even greater increases in kilograms of calf weaned in the next generation.

Similar considerations apply to dairy cattle, since most heifers still are not bred artificially. Hafs (1979) predicted an increase of 50 to 100% in numbers of dairy cattle inseminated artificially in the U.S. by 1990 if prostaglandins were available, in contrast to a decline if they were not available. Of the 1800 kg average increase in milk production per cow in the U.S.A. since 1958, approximately 450 of it can be accounted for by permanent genetic improvement achieved through selection of, and artificial insemination to, superior sires (Foote, 1982).

Thus, the development of $PGF_2\alpha$ and its analogues as agents for synchronizing oestrus is significant to the future of animal agriculture. But how have hormonal assays been used by contributors to this research and development effort? Probably first and foremost is the use of assays in developing the data to show that $PGF_2\alpha$ is safe for the animal and for the human population consuming meat and milk from treated animals. This effort included measurements of progesterone, oestradiol, glucocorticoids, LH, prolactin and growth hormone in plasma or serum of animals treated with $PGF_2\alpha$ (Hafs, Louis, Noden & Oxender, 1974; Lauderdale, 1979). All of these measurements were made by sensitive assays. Importantly, patterns of declines in progesterone and increases in oestrogen were similar to those seen at the end of the normal cycle (Louis, Hafs & Morrow, 1974) or after removal of the corpus luteum (Fogwell *et al.*, 1978). The latter workers showed that the rise in oestrogen was a function of the decline in progesterone and not a direct effect of $PGF_2\alpha$. The surge of LH was closely synchronized with the onset of oestrus (Welch, Hackett, Cunningham, Heishman, Ford, Nadaraja, Hansel & Inskeep, 1975), which might account in part for the fact that $PGF_2\alpha$ does not depress conception rate. Progestogens, which have been used sporadically for synchronization of oestrus for many years (Hansel & Beal, 1979), often do depress conception rate of first oestrus. Progestogens have been shown to alter both sperm transport (Hawk & Conley, 1971) and the degree of synchrony of the surge of LH with the onset of oestrus (Lewis, Bolt & Inskeep, 1974) in ewes.

Establishing the safety for the human population of the use of $PGF_2\alpha$ in animals required evaluation of content of the hormone and its metabolites in muscle, visceral organs and milk of treated animals as well as their rates of disappearance from plasma (Neff, 1979). These measurements could be made efficiently and in the numbers required only with radioimmunoassays. The important findings were (a) that $PGF_2\alpha$ and its major metabolites were transient substances in treated animals, returning to basal levels in plasma by 24 hours after intramuscular injection, (b) that less than 1% of the dose of $PGF_2\alpha$ went into milk during the 97 hours immediately posttreatment, and (c) that amounts in muscle at the injection site were equal to amounts in non-treated areas by 48 to 72 hours after injection (Neff, 1979). Of course, hormonal measurements were not in themselves sufficient to establish safety. A complete series of toxicological evaluations with higher doses was required (Goyings, 1979) including neurological, histopathological, physiological and haematological observations.

Assays have been utilized in determining the efficacy of $PGF_2\alpha$ and in devising means to improve efficacy. In early studies in cattle (Inskeep, 1973) it was observed that progesterone did not always decline to basal concentrations after administration of $PGF_2\alpha$ and that even if progesterone did decline, some animals were delayed in returning to oestrus. These animals often had small follicles and concentrations of oestrogen in plasma were quite variable (Fogwell, *et al.*, 1978). It was suspected that some animals were not producing enough oestrogen to initiate behavioral oestrus and/or release of LH. Because small doses of oestradiol benzoate had been effective in increasing the precision of synchronization of oestrus after progestogens (Ulberg & Lindley, 1960; Hansel, Schechter, Malven, Simmons, Black, Hackett & Saatman, 1975), workers at West Virginia University began to investigate whether synchronization with $PGF_2\alpha$ could be improved by injection of 400 µg oestradiol benzoate 40 to 48 hours after $PGF_2\alpha$ (Welch *et al.*, 1975). In a series of studies utilizing over 2000 animals (Inskeep, Dailey, James, Peters, Lewis & Welch, 1980), it was found that this addition of oestrogen to the treatment regimen increased the proportion of animals in oestrus by 84 hours after $PGF_2\alpha$ by 20 to 23 percentage points in lactating beef cows, 15 to 21 percentage points in beef heifers, and 7 percentage points in dairy heifers. Oestradiol benzoate had no effect in dry beef cows, which had a high response (over 90%) to $PGF_2\alpha$ alone. Use of the oestrogen im-

proved pregnancy rate to timed breeding (at 80 hours after $PGF_2\alpha$) by 11 percentage points in beef herds and by 15 percentage points in dairy heifers.

Uterine secretion of the prostaglandins

Workers in our laboratory have utilized radioimmunoassays to characterize the patterns of uterine secretion of $PGF_2\alpha$ throughout the oestrous cycle and early pregnancy in sheep (Inskeep & Murdoch, 1980). Joseph Ottobre has determined recently that the first peaks of $PGF_2\alpha$ on days 11 to 13 of the cycle are timed by previous exposure to progesterone for 8 days. He has obtained evidence that the final peaks of $PGF_2\alpha$ on days 15 and 16 are a result of, or timed by, the decline in progesterone in response to the earlier rises in $PGF_2\alpha$ and have ceased by the onset of oestrus. Neither of these series of peaks requires any rise in oestradiol-17β (Ottobre & Inskeep, 1981), which fits well with the observations by Fairclough, Smith & Peterson (1976) that antiplasma to oestradiol did not delay luteal regression in ewes. Thus the known effects of oestradiol on secretion of $PGF_2\alpha$ (reviewed by Inskeep & Murdoch, 1980) would appear to be pharmacological with respect to the oestrous cycle in the ewe. This may not be true for the cow (Cowley, Ireland, Wortman & Fogwell, 1979) since destruction of the follicles by x-irradiation caused a definite delay of luteal regression in that species, compared to only a slight delay in the ewe (Karsch, Noveroske, Roche, Norton & Nalbandov, 1971). On the other hand, Gengenbach, Hixon & Hansel (1977) observed that oestradiol and $PGF_2\alpha$ were more effective in causing luteolysis in hysterectomized ewes than either hormone alone. So even in the cow, the need for oestrogen may be for an effect independent of release of $PGF_2\alpha$.

This degree of understanding of patterns of $PGF_2\alpha$ may not, in itself, have any practical implications for animal agriculture, but an important sequel of the story does have such implications. Estimates of the proportions of embryonic death that might be due to failure of luteal maintenance vary (Hawk, 1979) but certainly luteal insufficiency is a significant problem. Early pregnancy does not suppress secretion of $PGF_2\alpha$ in ewes (Lewis *et al.*, 1977), yet the corpora lutea are maintained. High concentrations of $PGF_2\alpha$ were seen in uterine venous plasma on days 15, 16 and 18 of pregnancy even though progesterone had not declined in response to the high $PGF_2\alpha$. This might mean that $PGF_2\alpha$ has a role in establishment of pregnancy which is more important than its luteolytic effect. Assays of ovine uterine venous plasma, endometrium and uterine fluid (Ellinwood *et al.*, 1979; Silvia *et al.*, 1981) and of both bovine and ovine embryos (Lewis, Thatcher, Bazer & Curl, 1982; Marcus, 1981) have provided evidence that another prostaglandin, PGE_2, might be involved in preventing luteolysis at this critical time in early pregnancy. PGE_2 in uterine venous plasma increased by day 13 in pregnant ewes (Silvia *et al.*, 1981), while $PGF_2\alpha$ and 6-keto-PGFα did not vary between pregnant and non-pregnant ewes. Thus PGE_2 and perhaps other PG's of the E series (Magness, Huie, Hoyer, Hueckstead, Reynolds, Seperich, Whysong & Weems, 1981; Kimball & Lauderdale, 1975) might become useful in reducing pregnancy wastage due to luteal insufficiency. The exact impact that such a development would have on animal agriculture remains to be established.

The Development of GnRH for Treatment of Cystic Ovaries in Dairy Cattle

Higher producing and more frequently milked dairy cattle often develop cystic ovaries. In one study, cows with follicular cysts produced more milk in that lactation than in the previous one (Johnson, Legates & Ulberg, 1966), but the delay in rebreeding that usually results with cystic ovaries produces an economic loss to the dairyman. The incidence of cystic ovaries varies widely from herd to herd and with time within herd. In one recent study (Whitmore, Hurtgen, Mather & Seguin, 1979) it was 9,4% in 2393 cows in 25 herds in Minnesota, with 48% of those cases occurring after 60 days postpartum.

Treatments for cystic ovaries have progressed from manual rupture of the cysts to injection of gonadotropins, usually human chorionic gonadotropin (hCG), to the current treatment of choice, injection of GnRH, and finally to injection of GnRH, followed 9 days later by injection of $PGF_2\alpha$ (Kesler, Garverick, Caudle, Bierschwal, Elmore & Youngquist, 1978). Recovery from cystic ovaries has been reported to average 29% spontaneously (Whitmore *et al.*, 1974) or 21% after saline (Bierschwal, Garverick, Martin, Youngquist, Cantley & Brown, 1975) and 74 to 76% after a single treatment with GnRH (Bierschwal *et al.*, 1979). Repeated treatments with GnRH were effective (78 and 66% of previously unresponsive cows recovered after second and third treatments, respectively), perhaps reflecting the fact that GnRH is less antigenic than hCG (Whitmore *et al.*, 1979).

Hormonal assays have been used extensively in the development and testing of synthetic GnRH as a treatment for cystic ovaries. Assays for LH (which of course were required to establish the existence of the endogenous luteinizing hormone releasing hormone) have been used to describe the pattern of release of LH in response to GnRH (Kesler, *et al.*, 1978). Assays for progesterone have been used to determine whether the cysts or other ovarian follicles were luteinized (Cantley, Garverick, Bierschwal, Martin & Youngquist, 1975; Kesler *et al.*, 1978) in response to treatment. Whitmore *et al.*, (1979) have pointed out that rectal palpaltion is not sensitive enough to be sure whether cysts have luteinized in response to GnRH. A third assay, that for testosterone, revealed that concentrations of that hormone in plasma were not different in cows with ovarian cysts from the range of values observed during the oestrous cycle (Kesler, Garverick, Caudle, Bierschwal, Elmore & Youngquist, 1979). Assays for the releasing hormone itself have received limited use to date but will be necessary for a complete understanding of the hypothalamic control of release of LH.

Progesterone in milk

One of the basic findings which has led to a more accurate assessment of the reproductive status of dairy cows during lactation was the discovery of progesterone in milk (Williams, 1962). Little use was made of the knowledge that milk contained progesterone until the development of radioimmunoassay techniques. Application can be expected to be expanded by the use of enzyme-immunoassay; the use of this method for progesterone has just been described by Arnstadt & Cleere (1981). Details of the procedure for radioimmunoassay and some of the applications of the technique have been reviewed recently (Foote, 1979; Karg, Claus, Gunzler, Rattenberger, Hahn & Hocke, 1980; Kalis & Van de Wiel, 1980; Lamming, 1980). Examples from cows in the West Virginia University dairy herd (E.M. Meisterling & R.A. Dailey, unpublished) of the diagnostic uses of milk progesterone, along with data on oestrus, breeding and palpable ovarian structures, are presented in Figure 1. The period of increased progesterone in milk corresponds to the duration of increased progesterone in the plasma (not shown) in each case, but progesterone occurs in milk at a higher concentration. The upper panel contains a profile seen in many cows prior to achieving normal cycles of behavioral oestrus and ovulation. An abbreviated secretion of progesterone of low magnitude

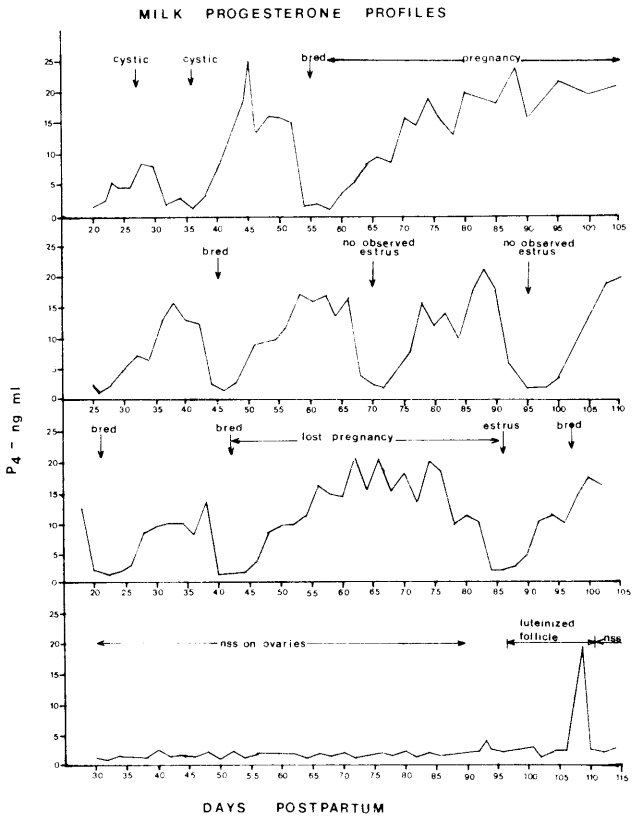

Figure 1. Profiles of milk progesterone (P4) in four individual dairy cows in the West Virginia University herd. No significant structures on the ovary is denoted nss.

precedes a normal luteal pattern. Oestrus usually is not observed until after the second interval of secretion of progesterone and corroborates the well known phenomenon of silent ovulation. The short luteal phase, which is similar to the short luteal phase preceding the onset of puberty, was not accompanied by a readily palpable luteal structure in the ovaries. In several studies, an association has been detected between concentrations of progesterone in plasma and subsequent fertility (Folman, Rosenbert, Herz & Davidson, 1973; Corah, Quealy, Dunn & Kaltenbach, 1974; Holness, Ellison, Sprowson & Carvalho, 1977). One group has reported that the level of progesterone in plasma in the luteal phase prior to breeding must equal 3 ng/ml to assure normal fertility in dairy cows (Carstairs, Morrow & Emery, 1980).

Some common bases for problem breeding in dairy cows are illustrated in the second and third panels. First is the failure to detect oestrus in cows which previously and subsequently show normal luteal function (panel 2). Zemjanis, Fahning and Schultz (1969) have estimated that 90% of the cows called anoestrus by dairymen actually are undergoing cyclic ovarian activity. Several researchers (e.g. Hurnik, King & Robertson, 1975) have demonstrated that the principal defect lies in the failure of the dairyman to observe cows for behavioral oestrus. D.A. Coleman and R.A. Dailey (unpublished) found that only 50% of dairy herdsmen surveyed in West Virginia had a planned program for detection of oestrus. A second problem is the breeding of cows during the luteal phase rather than at a time of low progesterone (panel 3, third breeding). This managerial mistake has been reported to occur in as many as 20% of the cows inseminated during a large trial in New York (Foote, 1979). In spite of the fact that there are environmental effects which can suppress oestrous activity (Thatcher, Roman-Ponce & Buffington, 1978), these managerial problems have strong implications for the extension dairy specialist. Emphasis in the educational program must be placed upon the need for and the value of very simple and fundamental procedures, proper animal identification and proper programs for detection of oestrus, if management is to be effective in obtaining and maintaining a high level of reproductive efficiency.

True "cow" problems, as opposed to human mistakes, are illustrated in the third and fourth panels of Figure 1. One (panel 3) is the failure to carry a calf. A presumption of embryonic loss, as evidenced by an extended period from breeding to next oestrus, can be supported by frequent assays of progesterone in milk; continued high values preclude failure to detect oestrus as the problem and verify an extended luteal phase. Hawk (1979) calculated that early embryonic losses accounted for 15% of infertility in dairy cattle. The cow depicted in panel 4 is truly anovulatory. These cows do not represent a large portion of most herds (1 to 7% in the studies summarized by Lamming, 1980), but still they are of economic consideration. The basis for this condition is unknown; however, during weekly rectal palpations, the ovaries of

these anoestrous cows had little follicular activity.

Another problem in which a knowledge of progesterone in milk is helpful is the cow with cystic ovaries. Often one cannot readily discern by palpation whether or not a structure embedded within the ovary is relatively thin-walled or is a luteinized follicle. Measurement of progesterone in the milk (panel 1) allows the veterinarian to prescribe a treatment for the follicular cyst (GnRH or hCG) or to recognize the presence of the luteinized structure. Thus, progesterone in milk can be utilized to distinguish cows with physiological problems from problems associated with management. Research into the causes of embryonic loss as well as the search for effective treatments for cystic and anoestrous cows can be enhanced by the use of assays for progesterone in milk.

One of the more rapidly adopted and utilized aspects of progesterone in milk – pregnancy diagnosis – is illustrated in panels 1, 2 and 3 of Figure 1. A low concentration of progesterone in milk at the time of breeding followed by a high value from a sample taken 22 to 24 days later from a cow not observed in heat during the interim period allows a reliable positive test of pregnancy. The availability of the assay for progesterone in milk has offered a relatively routine procedure for earlier diagnosis of pregnancy than can be obtained by rectal palpation. Theoretically, the open cow should be detected 14 to 25 days earlier than by palpation if the herd veterinarian visits every two weeks or 28 to 40 days earlier if he visits monthly.

Earlier estimates of nonpregnancy should result in substantial savings. In beef cattle, it has been estimated by Inskeep & Peters (1982) that full application of pregnancy diagnosis by rectal palpation would save wintering costs totalling as much as $1,139,000,000 annually in the U.S.A. alone. In the Allegheny Highlands Project, an attempt by West Virginia University to encourage adoption of technology including reproductive management in production of beef cattle and sheep, 75% of the potential reduction in nonpregnant cows wintered was achieved on 60 farms with whose owners the project staff had worked closely for 5 to 8 years. Calving percentages for cows wintered on these farms averaged 94,5%. One of the recommended approaches in addition to pregnancy testing was a restricted breeding season. Breeding the dairy herd and replacement heifers in a single 60-day period has been effective in providing both high conception rates and low calf mortality at the Henderson Research Station in Zimbabwe (Geoffrey Sprowson, personal communication).

Pregnancy diagnosis in the pig

Analysis of progesterone in milk as a diagnostic tool for pregnancy was discussed earlier for the cow and has been studied in several other species (Booth, 1980). In contrast, the measurement of oestrone sulfate has greater promise for practical use for early detection of pregnancy in the pig. This steroid becomes detectable in plasma (Robertson & King, 1974) or serum (Hattersley, Drane, Matthews, Wrathall & Saba, 1980) by the 16th to 18th day of gestation and increases to a maximum between days 23 and 30. Methods for rapid analysis of oestrone sulfate have been developed which allow use of this knowledge on a practical basis. Guthrie & Deaver (1979) have described an assay for total oestrone in jugular plasma which detected differences among pregnant and nonpregnant gilts as early as day 18. They proposed that total oestrone would be more accurate as a test for pregnancy than progesterone on days 22 to 25 (day of insemination was designated as day 1). More recently, Saba & Hattersley (1981) have described a rapid method for direct estimation of oestrone sulfate in pig serum. With this method, estimates of concentrations of oestrone sulfate in sera from 87 pregnant sows on days 26–29 averaged 2,7 ± 1,5 ng/ml and were never below 0,5 ng/ml. Of 7 nonpregnant sows, only one had a detectable concentration of oestrone sulfate, that being 0,3 ng/ml. Oestrone sulfate was stable in pig blood at room temperature for 7 days, so shipment and storage of samples would not be a problem. The assay could be completed easily within a single working day. In earlier work by a number of authors, cited by Robertson & King (1974), total oestrone increased in urine of sows during the first month of gestation, so it might be possible to adapt the methods of Guthrie & Deaver (1979) or Saba & Hattersley (1981) to analysis of urine for greater practicality and ease of sampling.

Using hormonal assays to understand reproductive failures

A classic example of the effective use of hormonal assays in conjunction with other measures of physiological function is the series of papers by D. Wentzel and his colleagues which appeared in Agroanimalia in 1974 through 1976 (see especially volume 7, pages 24–47, 1975). These workers set out to study the habitually-aborting Angora doe. First they determined that some animals aborted dead foetuses and some aborted live or fresh foetuses and that abortion was brought on by a reduction in energy nutrition. Next they showed that those does aborting live or fresh foetuses had low concentrations of progesterone in the corpora lutea and that these corpora were histologically regressed at the time of abortion. Application of hormonal assays to plasma collected before and during energy-nutritional stress revealed that the aborting does had higher concentrations of oestrogen in plasma than does carrying normal foetuses to term. When nutrition was reduced at 90 days of gestation, oestradiol-17β increased 4-fold in normal does, 7-fold in does aborting fresh foetuses and 9-fold in does aborting dead foetuses (Wentzel, Morgenthal & Van Niekerk, 1975b). Concentrations of corticosteroids in does expelling fresh foetuses resembled those in normal does, while corticosteroids were high for some time preceding foetal death in does aborting dead foetuses (Wentzel, Morgenthal & Van Niekerk, 1975a). No changes

in plasma thyroxine occurred in response to the nutritional stress (Wentzel & Botha, 1976). Administration of diethylstilbestrol (or foetal administration of androstenedione, which in turn increased plasma oestrogen) caused abortion of live foetuses and administration of cortisone acetate caused abortion of dead foetuses. These findings confirmed the hormonal diagnosis of the 2 types of abortion in response to reductions in dietary energy. Subsequently, Wentzel, Le Roux & Botha (1976) found that blood glucose decreased in response to nutritional stress and concluded that this might be the trigger of the endocrinological changes related to abortion.

Quirke, Hanrahan & Gosling (1981) have examined hormonal patterns around synchronized oestrus in ewe lambs and adult ewes in a search for potential reasons why lambing rate of ewe lambs mated at a single oestrus is lower than in adults. Except for a shorter interval from onset of oestrus to the beginning of the surge of LH in the lambs, characteristics relative to LH were similar. The major difference observed was that concentrations of total oestrogens in plasma in lambs were essentially twice those found in the adult ewes between 12 and 36 hours after removal of cronolone sponges. Since reduced quality of cleaved ova from ewe lambs had been observed in previous work, the authors proposed that the high oestrogen may have created detrimental conditions in the developing follicle or in the reproductive tract during the interval between ovulation and the 8 to 16 cell stage of cleavage. This proposal is consonant with findings of Butcher and his colleagues in rats with delayed ovulation (Butcher & Page, 1981).

The conception rates associated with artificial breeding of beef cattle are often lower than those observed in natural breeding programs. Investigators from the U.S.D.A. Range Livestock Experiment Station at Miles City, Montana, utilized hormonal assays of LH in conjunction with observation of behavioural oestrus and ovarian palpation in an effort to determine endocrine differences between natural and artificial mating stimuli (Randel, Short, Christensen & Bellows, 1973). At the first detection of oestrus, cows were removed from the observation lots (in which they were maintained with oestrogenized cows) and given no further treatment or the following stimuli: AI with no clitoral stimulation; AI with 10 sec of clitoral stimulation, mating 3 times in succession by a intact bull; or maintenance in a separated lot with an epididymectomized bull and being allowed to breed once. Cervical stimulation, either by AI or by natural service decreased the time from the onset of oestrus to the peak of release of LH by approximately 4,5 h. The magnitude of the surge of LH did not differ among groups. Clitoral stimulation decreased the interval from the onset of oestrus to ovulation by 4,3 h. These investigators concluded that ovulation might be accelerated in the cow by the appropriate mating stimuli. These findings were then applied in a study (Randel, Short, Christensen & Bellows, 1975) in which the effect of 10 sec. of clitoral massage on conception rates of artificially inseminated cows was investigated. In this trial 1183 beef females received either 10 sec. of clitoral massage or no clitoral massage following AI. The effect of stimulation upon AI conception rates was positive and significant, increasing these rates by 6,5 percentage points. Thus a hypothesis predicated upon hormonal analyses resulted in a practical management recommendation, to use 10 sec. of clitoral stimulation following AI in beef cattle.

Understanding the attainment of puberty

Puberty in female domestic mammals is marked by the first expression of oestrus with ovulation. By measuring levels of progesterone in plasma on a frequent basis, Gonzalez-Padilla, Wiltbank & Niswender (1975) were able to show that prior to the first oestrus, the beef heifer has one or two (7- to 9-day) periods of secretion of low concentrations of progesterone prior to first oestrus. Sampling every other day, and removing ovaries when progesterone was high, Berardinelli, Dailey, Butcher & Inskeep (1979) found that the source of the abbreviated bouts of progesterone was the ovary, rather than the adrenal glands as has been proposed for the rat (Ramaley & Bunn, 1972). Further, the ovarian structures responsible for the 2 periods of luteal activity could not be palpated rectally and were not visible on the surface of the ovary at laparotomy midway during the shortened period of luteal function. Histological examination of the ovaries revealed a luteal structure embedded within the ovary, averaging less than 6 mm in diameter. Although the mechanism of this action of progesterone has not been clarified, Berardinelli (1979) was able to induce puberty in 70% of peripuberal heifers within 3 days after 7 daily injections of 20 mg of progesterone, while only 24% of control heifers reached puberty within 10 days after seven daily injections of corn oil. Thus, the abbreviated exposure to progesterone may be required for attainment of puberty in the beef heifer.

In ewe lambs, Fitzgerald & Butler (1978) and Ryan & Foster (1979) observed a transient (1 to 4 days) elevation of plasma progesterone prior to puberty. Robinson (1968) reported that a period of progesterone priming was necessary for the full expression of oestrus in ewes. Berardinelli, Dailey, Butcher & Inskeep (1980) monitored progesterone daily in ewe lambs. Laparotomy performed during the transient rise revealed a small luteal structure on the ovary. Venous drainage of that ovary contained elevated levels of progesterone compared to venous effluent from the contralateral ovary. In many instances, an "ovulation" stigma was observed on the luteal structure (D.H. Keisler and R.A. Dailey, unpublished). Removal of the luteal structure or the ovary containing it after a single day of increased progesterone, did not affect type of, or time to, subsequent luteal activity differently than did removal of the contralateral ovary or sham operation (Keisler *et al.*, 1980). The transient rise in progesterone may not have to exceed one day in duration in lambs or it may serve as little more than a signal that the maturational process has

begun. In unpublished studies, these workers have shown that the lifespan of the transitory structure can be increased by hysterectomy. These results imply either enhanced secretion of a luteolytic agent by the uterus or heightened sensitivity of the luteal body to a luteolytic agent. The latter has been shown in prepuberal pigs which were induced to ovulate (Rampacek, Kraeling, Kiser, Barb & Benyshek, 1979; Puglisi, Rampacek, Kraeling & Kiser, 1979).

Ryan & Foster (1979) have hypothesized that the onset of puberty is characterized by an increased frequency of release of LH from the pituitary. By sampling daily, Keisler *et al.*, (1980) have found increases in the amplitude of pulses of LH and no change in the frequency of these pulses during attainment of puberty. Release of LH as episodic bursts occurred on the average every 56 minutes. Apparently, as proposed by Ryan & Foster (1980), the ewe lamb responds more readily to a circhoral delivery of LH to produce a surge of LH and a corpus luteum. Keisler & Dailey (1981) gave LH to prepuberal ewe lambs in various regimens. They found that hourly pulses of either 7,5 or 15 µg LH more often (in 5/6 and 4/6 ewes, respectively) produced subsequent increases in progesterone and that these increases persisted longer and reached higher levels than were obtained with other modes of administration of LH (1/5, 1/6 and 3/6 for pulses of 30 µg/3h and constant infusions of 15 µg/h respectively). However, not one of these treatments was followed consistently by either oestrus or further increases of progesterone, thus the full attainment of puberty had not been set into motion. If progesterone is not required (or is required for only a short time) and circhoral LH patterns are insufficient, then attention must be turned to the roles of other hormones as mediators of the process of puberty. Only when these roles and the genetic and environmental factors controlling them are understood can the best management systems for optimum attainment of puberty be developed and applied effectively.

Studies in anoestrous animals

Hormonal assays have been used to characterize the endocrine status of the cow, sow and ewe during postpartum anoestrus. Wetteman (1980) reviewed this area and emphasized the limited success obtained with treatments designed to induce ovulation or initiate cyclic ovarian activity in anoestrous animals. The duration of anoestrus in cattle varies with breed, age, herd and nutritional status (Inskeep & Lishman, 1979). Nutrition is clearly a dominant factor in determining the postpartum interval to oestrus and ovulation (Dunn & Kaltenbach, 1980). Prepartum energy levels have been shown to be especially important in beef cattle. In high producing dairy cows, high protein diets shorten the interval to oestrus, but delay conception. The endocrine effects of nutrition, lactation, season and their interactions have been the subject of several studies (Wetteman, 1980; Edgerton, 1980; Christenson; 1980). However, it is only recently that hormonal patterns have been investigated in experiments utilizing both frequent sampling and treatments known to alter length of anoestrus. The practical importance of anoestrus is clear. Under the relatively mild environmental conditions of West Virginia, Inskeep & Lishman (1979) found that only 56% of lactating beef cows over 40 days post-partum were exhibiting cyclic oestrous or ovulatory activity at the onset of the breeding season.

In lactating, anoestrous beef cows, patterns of release of LH after GnRH were altered by reduced prepartum nutrition (Lishman *et al.*, 1979), temporary calf removal (Inskeep, Lishman, Butcher & Allison, 1977) or pretreatment with Synchro-Mate B (Smith, Amoss, Harms, Inskeep, Ellersieck & Wiltbank, 1981) and varied with size of the largest follicle (Lishman *et al.*, 1979; Inskeep *et al.*, 1977). On the other hand, subsequent luteal function was not related to the amount or pattern of release of LH in these studies, and was of short duration except when pretreatment with progestogen was provided (Pratt, Berardinelli & Inskeep, 1979; Sheffel, Pratt & Inskeep, 1980). Similarly, corpora lutea formed spontaneously at the first oestrus after early weaning had a short life span and could not support a pregnancy unless oestrus was preceded by treatment with progestogen (Ramirez-Godinez, Kiracofe, McKee, Schalles & Kittok, 1981).

An initial step toward understanding why induced corpora lutea fail has been undertaken by Kesler, Weston, Pimentel, Troxel, Vincent & Hixon (1981). Using an assay for progesterone and comparing induced corpora lutea on days 5 and 7 to corpora lutea taken on days 5 and 7 postoestrus from cows showing regular reproductive cycles, they found that dispersed cells from the induced glands failed to respond to LH. NcNeilly, Hunter, Land & Fraser (1981) have reported the same problem in the short-lived corpora lutea induced by GnRH in anoestrous ewes, even though ability to bind hCG was normal.

The use of radioimmunoassays has been required in the investigation of the mechanisms whereby environmental cues are translated into endocrine messages governing gonadal function. By quantifying LH, oestradiol and progesterone in a variety of experimental paradigms, Karsch and coworkers at the University of Michigan have attempted to elucidate the seasonality of reproduction in the ewe (Karsch, Goodman & Legan, 1980). These investigators have hypothesized that the feedback control of seasonal breeding is predicated upon a change in responsiveness of the system driving tonic LH secretion to the negative feedback action of oestradiol. During seasonal anoestrus the potency of the negative feedback effect of oestradiol is high. At the onset of the breeding season in the fall, the response of LH to the negative feedback of oestradiol diminishes and reproductive competency is restored. The essential component of this feedback scheme is a sustained increase in tonic LH release (Goodman,

Legan, Ryan, Foster & Karsch, 1981). When tonic LH is suppressed anoestrus prevails. Conversely, when the sustained increase of LH occurs, a breeding season follows. Ryan & Foster (1980) have extended this concept to the timing of attainment of puberty in the ewe. These investigators have concluded that a decrease in the response of LH to oestradiol inhibition initiates the sequence of endocrine events associated with first ovulation.

Endocrine differences among breeds or species

Comparison of Bos indicus and Bos taurus. Beef cattle production in subtropical and tropical regions relies heavily upon the incorporation of Zebu *(Bos indicus)* breeds and their crosses (Bazer, 1973; Reynolds, 1973). The positive influence of the Zebu cattle, as exemplified by adaptation to climatic conditions and resistance to internal and external parasites, on tropical beef production properties is well documented (Rhoad, 1955; Koger, 1963a; Koger, 1963b; Warnick, 1963). However, the greatest detrimental characteristic of *Bos indicus* is relatively low reproductive efficiency in comparison to European cattle, *Bos taurus* (Kincaid, 1957; Warnick, 1963; Plasse, 1973). The low reproductive efficiency of *Bos indicus* appears to be due to late maturity, seasonality of breeding, depressed conception rates, long gestations and prolonged postpartum anoestrus. Empirical comparisons of various physiological events between *Bos indicus* and *Bos taurus* have been useful in elucidating reasons for the discrepancy in fertility between these breedtypes. The Zebu cow has a shorter duration of behavioural oestrus (6,7 h; Plasse, Warnick & Koger, 1970) than the British breeds of cattle (13,6 to 21,1 h; Hansel & Trimberger, 1952; Trimberger & Hansel, 1955; Wiltbank, Shumway, Parker & Zimmerman, 1967). Further, Randel (1976) reported that the interval from the onset of oestrus to ovulation was significantly shorter in Brahman cattle (18,9 h) than in either Brahman x Hereford (29,0) or Hereford cattle (28,6 h). Corpora lutea of Brahman cattle were smaller than corpora lutea of British-bred cattle (Plasse, Warnick & Koger, 1968). Irvin, Randel, Haensley & Sorensen (1978) stated that "the Brahman corpus luteum is more difficult to detect and is less distinct due to apparent embedding within the ovary."

On the basis of these observed behavioural and morphological differences one might expect hormonal variations between breedtypes. It appears that not only the magnitude of changes in hormones but also the temporal patterns of timing of their release vary between breedtypes. Randel (1976) and Randel & Moseley (1977) reported lower serum concentrations of LH during the preovulatory surge in Brahman than in British-bred cattle. The phenomenon of release of LH was investigated further in 2 studies utilizing ovariectomized females. They were challenged with oestradiol-17β in one study and GnRH in another. Zebu cattle released less LH upon challenge with oestrogen than either Brahman x Hereford or Hereford heifers (Rhodes, Randel & Harms, 1978). In addition, a greater interval from injection of oestrogen to LH response was observed in *Bos indicus* than in *Bos taurus.* Griffin & Randel (1978) observed that Brahman cows released significantly less LH than Hereford cows in response to a maximal (500 μg) challenge with GnRH. These investigators concluded that hypothalamic/pituitary responsiveness differed between the species.

Differences in ovarian steroid concentrations between breeds are less well defined. The preovulatory surge of oestrogen attained peak concentrations 16 h earlier (in relation to behavioural oestrus) in Brahman than in Hereford cows (Randel, 1980). These results are consistent with those obtained in an earlier study by Rhodes & Randel (1977) in which Brahman cows had a longer interval from injection of exogenous oestrogen to the display of behavioural oestrus than either Brahman x Hereford or Hereford cows. Lower concentrations of progesterone in serum have been reported in Brahman than in Hereford on days 2 through 11 of the oestrous cycle (Randel & Moseley, 1977). Consonant with these findings, Rhodes & Randel (1981) reported that the capability of cultured luteal cells to secrete progesterone in response to a challenge with LH was greater in *Bos taurus* than in *Bos indicus.*

A tentative schedule summarizing the sequence of endocrine events leading to ovulation in 3 breedtypes is depicted in Figure 2. Some important management recommendations have been made based upon these findings. First, if artificial insemination is to be used successfully in Zebu cattle, modifications in the frequency of heat checks are required since the duration of standing oestrus is so short. The current recommendation for Zebu cattle is observation for behavioural oestrus 3 to 4 times daily. Second, the time of breeding in an A.I. program appears to require modification. Rather than using the "AM–PM" rule (Trimberger & Hansel, 1955), breeding at either the onset of oestrus or 6 h following the onset of oestrus appears to be most efficacious.

Injection of oestradiol benzoate after $PGF_2\alpha$, which increased conception rate to timed breeding in beef and dairy cattle in studies at West Virginia (Inskeep, Woloshuk & Dailey, 1981) was not effective in Africander cattle

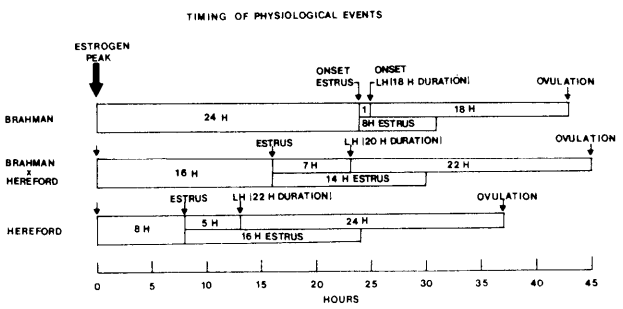

Figure 2. Effects of breed-type on various physiological parameters in cattle.

(Holness & Hurrell, 1977). Thus the timing of insemination or of the injection of oestrogen might need to be different for that breed, but Holness & Hurrell used higher doses of oestradiol benzoate than the 400 μg recommended by the West Virginia group.

In addition to the above recommendations, administration of hCG at breeding has been reported to increase conception rates in Zebu-based herds by as much as 10% (Randel, 1979). As stated previously, Zebu cattle have lower concentrations of serum LH during the preovulatory surge than Hereford cattle (Randel, 1976; Randel & Moseley, 1977). Whether the exogenous hCG augments the preovulatory surge of LH and thus stimulates subsequent luteal growth or merely substitutes for the LH surge at an appropriate time relative to insemination remains to be determined. In either case, exogenous hCG might cause conception in animals that would otherwise fail to conceive and be culled. This might perpetuate "substandard" reproductive characteristics in the offspring so that ovulation, conception and pregnancy might occur only with exogenous augmentation of gonadotropin in an increasing proportion of the population.

Potential relationships between LH and FSH and reproductive performance in sheep. In 1973, Land reported results from a study in which the potential relationships between male and female reproductive activity of sheep were investigated. He reported that testicular weight or testicular diameter was greater in rams from breeds in which the female counterparts had high ovulation rates. This led to the hypothesis that genetic selection for increased fertility or fecundity in the female might be based up on one or more reproductive characteristics of the male. During the same period Bindon (1973) observed that basal prepuberal concentrations of LH in serum were higher in ewes in flocks of Merino sheep that had been selected for fecundity than in unselected flocks. The prepuberal ewes in flocks with high ovulation rates had greater concentrations of LH than those in flocks showing low fecundity. Similarly, Land, Pelletier, Thimonier & Mauléon (1973) reported differences in concentrations of LH between breeds of sheep which had different ovulation rates. The more prolific breeds showed higher concentrations of LH than the less prolific breeds.

Several other studies have been published relating endocrine variations among breed-types with differences in ovulation rates. Romanov ewes, which have an ovulation rate of approximately 3, had a longer interval between the onset of oestrus and the release of LH and a greater concentration of LH in the plasma than either Ile-de-France or Prealpes ewes (Blanc, Courot, Pelletier & Thimonier, 1975). Recently, Cahill, Saumande, Ravault, Blanc, Thimonier, Mariana & Mauléon (1981) presented a composite temporal sequence for the hormonal and behavioural patterns of Romanov (highly prolific) and Ile-de-France (average prolific) ewes, relative to synchronized oestrus and the surge of LH. Differences among these breeds were of the same types observed in cattle (Figure 2). The interval from the fall in progesterone to oestrus was shorter by 13 h in the Romanov, which showed oestrus 18 h before the highest concentrations of oestrogen and 20 h before the surge of LH. In contrast, Ile-de-France ewes reached their highest concentrations of oestrogen about 4 h before onset of oestrus and onset of oestrus preceeded the surge of LH by only 7 h. The peak discharge of oestradiol was higher in Romanov, apparently due to the greater number of follicles in that breed. Both breeds showed a surge of FSH 2 h after the surge of LH. A second surge of FSH occurred 20 h after LH in the Romanov and 28 h after LH in the Ile-de-France. Follicular development was correlated with the magnitude of the second surge of FSH, but was not related to LH. Thus genetic differences in hormonal patterns may be associated with differences in fecundity of breeds.

The concentrations of LH in the plasma of young animals have been proposed to be an indicator of potential breeding value. As an animal matures, concentrations of LH become extremely variable and this could preclude the use of LH as an indicator of reproductive effficiency. This drawback might be overcome by using challenge with GnRH as a means for assessing release of LH and reproductive function. Presumably the induced release of LH by GnRH would overcome short term variations which might mask physiological differences. In fact, Hanrahan, Quirke & Gosling (1981) did find a lower coefficient of variation in concentrations of LH after GnRH (12%) than in basal concentrations (78%) in ewe lambs (Hanrahan, Quirke & Gosling, 1977). However, conflicting results (Bindon, 1975; Carr, Land & Sales, 1975) have been reported using this tool. Recently, Hanrahan *et al.*, (1981 found no breed differences in response to GnRH in male lambs, but did find a tendency for higher response in Galway lambs of both sexes from a strain selected for high litter size compared to a control strain. On the other hand, females of 2 prolific breeds showed a lower response to GnRH than those of three less prolific breeds or strains.

Hormonal patterns in the male

While this discourse has centered on female reproduction, assays of microquantities of steroids and peptides have been equally important in understanding the endocrine regulation of male reproduction. In fact, Desjardins (1981), in his review of endocrine signaling in the male, credits studies using these techniques for offering "unequivocal evidence that reproductive hormones are not secreted in a steady-state fashion in the male, but rather in an irregular, intermittent and pulsatile manner that is dictated, in part, by relevant environmental cues". Desjardins described how assays of hormones have helped to characterize the roles of anti-Müllerian hormone, testosterone and dihydrotestosterone in male sexual differentiation and to detect

endocrine defects which lead to disorders im morphogenesis and function. He reviewed changes in secretion of gonadotropins at puberty and in adult and ageing males, including diurnal patterns in man and changes associated with the breeding season in sheep. Desjardins emphasized the pulsatile nature of gonadotropin secretion and considered the evidence that input of GnRH into the pituitary portal system is a frequency coded phenomenon. He also considered the modulation of secretion and action of these factors by steroids.

One aspect of male reproduction with great practical importance that is under hormonal regulation is libido. It has been generally accepted that testosterone is the main hormonal factor influencing male sexual behaviour. Whether differences in sexual behaviour are due more to variations in concentrations of testosterone or to differences in tissue responsiveness to testosterone has not been settled (Chenoweth, 1981). It is clear that some factors other than circulating concentrations of androgens affect libido. An interesting approach in the investigation of male sexual behaviour has utilized the rough-skinned newt *Taricha granulosa.* Seasonal variations in testosterone and dihydrotestosterone were correlated with exhibition of courtship but plasma concentrations of androgens and sexual behaviour in individual newts within season were not positively correlated (Moore & Muller, 1977). Treatment with androgens did not induce sexual behaviour in inactive males. Addition of either arginine vasopressin or arginine vasotocin was capable of restoring sexual behaviour in inactive males which would not respond to testosterone or dihydrotestosterone alone (Moore & Zoeller, 1979). Miller & Moore (1981) have recently obtained evidence that low doses of LHRH stimulated courtship and an antagonistic analog of LHRH blocked this effect. It would appear that examination of the neuropeptides should be fruitful in improving our understanding of male sexual behaviour in mammals, but this area has received limited efforts to date.

The withdrawal of diethylstilbestrol as a growth stimulant for feedlot cattle in the U.S.

Diethylstilbestrol (DES), a growth-promoting synthetic oestrogen, was used in the U.S.A. for many years as a means to reduce total consumption and cost of feed in growing and fattening cattle. It increased body weight gain by 10 to 12% , thus saving 500 million dollars for American consumers and providing them with 2,2 more kilograms of beef per person on an annual basis (CAST Report 79, 1979). During the period 1973 to 1976, a chemical method for determining concentrations of total DES (DES and metabolites) was used to assay 9426 samples of beef liver. Levels of total DES greater than 0,5 parts per billion were detected in 0,6 % of the total number of samples (CAST Report 66, 1977). This was apparently due to failure of beef producers to observe the prescribed withdrawal period for DES.

The Delaney Clause in the U.S. Code provides that no carcinogen may be used in feed if a residue can be found by an approved method in any edible portion of the animal. Therefore, the Food and Drug Administration took steps to ban DES and eventually succeeded. This occurred despite compelling evidence that the amount of synthetic oestrogen that possibly could be consumed in human diets from ingestation of beef muscle or liver was minute compared to either endogenous oestrogens in the human body or amounts of plant oestrogens consumed by animals and man. While animal scientists can argue that the decision was not a rational one, since the link of DES to cancer was tenuous at best and based mainly upon occurrence of a rare type of cancer in daughters of women who had received up to 300 mg per day of DES (CAST Reports 66, 1977; 82, 1980), it was a legal decision. The need to consider banning DES probably would not have arisen, if producers had maintained the legal use of DES by adhering to the prescribed withdrawal period. It is of interest, also, that in the final order withdrawing approval of the marketing of DES, one reason given by then Commissioner of the Food and Drug Administration, Donald Kennedy, was the lack of an approved assay method. This occurred because they had revoked approval of the mouse uterine method previously approved (U.S. Federal Register, 1979).

This example may not be universally applicable, but it does show where hormonal assays have had a negative role in short-term economics of animal agriculture. One would hope that in the long run, even safer methods, perhaps taking greater advantage of the animals' natural hormonal milieu, would be developed. For example, the synthetic progestogen, melengestrol acetate, has growth promoting activity only in the intact female (CAST Report 66, 1977). Perhaps growth stimulants which are effective in the intact male will be developed. It is likely that hormonal assays will be required for such a development. If other growth stimulants for steers are withdrawn, intact or short scrotum males might rapidly become the animals of choice for the feedlot industry in the U.S.A.

As this is written, the European Economic Community has adopted proposals that limit administration of hormones and thyrostatic substances to meat animals to therapeutic use by veterinarians. Use for promotion of growth is specifically banned. These regulations, numbers 920 and 922 of the Commission of the European Communities, establish tolerances for endogenous hormones as "the level which is normally found in an animal of the same type and condition". Tolerance for all other substances will be the level of detection. Regulations will apply to all imported meats as well as those produced in the member countries.

Concluding remarks

As indicated at the outset, examples presented in the foregoing discussion are only illustrative of the value of

hormonal assays to animal agriculture. One can quickly list as many important areas that have not been covered as have been discussed. No consideration has been given to the value of our knowledge of the endocrinology of parturition (First, 1979) or the potential value of an understanding of the hormonal changes within the preovulatory follicle (Murdoch, Dailey & Inskeep, 1981). The endocrinology of the digestive system has been neglected by the nutritional research community and this area represents fertile ground for future studies. The avian species have not been included here, but hormonal assays are now being used extensively in studies of a variety of avian problems.

In the process of preparing this review, the authors have become convinced: (1) that data obtained with hormonal assays can be of tremendous importance to animal agriculture, (2) that these assays are powerful tools when used properly and (3) that much of the impact of use of these assays is still to come. This latter point will be particularly true with the indigenous animals of developing countries, where ruminants such as goats and water buffaloes, for example, present a number of important problems for study.

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References

AKBAR, A.M., NETT, T.M. & NISWENDER, G.D., 1974. Metabolic clearance and secretion rates of gonadotropin at different stages of the oestrous cycle in ewes. *Endocrinology* 94, 1318.

ANDERSON, J., 1944. The periodicity and duration of oestrus in Zebu and grade cattle. *J. Agric. Sci.* 34, 57.

ARNSTADT, K.I. & CLEERE, W.F., 1981. Enzyme-immunoassay for determination of progesterone in milk from cows. *J. Reprod. Fertil.* 62, 173.

BAKER, B.S., LEWIS, P.E., COLYER, D.K., WOODSON, F.W., INSKEEP, E.K. & MAXWELL, R.H., 1981. *West Virginia University-Allegheny Highlands Project, 1970–1979.* 87 pp.

BAZER, F.W., 1973. A twenty year summary of heterotic effects on reproduction in Florida cattle. In: *Crossbreeding Beef Cattle,* M. Koger, T.J. Cunha and A.C. Warnick (Ed.) Series 2. University of Florida Press, Gainesville, Florida. pp. 128–134.

BERARDINELLI, J.G., 1979. Factors involved in the attainment of puberty in female domestic ruminants. Ph.D. Dissertation. West Virginia University, 137 pp.

BERARDINELLI, J.G., DAILEY, R.A., BUTCHER, R.L. & INSKEEP, E.K., 1979. Source of progesterone prior to puberty in beef heifers. *J. Anim. Sci.* 49, 1276.

BERARDINELLI, J.G., DAILEY, R.A., BUTCHER, R.L. & INSKEEP, E.K., 1980. Source of circulating progesterone in prepubertal ewes. *Biol. Reprod.* 22, 233.

BIERSCHWAL, C.J., GARVERICK, H.A., MARTIN, C.E., YOUNGQUIST, R.S., CANTLEY, T.C. & BROWN, M.D. 1975. Clinical response of dairy cows with ovarian cysts to GnRH. *J. Anim. Sci.* 41, 1660.

BINDON, B.M., 1973. Genetic differences in plasma luteinizing hormone of the prepubertal lamb. *J. Reprod. Fertil.* 32, 347 (Abstr.).

BINDON, B.M., 1975. Ovulation in ewes selected for fecundity: effect of synthetic GnRH injected on the day of oestrus. *J. Reprod. Fertil.* 44: 325.

BLANC, M.R., COUROT, M., PELLETIER, J. & THIMONIER, J., 1975. The conception of plasma LH in ram lambs of different breeds at several ages. *In Reproduction in Domestic Animals.* H.H. Cole and P.T. Cupps (Ed.) Third Ed., Academic Press, New York p.595.

BLISS, C.I., 1952. *The Statistics of Bioassay.* Academic Press, New York.

BOLT, D.J. & ROLLINS, R., 1976. Increased plasma progesterone during infusion of epinephrine, norepinephrine or isoproterenol in luteal phase ewes. *Proc. 9th Ann. Meet. Soc. Study Reprod.* Abst. 43, p. 37.

BOOTH, J.M., 1980. Milk progesterone pregnancy testing in cattle and other species. *Ninth Int. Congr. Anim. Reprod. Artif. Insem.* II, 109.

BUTCHER, R.L. & PAGE, R.D., 1981. Role of the ageing ovary in cessation of reproduction. In: *Dynamics of Ovarian Function,* Ed. by N.B. Schwartz and M. Hunzicher-Dunn. Raven Press, New York. pp. 253–271.

CAHILL, L.P., SAUMANDE, J., RAVAULT, J.P., BLANC, M., THIMONIER, J., MARIANA, J.C. & MAULEON, P., 1981. Hormonal and follicular relationships in ewes of high and low ovulation rates. *J. Reprod. Fertil.* 62, 141.

CANTLEY, T.C., GARVERICK, H.A., BIERSCHWAL, C.J., MARTIN, C.E. & YOUNGQUIST, R.S., 1975. Hormonal responses of dairy cows with ovarian cysts to GnRH. *J. Anim. Sci.* 41, 1666.

CARR, W.R., LAND, R.B. & SALES, D.I., 1975. The effect of gonadotrophin releasing hormone (GnRH) on plasma levels of luteinizing hormone (LH) in lambs of breeds with high and low ovulation rates. *Ann. Biol. Anim. Biochim. Biophys.* 16, 167 (Abstr.).

CARSTAIRS, J.A. MORROW, D.A. & EMERY, R.S., 1980. Postpartum reproductive function of dairy cows as influeced by energy and phosphorus status. *J. Anim. Sci.* 51, 1122.

CHENAULT, J.R., THATCHER, W.W., KALRA, P.S., ABRAMS, R.M. & WILCOX, C.J., 1975. Transitory changes in plasma progestion, oestradiol and luteinizing hormone approaching ovulation in the bovine. *J. Dairy Sci.* 58: 709.

CHENOWETH, P.J., 1981. Libido and mating behaviour in bulls, boars and rams. A review. *Theriogenology* 16, 155.

CHRISTENSON, R.K., 1980. Environmental influences on the postpartum interval. *J. Anim. Sci.* 51 (Suppl. 2), 53.

CHRISTIAN, L.E., EVERSON, D.O. & DAVIS, S.L., 1978. A statistical method for detection of hormone secretory spikes. *J. Anim. Sci.* 46, 699.

CORAH, L.R., QUEALY, A.P., DUNN, T.G. & KALTENBACH, C.C., 1974. Prepartum and postpartum levels of progesterone and oestradiol in beef heifers fed two levels of energy. *J. Anim. Sci.* 39, 380.

COUNCIL FOR AGRICULTURAL SCIENCE AND TECHNOLOGY, 1977. Report No. 66. Hormonally active substances in foods: A safety evaluation. March, 1977, 46 pp.

COUNCIL FOR AGRICULTURAL SCIENCE AND TECHNOLOGY, 1979. Report No. 79. Impact of government regulation on the beef industry. October, 1979, 37 pp.

COUNCIL FOR AGRICULTURAL SCIENCE AND TECHNOLOGY, 1980. Report No. 82. Foods from Animals: quantity, quality and safety. March, 1980, 63 pp.

COWLEY, J.L., IRELAND. J.J., WORTMAN, J.A. & FOGWELL, R.L., 1979. Luteal function in cows following destruction of ovarian follicles at midcycle. *J. Anim. Sci.* 49 (Suppl. 1), 289.

DAILEY, R.A. & NEILL, J.D., 1981. Seasonal variation in reproductive hormones of rhesus monkeys: Anovulatory and short luteal phase menstrual cycles. *Biol. Reprod.* 25, 560.

DESJARDINS, C., 1981. Endocrine signaling and male reproduction. *Biol. Reprod.* 24, 1.

DUNN, T.G. & KALTENBACH, C.C., 1980. Nutrition and the postpartum interval of the ewe, sow and cow. *J. Anim. Sci.* 51 (Suppl. 2), 29–39.

EDGERTON, L.A., 1980. Effect of lactation upon the postpartum interval. *J. Anim. Sci.*, 51 (Suppl. 2) 40.

ELLINWOOD, W.E., NETT, T.M. & NISWENDER, G.D., 1979. Maintenance of the corpus luteum of early pregnancy in the ewe. II. Prostaglandin secretion by the endometrium *in vivo* and *in vitro*. *Biol. Reprod.* 21, 845.

FAIRCLOUGH, R.J., SMITH, J.F. & PETERSON, A.J., 1976. Passive immunization against oestradiol-17 β and its effect on luteolysis, oestrus and ovulation in the ewe. *J. Reprod. Fertil.* 48, 169.

FITZGERALD, J.A. & BUTLER, W.R., 1978. Reproductive hormone pattern from birth to puberty in ewe lambs. *Biol. Reprod.* 18 (Suppl. 1), 55A.

FIRST, N.L., 1979. Mechanisms controlling parturition in farm animals. *Anim. Reprod. BARC Symposium* 3, 215–257.

FOGWELL, R.L., WEEMS, C.W., LEWIS, G.S., BUTCHER, R.L., & INSKEEP, E.K., 1978. Secretion of steroids after induced luteal regression in beef heifers: Effects of $PGF_2\alpha$ and removal of corpora lutea. *J. Anim. Sci.* 46: 1718.

FOLMAN, Y., ROSENBERG, M., HERZ, Z., & DAVIDSON, M., 1973. The relationship between plasma progesterone concentration and conception in post-partum dairy cows maintained on two levels of nutrition. *J. Reprod. Fertil.* 34, 267.

FOOTE, R.H., 1979. Hormones in milk that may reflect reproductive changes. *Anim. Reprod. BARC Symposium* 3, 111.

FOOTE, R.H., 1982. Artificial insemination and sperm storage. In: *New Technologies In Animal Breeding,* ed. B.G. Bracket, Academic Press, New York.

GENGENBACH, D.R., HIXON, J.E. & HANSEL, W., 1977. A luteolytic interaction between oestradiol and prostaglandin $F_2\alpha$ in hysterectomized ewes. *Biol. Reprod.* 16, 571.

GILL, J.L. & HAFS, H.D., 1971. Analysis of repeated measurements of animals. *J. Anim. Sci.* 33, 331.

GONZALES-PADILLA, E., WILTBANK, J.N. & NISWENDER, G.D., 1975. Puberty in beef heifers. I. The interrelationship between pituitary, hypothalamus, and ovarian hormones. *J. Anim. Sci.* 40, 1091.

GOODMAN, R.L., LEGAN, S.J., RYAN, K.D., FOSTER, D.L. & KARSCH, F.J., 1981. Importance of variations in behavioural and feedback actions of oestradiol to control of seasonal breeding in the ewe. *J. Endocrinol.* 89, 229.

GOYINGS, L.S., 1979. Evaluation of safety of prostaglandin in cattle. In: *Proc. of the Lutalyse Symposium,* ed. J.W. Lauderdale and J.H. Sokolowski, The Upjohn Co., Kalamazoo, pp. 44–50.

GRIFFIN, J.L. & RANDEL, R.D., 1978. Reproductive studies of Brahman cattle. II. Luteinizing hormone patterns in ovariectomized Brahman and Hereford cows before and after injection of gonadotropin releasing hormone. *Theriogenology* 9, 437.

GUTHRIE, H.D. & DEAVER, D.R., 1979. Oestrone concentration in the peripheral plasma of pregnant and non-pregnant gilts. *Theriogenology* 11, 321.

HAFS, H.D., LOUIS, T.M., NODEN, P.A. & OXENDER, W.D., 1974. Control of the oestrous cycle with prostaglandin $F_2\alpha$ in cattle and horses. *J. Anim. Sci.* 38 (Supp. 1), 10.

HAFS, H.D., 1979. Potential impact of prostaglandin on prospects for food from beef and dairy cattle. In *Proc. of the Lutalyse Symposium,* ed. J.W. Lauderdale and J.H. Sokolowski, The Upjohn Co., Kalamazoo pp. 9–14.

HANRAHAN, J.D., QUIRKE, J.F. & GOSLING, J.P., 1977. Genetic and non-genetic effects on plasma LH concentrations in lambs at 4 and 8 weeks of age. *J. Reprod. Fertil.* 51, 343.

HANRAHAN, J.P., QUIRKE, J.F. & GOSLING, J.P., 1981. Effect of lamb age, breed and sex on plasma LH after admission of GnRH. *J. Reprod. Fertil.* 61, 281.

HANSEL, W. & TRIMBERGER, G.W., 1952. The effect of progesterone on ovulation time in dairy heifers. *J. Dairy Sci.* 35, 65.

HANSEL, W., SCHECHTER, R.J., MALVEN, P.V., SIMMONS, K.R., BLACK, D.L., HACKETT, A.J. & SAAIMAN, R.R., 1975. Plasma hormone levels in 6-methyl-17-acetoxyprogesterone and oestradiol benzoate treated heifers. *J. Anim. Sci.* 40, 671.

HANSEL, W. & BEAL, W.E., 1979. Ovulation control in cattle. *Anim. Reprod. BARC Symposium* 3, 91.

HATTERSLEY, J.P., DRANE, H.M., MATTHEWS, J.G., WRATHALL, A.E. & SABA, N. 1980. Estimation of oestrone sulphate in the serum of pregnant sows. *J. Reprod. Fertil.* 58, 7.

HAWK, H.W. & CONLEY, H.H., 1971. Loss of spermatozoa from the reproductive tract of the ewe and intensification of sperm 'breakage' by progestogen. *J. Reprod. Fertil.* 27, 339.

HAWK, H.W., 1979. Infertility in dairy cattle. *Anim. Reprod. BARC Symposium* 3, 19.

HOLNESS, D.H., ELLISON, J.A., SPROWSON, G.W. & de CARVALHO, A., 1977. Aspects of fertility in Friesland dairy cows with particular reference to the concentration of progesterone in peripheral plasma. *Rhod. J. Agric. Res.* 15, 109.

HOLNESS, D.H. & HURRELL, A.D., 1977. Fertility in cows after synchronisation of oestrus with prostaglandin in $F_2\alpha$ and oestradiol benzoate. *S. Afr. J. Anim. Sci.* 7, 27.

HURNIK, J.F., KING, G.J. & ROBERTSON, H.A., 1975. Oestrous and related behaviour in postpartum Holstein cows. *Appl. Anim. Ethol.* 2, 55.

INSKEEP, E.K., DAILEY, R.A., JAMES, R.E., PETERS, J.B., LEWIS, P.E. & WELCH, J.A., 1980. Oestradiol benzoate improves synchronization of oestrus in cattle with prostaglandin $F_2\alpha$ *Proc. IX Int. Congr. Anim. Reprod. AI.* 3, 138.

INSKEEP, E.K. & LISHMAN, A.W., 1979. Factors affecting postpartum anoestrus in beef cattle. *Anim. Reprod. BARC Symposium* 3, 277.

INSKEEP, E.K., LISHMAN, A.W., BUTCHER, R.L. & ALLISON, S.M.J., 1977. Effect of short-term removal of the calf on release of LH, ovulation and CL function following GnRH in beef cows. *J. Anim. Sci.* 45 (Suppl. 1), 171.

INSKEEP, E.K. & MURDOCH, W.J., 1980. Relation of ovarian functions to uterine and ovarian secretion of prostaglandins during the oestrous cycle and early pregnancy in the ewe and the cow. *Int. Rev. Physiol.* 22, 325.

INSKEEP, E.K. & PETERS, J.B., 1982. Economic benefits of reproductive management, synchronization of oestrus and artificial insemination in beef cattle and sheep. In: *New Technologies in Animal Breeding,* ed. B.G. Brackett, Academic Press, New York, pp. 243–254.

INSKEEP, K., WOLOSHUK, J.M. & DAILEY, R.A., 1981. Synchronizing oestrus with prostaglandin $F_2\alpha$ plus oestradiol benzoate. *Proc. 15th Annual Conference on Artificial Insemination of Beef Cattle.* Jan. 17, 1981 Denver, Colorado. pp. 13–16.

IRVIN, H.J., RANDEL, R.D., HAENSLEY, W.E., & SORENSEN, Jr., A.M., 1978. Reproductive studies of Brahman cattle. III. Comparison of weight, progesterone content, histological characteristics and 3β-hydroxysteroid dehydrogenase activity in corpora lutea of Brahman, Hereford and Brahman X Hereford heifers. *Theriogenology* 10, 417.

JOCHLE, W., 1972. Seasonal fluctuations of reproductive functions in zebu cattle. *Int. J. Biometeorology.* 16, 131.

JOHNSON, A.D., LEGATES, J.E. & ULBERG, L.C., 1966. Relationship between follicular cysts and milk production in dairy cattle. *J. Dairy Sci.* 49, 865.

KALIS, C.H.J. & VAN DE WIEL, D.F.M., 1980. Relationship of clinical examinations to milk progesterone profiles. *Ninth Int. Congr. Anim. Reprod. Artif. Insem.* 11, 125.

KARG, H., CLAUS, R., GUNZLER, O., RATTENBERGER, E., HAHN, R., & HOCKE, P., 1980. Milk progesterone assay for assessing cyclicity and ovarian dysfunction in cattle. *Ninth Int. Congr. Anim. Reprod. Artif. Insem.* 11, 119.

KARSCH, F.J., NOVEROSKE, J.W., ROCHE, J.F., NORTON, H.W. & NALBANDOV, A.V., 1970. Maintenance of ovine corpora lutea in the absence of ovarian follicles. *Endocrinology* 87, 1228.

KARSCH, F.J., GOODMAN, R.L. & LEGAN, S.J., 1980. Feedback basis of seasonal breeding: test of an hypothesis. *J. Reprod. Fertil.* 58, 521.

KEISLER, D.H., DAILEY, R.A. & INSKEEP, E.K., 1980. Effects of luteal tissue and/or progesterone on first oestrus in ewe lambs. *J. Anim. Sci.* 51 (Suppl. 1), 290.

KEISLER, D.H. & DAILEY, R.A., 1981. Effect of luteinizing hormone administrations on puberty in ewe lambs. *J. Anim. Sci.* 53 (Suppl. 1), 336.

KESLER, D.J., GARVERICK, H.A., CAUDLE, A.B., BIERSCHWAL, C.J., ELMORE, R.G. & YOUNGQUIST, R.S., 1978. Clinical and endocrine responses of dairy cows with ovarian cysts to GnRH and $PGF_2\alpha$. *J. Anim. Sci.* 46, 719.

KESLER, D.J., GARVERICK, H.A., CAUDLE, A.B., BIERSCHWAL, C.J., ELMORE, R.G. & YOUNGQUIST, R.S., 1979. Testosterone concentrations in plasma of dairy cows with ovarian cysts. *J. Dairy Sci.* 62, 1825.

KESLER, D.J., WESTON, P.G., PIMENTEL, C.A., TROXEL, T.R., VINCENT, D.L. & HIXON, J.E., 1981. Diminution of the *in vitro* response to luteinizing hormone by corpora lutea induced by gonadotropin releasing hormone treatment of postpartum suckled beef cows. *J. Anim. Sci.* 53, 749.

KIMBALL, F.A. & LAUDERDALE, J.W., 1975. Prostaglandin E_1 and $F_2\alpha$ specific binding in bovine corpora lutea: comparison with luteolytic effects. *Prostaglandins* 10: 313.

KINCAID, C.M., 1957. Calving percentage in S–10 Experiment Station herds based on a survey of all herds. *Ann. Meeting S–10 Tech Comm. Rept.*, Sept. 1–4, 1957, Gainesville, Florida.

KOGER, M., 1963a. Breeding for the American tropics. In: *Crossbreeding Beef Cattle.* T. Cunha, M. Koger and A.C. Warnick (Ed). University of Florida Press, Gainesville, Florida. pp. 41–46.

KOGER, M., 1963b. Breeding for Florida. In: *Crossbreeding Beef Cattle.* Cunha, M. Koger and A.C. Warnick (Ed). University of Florida Press, Gainesville, Florida. pp. 47–53.

LAMMING, G.E., 1980. Milk progesterone for assessing response to treatment of sub-fertile cattle. *Ninth Int. Congr. Anim. Reprod. Artif. Insem.* II, 143.

LAND, R.B., 1973. The expression of female sex-limited characters in the male. *Nature (London)* 241, 208.

LAUDERDALE, J.W., 1979. Effect of prostaglandin $F_2\alpha$ on the endocrine system of the bovine. In: *Proc. of the Lutalyse Symposium,* ed. J.W. Lauderdale and J.H. Sokolowski, The Upjohn Co., Kalamazoo, pp. 42–43.

LEWIS, G.S., WILSON, JR., L., WILKS, J.W., PEXTON, J.E., FOGWELL, R.L., FORD, S.P., BUTCHER, R.L., THAYNE, W.V. & INSKEEP, E.K., 1977. $PGF_2\alpha$ and its metabolites in uterine and peripheral venous, plasma and endometrium of ewes during early pregnancy. *J. Anim. Sci.* 45, 320.

LEWIS, G.S., JENKINS, P.E., FOGWELL, R.L. & INSKEEP, E.K., 1978. Concentrations of prostaglandings E_2 and $F_2\alpha$ and their relationship to luteal function in early pregnant ewes. *J. Anim. Sci.* 47, 1314.

LEWIS, G.S., LISHMAN, A.W., BUTCHER, R.L., DAILEY, R.A. & INSKEEP, E.K., 1981. Factors affecting function of induced corpora lutea in postpartum anoestrous ewes. *J. Anim. Sci.* 52, 1122.

LEWIS, G.S., THATCHER, W.W., BAZER, F.W. & CURL, J.S., 1982. Metabolism of arachidonic acid *in vitro* by bovine blastoupts and endometrium. *Biol. Reprod.* (submitted).

LEWIS, P.E., BOLT, D.J., & INSKEEP, E.K., 1974. Pattern of luteinizing hormone release in progestin-treated ewes. *J. Anim. Sci.* 38, 1294.

LISHMAN, A.W., ALLISON, S.M.J., FOGWELL, R.L., BUTCHER, R.L., & INSKEEP, E.K., 1979. Corpora lutea in underfed postpartum anoestrous beef cows. *J. Anim. Sci.* 48, 867.

LOUIS, T.M., HAFS, H.D., & MORROW, D.A., 1974. Intrauterine administration of prostaglandin $F_2\alpha$ in cows: Progesterone oestrogen, LH, oestrus and ovulation. *J. Anim. Sci.* 38, 347.

MAGNESS, R.R., HUIE, J.M., HOYER, G.L., HUECKSTEADT, T.P., REYNOLDS, L.R., SEPERICH, G.J., WHYSONG, G. & WEEMS, C.W., 1981. Effect of chronic ipsilateral or contralateral intrauterine infusion of prostaglandin E_2 (PGE_2) on luteal function of unilaterally ovariectomized ewes. *Prostaglandins and Medicine* 6, 389.

MARCUS, G.J., 1981. Prostaglandin formation by the sheep embryo and endometrium as an indication of maternal recognition of pregnancy. *Biol. Reprod.* 25, 56.

McNEILLY, A.S., HUNTER, M., LAND, R.B. & FRASER, H.M., 1981. Inadequate corpus luteum function after the induction of ovulation in anoestrous ewes by LH–RH or an LH–RH agonist. *J. Reprod. Fertil.* 63, 137.

MIDGLEY, A.R., NISWENDER, G.D. & REBAR, R.W., 1969. Principles for the assessment of the reliability of radioimmunoassay methods (precision, accuracy, sensitivity, specificity). *Karolinska Symposia on Research Methods in Reproductive Endocrinology* 1, 163.

MILLER, L.J. & MOORE, F.L., 1981. LHRH stimulation of male sexual behaviour in an amphibian. *Biol. Reprod.* 24 (Suppl. 1), 59.

MOORE, F.L., & MILLER, C.H., 1977. Androgens and male mating behaviour in rough-skinned newts, *Taricha granulosa. Horm. Behav.* 9, 309.

MOORE, F.L., & ZOELLER, R.T., 1979. Endocrine control of amphibian sexual behaviour: evidence for a neurohormone-androgen interaction. *Horm. Behav.* 13, 207.

MURDOCH, W.J., DAILEY, R.A., & INSKEEP, E.K., 1981. Preovulatory changes in prostaglandins E_2 and $F_2\alpha$ in ovine follicles. *J. Anim. Sci.* 53, 192.

NEFF, A.W., 1979. Evaluation of prostaglandin content in edible tissue. In: *Proc. of the Lutalyse Symposium*, ed. J.W. Lauderdale and J.H. Sokolowski, The Upjohn Co., Kalamazoo, pp. 51–63.

NEILL, J.D., DAILEY, R.A., TSOU, R.C. & REICHERT, L.E., Jr., 1977. Immunoreactive LH-like substances in serum of hypophysectomized and prepubertal monkeys: Inactive in an *in vitro* LH bioassay. *Endocrinology* 100, 856.

OTTOBRE, J.S. & INSKEEP, E.K., 1981. Pattern of utero-ovarian venous prostaglandin (PG) $F_2\alpha$ following removal of oestrogen in ewes. *J. Anim. Sci.* 53 (Suppl. 1), 355.

PEXTON, J.E., FORD, S.P., WILSON, L., Jr., BUTCHER, R.L., & INSKEEP, E.K., 1975a. Prostaglandins F in uterine tissue and venous plasma of ewes with intrauterine devices. *J. Anim. Sci.* 41, 144.

PEXTON, J.E., WEEMS, C.W. & INSKEEP, E.K., 1975b. Prostaglandins F in uterine venous plasma, ovarian arterial and venous plasma and in ovarian and luteal tissue of pregnant and nonpregnant ewes. *J. Anim. Sci.* 41, 154.

PLASSE, D., WARNICK, A.C., & KOGER, M., 1968b. Reproductive behaviour of *Bos indicus* females in a subtropical environment. I Puberty and ovulation frequency in Brahman and Brahman x British heifers. *J. Anim. Sci.* 27, 94.

PLASSE, D., WARNICK, A.C., & KOGER, M., 1970. Reproductive behaviour of *Bos Indicus* in a subtropical environment. IV. Length of oestrous cycle, duration of oestrus, time of ovulation, fertilization and embryo survival in grade Brahman heifers. *J. Anim. Sci.* 30; 63.

PLASSE, D., 1973. Basic problems involved in breeding cattle in Latin America. In: *Crossbreeding Beef Cattle.* M. Koger, T.J. Cunha and A.C. Warnick (Ed.). Series 2. University of Florida Press, Gainesville, Florida. pp. 383–394.

PRATT, B.R., BERARDINELLI, J.G. & INSKEEP, E.K., 1979. The effects of progestogen and oestradiol-17β on the life span of HCG-induced corpora lutea in the postpartum beef cow. *J. Anim. Sci.* 49 (Suppl. 1), 133.

PUGLISI, T.A., RAMPACEK, G.B., KRAELING, R.R. & KISER, T.E., 1979. Corpus luteum susceptibility to prostaglandin $F_2\alpha$ ($PGF_2\alpha$) luteolysis in hysterectomized, prepuberal and mature gilts. *Prostaglandins* 18, 257.

QUIRKE, J.F., HANRAHAN, J.P. & GOSLING, J.P., 1981. Duration of oestrus, ovulation rate, time of ovulation and plasma LH, total oestrogen and progesterone in Galway adult ewes and ewe lambs. *J. Reprod. Fertil.* 61, 265.

RAMALEY, J.A. & BUNN, E.L., 1972. Seasonal variations in the onset of puberty in rats. *Endocrinology* 91, 611.

RAMIREZ-GODINEZ, J.A., KIRACOFE, G.H., McKEE, R.M., SCHALLES, R.R. & KITTOK, R.J., 1981. Reducing the incidence of short oestrous cycles in beef cows with norgestomet. *Theriogenology* 15, 613.

RAMPACEK, G.B., KRAELING, R.R., KISER, T.E., BARB, C.R. & BENYSHEK, L.L., 1979. Prostaglandin F concentrations in utero-ovarian vein plasma of prepuberal and mature gilts. *Prostaglandins* 18, 247.

RANDEL, R.D., 1976. LH and ovulation in Brahman, Brahman x Hereford and Hereford heifers. *J. Anim. Sci.* 43, 300. (Abstr.).

RANDEL, R.D. & MOSELEY, W.M., 1977. Serum luteinizing hormone surge and progesterone near oestrus in Brahman compared to Brahman x Hereford and Hereford heifers. *J. Anim. Sci.* (Suppl. 1) 45, 199 (Abstr.).

RANDEL, R.D., 1979. Increasing artificial insemination conception rates in Brahman cattle by injecting 1500 I.U. Human Chorionic Gonadotropin at insemination. *J. Anim. Sci.* 49 (Supplement 1), 41 (Abstr.).

RANDEL, R.D., 1980. Total serum oestrogens before and after oestrus in Brahman, Brahman x Hereford and Hereford heifers. *J. Anim. Sci.* 51 (Supplement 1), 2 (Abstr.).

RANDEL, R.D., SHORT, R.E., CHRISTENSEN, R.K., & BELLOWS, R.A., 1973. Effect of various mating stimuli on the LH surge and ovulation time following synchronization of oestrus in the bovine. *J. Anim. Sci.* 37, 128.

RANDEL, R.D., SHORT, R.E., CHRISTENSEN, R.K., & BELLOWS, R.A., 1973. Effect of clitoral massage after artificial insemination on conception in the bovine. *J. Anim. Sci.* 40, 119.

REYNOLDS, W.L., 1973. Reproduction of Brahman, Angus, Africander and their crosses at Jeanerette, Louisiana. In: *Crossbreeding Beef Cattle, Series 2.* M. Koger, T.J. Cunha and A.C. Warnick (Ed.). University of Florida Press, Gainesville, Florida. pp. 135–142.

RHOAD, A.O., 1955. Procedures used in developing the Santa Gertrudis breed. In: *Breeding Beef Cattle for Unfavorable Environments.* A.O. Rhoad (ed.). University of Texas Press, Austin. p. 203.

RHODES, R.C., III, & RANDEL, R.D., 1978. Reproductive studies of Braham cattle. I. Behavioral effect of various dose levels of oestradiol-17β upon ovariectomized Brahman, Brahman x Hereford and Hereford cows. *Theriogenology.* 9, 429.

RHODES, R.C., III, RANDEL, R.D., & HARMS, P.G., 1978. Reproductive studies of Brahman cattle IV. Luteinizing hormone levels in ovariectomized Brahman, Brahman x Hereford and Hereford cows following a 20 mg dose of oestradiol-17β. *Theriogenology,* 10, 429.

RHODES, R.C., III, & RANDEL, R.D., 1981. Effect of breed and season on the *in vitro* capability or enzymatically dispersed luteal cells to release progesterone upon LH challenge. *J. Anim. Sci.* 53 (Suppl. 1), 29 (Abstr.).

RHODES, R.C., III, RANDEL, R.D., & LONG, C.R., 1981. Corpus luteum function in the bovine: *in vivo* and *in vitro* evidence for both a seasonal and breed type effect. *J. Anim. Sci.* (submitted).

ROBERTSON, H.A. & KING, G.J., 1974. Plasma concentrations of progesterone, oestrone, oestradiol-17β and of oestrone sulphae in the pig at implantation, during pregnancy and at parturition. *J. Reprod. Fert.* 40, 133.

ROBINSON, T.J., 1968. The synchronization of the oestrous cycle and fertility. *Sixth Int. Cong. Anim. Reprod. Artif. Insem.*, Paris, II, 1347–1383.

ROBINSON, T.J., 1974. The present status of applied reproductive physiology in animal production. *Proc. N.Z. Soc. Anim. Prod.* 34: 37.

RYAN, K.D. & FOSTER, D.L., 1979. Mechanisms governing onset of ovarian cyclicity at puberty in the lamb. *Ann. Biol. Anim. Bioch. Biophys.* 19, 1369.

RYAN, K.D. & FOSTER, D.L., 1980. Neuroendocrine mechanisms involved in the onset of puberty in the female: Concepts derived from the lamb. *Fed. Proc.* 39, 2372.

SABA, N. & HATTERSLEY, J.P., 1981. Direct estimation of oestrone sulphae in sow serum for a rapid pregnancy test. *J. Reprod. Fert.* 62, 87.

SHEFFEL, C.E., PRATT, B.R. & INSKEEP, E.K., 1980. Effect of progestogen and PMSG or FSH on induced corpora lutea in cattle. *J. Anim. Sci.* 51 (Suppl. 1), 328.

SILVIA, W.J., OTTOBRE, J.S. & INSKEEP, E.K., 1981. Concentrations of prostaglandins E_2, $F_2\alpha$ and 6-keto-prostaglandin $F_1\alpha$ in the utero-ovarian venous plasma of early pregnant and cycling ewes. *Biol. Reprod.* 24 (Suppl. 1), 99A.

SMITH, M.F., AMOSS, M.S., HARMS, P.G., INSKEEP, E.K., ELLERSIECK, M.R. & WILTBANK, J.N., 1981. Pituitary response to gonadotropin releasing hormone following steroid treatment and/or 27 hr calf removal. *J. Anim. Sci.* 53 (Suppl. 1), 367.

THATCHER, W.W., ROMAN-PONCE, H. & BUFFINGTON, D.E., 1978. Environmental effects on animal performance. In: *Large Dairy Herd Management.* Ed. C.J. Wilcox, University Presses of Florida Gainesville, pp. 219–230.

TOMAR, N.S., 1966. Effect of the season of insemination on conception rates in Hariana cows and Murrah buffaloes. *Indian J. Dairy Sci.* 19, 14.

TRIMBERGER, G.W. & HANSEL, W., 1955. Conception rate and ovarian function following oestrus control by progesterone injections in dairy cattle. *J. Anim. Sci.* 14, 224.

ULBERG, L.C. & LINDLEY, C.E., 1960. Use of progesterone and oestrogen in the control of reproductive activities in beef cattle. *J. Anim. Sci.* 19, 1132.

U.S. FEDERAL REGISTER 44 (185):54900, September 21, 1979.

WARNICK, A.C. 1963. Reproductive performance. In: *Crossbreeding Beef Cattle.* T. Cunha, M. Koger and A.C. Warnick (Ed.). University of Florida Press, Gainesville, Florida, pp. 82–87.

WEBB, D.W., HEAD, H.H. & WILCOX, C.J., 1969. Effect of age and diet on fasting blood and plasma glucose levels, plasma nonesterified fatty acid levels, and glucose tolerance in dairy calves. *J. Dairy Sci.* 52, 2007.

WELCH, J.A., HACKETT, A.J., CUNNINGHAM, C.J., HEISHMAN, J.O., FORD, S.P., NADARAJA, R., HANSEL, W., & INSKEEP, E.K., 1975. Control of oestrus in lactating beef cows with prostaglandin $F_2\alpha$ and oestradiol benzoate. *J. Anim. Sci.* 41, 1686.

WENTZEL, D., MORGENTHAL, J.C. & VAN NIEKERK, C.H., 1975a. The habitually aborting angora doe: IV. Adrenal function in normal and aborter does. *Agroanimalia* 7, 27.

WENTZEL, D., MORGENTHAL, J.C. & VAN NIEKERK, C.H., 1975b. The habitually aborting Angora doe. V. Plasma oestrogen concentration in normal and aborter does with special reference to the effect of an energy deficiency. *Agroanimalia* 7, 35.

WENTZEL, D. & BOTHA, L.J.J., 1976. Thyroid function in nutritionally-stressed pregnant Angora does. *Agroanimalia* 8, 163.

WENTZEL, D., LE ROUX, M.M. & BOTHA, L.J.J., 1976. Effect of the level of nutrition on blood glucose concentration and reproductive performance of pregnant Angora goats. *Agroanimalia*, 8, 59.

WETTEMANN, R.P., 1980. Postpartum endocrine function of cattle, sheep and swine. *J. Anim. Sci.* 51 (Suppl. 2), 2.

WHITMORE, H.L., HURTGEN, J.P., MATHER, E.C. & SEGUIN, B.E., 1979. Clinical response of dairy cattle with ovarian cysts to single or repeated treatments of gonadotropin releasing hormone. *J. Am. Vet. Med. Assn.* 174, 1113.

WILLIAMS, W.F., 1962. Excretion of progesterone and its metabolites in milk, urine and faeces. *J. Dairy Sci.* 45, 1541.

WILTBANK, J.N., SHUMWAY, R.P., PARKER, W.R., & ZIMMERMAN, D.R., 1967. Duration of oestrus, time of ovulation and fertilization rate in beef heifers synchronized with dehydroxyprogesterone acetophenide. *J. Anim. Sci.* 26, 764.

YATES, F.E., 1981. Analysis of endocrine signals: The engineering and physics of biochemical communication systems. *Biol. Reprod.* 24, 73.

ZEMJANIS, R., FAHNING, M.L. & SCHULTZ, R.H., 1969. Anoestrus: the practioner's dilemma. *Vet. Scope.* 14, 14.