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PHYSIOLOGICAL BASIS OF GENETIC VARIATION IN OVULATION RATE

BY

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ABSTRACT

Improvement of litter size (lambs born/ewes lambing) in sheep is difficult for several reasons. The experimental results on direct selection for litter size, coupled with generally low estimates of genetic parameters of litter size led to widespread interest in the use of indirect selection criteria for genetic improvement of litter size. It has been argued that ovulation rate is the main limiting factor to improve reproduction rate in sheep. The understanding of the physiological basis of genetic variation in ovulation rate would allow development of physiological criteria for genetic improvement of ovulation rate and development of reliable means of phenotypic modification of gonadal function. Literature on the indirect methods of selecting for litter size is reviewed with emphasis on the ovulation rate. Postulated physiological methanism of between breed variation in ovulation rate in sheep, the variation of the sensitivity of the hypothalamus/pituitary to negative feed-back of gonadotrophin release, is further investigated and its genetic nature is examined.

In passive immunization studies gonadotrophic releasing patterns were studied in 5 groups of Welsh Mountain sheep (10 each) before and after passive immunization against oestrone (E_1), oestradiol 17β (E_2), androstenedione (A), testosterone (T) and Control (C). Immunization against all four steroids increased the LH pulse frequency and mean LH levels. FSH levels were increased only in the group immunized against E_1 and E_2. However, after 12-15 hours the trophic and negative feed-back system tended to equilibrate

Immunization against all four steroids increased the ovulation rate significantly. It also slightly changed the oestrous cycle length. Number of animals showing oestrus also were affected by the high antibody titres. The lambing percentage was increased after immunization against testosterone. The half life of the four antibodies in the circulation varied from 12-16 days.

In the between breed study of the hypothalamic sensitivity to negative feed-back in Scottish Blackface and Finnish Landrace sheep the basal LH levels, LH pulse frequency and FSH levels were studied before and after insertion of silastic oestradial implants. The ovulation rate and the number of animals ovulating were studied after implant insertion. In this study the previous finding of between breed variation in hypothalamic/pituitary sensitivity to negative feed-back of gonadotrophin release was confirmed and extended the findings to LH pulse frequency, basal FSH levels and to intact animals.

In the mouse study the direct effect of the oestradiol 17β antiserum on the ovulation rate was studied. The heritability of the hypothalamic pituitary sensitivity to negative feed-back was estimated by offspring parent regression. Passive immunization against E_2 increased ovulation rate significantly. The heritability estimated was not significantly different from zero.

In the concluding section the results are discussed and prospects for further research are outlined. It is argued that the most fruitful approach for developing physiological selection criteria for increasing ovulation rate is to study the within breed or strain physiological basis of differences in ovulation rate.

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Chapter 1: Introduction

1.1 Reproduction is one of the most important considerations both in animal production and breeding. Improvement of litter size in polytocous livestock species offers one of the greatest opportunities for increasing both biological and economic efficiency (Blaxter, 1968; Dickerson, 1970). In addition to the direct economic impact of more offspring on profit large litters allow greater selection intensities for other traits and therefore have an important effect on genetic improvement.

In both wool (Turner, 1969) and meat (Meat and Livestock Commission, 1972) production systems female reproductive performance in sheep is a major breeding goal. Attempts to increase litter size by direct selection have had limited success, although the potential benefits from such increment have kept interest in its improvement high. Results from several selection experiments using different breeds of sheep show rather low response to selection, in the order of 0.01-0.02 lambs per year compared with the unselected controls (Clarke, 1972; Hanrahan and Timon, 1978; McGuirk, 1976; Turner, 1969; 1978). Selecting highly prolific ewes out of a large population made up of a number of breeds (Owen, 1976) has led to a new prolific strain, but its development has not been well documented. Difficulty in improvement of litter size in livestock and laboratory animals has been attributed to several reasons: the character is sex limited hence the selection intensity is low; it is discrete rather than continuous and has a low heritability although it has a high coefficient of variation; litter size can only be measured after the first litter which lengthens the generation interval. In addition in swine and mice, there are negative

environmental correlations between the daughter's and dam's litter size (e.g. Falconer, 1960). Lastly low reproduction rate itself limit the selection pressure that can be applied on the litter traits.

The experimental results on direct selection for litter size, coupled with generally low estimates of genetic parameters (see, Turner, 1969 for sheep, Legault, 1970, Ollivier and Bolet, 1981, Lush and Mollin, 1942 for pigs and Joakimson and Baker, 1977 for mice) of litter size led to a widespread interest in the use of indirect selection criteria for genetic improvement of litter size.

It has been argued (Bradford, 1972) that ovulation rate is the main limiting factor to improve reproductive rate in sheep. Land (1974) proposed the use of physiological traits related to litter size as direct selection criteria for improving litter size. Bradford (1972), Hanrahan (1974) and Land (1974) argued that litter size would increase as a correlated response to increase ovulation rate in sheep at least in the breeds with low ovulation rate. Work done in sheep, pigs and in the laboratory mouse show that there is considerable amount of genetic variation in ovulation rate.

This study was undertaken to investigate the underlying physiological mechanism of genetic variation in ovulation rate. Identification of physiological criteria for genetic improvement of litter size and development of reliable means of phenotypic modification of gonadal function (i.e. superovulation) are related longterm goals of the study.

The following section presents a review of pertinent literature available from the sheep, the pig and from the laboratory mouse.

1.2 Direct Selection for litter size

Current schemes for improving reproductive rate in sheep involve selection of females on their own performance (supplemented by information on female relatives) and selection of males on the basis of female relatives, generally on dams performance (MLC, 1972; Turner, 1976). The criterion for selection has been fecundity and assessed as the number of offspring at birth. Direct selection for increasing litter size in sheep has had limited success (Hanrahan and Timon, 1978; McGuirk, 1978; Turner, 1962 and Wallace, 1958). Owen (1976) has reported a development of a new prolific strain by selecting highly prolific ewes from a large population made up of number of breeds. Turner (1978) also observed a higher response to direct selection in reproductive rate in an initially selected population for fecundity and showed that the initial selection of the base population made a major contribution to the higher response to selection. However the details of the above two procedures have not been well documented. A similar approach has also been proposed for increasing litter size in pigs (Legault and Grand, 1976, Legault, Grand and Bolet, 1981) whereby replacement stock are selected from exceptionally prolific dams. In general response to direct selection in sheep has been low. Bradford (1972), Bindon and Piper (1976), Land (1974) and McGuirk (1976) also concluded that it would be difficult to achieve worthwhile gains in direct selection for reproductive rate.

Results of direct selection for litter size in pigs are few. However, Olliver (1973) reported a realised heritability estimate of 0.25 ± 0.37 after five generations of selection. In the same experiment after 10 generations of selection there was no significant

total response in numbers born in the first two litters (Olliver and Bolet, 1981). In view of the large sampling errors included in the estimates and the range of heritability estimates in the literature, the response to selection in pigs can be expected to be rather variable and low.

There have been several experiments to investigate the genetic control of litter size in mice (Falconer, 1960; Bateman, 1966; Bradford, 1968, Bakker, Wallinga and Politiek, 1976; Joakimson and Baker, 1977; Eisen, 1978). It was hoped that the results might have application, which could be applied to improve fertility in livestock species particularly in sheep and pigs. The experiments have varied in the selection cirteria used, the method of selection and population size. The realised heritabilities reported ranged from 13% to 22% with a mean of about 16%. The mice data show that the litter size can be changed by genetic selection but in sheep and pigs in view of the rather lower heritability estimates and the experimental results, response of the same rate are unlikely.

1.3 Heritability estimates

Heritability estimates reported for litter size in sheep are quite low. Young, Turner and Dolling (1963) found the heritability of number of lambs born at the two year old lambing to be negligible. However they reported a value of 0.35 for 3 year old lambings. Purser (1965) reported a much lower value for British hill sheep breeds. Reviews by Bradford (1972) and more recently Bindon and Piper (1976) both concluded that the upper limit of heritability of litter size in sheep is of the order of 15-20%. In general repeatability estimates

reported for sheep are also low. In some cases repeatability estimates are lower than the heritability. Bradford (1972) postulated that this might be due to a negative environmental correlation between consecutive records.

There are number of heritability estimates for litter size in pigs. There is good agreeemnt between the two studies involving the largest collection of data. Strang and King (1970) from 38,000 litters and Legault (1970) from 5,898 daughter dam pairs estimated the heritability to be 0.07±0.02 and 0.08±0.035 respectively. Lagault (1976) reviewed the available heritability estimates for pigs and his summary heritability estimates range from -0.11 to +0.59. He suggested that the most probable mean estimate to be 0.10. Smith and Strang (1979) re-analysed the data of Strang and King (1970) using half-sib analysis and the estimates of heritability obtained were lower than that obtained from daughter dam regression. In most instances where the heritability estimates are considerably higher than 0.10, these estimates are based on relatively few animals and/or are accompanied by large standard errors.

Falconer (1965), Bradford (1968) and Joakimson and Baker (1977) obtained realised heritability estimates of 0.17, 0.13 and 0.18 respectively for increasing litter size in mouse selection experiments. Some attempts have been made to explain the reasons for the low heritability of litter size. The most common explanation is that litter size, being a fitness related trait, has long been subjected to natural selection and as a consequence the genetic variability has been reduced. Falconer (1960) suggested that with mice a negative environmental correlation exists between daughter's and dam's litter sizes. Under this hypothesis one would expect little similarity between dam and daughter but relatively more

between daughter and granddam and among half-sibs. Revelle and Robison (1973) in an attempt to distinguish between possible genetic and maternal effects in gilt litters compared heritability estimates based on daughter-dam regression (.13±.06, 750 pairs) and grand daughter-granddam regression (.28±.26, 530 pairs). Although the results were not conclusive in view of the high standard errors of the estimates, the mean estimate provided some evidence of a negative maternal effects. Later these findings were confirmed by other workers (Alsing, Krippl and Pirchner, 1979; Vangen, 1980). Nelson and Robison (1973) also demonstrated negative environmental correlation between the mother's and daughter's litter size using cross fostering experiment in pigs. Vangen (1980) recently obtained higher heritabilities of litter size from daughter-granddam than daughter-dam regression, although with large standard errors, and argued that this resulted from the negative maternal correlation between generations. He also indicated a declining negative covariation between the mother's litter and offspring's litter size with increasing parity. Falconer (1964) indicated that the negative covariation between the mother's and daughter's litter size arise primarily through effects on the ovulation rate of the daughter.

1.4 Indirect selection for litter size

The earliest literature on the indirect methods of selection for increasing prolificacy are on the traits which had no known physiological relationships with the reproductive traits.

1.4.1 Face cover in sheep

Several workers reported phenotypic relationships between

face cover and prolificacy (Terril, 1949; Shelton, 1957; Fail and Dun, 1965, Hayland and Turner, 1966). However, Kennedy (1959) reported negligible phenotypic and genetic correlations between face cover and reproductive rate. Turner (1969) concluded from the available literature that the face cover is not of any value as an indirect selection criteria to raise reproductive rate in sheep.

1.4.2 Degree of skin wrinkles and skin folds

There are a number of reports on the relationship between skin wrinkles and reporductive rate in merino sheep (Dun, 1964; Dun and Hamilton, 1965; Fowler and Dun, 1966). Using photographic standards Kennedy (1959) reported a significant negative phenotypic correlation but a positive non-significant genetic correlation between skin wrinkles and the number of lambs born. Dun and Hamilton (1965) analysing the results with hot room experiments concluded that the wrinkled ram contributed to the lowered reproductive rate as they are unable to control the scrotal temperature if joined under hot conditions.

1.4.3 Fleece weight

Attempts have been made to calculate the genetic correlation of fleece weight with reproductive rate in sheep but the estimates are not consistent in sign. Young and Turner (1965) reported a negligible correlation between wool weight at 15-16 months and the number of lambs born or weaned in 2-4 year Merino ewes. A number of workers have reported negative genetic correlations between fleece weight and the number of lambs born (Shelton and Manzies, 1969; Ray and Chang, 1955). Genetic correlation estimates from British breeds were quite

variable and low (about 0.1). All these estimates were not significant but most of them were consistently negative.

1.4.4 Body weight

There is general agreement in the literature that body weight is important in relation to reproductive rate in sheep. Young, Turner and Dolling (1963) and Purser, (1965) reported the genetic correlation between ewe weight and lambs born to be 0.20 and 0.57 respectively. However Turner (1969) working on available genetic correlations and heritability estimates for the two traits showed that direct selection is more effective in increasing litter size than the indirect selection on body weight. Moreover increasing body size would not be acceptable as a selection criterion for increasing prolificacy because of the increased maternal feed cost.

1.4.5 Cannon bone length

Purser (1980) reported 20% more lamb production in a Scottish Blackface line selected for higher cannon bone length, compared with the control unselected line. He also pointed out the similar association between breeds. Many of the breeds with long thin bone structure (of which Finnish Landrace is an extreme example) are highly prolific with low lamb mortality. Other breeds with short thick bone structure (such as British down breeds) are less prolific. However there were differences in body size in the two lines, the long cannon bone line being heavier.

1.4.6 Single genes

The majority of reproductive characters can be assumed to be controlled by a large number of genes each having a small effect. A small number of associations have however been demonstrated between reproductive rate and single loci with two or more alleles.

Association between the reproductive performance of sheep with readily distinguished haemoglobin (Hb) types has been investigated in some sheep breeds. King, Evans, Harris and Warner (1958) found in a flock of Scottish Blackface that the ewes carrying the gene for B Hb type (Hb B) produced more lambs at weaning than other types although Hb.B gene was not common in that breed. In a flock selected for high and low fecundity Hb type A ewes produced fewer lambs than the Hb type B and AB ewes (Evans and Turner, 1965). Subsequently the above finding was confirmed by several workers (Bernoco, 1967, 1968, Dassat and Bernoco, 1968, Seth 1968, Arora, Acharya and Kakar, 1971). Recently same findings was repeated with very large number of records (Hall and Purser, 1979). From the available literature it appears that selection for Hb.B allele could increase prolificacy in sheep. However there are genotype x environment interaction in Hb type and reproduction (Obst and Evans, 1970). It has been postulated, using data from the original CSIRO sample of 14 Booroola ewes and their 19 daughters that exceptional fecundity of this breed is due to a major gene or closely linked group of genes (Piper and Bindon, 1980). The segretation criterion was a set of triplets at least once in the lifetime of a ewe.

In pigs also some associations have been shown between reproduction and single loci with two or more segregating alleles. For example, matings

involving heterozygotes for the "G" allele at the serum transferin locus (Tf^G) have been shown to reduce litter size by 1.4 pigs, possibly due to linkage with a lethal gene (Imlah, 1970). More recent studies with halothane test have shown a reduction in litter size in halothane positive recessive homozygote (HAL^{nn}) animals (Webb, 1980). Single gene associations may be a potentially useful aid to selection for reproduction as such genes can be identified in both sexes at a very early age (Smith and Webb, 1980).

1.5 Characters having direct physiological relationships with reproductive traits

Land (1974) discussed the possibility of using the underlying physiological variables in genetic improvement of reproductive performance. Reproduction is sex limited but not sex linked, although phenotypic variation is exhibited by only one of the sexes, genetic variation exists in both. It is possible that this genetic variation gives rise to physiological variation even in animals where it is not being expressed as litter size. Osmond (1980) developed this approach for selecting for milk production in cows, a trait which again has a restraint of sex limitation and a long generation interval.

It is possible to relate prolificacy of males and females in view of the common physiological pathways underlying the expression of male and female reproductive activities. It is well known that the same gonadotrophic hormones, follicle stimulating hormone (FSH) and Luteinizing hormone (LH) are controlling the reproductive activities in both sexes. Common physiological components in reproduction in males and females have been considered as a mean of overcoming the problem of

sex limitation (Land, 1974).

1.6 Male characteristics

Land (1973) working with mice highlighted the fact that the female reproductive characters are not sex limited and showed that male criteria could be used to select for female prolificacy.

1.6.1 Testis Growth

The comparison of testis growth of lambs from breeds and crosses with varying reproductive characters provided the first information on the covariation between male and female reproductive characteristics (Land, 1973). From this information he suggested that testis growth in lambs may be used as a simple measurement of circulating gonadotrophins. Carr and Land (1975) found a significant correlation between plasma LH and testis diameter. They suggested that combination of plasma LH levels and testis diameter may form a basis of a suitable selection criterion for selecting males for prolificacy. Hanrahan (1974) and Land and Sales (1974) concluded that testis weight and testis diameter are associated with natural prolificacy. Although between breed studies show such an association, similar association have not been demonstrated within breeds (Bindon and Piper, 1976)

Bindon and Piper (1976) indicated that the testis growth in the male is the most promising character for several reasons: it may be measured directly in the male, it may be measured early in life, measurement is simple and it's heritability and genetic correlation with ovulation rate are high in the mouse, initial estimates for sheep also appear high and it is known to be directly dependent on FSH and LH

and in this sense it is the counterpart of ovulation rate in ewes.

Preliminary work by Land (1977) indicated a correlated response in percentage ovulation rate and litter size on selection for testicular growth in sheep. However, Land (1978) subsequently stated that although four generations of selection for testicular diameter led to a change in 2.6 standard deviations in the trait, by far the most notable change in female reproductive performance was an early onset of breeding season. When the litter size responsed to long term selection, Hanrahan and Quirke (1977) did not observe any associated change in testis diameter up to 17 weeks of age.

There is also evidence in pigs (Proud, Donovan, Kinsey, Cunningham and Zimmerman, 1970) that the selection for ovulation rate in sows resulted in a corresponding change in adult testis size of boars. In mice when the selection was done on testis weight a response was observed in testis weight, but the correlated response in ovulation rate was not accompanied by a response in litter size. The genetic correlation between the two traits and the heritability of the testis size were both in the region of 0.5 (Islam, Hill, and Land, 1975). In a mouse selection experiment positive correlated response was observed in testis weight following selection for litter size (Joakimsenand Baker, 1977).

1.6.2 Sexual Behaviour

Intensity of sexual behaviour in adult and young rams of different breeds was associated with the reproductive performance of the breed (Land, 1970; Land and Sales, 1977). However, Land (1978) from within breed data indicated thay it would be difficult to incorporate male behaviour as a quantitative criteria for a selection programme.

Although some of the male characteristics have been shown to be correlated with female reproduction none of the characters have shown to be directly associated with litter size.

1.7 Female characteristics

A number of characteristics related to reproduction have been studied in order to understand the underlying physiological basis of genetic variation in reproduction. Identification of such criteria would allow animals to be selected on the physiological characteristics for increasing ovulation rate and thereby the litter size.

1.7.1 Ovulation rate

The number of young born per litter is determined by ovulation rate (excepting monozygote twinning) and also the losses between fertilisation and parturition. Bradford (1972) concluded that the number of ova shed and fertilised is the limiting factor to the present level of reporductive performance in sheep. Selection for increased litter size in sheep led to a positive correlated response in ovulation rate (Packham and Triffit, 1966; Bindon, Chang and Turner, 1971; Hanrahan, 1980) indicating a genetic correlation between ovulation rate and litter size in sheep. Robertson (1976) showed that the litter size differences between Booroola Merino x purebred Merino and purebred Merino was due to differences in ovulation rate. Thus, within breed selection response, breed and crossbred differences in litter size have been accounted for by the corresponding ovulation rate differences. Furthermore Falconer (1963) and Joakimsen and Baker (1977)

reported that the positive response to direct selection for litter size in mice was due mainly to a correlated response in ovulation rate rather than decreased number of loss of fertilised ova. The increase in litter size obtained by superovulation (Newton, Betts & Large, 1970) and transfer of embryos in excess of normal ovulation rate (Moore, 1968, Bradford, Taylor, Quirke and Hard, 1974, Hanrahan, 1980) also provided evidence that the ovulation rate is the primary limiting factor to the litter size in sheep. Hanrahan (1974) discussed the use of ovulation rate as a selection criteria for increasing litter size in sheep by using the technique of laparoscopy to record the ovulation rate. This technique can be used to record the natural ovulation rate without having adverse effects on the subsequent fertility. Ovulation rate as a selection criteria presents two definite advantages over litter size. Repeatability and heritability of ovulation rate are probably higher than the litter size and also the trait could be assessed in sheep earlier than litter size. Hanrahan (1974) theoretically studied the genetic gain in litter size achieved on selecting for ovulation rate on three repeated measurements and predicted that the genetic gain in litter size is at least two fold higher than achieved by direct selection, on the assumption that uterine capacity is not limiting, the genetic correlation between early and subsequent ovulation within a season is close to unity and the repeatability of ovulation rate is high. There are quite a number of repeatability estimates reported for ovulation rate and they are remarkably different both between and within breeds.

Hanrahan (1976) did not find significantly different repeatability estimates, for between and within years, and he pooled the

estimates. His results are given in Table 1.0 with corresponding repeatability estimates for litter size.

Table 1.0. Repeatability of ovulation rate and litter size in four sheep breeds (from Hanrahan, 1976).

Breed	Ovulation rate		Litter size	
	r	df	r	df
Finnish Landrace	0.66**	109	0.07	188
Galway	0.15*	135	0.20*	707
Fin Galway	0.15*	79	0.06	99
High Fertility	0.78*	32	0.13	79

*P<0.5 **P<0.01

(Repeatability estimates for the ovulation rate are pooled estimates, between and within years).

Although repeatability estimates on ovulation rate in sheep have been available for a long time (McDonald and Chang, 1966; Hullet and Foote, 1967), most of the genetic studies on ovulation rate have been started quite recently. Hanrahan (1980) published the heritability estimates for ovulation rate in sheep. His results are shown in the table (1.1). Half-sib analysis yielded rather imprecise estimates due to the low family size. However, it shows that there is considerable amount of genetic variability, fairly high heritability, and that mass selection for increased ovulation rate could be possible.

Table 1.1. Summary of heritability estimates for ovulation rate in Finnish Landrace (from Hanrahan, 1980).

Source	Estimate	df
Daughter-dam regression	0.45±0.07	212
Half-sib analysis (one record per ewe)	0.38±0.26	126
Half-sib analysis (More than one record per ewe)	0.53±0.29	115

He also reported a rather asymmetrical frequency distribution of ovulation rate in Finnish Landrace breed and response to selection after one generation of selection. Results obtained in the upward direction is in consistent with the estimated heritability. His results for the Galway breed with a limited amount of data indicate significant additive genetic variability, although the precision of the estimate is rather poor.

Work done in mice and pigs also indicates additive genetic variability in ovulation rate. The number of Corpora-Lutea (C.L.) present in the ovary on day 7-8 of gestation (Bradford, 1969) and the number of eggs present in the ampulla on the morning of mating (Land and Falconer, 1969) were the criteria for selection for ovulation rate in mice. On direct selection Bradford (1969) with a single upward line for 6 generations and Land and Falconer (1969) with high and low lines after 12 generations, reported realised heritability estimates of 0.1 and 0.3 respectively. The latter authors also reported a heritability estimate of 0.22±.19 for multiparous females, from the correlation between paternal halfsibs. In both of these experiments litter size did not change as a correlated response. Five generations of selection for

higher ovulation rate has been applied in a multibreed synthetic pig line (Zimmerman and Cunningham, 1975). Ovulation rate was recorded at laparotomy as the number of C.LS after the second oestrus in the gilts. Realised heritability was estimated by three methods and the estimates were more than 40%. However, even after nine generations of selection the correlated response in litter size was not significant being 0.06±0.07 young per generation (Cunningham, England, Young and Zimmermann, 1979).

From the available literature it is clear that there is considerable amount of genetic variability in the above mentioned species. However the selection for increased ovulation rate has not produced correlated responses in litter size in mice and pigs, but it seems likely that in sheep increased ovulation rate would result in an increase in litter size. Recadeau, Razungles, Egehenne and Tehamitchian - (1975) found no evidence of heterosis on ovulation rate in a study involving Romanov, Berrichon de Cher and their F_1, F_2 and backcross ewes. Land, Pelletier, Thimonier, and Mauleon (1973) and Piper and Bindon (1978) also found no evidence of heterosis in crosses involving the Romanov and Booroola, respectively. In contrast, Hanrahan (1980) working on Galway and Finnish Landrace crosses reported heterosis for ovulation rate. The estimated heterosis was about 31% of the F_1 mean. He also reported that the results were consistent with the observations for litter size obtained from the F_1 and F_2 comparisons. King (1974) in a review paper indicated that in pigs the large benefit in litter size derived from crossbred female must have been due to an improvement in embryonic viability rather than ovulation rate. Johnson and Omtvedt (1975) also reported heterosis for ovulation rate in pigs but the

results were not significant. In contrast to these results Squires Dickerson and Mayer (1952) reported that crossbred gilts from Poland, China and Hampshire lines and outbred Durocs had 1.19 (P<0.1) more ova per gilt than the average of purebreds. Furthermore inbreeding depression has also been reported in pigs (Squires et al., 1952; King and Young 1957). In mice inbreeding depression has not been observed in ovulation rate (McCarthy, 1963; Falconer and Roberts, 1960). However (Kumar, 1981) reported significant heterosis for ovulation rate in mice.

1.7.2 Ovarian response to exogenous gonadotrophin

Pregnant mare serum gonadotrophin (P.M.S.G.), has been known for a long time to induce ovulation both in domestic and laboratory animals.

Part of the variation in ovulation rate is also influenced by the activity of circulating gonadotrophins and ovarian sensitivity to these hormones. McLaren (1962) found that selection for increased litter size changed ovulation rate as a result of altered ovarian sensitivity to FSH. Fowler and Edwards (1960) administered PMSG to mice selected for large and small body size in doses that induced approximately their natural ovulation rate. Because of the different doses of PMSG required, they concluded that FSH secretion was different in two lines. However another explanation to their results is that the selection changed ovarian sensitivity to FSH.

When the endogenous gonadotrophins were removed by hypophysectomy, mice selected for higher ovulation rate gave a significantly higher ovulation rate than the controls to a standard dose of PMSG (Bindon

and Penngeirk, 1974). However, Edwards (1962) found no difference in the unit potency of gonadotrophins in mice selected for large and small body size. Durrant, Eisen and Ulberg (1980) studying the response to PMSG in mice selected for combinations of high and low body size and litter size, also suggested that the observed differences in ovulation rate were due to the differences in the ovarian sensitivity to gonadotrophins. Spearow (1980) also reported a higher response in PMSG induced ovulation rate in a mouse line with higher ovulation rate. In sheep high fecndity Merinos responded more than their low fecundity controls to injected PMSG (Bindon, Chang and Turner, 1971). Romney ewes selected for fecundity also behave in the same way (Smith, 1976).

In mice, Land and Falconer (1969) demonstrated genetic variation in the number of ova shed in response to PMSG. Selection applied to ovulation induced by 4.i.u. of PMSG led also to a response in both directions, with a realised heritability of 22%. Genetic correlation estimated between natural and induced ovulation in primiparous females was 0.33. Although the trait was responding to selection, there was no correlated change in litter size. The low genetic correlation between natural and induced ovulation shows that the induced ovulation can not be used as a criterion for selection for natural ovulation rate and it could not be relied on as a valid comparison of the natural ovulation rates of different strains or breeds. In addition the induction of ovulation by PMSG is not exactly comparable with the natural ovulation mechanism (see review by Richard, 1980). For example, Hay and Moor (1977) found that PMSG also acts by preventing atresia of the natural follicles.

1.7.3 Ovarian follicular population

Some attempts have been made to understand the genetic control of ovulation rate by studying the correlations between the ovarian follicular populations and the ovulation rate. Within breed and between breed studies have shown a negative relationship between primary oocyte population and ovulation rate (Jones and Krohn, 1961; mice; Spearow, Geschwind and Bradford, 1979, mice; Land, 1970, Sheep, Cahill, Mariana and Mauleon, 1979, sheep). Land (1970) also found that the ewe lambs from breeds with higher ovulation rates, have more antral follicles at birth. When the total ovarian follicular populations were studied (Cahill, Mariana and Mauleon, 1979), there were half as many small follicles but 1.5- 2 times more large follicles in the more prolific Romanov than in less prolific Ile-de-France. The number of antral follicles were approximately the same in both breeds and do not explain the differences observed in ovulation rate between breeds (Cahill, 1978). Cahill (1979) also showed that in sheep antral follicles are under the immediate control of FSH from the pituitary and the second FSH peak on day 2 of the cycle was correlated with the number of antral follicles present some 17 days later. It was found that the high fecundity ewes have more follicles in the growth phase than low fecundity ewes and thus more follicles available to be stimulated for ovulation. However, Turnbull, Land and Searamuzzi (1977) suggested that this arises from a slower follicular growth in the early stages and more rapid passage through the preovulatory stage in sheep with low ovulation rate.

Spearow, Geschwind and Bradford (1979) have studied the follicular population in two mouse lines originated from a common base population. They reported that the line selected for higher litter size had heavier

ovaries at 4-8 weeks of age and had significantly more preovulatory antral follicles (type 6) than in the control line. Their data indicated that selection for litter size has increased the ovulation rate by increasing the number of the specific follicle types (i.e. follicle type 6) but not by decreasing the incidence of atresia. Cahill (1979) also suggested that atresia was similar in high and low ovulation rate breeds. Both from within and between breed studies it is clear that high ovulation rate animals have more preovulatory antral follicles and hence ovulate more ova than the low ovulation rate animals.

1.7.4 Breed differences in embryo survival and uterine capacity

Uterine capacity and embryo survival are also important factors to be considered in selecting for higher ovulation rate or superovulating for increasing litter size. Egg transfer procedures allow comparison of embryo survival which is uncomplicated by adjustment for fertilization rate and ovulation rate. Egg transfer studies by Moor (1968) and Bradford, Taylor, Quirk and Hart (1974) suggested that uterine capacity is not a limiting factor for increasing litter size in sheep. On an egg transfer study involving low and high fertility breeds Hanrahan and Quirk (1977) found that there is no evidence of significant differences among the recipient breeds in embryo survival. Within breed studies also show that there are no significant differences in embryo survival. Bindon, Piper, Cheers and Curtis (1978) have reported no difference in egg survival when 3 eggs were transferred to Merino and Booroola Merino ewes which had ovulation

rates of 1.46 and 4 respectively. Several other experiments involving egg transfer, both between and within breeds gave similar results (Lowson and Rowson, 1972; Bradford, Taylor, Quirk and Hart, 1974; Trouson and Moor, 1972; Piper and Bindon, 1979; Hanrahan, 1980). From the consideration of a range of breeds studied it also seems that there is little evidence for an association between uterine capacity and natural ovulation rate. Working with large number of published data Taylor, Bradford, Land and Hanizdo (Unpublished, referred to by Land and Wilmut 1977) concluded that at least the breeds with low ovulation ratescan carry more embryos than normal. Hanrahan (1980) stated that it may be concluded that selection for ovulation rate in Galway breed can be contemplated without concern as to the ability of the breed to support more ova than usually encountered by this breed. However an existance of curvilinear relationship between litter size and ovulation rate has been shown in Finnish Landrace. The data from literature also provide a clear demonstration of the decline in egg survival as the number of fertilised ova per uterus increases.

The available data indicate that the genetic variation in the ability of uterus to support a given number of embryos (uterine capacity) is not an important component of breed difference in litter size. It seems unlikely, therefore the genetic variation in uterine capacity is important within a breed. Hanrahan (1980) from his own results and other published results concluded that there is very little genetic variation among ewes or embryos in survival rate and hence the genetic correlation between ovulation rate and litter size may be taken to equal to unity in sheep.

1.7.5 Number of oestrus periods

Several workers have shown that the number of oestrus periods in sheep is correlated with litter size and it's components such as ovulation rate. Chang and Ray (1972) estimated the genetic correlation between the duration of the first breeding season "the number of hogget oestruses" and ewe reproduction rate. Depending on the method of investigation the estimates varied from 0.5 to 0.9. Subsequently using a larger set of data from the same breed the genetic correlation estimated between the hogget oestruses and the subsequent reproduction was zero (Baker, Clarke, Carter and Diprose, 1981). Based on these results the validity of using these hogget traits as means of indirect selection for reproductive performance is questioned. Furthermore, the litter size of Welsh mountain ewes was found to be genetically independent of the onset of the breeding season (Purser, 1972). Bindon and Piper (1976) indicated that the highly prolific ewes have a higher total number of cycles to first joining than the low prolific ewes and also that the highly prolific ewes show first oestrus at an early age. They estimated the heritability of total oestrus records to first joining and it was 0.56 ± 0.10 (120 df for sires with an average 8.06 daughters). High prolific sheep breeds are known to have a longer breeding season than the low prolific breeds. This has been documented for the Romanov (Thomonier and Mouleon, 1969) and Finnish Landrace sheep (Wheeler and Land, 1977). The highly prolific Dahman breed is also known to breed at any time of the year (Bouix and Kadiri, 1975). Within breed studies also show the same trend. High prolific merino flocks showed 40% more oestruses than low prolific flocks (Turner, 1962). A high proportion of Booroola merinos have been shown to ovulate at all months of the year (Bindon and Piper, 1976) and

the Booroola has 40% more oestrous cycles per year than control Merinos.

1.7.6 Duration of oestrus

Both Finnish Landrace (Land, 1970; Hanrahan and Quirke, 1975) and Romanov (Desvignes, 1971) have significantly longer duration of oestrus than the low fecundity breeds. Within breed studies have also shown significant positive phenotypic correlations between length of oestrus and ovulation rate and litter size. Merino ewes with longer duration of oestrus produced more lambs than the others (Dunlop and Trallis, 1964). Land (1970) estimated within year regression of litter size on duration of oestrus to be 0.011 lambs per hour of oestrus and the correlation between the traits to be 0.18. By contrast, the duration of oestrus in Booroola Merino is not significantly longer than control Merino (Bindon and Piper, 1980). Hanrahan (1974) estimated the repeatability of duration of oestrus in Finnish Landrace after synchronisation of oestrus and it was relatively high (unweighted mean value = 0.53 for ewes). Schinaler and Amir (1972) obtained a repeatability estimate of 0.66 for natural oestrus and suggested that there might be a reasonably high heritability for duration of oestrus. Although repeatability estimates for duration of oestrus are high there are no heritability estimates. In practise recording of oestrus is difficult.

1.7.7 Hormonal events in the oestrus cycle

Before reviewing the quantitative endocrinological characters in relation to ovulation and fecundity it is relevant to describe some key endocrine events associated with oestrus cycle. The serum gonadotrophin and gonadal steroids during the oestrus cycle and their

control have been examined in several species by Gay, Midgly and Nieswender (1970), in mice by Murr, Geschwind and Bradford (1972), in sheep by Foster, Lemons, Jaffe and Nieswender (1970) and in pigs by Stanemfldt, Arkins, Ewing and Morrissette (1969). The subject has been reviewed by Greenwald (1978). While the absolute concentrations of these hormones differ greatly between species, the pattern of gonadotrophin release and their control show remarkable similarities.

The organisation of a single 16-17 day oestrous cycle of the sheep is illustrated schematically in Fig. 1.0. The cycle basically consists of two phases, 12-13 day luteal phase and 3-4 day follicular phase (preovulatory period). Progesterone is the predominant ovarian hormone during the luteal phase and its circulating plasma profiles reflects the secretory activity of the corpus luteum in relation to its formation and lysis (Quirk, Hanrahan and Gosling, 1979). The secretory activity of the corpus luteum is governed by the stimulatory factors from the pituitary and inhibitory factors from the uterus (Nalbandov, 1973; Hansel, Concannon and Lukaszewska, 1973; Galding, 1974). The flucturating plasma oestradiol originates almost exclusively from the ovarian follicle, several follicles develop and undergo atresia during each cycle (Smeaton and Robertson, 1971; Baird and Scaramuzzi, 1976). The pattern of circulating luteinizing hormone (LH) reflects the operation of two separate regulatory systems, a tonic system operating during most of the luteal phase of the cycle when LH is secreted in a pulsatile manner with relatively low amplitudes and a surge system of release which generates the LH surge prior to ovulation (Golding, Blockey, Brown, Cat and Cumming, 1976; Scaramuzzi, Tilson, Thorneyeroft and Caldwell 1971; Cahill, 1979). Follicle stimulating hormone (FSH) follows a somewhat similar circulating patterns to that of LH. However.

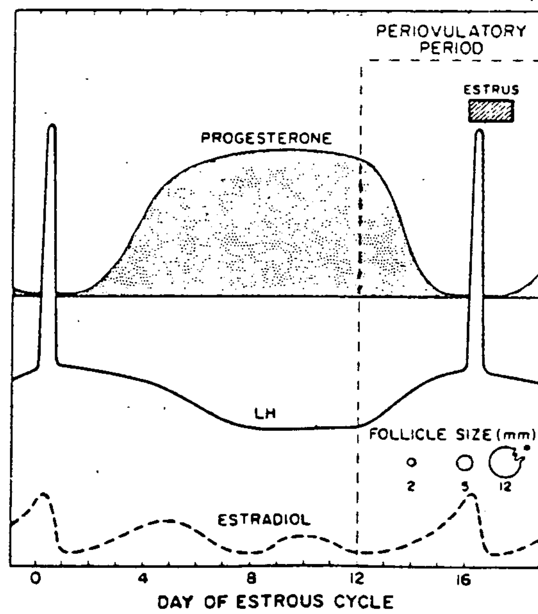


Figure 1.0. Schematic representation of the 16 day oestrous cycle of the ewe (from Legand and Karsch, 1979).

it does not form a pulsatile releasing pattern (Walton, McNeilly, McNeilly and Cunningham, 1977; Pant, Hopkinson and Fitzpatrick, 1977). In addition to the preovulatory FSH surge that coincides with LH surge, a second FSH surge occurs approximately 20-30 hours after the preovulatory LH surge (Bindon, Blane, Pelletier, Terqui, Thimonier, 1979; Cahill, 1979). In addition to oestradiol-17β and progesterone the sheep ovary secretes at least seven more steroids (Baird, 1978) including androgens such as androstenedione and testosterone.

1.7.8 Quantitative endocrinological estimates

Attempts have been made to study the quantitative endocrinological relationships between plasma gonadotrophins and gonadal hormones with ovulation rate in sheep in order to identify the genetic differences in prolificacy. Land, Crighton and Lamming (1972) studied the leutinizing hormone levels in the pituitary gland and urine of ewes with extremely different ovulation rates. Results indicated that the LH levels are related to ovarian activity of the breed type. Thimonier Pelletier and Land (1971) showed that the oestrogen induced plasma LH levels in both male and female lambs are related to their breed ovulation rate. Bindon and Turner (1974) working with high and low prolific merino strains around 30 days of age and Blanc, Courote, Pelletier and Thimonier (1975) using high and low prolific breeds found positive relationships between the LH levels and ovulation rate but the results were not consistent with single or repeated measurements. However Echternkamp and Luster (1976) and Hanrahan (1975) did not find differences in plasma LH levels of young lambs of different breeds. The incompatibility of the results were discussed by Land and Carr (1978).

They concluded that the different breeds go through their plasma LH rise at different ages and showed that the comparisons of LH levels at the different stages of prepubertal LH rise could even lead to a reverse ranking of the animals to their ovulation rate because of age and breed interaction.

Carr and Land (1975) estimated the repeatability of the concentration of consecutive plasma LH samples to be 0.12. Although the repeatability of LH levels in plasma is low, significantly different basal LH levels have been reported on day 1 and 8 of the oestrous cycle in breeds with differing ovulation rates (Land, Pelletier, Thimonier and Mauleon, 1973). However, the concentration of LH in peripheral plasma during the oestrous cycle was found to be similar in breeds of sheep differing three fold in ovulation rate (Land, Pelletier, Thimonier and Mauleon, 1973). Cahill (1979) also reported similar plasma LH profiles at all the stages of the oestrous cycle in three breeds with different ovulation rates. In general there is very little association between plasma LH concentration and ovulation rate in adult sheep.

Because of low repeatability of plasma LH concentration, being only 0.12 (Carr and Land, 1975) over a range of ages and breeds alternative approaches of using LH response to exogenous oestrogens (Land, 1974, Bindon, Ch'ang and Evans, 1974) and to leutinizing hormone releasing hormone (LH-RH) (Carr and Land, 1975) have been suggested. The latter authors also developed a simple method to detect the differences in response to LH-RH with small number of blood samples. The association between the ovulation rate and response to LH-RH

has been reported in between breed studies (Land and Carr, 1976, 1978). Furthermore the same workers reported genetic variation within a synthetic sheep breed in response to LH-RH and observed a response to selection the realised heritability obtained being 0.33 (Carr, Land and Sales, 1980).

Several workers studied the LH levels around ovulation (Land, Pelletier, Thimonier and Mauleon, 1973; Bindon, Thimonier, 1979; Quirke, Hanrahan and Gosling, 1979). All the breeds studied had similar peak LH values prior to ovulation. However there were consistent differences in the timing of the LH discharge in relation to the onset of oestrus, high fecundity breeds having a longer interval between onset and peak LH levels. In contrast, the Booroola sheep does not differ from the control Merino with respect to the time interval between the onset of oestrous and LH discharge, (Bindon and Piper, 1980). However the above correlation has been considered to be simply an association with and not a determinant of ovulation rate (Cahill, Mariana and Mauleon, 1979). Furthermore this is a laborious parameter to measure and it has no advantage over direct measurement of ovulation.

In comparing the Merino strains selected for high and low prolificacy Findlay and Bindon (1976) found substantially higher FSH levels in 30 day old lambs from the prolific strain. However, Bindon, Blanc Pelletier, Terquin and Thimonier (1979) could not detect any differences in the levels of FSH in ewes to explain the differences observed in ovulation rate. Cahill (1979) also did not find any differences in the over-all profiles of FSH in two different breeds, but he reported a significant difference ($P<0.05$) in the second FSH peak that occurs after the preovulatory LH peak in Romanov and

Ile-de-France ewes. The second FSH peak also commenced earlier in Romanovs. Even with repeated sampling (10 minutes interval for 4 hours) on day 1-2, 9 and 16-17 of the œstrous cycle, no significant difference was detected in peripheral plasma FSH in Booroola and control merinos (R.J. Scaramuzzi unpublished data, referred to by Bindon and Piper, 1980). Spearow (1980) also reported that the differences in ovulation rate in mouse lines achieved by selecting for higher litter size and growth rate, could not be explained by the differences in peripheral gonadotrophic hormones. Available results indicate that the plasma gonadotrophin levels do not explain the between or within breed variation in ovulation rate in sheep and mice.

1.7.8 Feed-back hormones

Bindon et al., (1979) and Scaramuzzi and Land (1976) did not find differences in the pheripheral plasma oestrogen concentration in pro-oestrous period in ewes with different ovulation rates. However, Wheeler, Baird and Land (1977) observed between breed differences in the levels of oestridiol 17β secretion in ewes following ovarian autotransplantation but it is difficult to correlate these results with the events happening in the normal oestrous cycle. Although previous studies reported similar peripheral oestradiol levels Cahill (1979) reported higher oestradiol 17β levels in circulation almost throughout the oestrous cycle in the high prolific ewes than in the low prolific ewes. In addition the peak value of oestradiol 17β measured prior to the preovulatory LH peak was higher in Romanov ewes than in Ile-de-France ewes and it was correlated with the number of large follicles present. Detection of such differences may have been

possible because of the use of sensitive E_2 assay techniques.

Plasma progesterone has also been considered to be the primary negative feed-back hormone during the breeding season (Baird and Scaramuzzi, 1976; Hauger, Karsch and Foster, 1977). In sheep, physiological levels of oestradiol and progesterone each suppress the pulses of LH characteristic of tonic LH secretion, but do so by completely different mechanisms (Goodman and Karsch, 1980). A positive association has been demonstrated between plasma progesterone and prolificacy, in between breed studies (Bindon et al., 1979; Quirke et al., 1979). Cahill (1979) also reported a higher correlation between ovulation rate and luteal phase plasma progesterone. Although the results available on the feed-back hormones are limited it is likely that there are breed differences in feed-back hormones, more prolific breeds having a higher concentration in the circulation.

1.8 Variation in feed-back system

Although it has been known for nearly fifty years that the secretion of pituitary gonadotrophins is controlled in part by the steroid hormones of the ovary and testis, most of the studies on control of ovulation in mammals has been concentrated on the gonadotrophins and the ovary itself. Recently new insight has been gained into the feed-back inter-relationship between gonadal and gonadotrophic hormones, which govern the ovarian activity of the mammals (Karsch, Legan, Ryan and Foster, 1973).

Moore and Price (1932) presented evidence for a reciprocal relationship between gonadal steroids and pituitary gonadotrophic hormones. A mechanism which has subsequently called a "negative feed-back"

relationship provides an adequate conceptual basis to explain such findings as gonadal atrophy after prolonged administration of oestrogen or androgens in the intact animals (Greep, 1961) and pituitary hypertrophy and increased gonadotrophic secretion after gonadectomy (Blackwell 1971; Yamamoto, 1970). Hohlweg and Junkmann (1932) showed that the separation of hypophysis from the hypothalamus prevented the hypertrophy of the adenohypophysis after gonadectomy and proposed that the steroid sensitive "Sex centre" in the hypothalamus mediated the effect of gonadal steroid feed-back on the pituitary. Since then the concept of neurohormonal control of the adenohypophysis via the hypophysial portal system has been developed largely, by the work of Green (1951), Harris (1962) and their coworkers. The synthesis and release of the pituitary trophins is triggered by chemical mediators or "releasing hormones" secreted into the portal system at the median eminence and transferred to the hypophysial portal system.

The characteristic pattern of gonadotrophic secretion for instance the pattern of LH secretion during the oestrous cycle of the sheep, as described earlier seem to be the result of two functionally independent secretory systems (Goding et al., 1970; Scaramuzzi et al., 1971). It is accepted that a negative feed-back system operates between output of gonadal steroids and the "tonic" output of gonadotrophins (Brown and Grant, 1971; McCann, 1964).

Although longterm treatment with high dosage of gonadal steroids inhibit the pituitary gonadotrophic function brief treatment with low dosage of the female steroids facilitate releasing of gonadotrophins (see review by Everett, 1961). The mechanism of gonadotrophin release at the preovulatory period, the surge system, is

facilitated by the increasing circulating oestradiol (Goding, Catt, Brown, Kattenbaen, Cumming and Mole, 1969; Kursch and Foster, 1975). This process by which oestrogen elicits, rather than inhibits, the secretion of luteinizing hormone is referred to as the positive feed back action of gonadotrophins.

1.8.1 Modification of gonadal activity through changes in feed-back hormones

Classical method of demonstration of improvement of gonadal activity through negative feed-back involves hemicastration. Hunter (1787) demonstrated that the removal of one ovary did not affect the litter-size in pigs. Fenton (1970) showed in pigs unilateral ovariectomy doubled the ovulation rate in the remaining ovary. Similar phenomenon has been reported in mice (McLaren, 1966) and sheep (Sundaram and Stub, 1967; Land, 1973). Other methods such as immunization to nullify the biological activity of circulating gonadal hormones and controlled delivery of physiological quantities of reproductive hormones have been used to modify the feed-back effect of gonadal hormones.

1.8.2 Immunization against steroids

The concept of neutralization of biological activity of reproductive hormones was developed nearly forty years ago (see reviews by Collip, Selye and Thomson, 1940; Zondek and Sulman, 1942). Steroid hormones are relatively small compounds (Molecular weight 200-400) and as such do not have immunogenic properties. However preparation of steroid-protein conjugates (hapterns), that are immunogenic, (Ealanger, Borek, Beiser and Lieberman,

1959; Goodfriend and Seton, 1958), make it possible to produce relatively specific antibodies to steroid hormones. Such antisera have been extensively used to measure the steroids in biological fluids since the advent of radioimmunoassay (Abraham, 1969; Midgley, Nieswender and Ram, 1969). The action of antibodies against steroids to neutralise the biological activity of steroid hormones lies in their ability to bind hormones in the circulation and thus prevent the steroid performing their normal function in the target organs (Ferin, Zimmering, Lieberman and vande Wiele, 1968). Immunization against steroids can be achieved by two different methods, either active immunization, when the animal produces its own antibodies in response to steroid-protein conjugate, or passively when the antibodies are administered exogenously. Although active immunization allows the achievement of high antibody titres in the circulation for long periods the investigator has little control over the antibody titre achieved by the individual animals. On the other hand passive immunization allows more control over the antibody titre achieved by the animals. However, it suffers from the practical problem of administration of large quantities of antisera and also from the relatively short duration of the activity of the antibody depending on the half life of the immunoglobin administered. There is a considerable amount of literature on the biological effects of immunization against reproductive hormones in the male and the female (see reviews by Martensz, 1977, 1980).

1.8.3 Effect of immunization against steroids in female sheep

Active immunization against 17-oestradiol-6-0-carboxymethyloxime-BSA in female sheep during anoestrous season increased the plasma LH and FSH to values approaching those found in ovariectomised

animals (Martensz, Scaramuzzi, and Van Look, 1979). Similar results have also been reported in the ewe during the breeding season after active immunization against oestradiol 17β (Pant, Dobson, Ward, 1978 and Scaramuzzi, Martensz and Van Look unpublished results). Plasma concentration of LH was also increased in the female rat (Hillier, Groom, Boyns and Cameron, 1975) after active immunization against oestradiol 17β. Active immunization also prevented ovulation and resulted in a marked disruption of the oestrus in rats (Hillier et al., 1975) and in sheep (Scaramuzzi, Baird, Clarke, Davidson, Martensz and Van Look, 1977). Similar treatment resulted in increased tonic LH secretion in sheep, however it stopped the ovulation and oestrus (Rawlings, Kennedy and Hanrieks, 1978). Similarly passive immunization of sheep against oestradiol 17β prevented the preovulatory LH surge, ovulation and oestrus activity (Fairclough, Smith and Peterson, 1976; Rawlings, Kennedy and Hendricks, 1979). Oestradiol 17β has been considered to be the primary steroidol component involved in the mechanism of negative feed-back in gonadotrophic secretion (Goding, Blockey, Brown, Catt and Cumming, 1975; Scaramuzzi, et al., 1971; Karsch and Foster, 1975). However attempts have been made to identify the other steroids involved in feed-back regulation.

Active immunization against oestrone-6 (O-carboxymethyl) oximelBSA in sheep resulted in an increase in plasma gonadotrophin levels to values similar to or even higher than those found in ovariectomised animals (Martensz and Scaramuzzi, 1979). However, these effects at least in part were attributed to neutralization of oestradiol 17β. Unlike the ewes immunized against oestradiol 17β most of the ewes continued to display oestrus and the ovulation rate was increased (Scaramuzzi, Baird, Clerk, Davidson, Martensz and Van Look (1977).

Furthermore, Scaramuzzi, Martensz and Van Look (1980) reported increased plasma LH and to a lesser extent FSH in female sheep actively immunized against oestone. The latter authors also suggested that the immunization against oestrone disrupt the reproductive function by interfering with the feed-back mechanism controlling gonadotrophin secretion. Rawlings, Kennedy and Henrricks (1979) also reported similar results.

Little is known about the function of androgens in the female, however they may be important determinants of sexual behaviour in female primates (Baum, Everitt, Herbert and Kenerne, 1977). Since the androgen can be converted to oestrogens (Baird, Horton, Longlope and Tair, 1968), the androgen may act my modifying the oestrogenic control of gonadotrophin secretion.

Active immunization of ewes against testosterone resulted in castrate-like patterns of LH levels without altering levels of FSH (Martensz and Scaramuzzi, 1975). These animals also do not show oestrus during the breeding season and the majority of the animals were also anovulatory (Scaramuzzi, 1979). In contrast to the results obtained in immunization against oestradiol-17β and oestrone elevated plasma FSH levels were reported in rats after active immunization against testosterone (Hillier, Groom, Boyns and Cameron, 1974). These animals also showed marked disruption of the oestrous cycle. Furthermore Australian workers did not observe an increase in ovulation rate in ewes after passive immunization against testosterone.

During the oestrous cycle, the pattern of ovarian secretion of androsteinedione is closely related to the secretion of oestradiol 17β

(Baird, Land, Scaramuzzi and Wheeler, 1976). Active immunization against androstenedione produce a differential effect on gonadotrophic secretion, resulting in an increase plasma LH concentration and a reduction in plasma FSH levels (Martensz, Baird, Scaramuzzi and Van Look, 1976; Scaramuzzi, Davidson and Van Look 1977; Martensz and Scaramuzzi, 1979). The animals continued to undergo normal oestrous cycles and the ovulation rate was increased (Scaramuzzi, Davidson and van Look, 1977; Van Look, Clarke, Davidson and Scaramuzzi, 1978). Furthermore when the ewes were mated after similar treatment the number of lambs/nursing ewe was increased (Van Look et al., 1978).

1.8.4 Control administration of exogenous steroids

Control delivery of physiological quantities of gonadal steroids through silastic implants (Karsch et al., 1973) has been used to study the feed-back effects of gonadal steroids (Legan et al., 1977). Silastic implant containing oestradiol 17β has led to a constant reduction in ovulation rate of normally prolific Finnish Landrace sheep (R.B. Land unpublished data) confirming the argument that the exogenous oestrogen may supplement the endogenous negative feed-back.

1.8.5 Genetic variation in negative feed-back system

The observation that LH release in ewes of high prolificacy was less sensitive to negative feed-back effect of oestradiol (Land, Wheelerand Carr, 1976) led to the argument that the variation in ovulation rate arose from variation in the sensitivity of the hypothalamus/hypophysis to negative feed-back. In view of similar peripheral plasma FSH and LH concentrations observed between breeds (as

described earlier) the hypothesis is that prolific sheep could tolerate higher concentrations of feed back hormones than could the hypothalamus/hypophysis of low prolific breed hence more follicular development is possible. It is further supported by the observation that the treatment with oestradiol during oestrous cycle had less effect on the ovulation rate of high prolific Finnish Landrace ewes than on the ovulation rate of less prolific Scottish Blackface ewes (Land, 1976).

1.9 CONCLUSIONS

The foregoing section reviews the indirect characters studied to improve ovulation rate and reproduction in mice, pigs and sheep. The indirect criteria that have been studied at the initial stages such as body weight, fleece weight and biochemical polymorphisms do not have any known direct physiological relationships with ovulation rate or with any other component of reproduction. More recent work on the physiologically related traits also do not explain the underlying physiological variation of genetic variation in ovulation rate. The present study seeks to identify an underlying physiological component of ovulation rate, sensitivity of the hypothalamus/pituitary to negative feed-back, which has already been shown to differ between breeds and postulated to be the causal component of genetic variation between breeds. Such a character would be more valuable (1) the higher its heritability, (2) the higher its genetic correlation with the ovulation rate, (3) the easier its measurement (4) if also measured in young animals. At present the measurement of variation of the hypothalamus/pituitary to negative feed-back is not easily done. However it is necessary to identify the genetic nature of the trait before pursuing further research to develop simple methods in measuring the trait.

Chapter 2: Assay methods

2.1 Radioimmunoassay of pituitary gonadotrophins

The pituitary glycoprotein hormones are a unique family consisting of two sub units called α and β, assembled to give a quarternary structure (Pierce, 1971; Sairam, 1978). The glycoprotein gonadotrophins of both pituitary and placental origin, from the same species have virtually identical subunit designated α. The β subunit which is hormone specific confers the biological and immunological specificity to the molecule. The majority of individual subunits are biologically inactive and this property causes some difficulties in the production of specific antisera demanded for radioimmunoassay. The antibody production in another species against these hormones could be directed against the antigenic determinants that are contributed by the α and β subunit portion of the molecule or by a unique conformation structure generated by the noncovalent linkage of the two subunits. Owing to similarities in the subunits, particularly α subunit, antibodies prepared against either follicle stimulating hormone (FSH) or luteinizing hormone (LH) could cross react with other gonadotrophin or with other pituitary hormones of the same type. This problem has been overcome by selection from available antisera which have been carefully selected for specificity.

2.2 LH double antibody radioimmunoassay

2.2.1 Introduction

Plasma LH concentrations were determined by double antibody radioimmunoassay described by Carr and Land (1975) with some modifications. A double antibody equilibrated system was used. Standards (or unknown samples) and anti-ovine LH antisera were incubated for 2-3 days at 4°, after which the labelled LH (^{125}ILH) was added and the mixture incubated for a further 2-3 days. Finally the antigen-antibody complex was precipitated using donkey anti-rabbit gamma-globulin antiserum. After an overnight incubation with the second antibody, 1ml of assay buffer was added, and the tubes were centrifuged 3000 r.p.m. at 4°C for 30 minutes and the supernatant decanted to waste. The precipitate was counted on an automatic gamma-counter with a paper tape output : the data from the tape were analysed by ABRO radioimmunoassay programme package based on the method of Rodbard and Lewald (1970) (see section on Statistical analysis of assays).

2.2.3 Materials

The antiserum used was rabbit anti-ovine LH antiserum validated for specificity and sensitivity by Martensz, Baird, Scaramuzzi and Van Look (1976). The neat antiserum was diluted 1:1000 in assay buffer and stored in 0.5ml aliquotes.

The assay buffer was 0.15 mol/l phosphate saline buffer, pH 7.5, containing 0.1% bovine serum albumin and 0.01% sodium azide.

Highly purified ovine LH (M4; Jutisz and Courte, 1968) was labelled with ^{125}I by chloramine T method (see section on method of labelling). The working soluton of labelled LH was prepared by diluting the appropriate quantity from the stock solution to 100mls in assay buffer. The dilution was done immediately before using. The standards used were prepared from dilutions of purified ovine LH (NIH-LH-S18). The stock solutions were prepared by dissolving 2.06mg in 100ml of assay buffer to give 20.6μg per ml. Working standards for use in assay were prepared by serial dilution of the stock solution in assay buffer.

The samples were stored inpolystyrene 3.0ml cups (Sarstedt) and the reaction tubes were Luckhan's LP4S.

2.2.4 Labelling of purified LH with ^{125}I

Highly purified LH (M4; Jutisz and Courte, 1968) was labelled with ^{125}I by a chloramine T method modified from that of Greenwood, Hunter and GLover (1963). Highly purified 5μg of LH M4 in 5ul of distilled water was added to a clean conical reaction vial. The following solutions were added successively to the reaction vial containing purified LH:

(a) 25ul 0.5m phosphate buffer pH 7.5

(b) 1μl of carrier free ^{125}I (Amersham-Radiochemical centre) followed by mixing the vial thoroughly.

(e) 20μl of 0.4% chloramine T and allowed to react for 2 minutes with shaking.

(d) 100μl of sodium metabisulphite 2.4mg/ml and mix thoroughly.

(e) 200μl of 1.0% potasium iodide and mixed thoroughly

The reaction mixture was counted in the mini-assay counter in a position to give maximum counts. After mixing the contents in the reaction tube the reaction mixture was passed through a 1.5 x 28cm column of sephadex G-50 saturated with bovine serum albumin. The reaction tube was washed with 2 x 200μl of potassium iodide and washing put into the column. The column was allowed to run using phosphate buffer containing 1% bovine serum albumin and 0.01% sodium azide, collecting approximately 25 x 1ml fractions. Then each fraction was counted in a mini-assay counter to give the maximum counts. The tube showing the maximum activity in the first peak off the column was made up to 10ml in assay buffer. This was called the concentrated label and stored at $4^{o}C$. The concentrated label was diluted again in assay buffer immediately before adding to the assay reaction so that 100μl of diluted tracer gave approximately 200ng (i.e. 20,000-25,000 counts per 10 seconds).

2.2.5 Assay method

Using an analmatic sample preparation unit 2 replicates of 200μl were picked from the standard or unknown plasma samples and dispensed into

the reaction tubes, each washed through with 500μl of assay buffer containing rabbit antiserum to ovine LH (final dilution of 1:550,000). Tubes containing this mixture were incubated for 2-3 days at 4°C. Each incubation mixture for the standards contained NIH-LH-S18 in 200μl assay buffer (equivalent to 0.1024, 0.2048, 0.4096, 0.60, 0.819, 1.20, 1.683, 2.56, 3.84, 5.12 and 7.68 ng/ml), while each tube for unknown contained 200μl plasma. Each standard was run in triplicate and each unknown in duplicate. After the first incubation 100μl tracer (I = ^{125} ILH) containing 200pg of ^{125}ILH was added. This solution was added by an automatic device (Microlab, Hamilton). This mixture was then mixed and incubated for 2-3 days at 4°C. Pooled normal rabbit serum (100μl, final dilution 1:200) was then added; followed by donkey antiserum to rabbit gamma-globulin (200μl, final dilution 1:200 with 1:10 EDTA in assay buffer; Guildhay antiserum, University of Surrey). All tubes were mixed in a vortex mixture after adding each reagent. After a further overnight incubation at 4°C 1ml assay buffer was added to each tube as a prewash and they were centrifuged at 1200g for 30 min. in a refrigerated centrifuge at 4°C. The supernatant was decanted to waste immediately after centrifugation, any remaining droplets being aspirated from the rim of the tube. These tubes were counted in an automatic gamma-counter for 100 seconds.

2.2.6 Assay optimization

Optimum sensitivity of the assay was obtained with 20-30% binding of labelled antigen. This was obtained by diluting the neat antiserum to 1:550,000 dilution.

The dilutions of standards used were arranged to fall within the limits of 10-90% B/BO, where B is the amount of tracer bound when the unlabel antigen LH is present in the standard, Bo is the amount of tracer bound when no unlabel antigen is present in the reaction mixture.

As most of the unknown LH concentrations fell within the range of standards the unknown plasma samples were also diluted in the same way as in the standards in the reaction mixture. A plasma volume of 200μl was found to be the compatible plasma volume in the reaction mixture to obtain minimum plasma effect.

2.3 FSH Radioimmunoassay

As it was expected to detect relatively small variations in circulating FSH patterns after modification of hypothalamic pituitary steroid feed-back system, it was necessary to use a very specific and sensitive assay system to measure FSH levels in the experimental plasma samples. A series of experiments were carried out to find out the most sensitive and specific FSH assay. Three different antisera (M91, M94 and Sairam were tested with two different purified FSH preparations (FSH, Papkoff and FSH, Sairam) labelled by two different methods (i.e. Chloramine T and Iodogen). From this experiment, the antiovine rabbit antiserum obtained from Dr. M.R. Sairam was found to be the most appropriate. Antisera were compared for the slope of the standard curve, minimum detectable dose and potency estimates for plasma samples with very high LH values. The results of this experiment aresummarised in table 2.1. Sairam) (1978) reported that this antiserum is conformation dependant and

Table 2.1 Results of the FSH experimental assays with different labels and antisera

Antiserum	M91	M94	Sairam	M91	M94	Sairam	M91	M94	Sairam	M91	M94	Sairam
Antigen	P-FSH(Chl.T)			P-FSH (Iodogen)			S-FSH(Chl.T)			S-FSH(Iodogen)		
% Binding	19.7	25.1	32.9	11.4	13.5	18.5	36.8	42.8	51.2	21.5	39.6	12.7
Slope	-1.20	-1.41	-1.44	-0.97	-0.64	-1.20	-1.12	-1.31	-1.43	-0.91	-0.83	-1.92
Y Intercept	5.0	5.5	4.8	4.5	3.1	4.2	4.8	5.4	5.1	4.2	3.8	5.1
Jutisz M4 RP	13.6	13.1	10.0	14.7	16.1	11.6	13.7	13.0	11.3	14.1	14.1	8.6
FSH Potency Ov ewe ng/ml	569	577	423	922	1045	579	581	671	462	677	650	425
FSH Potency LRF ewe ng/ml	89	96	86	232	254	139	88	96	92	107	93	100
Min detectable dose ng/ml	52	46	2.6	9.6	160	3.8	51	48	8.5	49	120	7.0

Jutisz M4 = Purified LH
Ov Ewe = Ovarietomised ewe plasma
LRF Ewe = Ewes treated with LRF, plasma

required the proper recombination of α and β subunits for maximum reactivity, isolated α subunits are essentially inactive and the hormone specific β subunit is weakly reactive, it showed species specificity and very low cross-reactivity in the order of about 0.05% with highly purified ovine LH and bovine thyrotrophin. Even this small cross-reaction was attributed to possible contamination of FSH. Maximum sensitivity in a double equilibrated double antibody assay system is generally obtained with 20-30% binding of tracer in the absence of free antigen (i.e. zero standard) when the system has 200pg labelled antigen (i.e. 20,000 cpm) in the reaction mixture. As the above antiserum was validated at the dilution of 1:48,000 at which the antibody bound 60% of tracer (^{125}IFSH) and the system had 1ng tracer (i.e. 1000,000 cpm) and the incubation was performed at 22°C, it was necessary to carry out series of experimental assays at a higher antibody dilution and with 200pg ^{125}I labelled FSH. When the system was adjusted to optimum sensitivity and precision it was found that LH did indeed cross-react with the antiserum. In the experimental assays lebelles prepared by two different methods (i.e. chloromine T and lactoperoxidate method) were used. When the plasma samples with high concentrations of LH were assayed at different dilutions in the experimental assays, the plasma samples gave nearly parallel curves to the standard curve. When the results were plotted, logit B/Bo agains log dose of unlabelled antigen or percentage B/Bo agains log dose of unlabelled antigen it indicated a higher degree of cross reactivity with LH (see figure 2.1). However by adding LH (NIH-LH-M4) at the concentration of 5ng/ml to the antibody diluted up to 1:350,000, the curve for plasma samples with high LH became almost flat and percentage binding decreased

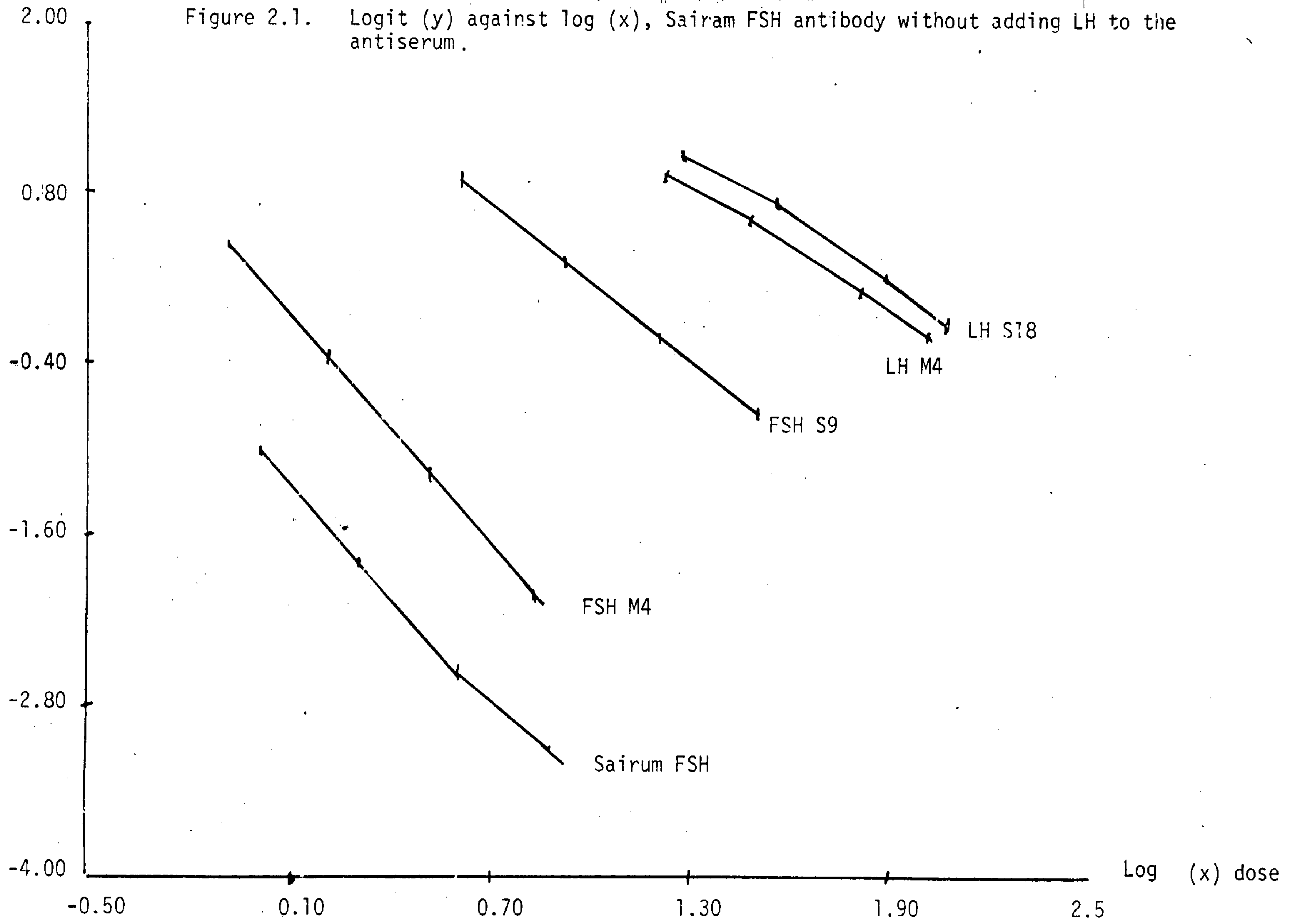


Figure 2.1. Logit (y) against log (x), Sairam FSH antibody without adding LH to the antiserum.

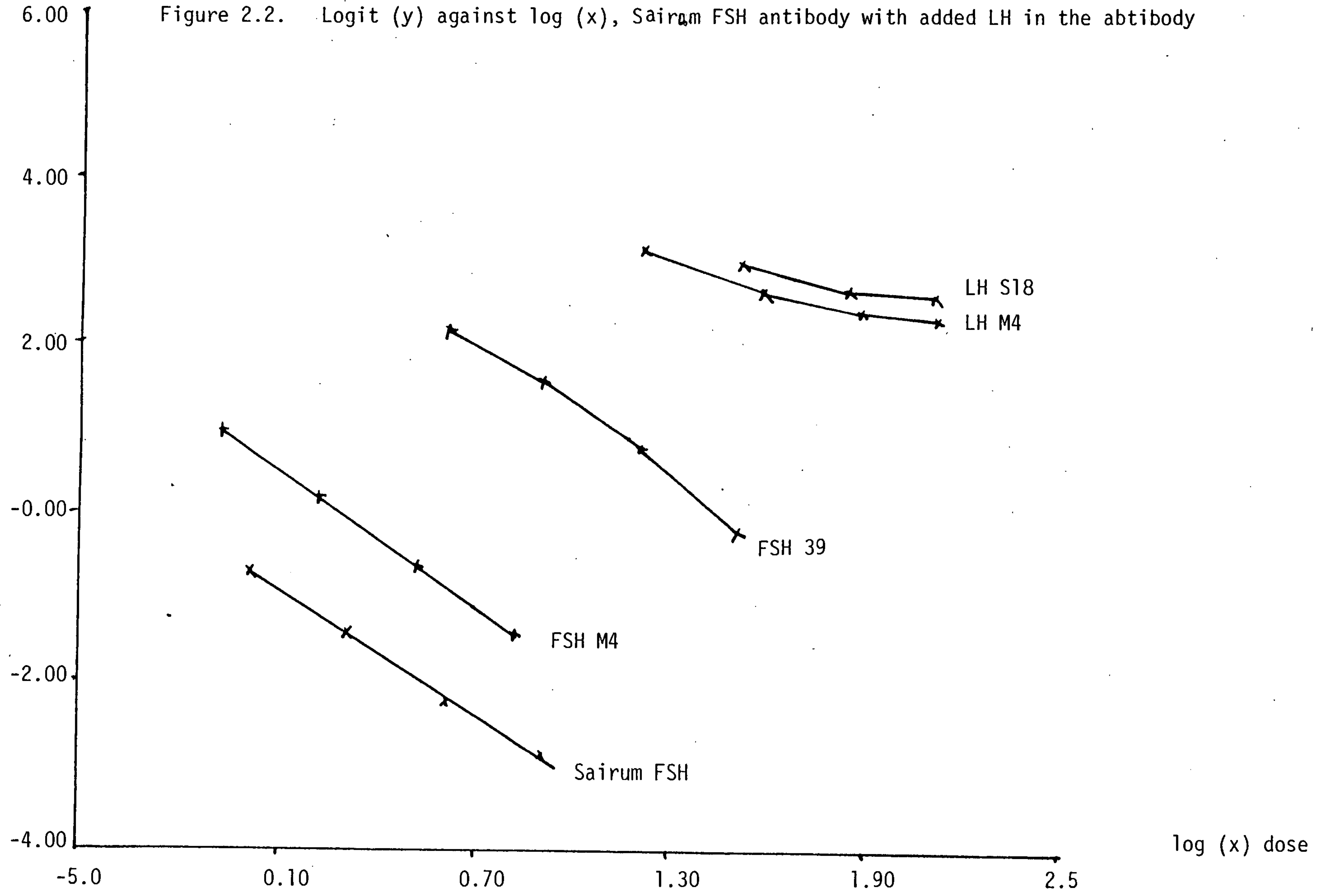


Figure 2.2. Logit (y) against log (x), Sairum FSH antibody with added LH in the abtibody

significantly (see figure 2.2). It indicated a reduction in cross reactivity and the minimum detectable dose was significantly improved. Based on the above results it was decided to add LH (NIH-LH-M4) to the antiserum in order to minimise the cross-reactivity with LH when the antiserum was diluted to obtain a sensitive assay. Labelling by lactoperoxidase method produced a superior label than the chloramine T method and it improved the minimum detectable dose significantly.

2.3.1 Materials

The antiserum was a rabbit anti ovine FSH antiserum (Sairam 1978). The neat antiserum was diluted in 1:100 in assay buffer and stored frozen in 1ml aliquotes.

The assay buffer was 0.15ml/l phosphate saline buffer pH 7.5, containing bovine serum albumin (5g/l) and sodium azide (100mg/l).

Highly purified ovine FSH (Sairam) was labelled with ^{125}I by lactoperoxidase method (see the section on method of labelling).

The working solution of tracer was prepared to obtain 200pg ^{125}I FSH in 100ul of assay buffer by diluting the stock solution in assay buffer. The FSH standard was NIH-FSH-S18. The stock standard solution was prepared by dissolving 1.14mg FSH in 100ml of assay buffer to give 11.4ug FSH per ml. Working standards for use in the assay were prepared by serial dilution of stock solution in assay buffer. The sample tubes were polystyrene 3.0ml cups (Sarstedt) and the reaction

tubes were Luckham's LP4'S.

2.3.2 Method

Plasma FSH levels were determined by a similar system of double equilibrated double antibody radioimmunoassay as described earlier for the LH assay.

Automated analmatic sample preparation unit was used to dispense the assay. The volume of plasma or standard FSH used was 200ul. Each incubation mixture for standards contained NIH-FSH-S18in 200µl assay buffer (equivalent to 16.6, 25.0, 33.33, 50.00, 66.67, 100.00, 200.00, 400.00 and 800.00 ng/ml). The dilution of Sairum antiserum used in the assay was 1:200,000. Each reaction tube contained 200pg of radioactive FSH (^{125}IFSH).

2.3.3 Assay optimization

Optimum sensitivity of the assay was obtained with 20-30% binding of the labelled antigen. This was obtained by diluting the neat anti-serum to 1:200,000. The dilutions of standards used were arranged to fall within the limits of 10-90% B/Bo.

As most of the unknown concentrations fell within the range of standards the unknown plasma samples were also diluted in the same way as for the standards in the reaction mixture. A plasma volume of 200ul was found to be the compatible plasma volume in the reaction mixture to obtained the minimum plasma effect.

2.3.4 Labelling of purified FSH with ^{125}I

Highly purified ovine FSH (Sairam, 1978) was labelled with ^{125}I by the lactoperoxidase method. Buffers used for this technique were prepared without preservatives (i.e. sodium azide or Thiomersilate).

Highly purified 5μg of FSH in 5μl distilled water was added to a clean conical reaction vial. The following solutions were added successively to the reaction vial containing the purified FSH preparation.

(a) 20μl 0.075M PSB.

(b) 1 μl of carrier free ^{125}I (Amersham-Radiochemical centre) and mixed thoroughly by tapping the vial.

(c) 10μl of lactoperoxidase (0.5mg/ml in 0.75 M PSB)

(d) 10μl H_2O_2 (50μl of 30% H_2O_2 in 750ml distilled water) and allowed to react for 3 minutes.

The reaction mixture is counted in themini-assay counter in a position to give the maximum counts. After mixing the counts in the reaction tube the reaction mixture was passed through a 1.5 x 28cm colume of sephadex G50 saturated with bovine serum albumin and continued as with the chloramine T method.

2.4 The capacity of jugular venous plasma to bind exogenous titrated steroids

The binding of the respective steroid (oestradiol 17β, oestrone

androsternedione or testosterone) by jugular venous plasma from the ewes passively immunized against steroids was determined by incubation of plasma with tritiated steroids at 4°C and separation of the free and bound steroids by charcoal absorption according to the method of Scaramuzzi, Corker, Young and Baird (1975).

^{3}H-1, 2-testosterone was obtained from New England nuclear. ^{3}H-2-4,6,7-oestrone, ^{3}H-6,7-oestradiol and ^{3}H-1,2,67-androstenedione were obtained from the radiochemical centre, Amersham. All steroids were reconstituted in ethanol and stored undiluted at 4°C until used.

A range of dilutions of plasma were prepared in phosphate gelatin buffer (PGB). Duplicate aliquotes (0.1ml) were incubated overnight at 4°C with 0.1ml of the tritiated steroid and 0.1ml of PGB. The tritiated steroid solution was diluted in PGB to give 15-20 pg/0.1ml. The free and bound steroids were separated by the addition of 1ml of a charcoal suspension (250mg charcoal and 25mg dextran/100ml of PGB). After standing for 15 minutes, the tubes were centrifuged at 3000 rpm for 30 minutes and the supernatant decanted to scintilation vial containing 10ml of scintillant (Miniria) and the readioactivity measured. The titre of steroid antibodies, defined as the initial dilution of plasma which bound 50% of the radioactive tracer, was determined from a graph of the percentage radioactivity bound (Y-axis) against the dilution of plasma (log-scale) (X-axis).

2.5 Cross reactivity

The cross-reactivity of the antisera used in passive immunization were determined by the method described by Abraham, Odell, Edwards and Purdy (1970).

2.6 Statistical analysis of assays

2.6.1 Radioimmunoassay programme description

All assay counting data were punched on to paper tape by the Wallace Autogamma Spectrometer and analysed using the ABRO radioummunoassay data processing programme package. The package consists of four programmes.

The assay parameters, each identified by a directive are input from a terminal. Parameters not presented are assigned default values. Assay counts are input from paper tape and stored after preliminary checking. The output from programme 1 comprises errors in the parameter list or in assay counts, list of parameters including default values, list of assay counts and statistics for batch and overall standards.

In programme 2 the standard counts are combined with the concentration level information from the input parameters. A calibration curve is calculated by applying the logit-log transformation and iterative weighted least squares regression analyses (Rodbard and Lewald, 1970) to each batch of standards and to the overall standards. The control counts are treated in the same manner. The "edit" option allows the user to select or reject the tube counts for analysis thus overruling or supplementing the screening process. A Scatchard plot is available if requested. The output from programme 2 includes the results of unweighted and weighted regression and a plot of logit (Y) V log (X) for each batch of standards and overall standards (where Y = response variable and X = dose) and for control.

Programme 3 uses analysis of variance to examine the calibration curves calculated by programme 2. Each batch standard curve and overall curve are tested for linearity and the batches are tested for equality. The batch standard and control curves are combined and tested for linearity. The overall standard and control curves are tested for parallelism. A further option calculates optimum assay parameters for maximum sensitivity based on the results of scatchard analyses in programme 2.

In programme 4 potency estimates are calculated for each unknown sample. Upper and lower 95% confidence limits are calculated based on the pooled residual variance of the standard curve and the counts for the samples.

2.6.2 Data checking

The output from program 1 allows easy detection of obvious outliers among the standards or of counts of above zero standard below blank count for which no interpolation would be possible. These counts can be rejected. By running zero-standards at points throughout the assay, a plot of their counts can be obtained in programme 1 allowing easy visual assessment of drift. No significant drift occurred in any of the LH or FSH assays by this assessment, and the equality of the two standard batches was accepted for all assays on an F test.

The F test of homogeneity for the replicates of unknown samples generally has degree of freedom $n_1 = 1$, $n_2 = 50$ in all assays. Although an F value of 5.0 is significant at 5% level on these degrees of

freedom, many greater than this will occur when large numbers of independent tests are performed (say 300 per assay). Thus the cut-off value for F was taken as 10.0 and unknown samples with F value exceeding 10.0 for homogeneity were rejected and re-assayed.

Quality control, linearity, parallelism and slope of dose response curve.

(a) LH assays : Linearity of the overall standard curve and parallelism of the standard and control curves were achieved in all assays (as judged by non-significant F test for non-linearity, non parallelism etc). The slope of standard curve (weighted regression of logit Y on $\log_e$ x, as described) lay between -0.97 and -1.03 in all assays. Mean coefficients of variation for standard counts within assays ranged from about 2.0% for zero standards to about 7.0% for the top standard.

2.6.3 FSH assays

Linearity of the overall standard curve was not achieved in two assays and those two assays were repeated. Parallelism of the standard and control curves were achieved in all the assays. The slope of the standard curve lay between -0.97 and -1.01. Coefficient of variation for standard counts lay in the same range as LH assays.

2.6.4 Quality control

Between and within assay variation. For each hormone assay system between and within assay variation was estimated as follows:

with each batch of standards a pooled ovine plasma sample was run in triplicate at concentrations of 12.5%, 25%, 50% and 100%. Thus two potency estimates were obtained for this sample in each assay, each derived from 12 tubes but on different sections of the standard curve.

All these potency estimates for all assays were tabulated and a one way analysis of variance was performed. For any pair of replicates of an unknown sample, a mean potency estimate can be obtained with expected variance $\sigma^2 e$, the within assay error variance. For any pair of replicates in separate assays, the potency estimate will have the same mean but an expected variance of $\sigma^2 e + \sigma^2 A$, where $\sigma^2 A$ is the between assay component. Estimates of σ^2_e and $\sigma^2 A$ were obtained by the analysis described which gave results of the form:

Source of variation	Expected mean square
Assay	$\sigma^2 e + 2\sigma^2 A$
Residual	$\sigma^2 e$

The coefficient of variation % between assays was taken as:

$$\frac{\sqrt{\sigma^2 e + \sigma^2 A} \times 100}{u}$$

Where u = mean potency estimate. The coefficient variation of within assays was taken as:

$$\frac{\sqrt{\sigma^2 e} \times 100}{u}$$

For LH assays, within assay variation was 7.9% and between assay variation was 10.1%

For FSH assays, within assay variation was 6.7% and between assay variation was 9.1%

2.6.5 Sensitivity (LH assays)

The hormone concentration corresponding to a level of two standard deviations away from the zero standard (minimum detectable dose) was between 0.123 and 0.243ng/ml in all assays, with a mean of 0.183ng/ml.

2.6.6 Sensitivity (FSH assays)

The hormone concentration corresponding to a level of two standard deviations away from the zero standard (minimum detectable dose) was between 26.35 and 34.36ng/ml in all assays, with a mean of 29.31 ng/ml.

Chapter 3: Modification of gonadal function through negative feed-back

3.1 Introduction

It is well known that the normal gonadotrophic stimulation is buffered through negative feed-back. Gonadotrophins from the pituitary stimulate the gametogenic and the hormonal activities of the gonads and the gonadal hormones in turn feed-back on the hypothalamus/ pituitary to control gonadotrophin release. At present improvement of gonadal function in the female depends on the use of exogenous gonadotrophins. High variation in response to exogenous gonadotrophins tends to limit such techniques to special circumstances such as superovulation for embryo transfer. However recently there has been much emphasis on the feed-back relationships between gonadal and gonadotrophic hormones in mammals (Karsch Legan, Ryan and Foster, 1977). Improvement of gonadal function through modification of negative feed-back of gonadal steroids has been considered (Land, 1979), and between breed variation in ovulation rate in sheep was postulated to arise from variation in sensitivity of the hypothalamus/pituitary to negative feed-back.

Biological activity of the circulating steroid hormones can be neutralised by using antibodies against steroids (Ferin, Zimmerring, Liberaman and Vande Wiele, 1968). Active immunization against androstenedione (Scaramuzzi et al, 1977) and oestrone (Marsensz et al 1978) increased the ovulation rate in ewes. Van Look et al (1978) also reported an increase in litter size after active immunization against androstenedione in sheep. However several workers have reported

disruption of the oestrous cycle and anovulation in sheep after active immunization against oestradiol 17β, oestrone and testosterone as described in the chapter 1. Active immunization does not allow control of the antibody titre achieved by individual animals because of the variation of immunogenic potency between animals (Oosterlee, 1981).

Gonadotrophic releasing patterns after active and passive immunization has been reported (Marsensz, 1977; Rawlings et al 1976). However most of such studies have been done either when the animals are anovulatory after the treatment or during the anoestrus season. When the animals are immunized passively against gonadal steroids without disrupting the oestrous cycle the changes observed in the gonadotrophic release have not been reported.

The work described in this chapter was undertaken to investigate the effect of passive immunization against oestrone, oestradiol 17β, androstenedione and testosterone on the plasma gonadotrophin patterns, ovulation rate, oestrus and oestrous cycle length. The possibility of increasing the litter size through modification of negative feed-back using passive immunization was also examined.

3.2 Materials and Methods

3.2.1 Experimental plan

Sixty Welsh Mountain ewes were divided into 5 treatment groups of equal size. One group was kept as control and the other groups were passively immunized against either oestrone (E_1), oestradiol 17β (E_2), androstenedione (A) or testosterone (T) at the

beginning of the breeding season. Each antiserum treatment group was subdivided into low and high levels and given x and 5x units of antiserum respectively at the first treatment. Before and after the first antiserum treatment gonadotrophin releasing patterns were studied. Animals were given antibody on day 10-12 of the first cycle and thereafter antibody treatment was given on day 11 of the oestrous cycle. Animals were further immunized for another two cycles with increasing antibody dose and thereafter kept untreated for two more cycles. After two untreated cycles animals were treated again with antiserum and at the next oestrus all the animals were hand mated. Throughout the experiment ovulation rate, oestrus and oestrous cycle length were recorded.

3.2.2 Animals

Animals used in this experiment were Welsh Mountain ewes. Sixty fertile 2½-3 year old Welsh Mountain ewes were brought to the Animal Breeding Research Organisation (ABRO) Field Laboratory, Roslin, Midlothian on 12th September 1979, from the ABRO farm, Rhydyglafes in North Wales. Animals were kept outside the paddocks throughout the experiment except when moved inside for pre-laparoscopy fasting, serial bleeding or antibody injection. To supplement grass, the animals received hay and cereal concentrates during the winter months. Animals had access to fresh drinking water all the time. Whenever the animals were fed inside they were given hay.

3.2.3 Antisera

Antisera raised against 17β-oestradiol-6-(O-carboxymethyl) oxime-bovine serum albumin (oestradiol-6-BSA), oestrone-6-(O-carboxymethyl)

oxime-bovine serum albumin (oestrone-6-BSA), androstenedione -11-α -hemisuccinyl-bovine serum albumin (androstenedione-11-BSA) and testosterone-3 (O-carboxymethyl)-oxime-bovine serum albumin (testosterone-3-BSA) were used in this experiment. Antisera made in castrate male sheep against each of the above conjugates namely oestrone (E_1), oestradiol 17β(E_2) androstenedrone (A) and testosterone (T) were kindly supplied by Dr. B.A. Morris of the University of Surrey. In each of the antisera the quantities obtained from different animals were pooled together to form a pooled antisera against E_1, E_2, A and T. Sodium azide had been added to antisera as a preservative. As sodium azide is toxic to animals, it was necessary to remove as much as possible from the antisera. All four pooled antisera were dialysed against normal saline in order to remove sodium azide.

Twenty to twnetyfive CM long sengments of celophane dialysis tubing (Visking, tubing, scientific Instrument Centre Ltd., London, diameter 0.5cm) were immersed in distilled water for 30 minutes before use. Tubes were filled with antiserum and the ends were secured with double knots. The tubes filled with antisera, were placed in 3 litre plastic containers filled with 2 litres of nomal saline (0.9% NaCl) for 30 hours at 4°C. During dialysis saline solutions in the buckets were changed twice. After a 30 hour dialysis antisera were stored in 300ml and 50ml screw capped glass bottles at -20°C until they were used.

Sample collected from each of the antibody pools after dialysis was used to determine the antibody titre and the cross-reactivity. The titres were determined by the method described in chapter 2 and the results are shown in Table 3.0. The cross reactivity was determined

Table 3.0: Antibody titre of the antisera used in passive immunisation

ANTIBODY	TITRE
E_1	1:13,500
E_2	1:52,000
A	1:17,000
T	1:26,000

by the method described by Abraham et al 1970. Table 3.1 presents the cross reactivity of each antisera against the tested. The frozen

Table 3.1 Specificity of antisera to oestrone (E_1), oestradiol 17β (E_2), androstenedione (A) and testosterone (T) (percentage cross reaction).

COMPOUND TESTED	SPECIFICITY OF ANTISERA			
	E_1	E_2	A	T
OESTRONE	100	3	<0.001	<0.001
OESTRADIOL 17β	3.5	100	<0.1	<0.001
OESTRADIOL 17α	0.1	1	-	-
ANDROSTENEDIONE	<0.001	<0.001	100	0.5
DEHYDROANDROSTEROL	-	-	0.5	-
TESTOSTERONE	<0.001	<0.002	0.15	100
5α HYDROXYTESTOSTERONE	-	-	0.3	29
PROGESTERONE	<0.002	<0.002	<0.002	<0.002

samples of antisera were thawed immediately prior to injection in a water bath at 38°C. Care was taken to thaw only the required quantity of antiserum at each occasion. Samples thawed and stored were not used later.

3.2.4 Recording of oestrus

Rams with mating harness and crayon (Radford, Waston and Wood, 1960) were used throughout the experiment to detect ewes in oestrus. Vasectomised Finnish Landrace rams were used to detect oestrus as they are reported to be the most reliable breed for detecting sheep in oestrus (A. McGregor, personal communication). Ewes were run with two vasectomised Finnish Landrace rams. Animals were examined everyday between 0800-0900 hours and 1600-1700 hours. Ewes with colour marks on the rump were recorded as having been in oestrus and that day was counted as day zero of the oestrous cycle. Every ten days the colour mark of the raddle was changed.

3.2.5 Recording of ovulation rate

Ovulation rate was determined by direct observation of ovaries using a laparoscope. The technique was first successfully applied to record ovulation rate in ewes by the workers at Laboratoire de Physiologie de la Reproduction, Tours, France and has been described by Roberts (1968) and Thimomier and Mauleon (1969). Laparoscopies were performed between the 4th and 12th day after oestrus, this being the time when corpora lutea are largest and easily identified (Restall 1964). The ewes to be examined were brought in late afternoon of the previous day and starved of food and water. Immediately before laparoscopy animals were anaesthetised by using halothane (Flueothane ICI) through a gas mask.

When the animal was anaesthetised it was kept on a trolley with the ventral surface up and the legs were fixed securely to the trolley. When the animal was in that position the ventral and lateral abdominal wall was clipped from the mammary gland up to the umbilicus area by using electric clippers. The clipped surface was cleaned thoroughly by using a warm savlon solution (ICI) and dried with clean paper towels. Anaesthesia was maintained by using a gas mixture of halothane and oxygen applied through a face mask. The clipped abdominal wall was again sterilised with a Hibitane Solution (2:3:15 Hibitane (ICI), distilled water, industrial methylated spirit) and dried with sterilised paper towels. Leaving a trangular clean area outer abdomen was covered with sterilised paper drapes held in place with towel clips. The trolley was tilted from the rear end of the sheep (about 10^0) so that the abdominal wall was flat, in order that the trocar was less likely to penetrate the rumen or any other abdominal organ. Two skin incisions were made either side of the mid ventral line about 15cm anterior to the mammary gland and 10cm lateral to the midline. The incision on the right side was about 0 5cm long; the incision on the left side was about 1cm long. The incisions facilitates the insersion of the trocars and cannulæ easier. The small troca and cannula was inserted through the small (offside) skin incision the trocar withdrawn and the manupulator introduced through the smallcannula. The large trocar was inserted through the large incision on the skin. The trocar was withdrawn and the telescope and light source combination was inserted through the cannula. Nitrous oxide gas (British oxygen company) was introduced through the tube connected through the large cannula into the abdominal cavity at the rate of about 5 litres/minute (measured with an oxygen flow meter) until the abdomen sounded hollow and firm to

pressure. While observing the abdominal contents through the telescope the trolley was gradually tilted further, such that the animal's head was lowered. The omentum followed by the viscerae moved forward exposing the uterus. Location and examination of the ovary suspended on the broad ligament was facilitated by the manipulator inserted through the small cannula. If the trolley was tilted too far the uterus fell over the ovaries and it was difficult to observe ovaries. If the trolley was further tilted the bladder would fall over the uterus. In this event it was necessary to tilt the table to the opposite direction, back to the original position, such that the bladder, uterus, viscerae and omentum all fell back into place. The tilting porcess was then repeated. After examining all the surfaces of both ovaries the number of corpora lutea present in each ovary was recorded. The trolley was tilted back to the horizontal position and small cannula manipulator and telescope were withdrawn and as much nitrous oxide as possible was expelled. The large cannulawas withdrawn and optocal (Willington, medicals), bacteriostatic powder, was introduced into the skin incisions. The skin was then closed over with Michel clips, usually one for the small incision and two for the large incision. The clips were removed about 2 weeks later, or prior to the subsequent laparoscopy. The layers of the abdominal wall tended to slide over so that the holes were discontinuous. Four ml of Streptopen (Procanine penicilline - G and dihydrostreptomycin Glaxo, Laboratories) were injected into the rump muscles. The face mask was removed and the animal was lifted to the floor of the recovery pen. Animals were observed until recovery was certain and kept in the recovery pen with hay and water until late afternoon and then taken back to the paddock.

3.2.6 Synchronisation of oestrus

On 25th September (1978) progestagen impregnated vaginal sponges (Veramix, Upjohn) were inserted into the vagina of all the animals except for the animal 5FL003. Animal No. SFL003 had a small vaginal orifice and it was anaesthetised using halothane (Flueothane ICI) and a progesterone implant was introduced subcutaneously to the left axillary region. Vaginal sponges were removed from all the animals after 14 days (9th October in the afternoon). The progesterone implant from SFL003 was also removed at the same time under halothane anaesthesia. Two vasectomised Finnish Landrace rams with mating harnesses were introduced to the ewes. From the following morning onwards ewes were observed for oestrus twice daily and animals in oestrus were recorded. Of the 60 animals 50 animals showed oestrus on the 11th and 12th of October. Another 8 animals showed oestrus either on 9th October or after 12th October. Progesterone impregnated vaginal sponges were introduced to the 10 animals that did not show oestrus either on 11th or 12th October, on 19th October the sponges were withdrawn seven days later (on 26th October) and 100mg of prostaglandin $F_{2\alpha}$ (Estrumate ICI) was injected intramuscularly in order to synchronise oestrus of these 10 animals with the expected oestrus of the other 50 animals. All the animals were laparoscopied on 17th, 18 and 19th October and ovulation rate was recorded.

3.2.7 Antiserum units

As there are no proper data available on the passive immunisation against gonadal steroids in sheep the work done by Fairclough et al (1976) was taken as the basis for the antiserum treatment. Although it was intended to give a lesser dose than the latter workers, the appropriate

dose was not known, so that two lower doses were selected arbitrarily. The dose selected had a five fold difference at the initial antibody injection. A unit dose (x) of each antiserum is the volume of the particular antiserum that is equivalent to 5ml of antiserum with a titre of 1:52,000.

3.2.8 Serial bleeding

The fifty animals that came into oestrus on 11th and 12th October were randomly divided into five groups of equal size. The five groups were control (C) and treated with either anti-oestrone (TE), anti-oestradiol 17β (TE_2) anti-andradtenedione (TA) or anti-testosterone (TT) serum. Experimental animal groups are shown in Table 3.2. Animals were brought in on 21st October in late afternoon and both sides of their necks were clipped in order to facilitate easy bleeding from the jugular vain. Animals were allocated to a row of ten pens (7' x 10' each bedded with wood shavings). Each pen had five animals one animal randomly taken from each of the five treatment groups. Animals had access to hay _ad-libitum_ and fresh drinking water.

All these animals were in day 10-12 of the oestrous cycle on 22nd October. Starting from 0800 hours on 22nd October blood samples were collected from all the 50 animals every half hour until 1330 hours. Bleeding was by acute puncture of the jugular vain using a 26G x 1½" vacutainer needle (Beckton, Dickinson and Co.) into an evacuated heparinised 10ml glass tube. Ten ml. of blood was collected from each animal on each occasion. Blood samples were centrifuged in a refrigerated centrifuge at 4°C and plasma was separated and each sample

Table 3.2 Number of animals in each of the treatment group during the six oestrous cycles

OESTROUS CYCLE	LEVEL	TREATMENT GROUPS				
		TE_1	TE_2	TA_1	TT_1	C
1	L	5	5	5	5	10
	H	5	5	5	5	
2	L	6	6	6	6	12
	H	6	6	5	5	
3	L	5	6	6	6	12
	H	6	6	5	5	
4	L	5	6	6	6	12
	H	6	5	5	5	
5	L	5	6	6	6	12
	H	6	5	5	5	
6	L	4	5	5	5	10
	H	5	4	4	4	

L = Low level; H = High level

TE, TE_2, TA, TT and C are treated with anti-oestrone, anti-oestradiol 17β, anti-androstenedione, anti-testosterone and control respectively.

was stored in two separate 3ml polystyrene cups at $-20^{\circ}C$ until assayed. At 1400 hours all the animals were injected with either saline, anti-oestrone, anti-oestrodiol 17β, anti-androstenedione or anti-testosterone serum intravenously through the jugular vein. Control animals were injected with 50ml sterile normal saline. Other animals were given 'x' or '5x' volumes of anti-serum to the low and high levels respectively. Animals were restrained and the injections were given as slowly as possible in order to minimise cardiovascular reactions. Blood sampling was resumed at 1430 hours and continued every half hour until 0730 hours the following day. Blood samples were not collected from the animals at 1930, 2000, 0200 and 0230 hours in order to rest the animals. The bleeding schedule is schematically illustrated in Figure 3.0.

3.2.9 Antiserum treatment in the following cycles

After serial bleeding all the sixty animals were kept together in a single paddock with two raddled vascetomised rams. All 60 animals came into oestrus approximately 17 days after 11th October (around 27th October). From the ten animals that were not originally allocated to treatment groups, two animals were randomly allocated to each of the five treatments. Animals were treated in the following two cycles on day 11th of the oestrous cycle. Control animals had the same treatment as before. High level had 10x and 5x and the low level had 5x and 7½x units of antiserum respectively in the two successive cycles. Thereafter animals were kept untreated for two more cycles. After two untreated cycles two animals were randomly taken from each of the five groups to be treated with a mixed antiserum. All the animals from the previous four groups treated with

Figure 3.0. Blood sampling schedule for Welsh Mountain ewes

Date 22/10/79

Sample	1	2	3	4	5	6	7	8	9	10	11	12		13	14	15
Time	0800	0830	0900	0930	1000	1030	1100	1130	1200	1230	1300	1330	1400 ↑ Antibody Injection	1430	1500	1530

Sample	16	17	18	19	20	21	22			23	24	25	26	27	28	29
Time	1600	1630	1700	1730	1800	1830	1900	1930	2000	2030	2100	2130	2200	2230	2300	2330

Date 23/10/79

Sample	30	31			32	33	34	34	36	37	38	39	40	41
Time	0100	0130	0200	0230	0300	0330	0400	0430	0500	0530	0600	0630	0700	0730

antiserum were given 5x dose of their respective antisera. The actual volumes of antiserum given to each of the sub-groups in the four treated occasions are given in Table 3.3. The new treatment group formed was given a mixture of antiserum which contained 1.25 x units of antisera of each of the four antisera. The composition of a single dose of mixed antiserum is given in the Table 3.4. When the volume of antiserum given to an animal exceeded 100ml, treatment was given in two equally divided doses in the morning and afternoon. Oestrus was recorded throughout the experiment. Ovulation rate was recorded after each oestrus.

During the course of the experiment 4 animals died. One animal died during surgery. Three animals that were sick before death were autopsied by the Royal Dick Veterinary School, Edinburgh. Postmortum examinations revealed that two animals died of accute pasturella pneumonia and the other from an undiagnosed oesophagial haemorrhage.

3.2.10 Mating of ewes after last treatment

When the animals were found in oestrus after the last treatment ewes were hand mated twice, immediately after heat detection and approximately 12 hours later. Scottish Blackface rams were used for matings. All the ewes were managed as a group and the animals returning to oestrus after mating were recorded. Their lambings were recorded in July 1980.

3.2.11 Blood sampling for antibody titration

After the first antibody treatment a blood sample was collected from all the animals on day 13 of the cycle. In the following treated cycles blood samples were collected

Table 3.3: Volume (ml) of antiserum given to each of the levels in the four treated cycles.

OESTROUS CYCLE	Level	UNIT DOSE	TREATMENT GROUPS			
			TE_1	TE_2	TA_1	TT_1
1	L	X	19.25	5	15.29	10
	H	5X	96.25	25	76.45	50
2	L	5X	96.25	25	76.45	50
	H	10X	192.5	50	152.9	100
3	L	7½X	144.5	37.5	114.67	75.00
	H	5X	96.25	25	76.45	50
6	L	5X	96.25	25	76.45	50
	H	5X	96.25	25	76.45	50

Table 3.4: The composition of a single dose of mixed antiserum

ANTISERUM	VOLUME (ml)
E_1	24.06
E_2	6.25
A_1	19.11
T_1	12.5
	61.92

on day 5, 11 (immediately before antiserum treatment) and 13. In the untreated cycles samples were collected only on day 5 and 11.

3.2.12 Measurement of hormone levels

In the samples collected during the intensive bleeding LH and FSH levels were determined before and after antiserum treatment. There were 12 samples before the antiserum injection and 31 samples after the antiserum treatment.

Estimation of LH levels in the plasma samples was done by a modified double antibody radioimmunoassay method described by Carr and Land (1975). Plasma FSH levels were determined by a double-antibody radioimmunoassay method described in the Chapter 2. Samples collected from a single animal were run in a single assay for each hormone except for a small number (about 5%) which had to be reassayed. Samples collected from all the five animals in an individual pen were assayed together in a single assay. Groups of 15 samples were randomised in sequence for the assay.

3.2.13 Antibody titration

Titre of the antibody in the jugular venous plasma for the four antisteroid antibodies were determined by the method of Scaramuzzi et al (1975).

3.3 Statistical analysis

Statistical programme

The analysis of the data in this experiment was performed using statistical package GLIM (Royal Statistical Society, London).

The GLIM programme is an iterative maximum likelihood programme which may be used to fit linear models with various link functions. An identity link function gives the normal analysis of variance linear model with parameter estimation after one cycle of iteration.

3.3.1 Number of LH pulses

An objective method of determining the LH pulses was used (Brinkly, Willfinger, and Young, 1973; Wilfinger, 1974). For the purpose of determination of LH pulses the data collected from each animal was treated initially as a separate set of data. In each animal LH concentration was shown to be skewed to the right (significant coefficient of Skewness, q_1) due to small number of higher concentrations associated with LH pulses. Figure 3.1 illustrates the nature of the LH data from nine individual animals, one animal taken from each treatment group and level. Further analysis indicated that the data actually consisted of two populations of concentrations. To separate the plasma sample concentrations into their respective populations the sample values were ranked from the highest to the lowest concentrations. Higher values were removed one by one from the data set until q_1 was no longer significant. The critical value for the coefficient of skewness was taken as 0.062 which is tabulated as the 5% point based on the sample size of 30 (see table 34B of tables for statisticians and biometricians vol. 1970). The remaining data subset of smaller concentration values were normally distributed. This latter subset was designated the "baseline population". For each animal a cut off point was calculated as baseline + 2.5 standard deviations. The mean and the standard deviation was calculated for the baseline population, Successive concentrations

arrow indicate the time of treatment

- - - Control
——— Control

LH POTENCY ng/ml

0 2 4 6 8

0 5 10 15 20 25

TIME IN HOURS

- - - Oestrone low
——— Oestrone high

OESTRONE

LH POTENCY ng/ml

0 2 4 6 8

0 5 10 15 20 25

- - - Oestradial low
—— Oestradial high

LH POTENCY ng/ml

8

6

4

2

0

0 5 10 15 20 25

TIME IN HOURS

ANDROSTENEDIONE

- - - Androstenedione low
—— Androstenedione high

LH POTENCY ng/ml

10

8

6

4

2

0

0 5 10 15 20 25

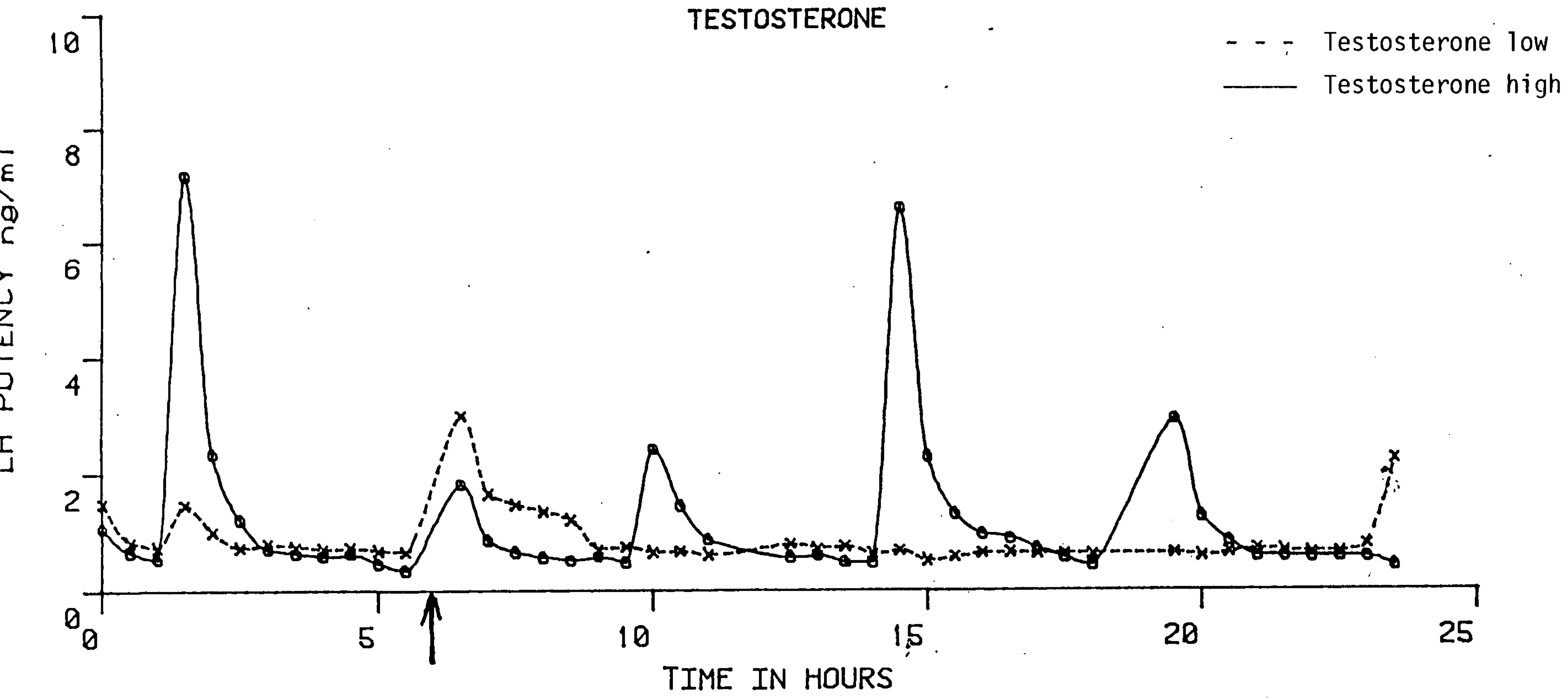


Figure 3.1

above the baseline which contained one or more concentrations that exceeded 2.5 standard deviations of the baseline were significant ($P<0.05$) and indicated the presence of a real surge. Where 3 or more consecutive samples lay above the cut off point the number of pulses in that set of samples was taken to be the number of peaks observed, two peaks being separated by a sample whose potency was at least one standard deviation less than the adjacent peak.

Presence of LH pulses was determined as above and the pulses were calculated for each animal for the first 12 samples and the subsequent 31 samples. Statistics obtained by this procedure were objectively derived and permitted the objective numerical description of each animal's LH pulse during the sampling period. Since the samples were collected every half hour; the number of true pulses occuring may differ considerably from the number recorded because of the missing peaks but it is assumed that the bias is consistant throughout all treatment groups.

LH pulses in the first 12 samples were analysed in all the treatments and levels to detect the presence of chance differences among treatments and levels. In the model treatments and levels within treatments were fitted as factors. In the analysis it was assumed that the number of LH pulses have a poission distribution. The GLIM programme was used to analyse the data with the poission error and with log link function.

LH pulses in the 31 samples collected after the antiserum treatment were also analysed in the same way as the first 12 samples. In the model treatment and levels within treatments were fitted as

factors. A treatment x level, interaction term was also fitted.

3.3.2 Mean LH concentrations

Another analysis was done to determine the differences in the mean LH levels in the treatment groups. As the consecutive samples are highly correlated every other sample was considered in this analysis. Samples collected every hour were subjected to analysis of variance. To bring the distribution of LH values closer to normality the LH data were transformed into logarithms. Therefore analysis of LH data were conducted on a log scale. In the model whole set of data were considered together. The total set of 43 samples were devided into five occasions as follows:

Sample 1-12	Occasion 1
Sample 13-18	Occasion 2
Sample 19-23	Occasion 3
Sample 24-32	Occasion 4
Sample 33-43	Occasion 5

After dividing the 43 samples to five occasions the design of the experiment is as follows.

		GROUP				
		1	2	3	4	5
Occasion	1	0	0	0	0	0
	2	0	1	2	3	4
	3	0	1	2	3	4
	4	0	1	2	3	4
	5	0	1	2	3	4

Group 1,2,3,4,5
Occasion 1,2,3,4,5

Treatment 5

(1) 0 = No antiserum
(2) 1 = Anti-E1
(3) 2 = Anti E2
(4) 3 = Anti A
(5) 4 = Anti T

For the purpose of the analysis all the cells with zero values were coded as treatment 1 and the cells with either 1,2,3 or 4 were coded as treatment 2,3,4 and 5 respectively. In the model treatment, occasion, levels within treatment and animals within group were fitted as factors. Treatment x occasion, treatment x level two way interactions and treatment x occasion x level three way interaction were also fitted with the main effects. In this analysis treatment x animal interaction was not considered.

3.3.3 Mean FSH concentrations

FSH potency data obtained in the first 12 samples collected before and 31 samples collected after the antiserum treatment were transformed into logarithms to bring the distribution closer to normality. Before analysing the total data set the first 12 samples from all the animals were subjected to analysis of variance to look for chance differences between the treatments and levels. In the model treatment, levels within treatment and animals within levels within treatment were fitted as factors.

In the final analysis all the 43 samples were considered together. The 43 samples collected during the sampling period were divided into 5 occasions as follows.

Sample 1-12	Occasion 1
Sample 13-18	Occasion 2
Sample 19-23	Occasion 3
Sample 24-32	Occasion 4
Sample 33-43	Occasion 5

After dividing the 43 samples to five occasions the design of the experiments was equivalent to mean LH analysis. There were 5 groups, 5 treatments and 5 occasions. Data were subjected to analysis of variance. Treatment, occasion, levels within treatment and animals within group were fitted as factors in the model. Treatment x occasion, treatment x levels two way interaction and treatment x occasion x level three way interaction terms were fitted with the main effects. In this analysis treatment x animal interaction term was not considered.

3.3.4 Ovulation rate

In order to investigate the chance differences between the treatments and levels the pre-treatment ovulation rate data were subjected to analysis of variance with the terms fitted for treatment and levels. In this analysis ovulation rate is assumed to have a normal distribution.

To test for any seasonal variation in ovulation rate the control animal ovulation rate data were subjected to an analysis of variance. The factors fitted were animals and occasions.

To investigate the effect of the antiserum treatment on the ovulation rate of the seven ovulations (occasions) the ovulation rate data from all the treatment were subjected to analysis of variance. In the model used treatment, occasions and animals were fitted as factors and the titres for each of the antisera (E_1,E2, A, T) were fitted as covariates. For the titres linear and quadratic regression terms were fitted.

The test for the effect of the mix antiserum given to a treatment

group of animals at the seventh occasion (ovulation) a separate analysis was done after coding those animals as 'mix'. Full model fitted for total ovulation data was fitted with the extra term 'mix' and the factor 'mix' was tested against the error from the full model.

3.3.5 Oestrous cycle length

For the analysis of oestrous cycle length for the six occasions (cycles), cycle length was considered to be normally distributed. Number of models were fitted to oestrous cycle length data. However the best fit was found in the model which included treatment, occasion, animal and treatment x occasion interaction.

3.3.6 Oestrus

In this experiment for the animals in the six occasions (oestrous cycles), animals were coded 1 for showing oestrus and 0 for not showing oestrus. The oestrous data were analysed using a binomial model. In the model treatment, occasion and animals within treatment were fitted as factors. A treatment x occasion interaction term also was fitted.

3.3.7 Lambing data

Two analysis were done for the lambing data. A GLIM analysis was done looking at the proportion of lambs born alive per ovulation observed. The terms fitted were treatment, ovulation rate and the interaction between the two terms. The interaction term was not significant and it was dropped from the model. The ovulation rate effect was substantially bigger and it was kept in the model. In this analysis a binomial model was used.

Two tables were prepared from the proportion of ovulation and lambs born in each group as follows:

Ovulation

	0	1	2	3
Control		10		
E1		4	4	1
E2	2	1	3	3
A		4	5	
T		5	5	
Mix		7	3	

Lambs

	0	1	2	3
Control	2	8		0
E1	3	5	1	0
E2	4	5		0
A	2	5	2	0
T		4	5	0
Mix	1	7	2	0

Various tests were carried out on these tables: either randomisation test of the whole table or Fisher's exact test of difference between each treatment and the control group.

3.3.8 Antiserum titre and half-life of antisera

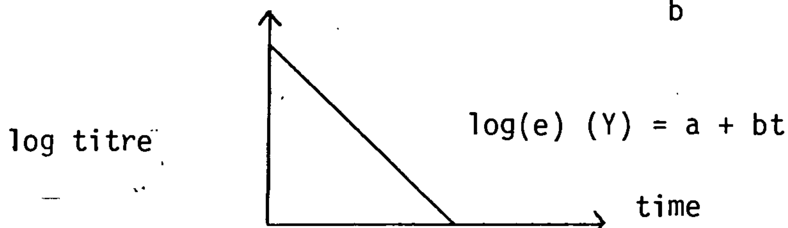
Antiserum titres obtained after the first antibody injection were subjected to analysis of variance in order to determine the

differences between treatments and levels.

The data used to estimate half-life are from the titres observed between day 13 of the 3rd oestrous cycle and day 11 of the 6th oestrous cycle, as the animals were not injected with antiserum during this perdiod. Considering the day of injection (11th day of the 3rd oestrous cycle) as day zero and assuming a 17 day oestrous cycle the titres considered were those measured on days 2, 11, 17, 28, 34, 45 and 51 after the injection. Thus there were seven occasions per animal and 12 animals per antiserum treatment group, each treatment being sub-divided into two levels of 6 animals. The two levels differed in the sequence of antiserum injections received during cycle 1-3. After the 3rd antiserum injection it was intended that the titres would be similar in the two levels. The object of this analysis was to investigate the rate of "decay" of the antiserum within the animals and to produce an estimate of half life of each of the antisera.

It was assumed that the change in titre over time of each of the antisera might be modelled by the function $y = e^{a+bt}$ where t is time, y is titre and e, a and b are constants.

$$\log y = a + bt \text{ and so } t = \frac{\log(Y) - a}{b}$$



Let b be defined as the rate of decay of antiserum. The half-life is defined as the time taken for the titre to halve in level and it can be shown that the half life is independent of the initial titre

Let to be a time when titre y was attained.

Let tl be a time when titre y/2 was attained

$$\text{Then half-life} = t1 - to$$

$$= \frac{(\log (Y/2)-a)-(\log(y)-a)}{b}$$

$$= \frac{1}{b} \log (\tfrac{1}{2})$$

$$= -(\frac{1}{b}) \log 2 \text{ which is}$$

independent of initial titre.

To estimate the half-life, log titre was regressed on time: the slope of the regression line and the associated standard error was estimated. The half-life was calculated as $(-\frac{1}{b}) \log 2$ with a standard error of $(S/b^2) \log 2$ where S is the standard error of the regression coefficient.

The statistical package GLIM was used to estimate the regression and each antiserum group was treated separately but in the same way. Regression lines were fitted to log (titre), different lines being fitted to each animal's response. The model may be summarised by:

$$Y_{ijk} = u + Le.An_{j(i)} + Le.An.Time_{k(ij)} + e_{ijk}$$

where Y_{ijk} is the log titre observed in the jth animal (AN) (j = 1,6) of the ith level (Le) (n=1,2) at the kth time (k=1,7). It is assumed that the error term (eijk) is normally distributed with mean zero and variance σ_e^2. Sub models of this model were fitted in order to produce F tests of parallelism of the slope of the lines for

animals within a level, of the lines for all the animals and of the slope of the two level lines produced after pooling the data to form one line for each level. Similarly F tests for the equality of the intercepts of those sets of lines were constructed.

3.4 Results

3.4.1 Number of LH pulses

When the LH pulses were examined in the first 12 samples (number of LH pulses estimated for 5 hours and 30 minutes) in all the levels (high and low) and treatment (control, E1, E2, A and T) there were no significant differences between the levels and treatments. As there are no group differences in the pretreatment LH pulses it shows that there are no chance differences among groups. The analysis of variance table is given in Table 3.5.

Table 3.5 Analysis of variance for pre-treatment LH pulse date

EFFECT	DF	MS	F. RATIO
Levels (high and low) within treatments	4	0.226	0.201
Treatments	4	0.297	0.248
Error	41	1.123	

In the analysis where the pulses occuring in the 31 samples after the treatment were considered there were no significant treatment effects on the number of LH pulses. However, the levels had a significant effect on the number of LH pulses. The analysis of variance table is

given in Table 3.6. The factor treatment also approached significance at 5% level. The fitted values for pulses from each of the levels and treatment shows higher number of pulses than the control indicating that the treatment increase the number of LH pulses. The fitted values for the pulses for each of the treatment and levels are given in Table 3.7

Table 3.6 Analysis of variance for post-treatment LH pulses

EFFECT	DF	MS	F. RATIO
Treatment	4	1.706	2.369
Levels (high and low) within treatment	4	1.947	2.704*
Error	41	0.720	

Table 3.7 Fitted values for number of LH pulses estimated for 15 hours after treatment, with standard error

CONTROL	E1	E2	A	T	
1.7 (0.41)	2.6 (0.72)	2.8 (0.75)	2.0 (0.63)	2.0 (0.63)	Low
	3.0 (0.77)	4.2 (1.13)	3.8 (0.87)	4.0 (0.89)	High

All the high levels had higher fitted values indicating a dose effect of the number of LH pulses.

3.4.2 Mean LH levels

In the analysis where mean LH values were tested for differences there were significant (P<0.01) animals and occasion effects. Treatment effect also approached 5% significance level. Treatment x level (High, low), treatment x occasion two way interaction and treatment x occasion x level (high, low) three way interactions were significant. Anyalsis of variance table is given in Table 3.8. Table 3.9 gives the individual animal LH (ng/ml) levels over the five occasions for each of the treatments and levels. Figure 3.2 shows the graph drawn for the fitted values for each of the level and occasions in the five treatment groups. In general mean LH levels increase after the treatment and it tends to come back to normal after 12-15 hours. As the levels do not behave in the same way statistically significant interactions are explainable.

Table 3.8 Analysis of variance for mean LH levels

Effect	DF	MS	F. ratio
Occasion	4	1.238	4.932**
Treatment	4	0.569	2.226
Animals within group	43	2.265	9.023**
Treatment x occasion	12	0.796	3.17**
Treatment x level (high,low)	14	0.738	3.119*
Treatment x level (high,low) x occasion	12	0.647	2.577*
Error	976	0.251	

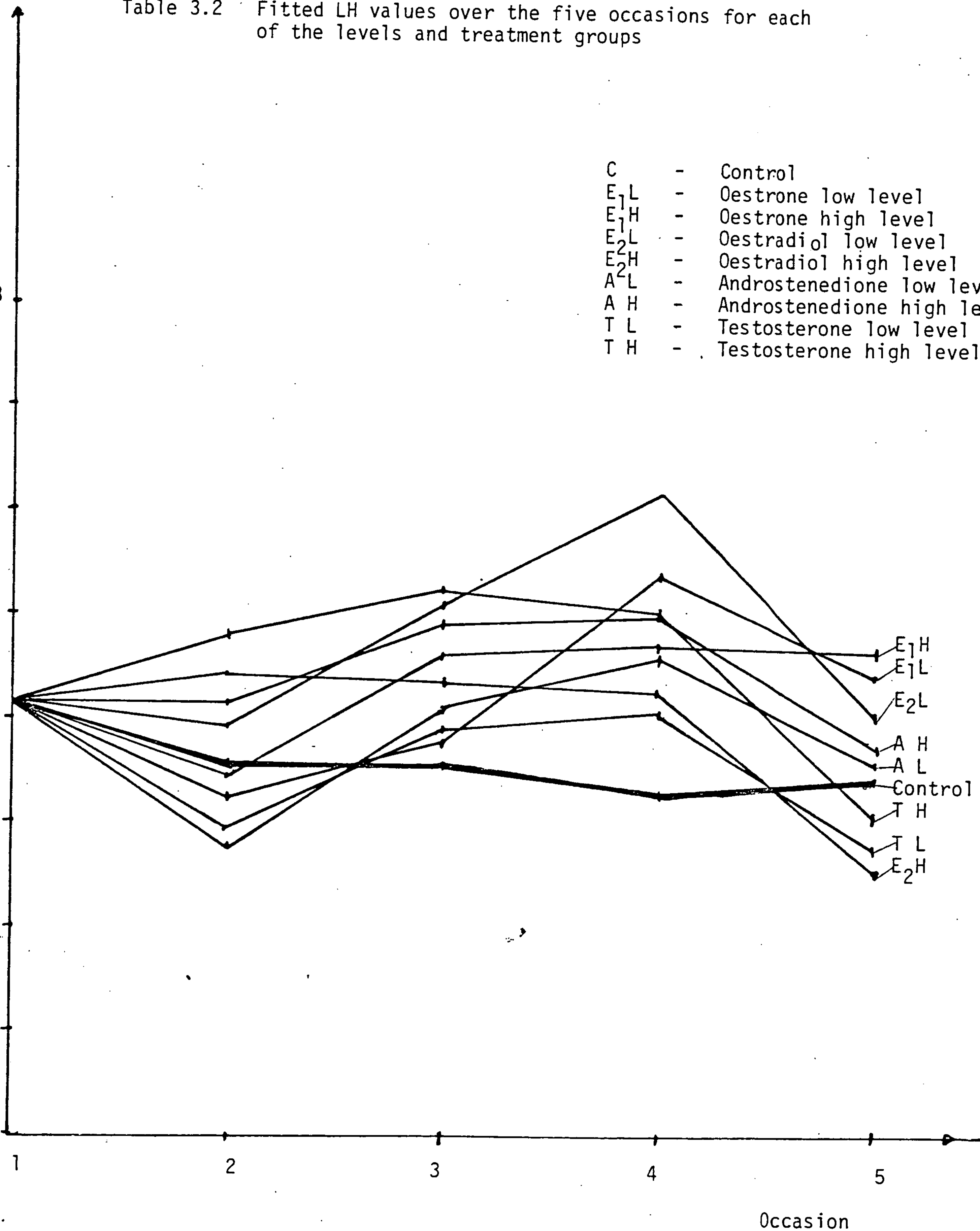


Table 3.2 Fitted LH values over the five occasions for each of the levels and treatment groups

Table 3. 9 Individual animal LH (ng/ml) levels over the five occasions for each of the treatments and levels

Treatment & animal no.	Occasion 1	2	3	4	5
Control 1	1.515	0.958	0.896	0.913	0.907
2	0.780	0.673	0.885	0.804	0.813
3	0.759	2.572	0.936	0.722	0.684
4	0.785	0.451	0.905	0.447	0.442
5	0.992	0.575	0.466	1.121	0.540
6	1.359	2.602	1.158	1.454	1.935
7	1.026	0.692	0.659	0.748	0.770
8	0.882	0.544	0.345	0.561	0.379
9	0.807	1.017	0.743	0.822	0.861
10	1.425	3.810	1.462	1.210	2.061
E_1 Low 11	1.966	0.705	1.255	0.824	0.680
12	1.276	1.116	1.845	1.513	0.992
13	0.848	0.972	0.732	1.009	1.093
14	0.865	0.472	0.552	1.029	0.568
15	1.871	1.249	0.943	3.588	2.388
E_1 High 16	2.276	2.272	1.950	2.330	3.030
17	1.006	0.770	1.371	1.784	1.898
18	0.867	0.899	0.762	1.200	1.542
19	1.319	0.900	1.434	3.246	3.429
20	0.824	0.691	1.131	1.136	0.852
Mean					
Control	1.031	1.389	0.841	0.880	0.939
E_1 Low	1.365	0.903	1.065	1.592	1.144
E_1 High	1.258	1.106	1.329	1.939	2.150

Treatment & animal no.	Occasion 1	2	3	4	5
E_2 Low 21	1.025	1.096	1.052	0.557	1.724
22	1.256	0.685	0.695	0.833	0.806
23	1.931	1.786	1.454	1.683	1.539
24	0.801	1.297	0.730	1.118	0.934
25	0.948	0.532	0.589	0.554	1.582
E_2 High 26	0.845	1.000	0.940	1.294	0.647
27	1.011	0.683	1.129	0.864	0.581
28	1.081	0.858	0.904	1.550	0.504
29	1.325	0.784	0.882	1.392	0.915
30	1.358	1.144	1.329	1.802	0.955
A Low 31	0.487	0.390	0.411	0.573	0.485
32	1.896	0.800	1.073	0.857	0.933
33	1.762	1.386	1.440	1.624	1.396
34	0.845	1.588	1.486	1.132	0.416
35	1.074	0.756	0.981	1.042	0.582
A High 36	0.821	0.586	0.821	0.586	0.918
37	1.238	1.315	0.884	0.920	1.545
38	0.919	1.069	1.685	1.219	0.892
39	0.939	1.871	1.509	1.353	1.689
40	0.969	0.895	1.163	1.199	0.961
Mean					
E_2 Low	1.192	1.079	0.804	0.949	1.317
E_2 High	1.124	0.892	1.036	1.380	0.720
A Low	1.213	0.984	1.078	1.045	0.762
A High	0.977	1.472	1.212	1.055	1.201

Treatment & animal no.	Occasion 1	2	3	4	5
T Low 41	1.234	1.895	0.792	0.698	0.352
42	1.393	1.274	1.174	1.565	1.051
43	0.893	0.437	0.772	0.603	0.420
44	0.313	0.493	0.319	0.780	0.400
45	0.866	1.847	0.745	0.638	0.637
T High 46	1.335	0.952	1.025	1.461	0.862
47	0.939	1.070	0.867	1.117	0.347
48	0.734	2.478	0.087	0.696	0.646
49	1.104	0.739	1.352	1.414	1.259
50	0.877	1.946	0.931	0.911	0.871
Mean					
T Low	0.939	1.189	0.760	0.856	0.572
T High	0.997	1.437	0.972	1.119	0.797

3.4.3 Mean FSH levels

When the FSH values from the first 12 samples (before treatment) were subjected to analysis of variance there were no significant treatment or level differences but the variation due to animals within levels within treatment was significant. Although with high animal variation there were no significant chance differences between treatments and levels. The analysis of variance table is given in Table 3.10.

Table 3.10 Analysis of variance table for pretreatment FSH data

Effect	DF	MS	F. ratio
Level (high and low)	4	0.961	0.972
Treatment	4	0.983	0.994
Animals within levels within treatment	41	0.988	5.581**
Error	484	0.177	

Levels and treatments were tested against animals and the animals were tested against error.

In the analysis where all 43 samples were considered there were significant effects due to animals, occasions and treatmen groups. It indicated that the antiserum treatment increased the FSH levels. The analysis of variance table is given in Table 3.11. Individual animal mean FSH (ng/ml) levels over the five occasions in the five treatments are given in Table 3.12. The fitted values for each of the occasion and levels are graphically presented in figure 3.3.

Figure 3.3. Fitted FSH values over the fine occasions for each of the levels and treatment groups.

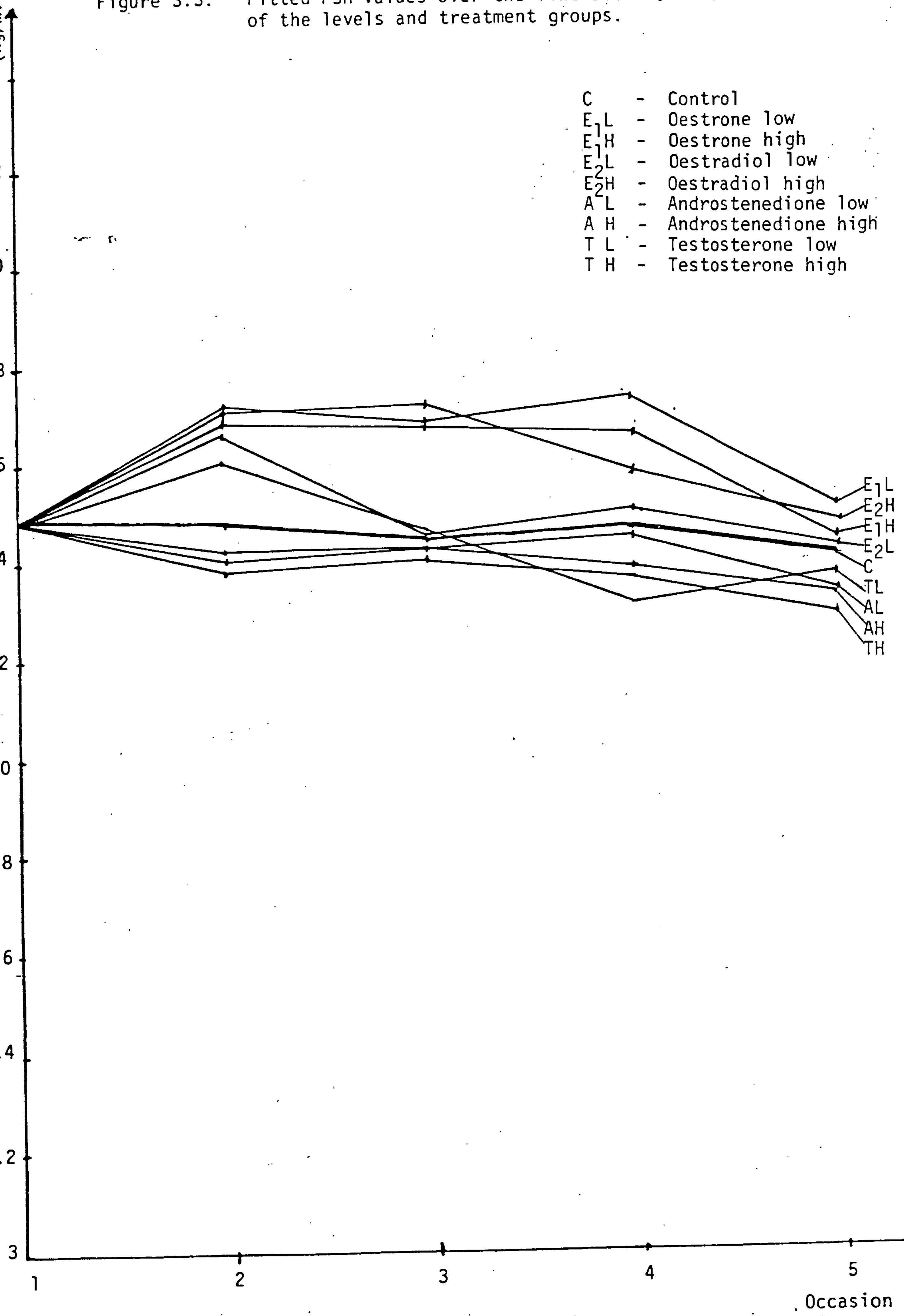


Table 3.12 Individual animal FSH (ng/ml) levels over the five occasions for each of the treatments and levels

Treatment & animal no.	Occasion 1	2	3	4	5
Control 1	62.12	65.86	70.05	66.00	66.36
2	80.46	73.89	70.44	57.08	57.96
3	96.51	97.47	113.7	96.94	82.27
4	105.0	120.2	117.4	102.3	95.76
5	86.19	117.3	110.3	93.72	95.94
6	186.3	216.3	208.1	186.7	218.6
7	84.23	110.8	98.64	79.69	90.28
8	79.17	80.00	82.01	76.03	70.14
9	91.92	106.4	95.57	67.98	88.96
10	154.7	185.7	176.8	143.9	182.6
E_1 Low 11	116.2	114.8	119.0	108.3	88.78
12	108.3	113.1	75.44	62.66	90.56
13	120.9	100.4	96.97	87.96	108.3
14	61.63	69.23	63.06	48.53	59.52
15	87.14	98.82	107.6	110.4	113.1
E_1 High 16	64.22	88.10	80.66	65.95	80.36
17	68.16	117.9	107.0	86.08	106.8
18	61.25	90.57	81.40	52.10	64.98
19	125.4	153.0	132.3	98.44	81.45
20	63.21	86.23	78.61	63.91	78.25
Mean					
Control	102.6	117.4	114.3	97.03	104.8
E_1 Low	98.82	99.27	92.41	83.57	92.05
E_1 High	76.44	107.16	95.99	73.29	82.36

Treatment & animal no.	Occasion 1	2	3	4	5
E_2 Low 21	77.39	83.86	88.76	71.65	70.83
22	90.56	102.5	89.41	97.14	116.0
23	58.20	72.71	74.84	71.51	77.28
24	178.8	184.1	180.1	166.3	155.0
25	75.80	77.62	68.78	53.81	55.61
E_2 High 26	74.81	83.94	93.25	91.69	77.35
27	83.00	131.4	135.7	89.76	68.65
28	87.60	108.9	99.84	81.82	99.02
29	67.39	83.63	76.46	59.83	55.83
30	66.27	82.61	75.31	58.71	54.33
A Low 31	83.16	92.43	83.18	64.55	58.52
32	87.67	82.44	67.52	65.32	85.70
33	75.16	101.3	98.61	76.58	56.84
34	86.30	79.80	89.48	94.00	96.30
35	82.60	76.67	77.66	74.58	62.40
A High 36	94.79	105.1	106.0	79.36	77.41
37	91.13	96.49	96.86	87.44	82.19
38	78.93	85.55	83.83	85.59	68.03
39	80.44	81.71	83.31	80.31	82.51
40	94.36	91.37	96.37	95.11	94.17
Mean					
E_2 Low	96.15	104.1	100.3	92.08	94.94
E_2 High	75.18	98.09	95.71	76.36	71.03
A low	82.91	86.52	83.29	75.01	71.95
A High	87.93	92.13	93.27	85.56	80.86

Treatment & animal no.	Occasion 1	2	3	4	5
T Low 41	108.9	107.8	105.8	110.1	90.89
42	90.43	90.89	101.4	101.4	93.10
43	144.9	142.3	141.1	137.7	136.6
44	75.38	85.62	95.80	84.09	78.21
45	74.50	94.28	92.37	80.84	71.68
T High 46	123.1	157.9	154.5	143.5	131.4
47	146.8	117.4	130.9	114.6	117.1
48	87.15	91.40	81.80	87.66	82.29
49	83.16	84.30	84.50	82.15	80.11
50	93.26	90.26	95.27	94.12	94.37
Mean					
T Low	98.82	104.17	107.29	102.75	94.06
T High	106.7	108.3	109.4	104.4	101.1

In general FSH values increase after treatment in the groups treated against oestrone and oestradiol 17β and it tends to come back to normal in the 5th occasion, after 12-15 hours. As the levels do not behave in the same manner over the five occasions the statistically significant treatment x occasion, treatment x level and treatment x level x occasion interaction terms are explainable.

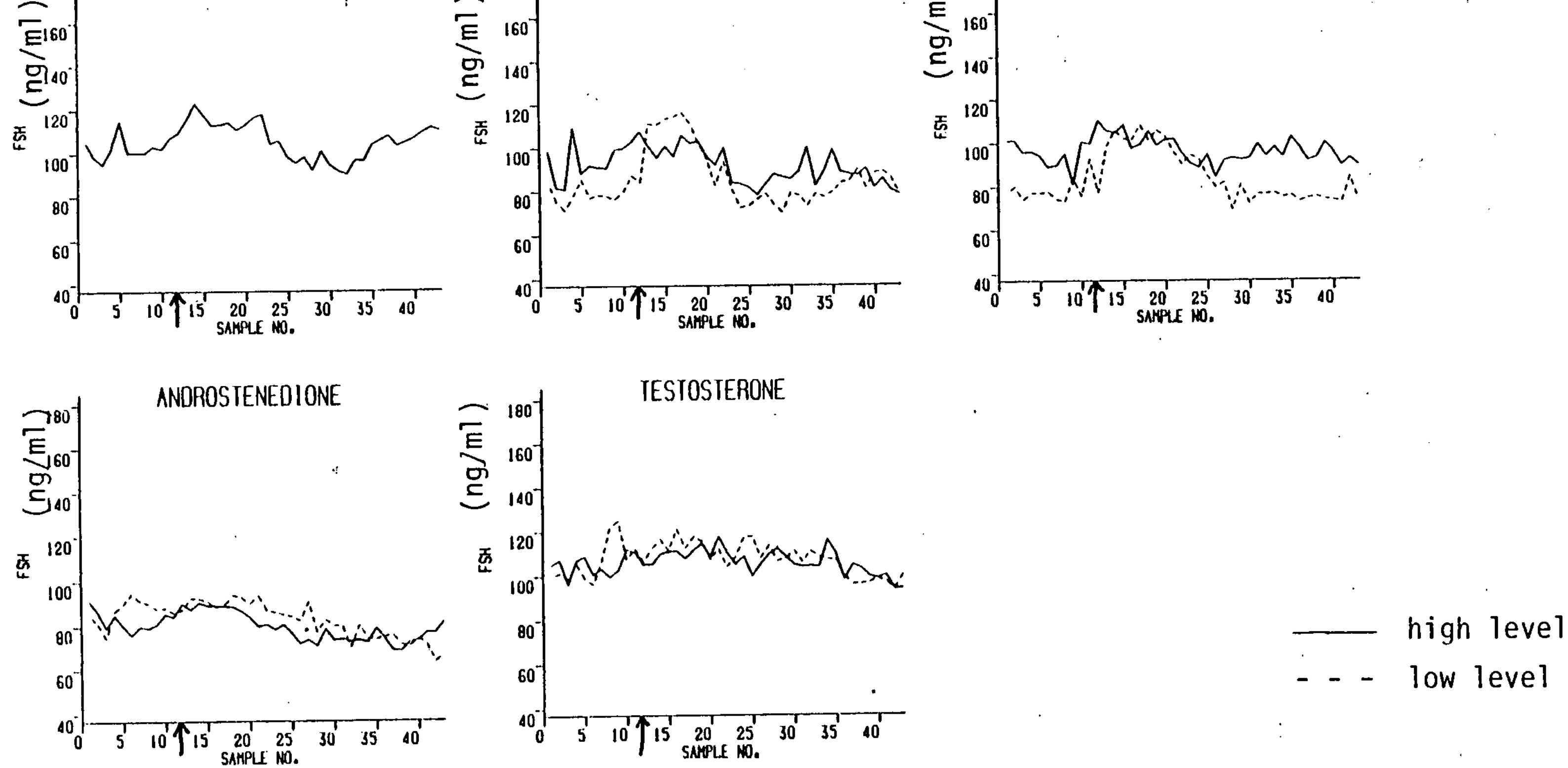
Table 3.11 Analysis of variance for FSH data (all 43 samples)

Effect	DF	MS	F. ratio
Occasion	4	1.835	83.409**
Treatment	4	1.257	57.136**
Animals within group	43	2.867	130.318**
Treatment x occasion	12	0.115	5.227**
Treatment x level (high,low)	4	0.157	7.136*
Treatment x level (high,low) x occasion	12	0.098	4.454**
Error	976	0.022	

The absolute FSH values are graphically presented in Figure 3.4

3.4.4 Ovulation rate

In the pretreatment ovulation rate data there were no significant differences between treatments and levels (high and low). It indicated that there were no chance differences between the treatments and levels. The analysis of variance table is given in Table 3.12A.



Arrow indicates the time of treatment

-FSH MEAN LEVELS

Figure 3.4 Graph of untransformed FSH values in the five treatment groups

Table 3.12A. Analysis of variance for pretreatment ovulation rate data

Effect	DF	M. square	F. ratio
Levels (high and low)	4	0.042	0.156
Treatments	4	0.131	0.488
Error	55	0.268	

When the control animal data were tested for season variation there were significant seasonal differences in ovulation rate. Towards the end of the breeding season the ovulation rate significantly reduced. There was also significant animal variation (P<0.01). The analysis of variance table is given in Table 3.13.

Table 3.13: Analysis of variance for seasonal differences in ovulation rate

Effect	DF	S. square	F ratio
Animal	11	0.568	3.527**
Occasion	5	0.587	3.645**
Error	55	0.161	

When all the ovulation rate data were subjected to analysis of variance there were significant (P<0.01) differences in ovulation rate between

the treatment groups. Analysis of variance table is given in Table 3.14.

Table 3.14: Analysis of variance, ovulation rate data from all the animals

Effect	DF	MS	F. ratio
Linear regression due to titre before the quadratic regression			
E1t	1	1.541	4.479**
E2t	1	0.071	0.210
At	1	0.009	0.026
TE	1	0.879	5.583**
Quadratic regression due to titre after the linear regression			
E1t2	1	0.414	1.232
E2t2	1	3.832	11.404**
At2	1	1.214	3.613**
Tt2	1	0.188	0.559
Treatment	4	0.094	0.109
Occasion	6	1.286	3.821**
Animal	55	0.859	2.552**
Error	330	0.336	

t = linear regression of ovulation rate on titre

t2= quadratic regression of ovulation rate on titre
Treatments were tested against the animal mean square and the other terms were tested against error mean square.

There were significant effects on ovulation rate due to E_1,E_2,A and T antisera. E_1 and T showed linear effects due to titre. E_2 and A show quadratic effects. There were no significant treatment effects for ovulation rate after removing regression effects due to titre. However the treatment effects were almost entirely explained by the titre regression terms. For the titres E2 and A had significant

quadratic effects ($P<0.01$) and E_1 and T had significant linear effects ($P<0.01$). The effect of occasion and animals were also significant. In general ovulation rate was increased after the treatment. Figures 3.5 and 3.6 show the graphs of ovulation rate plotted against each of the occasions (oestrous cycle). When the treatment is stopped ovulation rate is reduced. Towards the end of the breeding season ovulation rate is increased due to treatment. Figure 3.5 shows the ovulation rate results in comparison with the control group.
The fitted curves for the ovulation rates for each of the antisera titres are shown in Figures 3.7 and 3.7A.

When the mixed antiserum treated group was tested for effects of antiserum there was no significant effect on the mixture group. The analysis of variance Table is given in Table 3.15

Table 3.15 - Analysis of variance for 'mix' antiserum group

Effect	DF	S.square	M.square	F. ratio
'mix'	1	0.03	0.03	0.088
Error	329	111.025	0.337	

3.4.5 Oestrous cycle length

In the oestrus cycle length data out of the factors considered occasion had a significant effect ($P<0.01$). Treatment x Occasion interaction term was also significant. Although the effect of occasion was significant actual difference was small. The mean

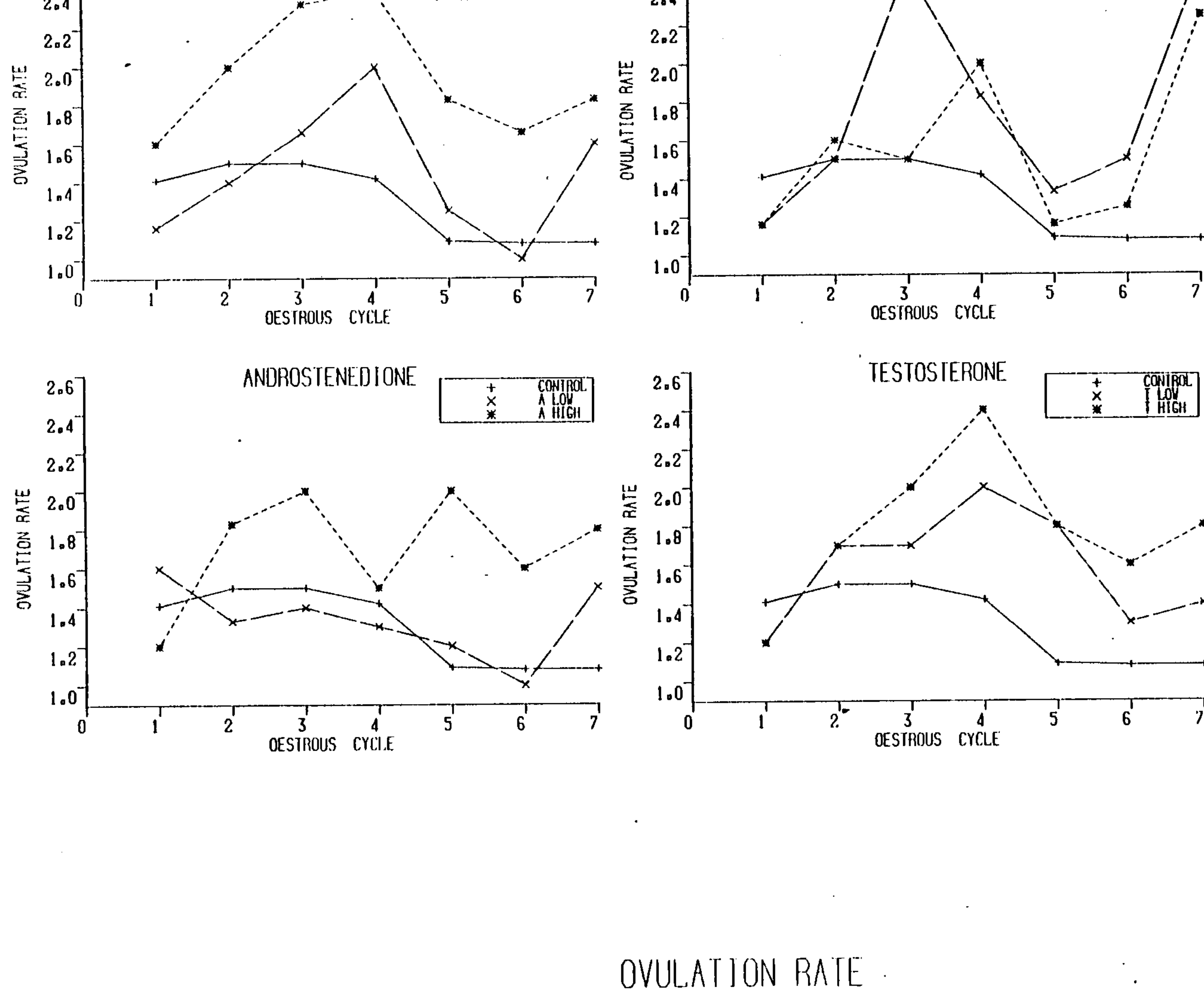


Figure 3.5. Graphs of ovulation rate means, in each treatment high and low levels are separated.

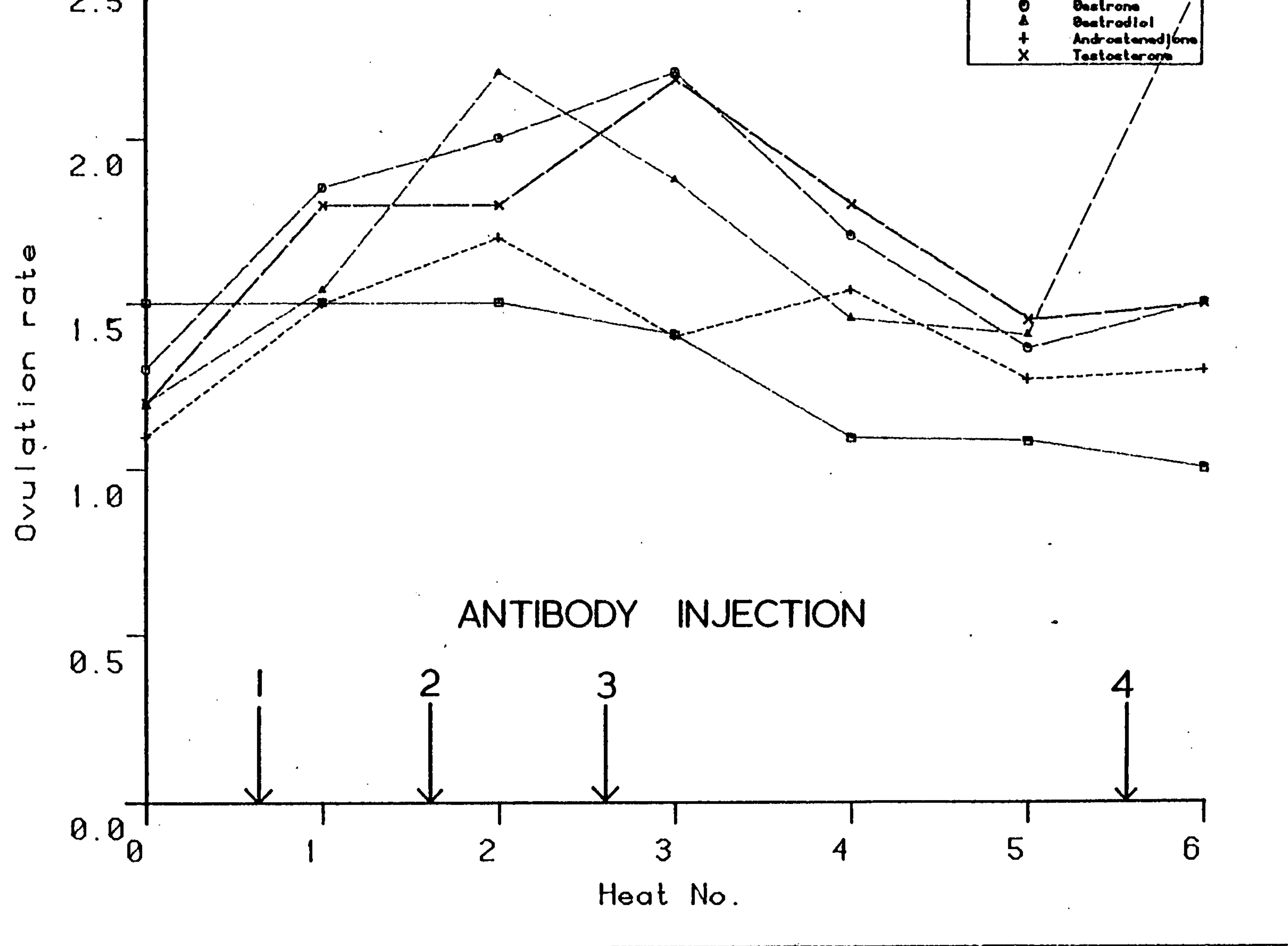
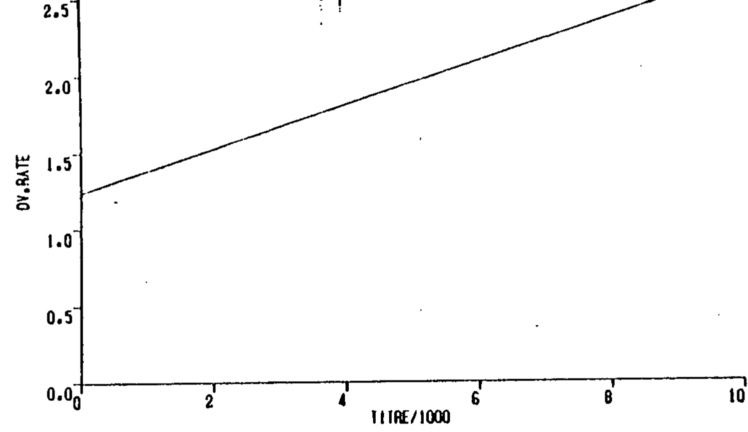
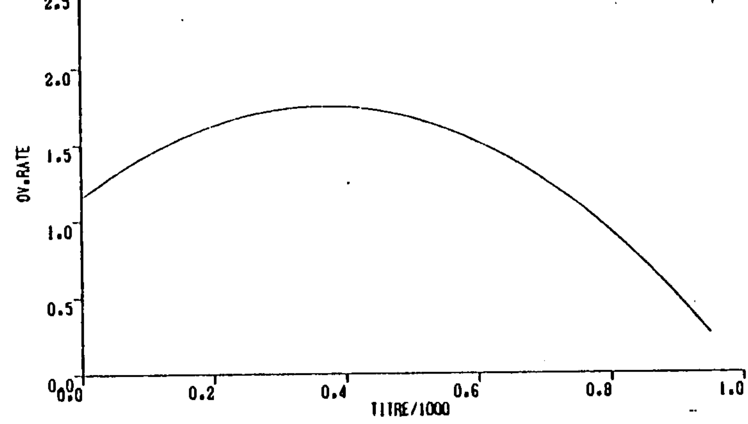
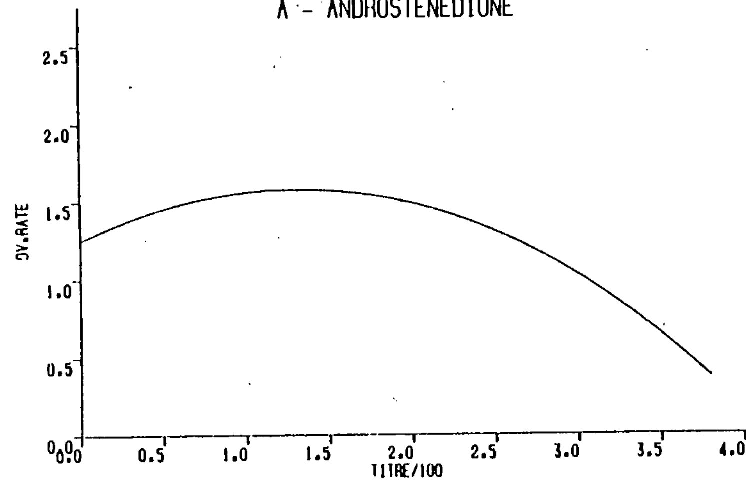


Figure 3.6. Graph of ovulation rate means, within each treatment high and low levels are pooled together.







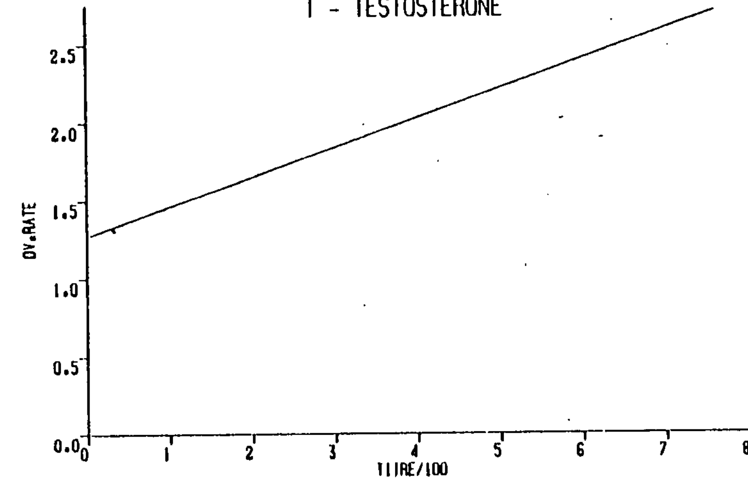
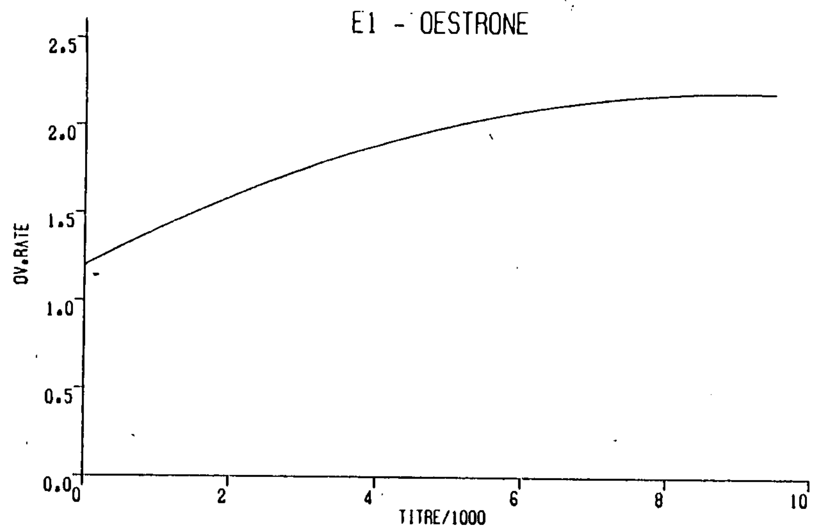
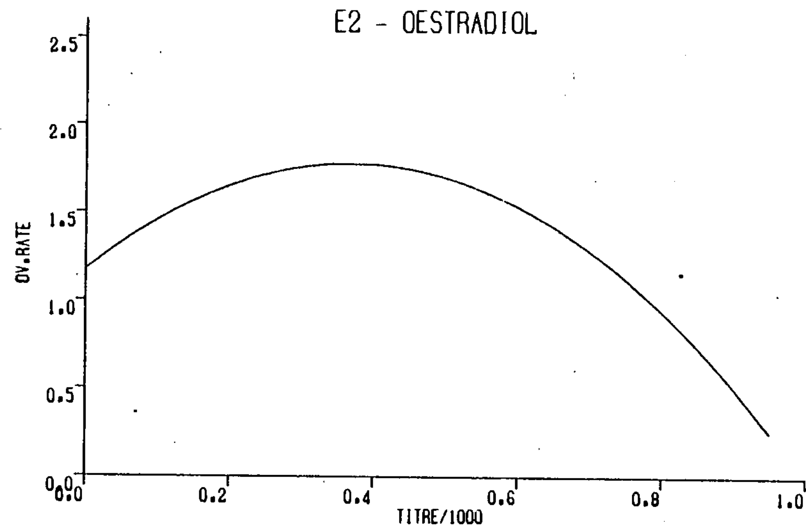
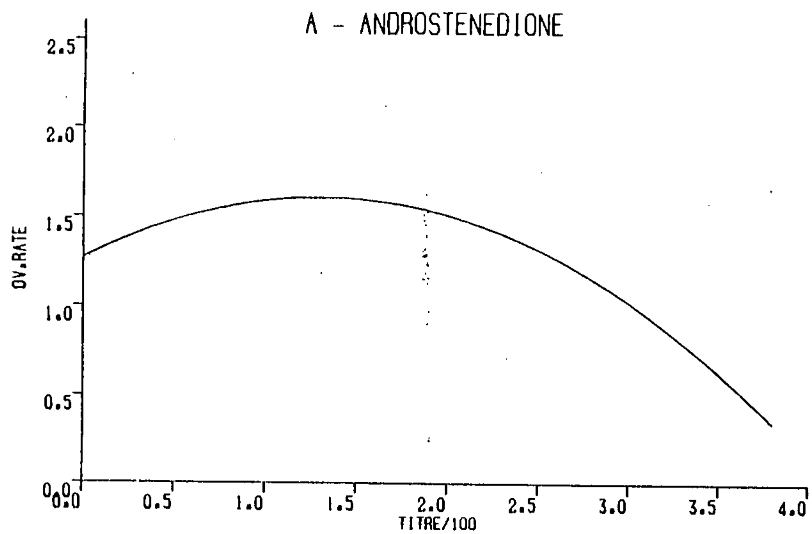
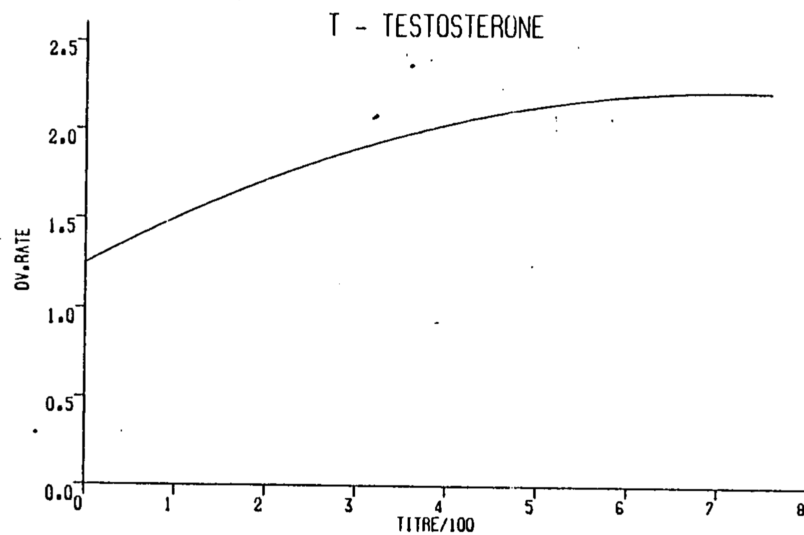


Figure 3.7. Fitted curves for ovulation rate on titre, curves are fitted only with significant regression terms.









FITTED CURVES FROM COMPLETE MODEL

Figure 3.7.A. Fitted curves for ovulation rate on titre on complete model.

cycle length of the control animals was 16.25±.26 days. The analysis of variance table for oestrous cycle length is given in Table 3.16.

Table 3.16. Analysis of variance for oestrous cycle length

Effect	DF	M. square	F. ratio
Occasion	5	6.046	8.563**
Treatment Group	4	2.394	1.913
Treatment Group x Occasion	20	1.377	1.930*
Animals	55	1.251	1.771
Error	199	0.706	

Fitted values for oestrous cycle length are given in Table 3.17.

Table 3.17 Fitted values for oestrous cycle length (days)

Control	16.25±.26
E_1	17.41±.37
E_2	17.54±.37
A	16.25±.37
T	16.58±.32

Table 3.17 continued

	Oestrous cycle					
	1	2	3	4	5	6
Control	16.25±.26	16.25±.37	16.12±.37	16.58±.38	16.02±.38	16.30±.21
E_1	16.25±.26	15.27±.54	15.42±.56	15.76±.55	15.97±.57	14.90±.65
E_2	16.25±.26	15.13±.57	15.03±.59	15.20±.59	16.20±.65	16.90±.57
A	16.25±.26	15.91±.25	16.33±.55	16.33±.55	16.87±.56	14.90±.65
T	16.25±.26	16.75±.52	16.98±.53	16.98±.53	16.36±.54	15.97±.54

3.4.6 Oestrus

In the analysis of oestrus data animals, treatment group, occasion and treatment group x occasion interaction were significant. Analysis of variance table is given in Table 3.18.

Table 3.18 - Analysis of variance for oestrus

Effect	DF	M. square	F. ratio
Occasion	4	10.942	45.402**
Treatment Group	4	11.051	6.421**
Treatment Group x Occasion	20	0.678	2.813 **
Animals	55	1.721	7.141**
Error	260	0.251	

The number of animals not showing oestrus in each of the treatment groups are shown in Table 3.19.

Table 3.19 Number of animals not showing oestrus in each of the treatment groups

Group	Oestrous Cycle 1	2	3	4	5	6
Control	0	0	0	0	1	0
E_1	0	3	3	2	4	0
E_2	1	5	4	3	7	0
A	0	0	1	2	2	0
T	0	0	0	0	1	0

In cycle 1-5 each cell represent 12 animals

In cycle 6 each cell represent 10 animals

All the treatments did not have a similar effect on the number of animals not showing oestrus. Out of the treatments testosterone treatment group has less effect on the oestrus and oestradiol 17β treatment group had the highest effect on the oestrus.

3.4.7 Lambing data

When the lambing data were examined as proportions of the ovulation rate in a binomial model there were significant differences between groups but the effect of ovulation rate was not significant. The analysis of variance table is given in Table 3.20.

Table 3.20 Analysis of variance for the lambing data

Effect	DF	M. Square	F. Ratio
Treatment group	5	3.014	2.892*
Ovulation rate	2	1.855	1.780
Residual	46	1.042	

The table prepared from the 7th occasion of ovulation was tested by using a randomisation test. There were clear differences in ovulation rate between the individual treatment group as a whole ($P<0.01$). The comparison of each treatment and the control also showed differences in each as follows E ($P<0.05$), E_2 ($P<0.01$), A ($P<0.05$), T ($P<0.05$) and mix ($P>0.05$).

The lambing data table as a whole was not significant and there were no significant differences between the individual treatment and the control except the group T ($P<0.05$). Table 3.21 illustrates the lambing data results.

Table 3.21 The number of lambs born to mating after the last treatment in each treatment

Group	Ovulation rate	Lambs per ewe	Ewes Lambing	Lamb per corpus luteum
C	1.0	0.80	8/10	8/10
E1	1.5	0.77	6/9	7/15
E2	2.28	0.55	5/9	5/16
A	1.5	1.0	7/9	9/14
T	1.5	1.5	9/9	14/14
Mix-antiserum	1.3	1.1	9/10	11/13

3.4.8 Antiserum titre and half life

When the antibody titres obtained after the first antibody injection was subjected to analysis of variance there were significant ($P<0.01$) differences in titre between treatments. The two levels also had significantly different titres in all four groups. Mean antibody titres during the 6 oestrous cycles are given in Table 3.22.

For the half-life data there was little evidence of variation between individuals within levels affecting the rate of decay and hence the half-life of E_1, A and T. Variation between animals within levels did significantly affect in the E_2 group. Rate of decay half-life of E_1, E_2 and A but not T were apparently affected by the sub-groups. The low E_1 and A sub-groups indicated a lower half-life for E_1 and A than the high sub-group while the reverse was the case for E_2. Table 3.23, 3.24, 3.25 and 3.26 give the half-life, analysis of variance for parallelism of slopes and equality of intercepts for E_1, E_2, A and T decay curves respectively.

Table 3.23 Half-life and test for parallelism of slopes for E_1 decay curves

Effect	DF	M. square	F. ratio	Pr.
Parallelism of slopes				
Animals within levels	9	0.005	0.454	P>5%
Animals	10	0.032	2.704	P<1%
Pooled levels	1	0.144	11.057	P<1%
Equality of intercept				
Animals within levels	9	0.009	0.748	P>5%
Pooled levels	1	0.0005	0.038	P<5%

Overall estimate of slope = 10.0457 ± 0.0010
Half-life E1 = 15.16 ± 0.33 days

Table 3.24 Half-life and tests for parallelism of slopes for E2 decay curves

Effect	DF	M. square	F. Ratio	Pr.
Parallelism of slopes				
Animals within level	10	0.058	3.763	P>1%
Animals	11	0.080	5.006	P<1%
Pooled levels	1	0.123	5.141	1% P<5%
Equality of intercepts				
Animals within levels	10	0.019	1.277	P>5%
Pooled levels	1	0.052	2.174	P>5%

Overall estimate of slope = -0.0514±.0011
Half life E2 = 13.50±0.28 days

Table 3.25 Half life and tests of parallelism of slopes for A decay curve

Effect	DF	M. square	F. Ratio	Pr.
Parallelism of slopes				
Animals within levels	9	0.154	1.462	P<5%
Animals	10	0.056	4.741	P<1%
Pooled levels	1	0.072	4.178	P<1%
Equality of intercepts				
Animals within levels	9	0.020	1.958	P>5%
Pooled levels	1	0.007	0.002	P>5%

Overall estimate of slope = -0.044±0.0010
Half life of A = 15.61±0.35 days

Table 3.22 Mean antibody titres during the 6 oestrous cycles

		CYCLE 1	CYCLE 2			CYCLE 3			CYCLE 4		CYCLE 5		CYCLE 6		
DAY		13	5	11	15	5	11	13	5	11	5	11	5	11	13
E_1	L	566	377	278	3121	2033	1533	5580	3580	2840	1600	1122	800	456	3476
	H	3020	2010	1570	5700	4616	3250	6133	3783	2716	1666	1466	945	564	3580
E_2	L	70	49	32	363	231	181	641	410	306	173	123	76	60	348
	H	288	202	154	760	528	370	698	411	301	190	113	69	52	340
A	L	19	14	9	913	82	43	186	118	89	54	37	17	14	103
	H	87	61	41	206	148	96	190	124	96	60	45	33	21	108
T	L	43	31	21	184	130	93	418	285	211	143	95	70	45	216
	H	171	118	88	416	263	194	406	290	222	140	110	71	50	221

The titre is defined as the initial dilution of plasma which bound 50% of the radioactive tracer (15-20 pg).

Table 3.26 Half-life and test for parallelism of slopes for T decay curve

Effect	DF	M. square	F. ratio	Pr.
Parellelism of slopes				
Animals within levels	9	0.014	1.337	P>5%
Animals	10	0.027	2.359	1% < P <5%
Pooled levels	1	0.016	1.148	P>5%
Equality of intercepts				
Animals within levels	9	0.005	0.526	P> 5%
Pooled levels	1	0.001	0.073	P> 5%

Overall estimate of slope = -0.0433±.0008
Half life T = 16.00±0.30 days

3.5 Discussion

The primary objective of the study of gonadotrophin levels is to investigate the changes observed in gonadotrophins after modification of the negative feed-back without blocking the positive feed-back effects. In general it may be noted that the absolute levels observed were comparable to those reported earlier for cyclic ewes in the luteal phase. For example the concentrations of LH and FSH in control groups are similar to those recorded previously for cyclic ewes (Cunningham, Symans and Saba (1975); Nett, Akbar and Nieswender, 1974).

Passive immunization against oestrone, oestradiol 17β, androstenedione and testosterone increase the plasma binding of the respective steroid. Passive immunization of female sheep against the above steroids produced an increase in the levels of plasma LH

characterised by pulsatile pattern of LH release. Active immunization against oestrone protein conjugate in sheep also increased the LH pulse frequency (Rawlings, Kennedy and Henrieks, 1979; Martensz et al 1979). In the latter experiments oestrous cycle was disrupted and the ovulation was stopped. The present results do not show disruption of the oestrous cycle. Active immunization against oestradiol 17β produced LH releasing pattern which was similar to that found in ovariectomised-hysterectomised sheep (Martensz et al 1976). In this present experiment when the negative feed-back is reduced by the antibodies against oestradiol 17β the change in pulse frequency is barely detectable. It is probable that when the negative feed-back is reduced without reducing the positive feed-back the change in pulse frequency is only marginal as the negative feed-back is not completely reduced as in the ovariectomy.

Increasing pulse frequency has been observed after active immunization against androstenedione (Martensz et al 1976) and testosterone(Scaramuzzi et al 1981). In this present study immunization against all four steroids increased the LH pulse frequency but the change in pulse frequency is dependant on the antibody titre. All the high antibody titre sub-groups had higher number of LH pulses showing that the LH pulse frequency increases with the antibody titre in the ciculation. When comparing the antibody titres achieved in the circulation in this experiment with other experiments (e.g. Martensz er al 1979) the antibody titres achieved are almost ten fold higher in the other experiments. In the other experiments higher antibody titres not only reduced the negative feed-back but also inhibited the positive feed-back and the oestrous cycle was disrupted.

It may be possible that in such a situation the change in pulse frequency is as high as that found in ovariectomy.

In the basal LH levels there was a marginal change of increase after passive immunization against all four steroids. The mean LH levels increase after passive immunization and it tends to drop down to the normal after 12-15 hours. When the negative feed-back level is reduced within the physiological limits it seems that the LH levels are only changed transiently and the system tends to equillibrate by increasing the number of follicles as in the case of unilateral ovarietomy (Findlay and Cumming, 1977).

Plasma FSH levels were increased after passive immunization against E_1 and E_2 but not with A and T. It is suggestive that oestrone and oestradiol 17β are involved in some way in controlling FSH secretion. The finding that FSH levels did not change after passive immunization against testosterone is in consistent with the finding of Martensz and Scaramuzzi (1979). There was no change in FSH after passive imminization against androstenedione. In contrast Martensz and Scaramuzzi (1979) found a decrease in plasma FSH after active immunization against androstenedione. In the experiment of Martensz and Scaramuzzi (1979) androstenedione immunized animals continued to cycle but the animals immunized against testosterone did not cycle. However, in the present experiment all the treated groups were cyclic. Although the FSH levels are increased after passive immunization against E_1 and E_2 the levels came back to normal within 12-15 hours. Again it is tempting to suggest that when the negative feed-back is reduced within physiological limits as in the case of unilateral ovariectomy the

changes observed in gonadotrophin levels are transcient and the trophic and negative feed-back system tend to equillibrated within few hours. The levels between the treatment behave differently from each other in increasing the FSH and coming back to normal levels. This finding is supported by the statistically significant treatment x level x occasion three way interaction.

Although antiserum used in this experiment have slight cross-reaction with other steroids (see Table 3.1) it is not possible to attribute changes observed in any of the groups due to cross-reaction alone as the cross-reactivities were very low.

There were significant differences in ovulation rate in all the four antiserum treatment groups. The differences between the groups are almost entirely explained by the antibody titres. Active immunization against oestrone (Rawlings et al 1979; Scaramuzzi et al 1980) disrupted the oestrous cycle and stopped the ovulation but in the proportion of animals that ovulated (Scaramuzzi et al 1980), ovulation rate was singificantly increased. If the negative feed-back is reduced without inhibiting the positive feed-back the ovulation rate can be increased. Although ovulation rate is significantly increased after passive immunization against oestradiol 17β in this experiment, passive immunization against oestradiol 17β (Fairclough et al 1976; Rawlings et al 1979) and active immunisation against oestradiol 17β (Rawlings et al 1978 and Scraamuzzi et al 1980) stopped the ovulation rate due to decreased positive feed-back. When the positive feed-back is reduced the pre-ovulatory LH surge is stopped and hence the ovulation is stopped. Increased ovulation rate observed in this experiment after passive

immunization against androstenedione is in agreement with the results obtained by Scaramuzzi et al (1977) and Van Look et al (1978). The high ovulation rate observed after passive immunisation against testosterone in this experiment has not been reported by the other workers. However, Scaramuzzi et al (1981) found over stimulation of the ovaries after active immunization against testosterone and ovulation was stopped. They found higher number of large non-atretic follicles in the ovary. The results of this experiment demonstrate that the ovulation rate in the ewes can be increased significantly by modification of the negative feed-back through passive immunization against gonadal steroids. Probably most of the other experiments reported with immunization against gonadal steroids reduced the positive feed-back beyond the physiological limits and the pre-ovulatory LH surge was inhibited and thereby the ovulation was stopped.

Although antibody titres reported in other experiments are not directly comparable with this experiment because of the differences in the method of antibody titration, in general the titres achieved in other experiments are higher than in this experiment. In the other experiments there were significantly higher cross reactions with other ovarian steroids.

Although similar amounts of antisera (units) were given to each of the groups on different occasions. The titres achieved in each of the antiserum groups were significantly different (see Table 3.22). In the case of oestrone the titres achieved were much higher than the other antibodies. The titre measured the amount of free antibody in the circulation. In the circulation normally oestrone has a lower

concentration than that of oestradiol 17β. During the oestrous cycle most of the time secretion rate of oestradiol is three times higher than the oestradiol (Baird, Goding, Ichikuwa, McCracken, 1968). The ratio of oestradiol 17β to oestrone was found to vary from 1:1 to 43:1 in ovarian blood (Moor, Barnette, Brown, Schindlet, Smith and Smyth, 1979) and 1:1 to 10:1 in jugular venous plasms (Short, McDonald and Rowson, 1963) in the pre-ovulatory period. As there is less oestrone antigen than the oestradiol in the circulation the high titre achieved in oestrone is explainable. In general titre for other three antisera agree with the ratio of plasma concentration of three antigens as reported by Scaramuzzi et al (1980).

The high ovulation achieved after passive immunization against gonadal steroids can be explained. Immunization against oestrogens resulted in generally increased plasma LH and FSH immediately after passive immunization and the levels tend to equilibriate within 12-15 hours. As the negative feed-back is reduced by the biological neutralisation of free steroids in the circulation, then the ovary could produce more follicles to conpensate for the reduced negative feed-back such as in unilateral ovariectomy. In this situation animals could produce more follicles without reducing the gonadotrophin stimulation as some of the free steroid molecules are bound by the antibodies. High gonadotrophic levels produced after passive immunization are also probably responsible for the increased follicular development. When the negative feed-back is reduced ovaries tend to produce more follicles until the negative feed-back system get equilibrated with the gonadotrophic stimulation, so that more follicles are formed and hence the ovulation rate is increased. Furthermore elevated

LH levels have an antimitotic effect on the granulosa cells (McNatty and Suwers, 1975) and might allow follicles capable of ovulating to remain viable for a long period (Turnbull; Land and Scaramuzzi, 1975) and hence more ovulations are possible.

Although it is not known whether androgens are directly involved in the negative feed-back system there are other possible ways that reduced androgen can increase ovulation rate. It has been suggested that the ovarian androgen increase the rate of follicular atresia (Louvet, Harman, Scheiber and Ross, 1975). It is possible that the binding of andorgens by antibodies in the follicular fluid and vascular compartment leads to reduction in the rate of antisera in follicles 3mm diameter and so increase the ovulation rate. Further more neutralisation of biological activity of androgens by antibody binding could interfere with oestrogen synthesis in the ovary, or with conversion of androgens to oestrogen in perihpheral tissue and the hypothalamus. Alternatively androgen itself may have a direct inhibitory action on the hypothalamus/hypophysis system. It is even possible the antibody against androstenedione and testosterone cross-react to a minor degree with oestrogen in vivo, although this cross reaction was shown to be less than 0.1% in an invitro test.

In the present experiment increased gonadotrophin levels tend to come back to normal within 12-15 hours after passive immunisation. However, Scaramuzzi et al., (1977) explained the higher ovulation rate in animals immunized against androstenedione from the increased level of LH secretion. Although gonadotrophin levels come back normal after 12-15 hours after first immunization it is difficult to speculate on the gonadotrophic levels after subsequent

immunizations.

From the fitted values it shows that the effect of immunization on the ovulation rate is curvilinear and the ovulation rate goes through a maximum with increasing titre and then it comes down. However from the fitted curves it shows that with oestrone and testosterone higher ovulation rate can be achieved than with oestradiol 17β and androstenedione. Although the quadratic regression of ovulation rate on titres of E_1 and T are not statistically significant that regression may be biologically important. It is possible that E_1 and T ovulation rate also have a curvilinear relationship with the titres.

Although the effect of antibodies are clearly shown when they were given separately, an effect of mixed antiserum was not clearly shown. Unit 5x of antiserum gave a significant increase in all the four antisera groups. When an antiserum was made by using 1.25 x units from each of the antisera the mixture did not have a significant effect on the ovulation rate.

Passive immunization did not change the oestrous cycle length significantly. However, occasion had a significant effect on the oestrous cycle length, but the differences in cycle length was very small. Scaramuzzi et al., 1977 and Scarmauzzi (1979) also did not observe a change in oestrous cycle length after active immunization against androstenedione. Previous work show that oestradiol antibodies do not have an effect on the luteolysis and corpus luteum function (Fairclough et al., 1976). However the change in oestrous cycle length observed in this experiment during the different oestrous

cycles (occasions) is difficult to explain. The change in oestrous cycle length in different occasions do not show a regular pattern between the treatment groups. This finding is supported by the statistically significant treatment group x occasion interaction.

The number of animals showing oestrus was reduced with the increasing titre in E_1, E_2 and A groups. When the antibody titre was increased at the 3rd oestrous cycle, thereafter the number of animals coming into oestrus was decreased. However, the decrease was less marked in the testosterone group. It suggests that testosterone has very little effect on the manifestation of oestrus. The positive feed-back was not reduced and the ovulation occurred continuously. It shows that the antibody titres are high enough to reduce behavioural oestrus in some animals but not high enough to reduce positive feed-back. Several other workers reported inhibition of oestrus after immunization against oestrone, oestradiol 17β and testosterone (Fairclough et al., 1976; Rawlings et al., 1979; Rawlings et al., 1978; Scaramuzzi, 1979).

Although lambing percentage was increased in groups immunized against androstenedione and testosterone and mixed antiserum (see Table 3.19) only the testosterone group had a significant effect. Van Look et al., (1978) reported an increase in lambing percentage after active immunization against androstenedione but the difference was not statistically significant. In this experiment also the group immunized against androstenedione did not increase the lambing percentage significantly. Although all the treatment groups had significantly increased ovulation rate the lambing percentage was significantly increased only in the testosterone group. E2 group had the highest

ovulation rate but it gave the lowest number of lambs per ewe. It may be possible that when the animals are passively immunized against E_1 and E_2 some of the essential functions of such steroids are inhibited, so the fertilisation and conception rate is reduced. However with anti-testosterone the lambing percentage is increased significantly ($P<0.05$) and each of the corpora lutea are represented as a lamb. If this technique is improved this method can be used to increase lambing percentage in sheep.

When the antibody was injected to the animals in the low and high sub-group with a five fold dose difference, the titre achieved was also had a five fold difference. It indicated that the antibody titre achieved in the circulation was dependant on the absolute dose administered and that the antibody titre in the circulation could be regulated by careful administration of antibodies. The half-life of antibodies to ovarian steroids in sheep have not been reported previously. In the four antisera studies half-life varies from 12-16 days. Since the half-life is relatively long compared to the oestrous cycle length the time of treatment with antiserum to increase the ovulation rate may not be very critical. Although in this experiment animals were always injected with antiserum on day 10-12 of the oestrous cycle recent studies indicate the time of treatment with antisera does not have a significant effect on ovulation rate (R.B. Land, unpublished data).

Chapter 4 : Hypothalamic/pituitary sensitivity to steroid negative feed-back in two different breeds

4.1 Introduction

The observation of the LH release in ewes of high prolificacy was less sensitive to the feed-back effects of oestradiol 17β (Land, Wheeler and Carr, 1976) led to the argument that the variation in ovulation rate arose from variation in the sensitivity of the hypothalamus/pituitary to the negative feed-back. With similar gonadotrophin levels found between breeds (Cahill, 1979) the hypothesis is that the prolific sheep could tolerate higher concentrations of feed-back hormones (e.g. Oestradiol 17β) allowing more ova to be shed. Treatment with oestradiol 17β during the oestrous cycle had less effect on the ovulation rate of high prolificacy Finnish Landrace ewes than on the ovulation rate of less prolific Scottish Blackface ewes (Land, 1976).

Development of methods of controlled delivery of physiological quantities of feed-back hormones through silastic implants (Karsch, Dierschke, Weick, Young, Hotchkiss and Knobil, 1973) has made it possible to ellucidate the functioning of feed-back effects of gonadal hormones in a number of species (Karsch et al., 1973; Gnodde, van, Dieten and Van Look, 1979). Silastic implants with gonadal steroids have been used successfully to investigate the negative feed-back effects of gonadal hormones in relation to seasonal breeding in sheep (Karsch, Legan, Hauger and Foster, 1977; Legan, Karsch and Foster, 1977).

When ewes are treated with subcutaneous oestradiol-17β silastic implants the effect on the ovulation rate is more marked in less prolific breeds than in the high prolific breeds (R.B. Land, unpublished data).

However, when the ovulation rate has been reduced after inserting silastic implants with oestradiol 17β it's effect on gonadotrophic release has not been reported. This experiment was undertaken to investigate the effect of oestradiol 17β implants on the gonadotrophic release in two breeds of sheep with differing ovulation rates. Scottish Blackface (Blackface) and Finnish Landrace (Finn) breeds were chosen for the large differences in ovulation rate (Blackface 1.3; Finn 3.0) (Wheeler and Land, 1977).

4.2 Materials and methods

4.2.1 Experimental plan

Finn and Blackface ewes were divided into three groups of equal size. Oestrus of the animals were synchronised and on day 10-12 of the oestrous cycle either an empty implant, an oestradiol implant or two oestradiol implants were introduced subcutaneously to the three groups within each breed. Before and after oestradiol implant insertion blood samples were collected for gonadotrophin assay. In the oestrus following the implant insertion their ovulation rate was recorded.

4.2.2 Animals

Twenty seven (27) cyclic animals from each of the breeds that were kept at ABRO Field Laboratory were selected. All were mature females aged 2½-8 years. The animals were kept outside in paddocks throughout the experiment except when moved inside for pre-laparoscopy fasting or serial bleeding. Most of the time all the animals were run

together as a group. Animals received hay and concentrates as required and had access to fresh drinking water all the time. Whenever they were fed inside they were given hay.

4.2.3 Recordings

Oestrus and ovulation rate were recorded by the method described in Chapter 3.

4.2.4 Preparation and insertion of oestradiol 17β implants

Implants releasing physiological quantities of oestradiol 17β were prepared by the method described by Karsch et al., (1973). Medical grade silastic tubing (0.132 x inches i.d. x 0.183 inches o.d., Dow, Corning) was cut out into lengths of 5.4cm. On each end 0.5cm length was marked out from the end of the tube. One end of the silastic tubing was sealed with 0.5cm plus of medical grade silicone elastomer adhesive (Dow, Corning, elastomer) by injecting the material to the end of the tube by a 2ml plastic syringe. When the plug became completely dry and firm, crystaline oestradiol 17β (sigma) was filled into the tube up to the second mark by inserting a small filter funnel into the unpluged end of the tubing and using a whirlmix in light contact with the tubing to bring the crystals down into the tube. After cleaning the unplugged end of the implant with a cotton bud the silastic tubing was pressed immediately above the oestradiol to evacuate the air and it was sealed as before with the elastomer and allowed to dry. Prior to insertion all implants were soaked in water for 12-24 hours to avoid the transitory peak in circulating oestradiol which follows placement of nonsoaked implants due to surface contamination.

Implants were soaked in methanol for 15 minutes immediately before insertion. These implants are reported to release 7-8 ug oestradiol 17β per day in vitro and to elevate serum oestradiol 17β levels to 3.5 pg/ml when inserted subcutaneously to ovariectomised sheep (Karsch and Foster, 1975).

The implants were inserted under anaesthesia in the following manner. Animals were anaesthetised using Halothane (Flueothane, I.C.I.) gas mask and then held on a table ventrally. The right axillary region was shaved with electric clippers, the surface cleaned with warm Savlon solution, wiped with a clean paper towel and disinfected with hibitane solution (2:3:15 Hibitane (I.C.I.), distilled water, industrial methylated spirit). The cleaned surface was wiped with a sterilized paper towel. An incision 1-2cm in length, was made and a subcutaneous pouch formed using a pair of tissue forceps. The pouch was extended under the skin to a length of about 5cm. The implant was inserted into the pouch and the incision closed with surgical silk. The animals were then transferred to a recovery pen and recovered within 3-5 minutes. The whole procedure took about 10 minutes.

4.2.5 Synchronization of oestrus

On 28th December 1979 progesterone impregnated vaginal sponges (Veramix, Upjohn) were inserted into the vagina of all the animals and removed after 12 days (9th January, 1980). All the animals had retained the vaginal sponges. Two vasectomised raddled Finnish Landrace rams were introduced to the ewes. From the following day onwards ewes were observed for oestrus twice a day, in the morning and

in the afternoon, and the animals in oestrus were recorded. Out of the 54 animals 50 animals came into oestrus from 10th to 14th of January 1981.

4.2.6 Recording of ovulation rate and serial bleeding

Twenty five Blackface ewes and twenty five Finn ewes that came into oestrus between 10th and 14th January were divided into three groups namely control (C), one implant (1I) and two implants (2I) balanced for age. The number of animals in each treatment group is shown in Table 4.1.

Table 4.1: Number of animals in each treatment group

Breed	Control	1 Implant	2 Implant
Blackface	8	8	9
Finn	8	8	9

Twentyfive animals that came into oestrus from 10th morning to 12th morning (first batch) were brought in on the afternoon of 21st January and both sides of their necks were clipped with electric clippers in order to facilitate easy bleeding from the jugular vein. Animals were allocated to six pens randomly within groups. On 22nd morning blood samples were collected from all the animals every 40 minutes from 0830 hrs until 1230 hrs. Animals were then taken to the surgery in groups between 1230-1400 hrs and empty implants or oestradiol 17β implants were inserted subcutaneously according to their treatment groups.

From 1510 hrs all the animals were bled every 40 minutes until 1110 hrs following day. On the afternoon of the 22nd the other 25 animals (second batch) that came into oestrus between the 12th afternoon and the 14th morning were brought in and blood samples were collected in the same way as in the previous group of animals. In each batch of animals there were approximately half of the animals from each treatment group within a breed. Figure 4.0 shows the blood sampling and implant insertion schedule.

After bleeding each group of animals were allowed to stay in pens until late afternoon and returned to the paddocks. The four animals that did not come into oestrus between 10th and 13th January were also given oestradiol implants according to their treatment group on day 10-12 of their oestrous cycle. Thereafter all the animals (54) were run together as a group and their next oestrus was recorded. Five days after recording the oestrus their ovulation rate was recorded by laparoscopy.

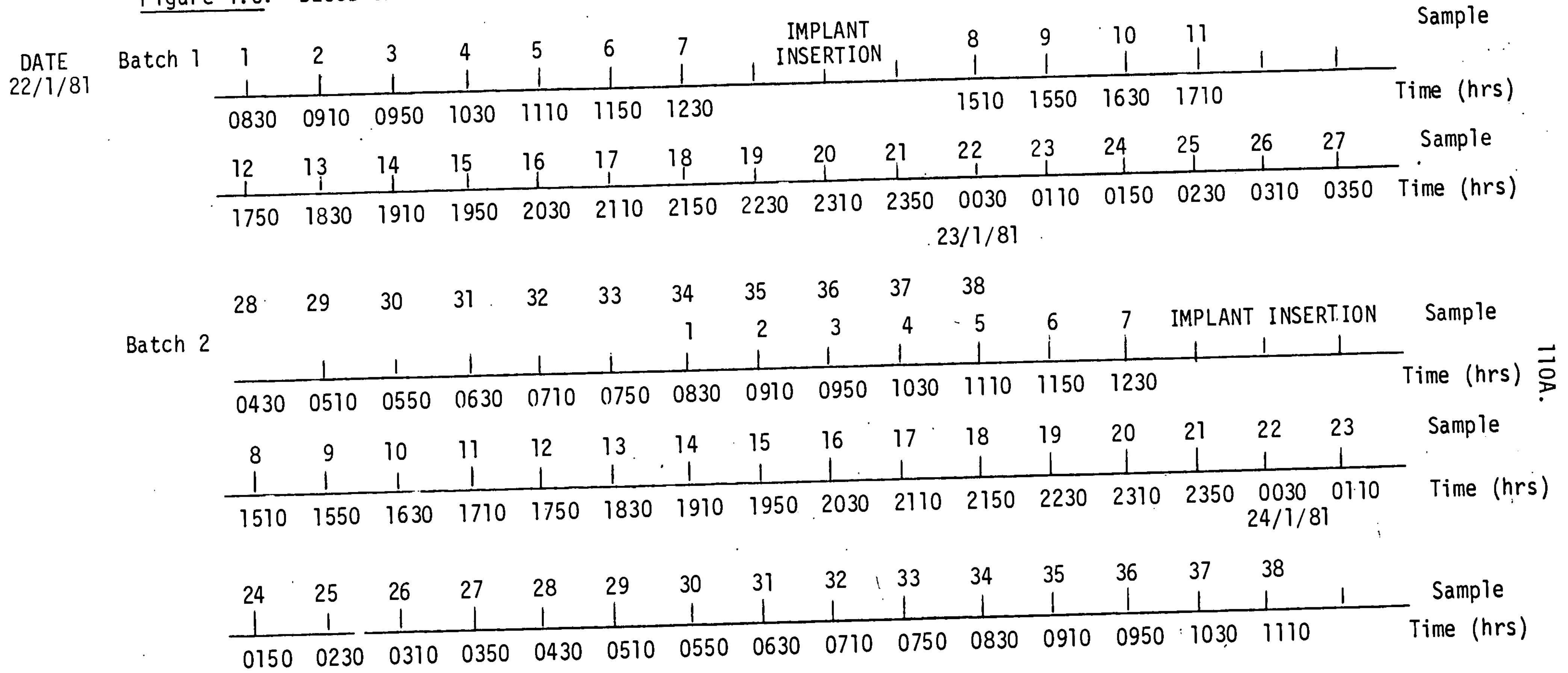
4.2.7 Hormone assays

LH and FSH concentrations were measured as described in Chapter 2. All the samples from one animal were run in a single assay for each hormone except for a small number (about 5%) which had to be re-assayed. Groups of fifteen samples were randomized in sequence for the assay.

4.3 Statistical Method

The "GLIM" statistical package (Royal Statistical Society, London) was used to analyse the data.

Figure 4.0: BLOOD SAMPLING - FINNISH LANDRACE AND BLACKFACE ANIMALS WITH OESTROGEN IMPLANTS



4.3.1 LH pulses

The number of LH pulses occurring before (8 samples) and after (30 samples) implant insertion was determined by the method described in Chapter 3. Pre-treatment LH pulses were analysed between the breeds and treatments to determine whether there was a breed difference before treatment and to test for any chance differences occurring among groups within breeds. In the model used breed and treatment were fitted as factors. A breed x treatment interaction was also fitted in the model. In the analysis it was assumed that the number of LH pulses had a Poissôn distribution. The GLIM programme was used to analyse the data with Poisson error and log link function.

LH pulses occurring in the 30 samples collected after the implant insertion also were analysed as in the first 8 samples. In the model breed and group were fitted as main effects. A breed x group interaction term was also fitted.

4.3.2 Mean LH levels

Another analysis was conducted to determine the differences in the mean LH levels in the two breeds and treatments. As the consecutive samples are highly correlated every other sample was considered in this analysis. Samples collected in every 80 minutes were subjected to analysis of variance. To bring the distribution of the LH values closer to normality the LH data were transformed into logarithms. In the model whole set of data, both pre-treatment and post-treatment was considered together. The 38 samples collected from each animal were divided into four occasions. The occasions were

as follows:

Sample 1-8	(8 samples)	Occasion 1
Sample 9-20	(12 samples)	Occasion 2
Sample 21-30	(10 samples)	Occasion 3
Sample 31-35	(8 samples)	Occasion 4

After dividing the 38 samples into four occasions the design of the experiment is as follows:

	Blackface			Finn		
	Group			Group		
	1	2	3	1	2	3
Occasion 1	1	1	1	1	1	1
2	1	2	3	1	2	3
3	1	2	3	1	2	3
4	1	2	3	1	2	3

1 = Control	1 = treatment 1	Group 1,2,3
2 = 1 implant	2 = treatment 2	
3 = 2 implant	3 = treatment 3	

For the purpose of the analysis all the cells with 1 were coded as treatment 1 and cells with 2 and 3 were coded as treatment 2 and 3

respectively. In the model breed, occasion, treatment and animals within group within breeds were fitted as factors. Treatment x occasion, breed x occasion, breed x treatment two way interactions and breed x occasion x treatment three way interaction terms were fitted. In this analysis treatment x animal interaction was not considered. Although samples were collected in two batches of animals the effect of batch was not considered in the model as all the animals in both batches were in day 10-12 of the oestrus cycle.

4.3.3 Mean FSH levels

FSH potency data obtained in the first 8 samples collected before and 30 samples collected after implant insertion were transformed into logarithms to normalise the data. Before analysing the total set the first 8 samples from all the animals were subjected to analysis of variance to look for breed differences and the chance differences among the treatment groups within breeds. In the model breeds, treatments and animals within treatment groups within breeds were fitted as factors. A breed x treatment group interaction term was also fitted with the main effects.

In the final analysis all the 38 samples were considered together. The 38 samples were divided into four occasions as for the mean LH levels. After dividing the 38 samples into four occasions the design of the experiment is equivalent to mean LH level analysis. Data were subjected to analysis of variance using a similar model as with the mean LH levels.

4.3.4 Ovulation rate

The ovulation rate data obtained after implant insertion from

the 54 animals were analysed using two error structures, normal and Poission error. The analysis which used the Poission error structure gave a better fit to the ovulation rate results. In the final analysis a Poission distribution was assumed to the ovulation rate data. Breed and treatment were fitted as main effects in the model. A breed x treatment interaction term was also fitted with the main effects. Fisher's exact test was used to test whether the number of animals ovulating after treatment was significantlydifferent in each breed.

4.5 Results

When the LH pulses were examined in the first 8 samples in the breeds and treatments, there were no significant differences between the two breeds or chance differences between the treatment groups within breeds. The analysis of variance table is given in Table 4.1:

Table 4.1: Analysis of variance of pre-treatment LH pulse data

Effect	DF	M.S.	F. ratio
Breed	1	0.555	0.616
Treatment	2	0.755	0.838
Breed x Treatment	2	1.780	1.977
Error	44	0.900	

In the analysis where the pulses occurring in the 30 samples after the treatment were considered there were no significant breed effect but there were significant treatment effect (P<0.05). Breed x treatment interaction was also not significant. The analysis of variance table is given in Table 4.2.

Table 4.2: Analysis of variance for LH pulse data after treatment

Effect	DF	M.S.	F ratio
Breed	1	0.72	0.670
Treatment	2	4.016	3.735**
Breed x treatment	2	2.413	2.244
Error	44	1.075	

The fitted values for pulses from each of the treatments show a lower number of pulses in the groups that were treated with oestradiol implants. The fitted values of pulses for each of the breed and treatment are given in Table 4.3.

Table 4.3: Fitted values of number of LH pulses in samples collected after implant insertion for 20 hours. Standard errors are given in brackets.

No. of implant	Blackface	Finn
0	2.63 (0.57)	1.63 (0.45)
1	1.50 (0.43)	1.50 (0.50)
2	0.70 (0.26)	0.88 (0.27)

All the groups treated with oestradiol 17β implants had a lower number of LH pulses indicating an effect from the implant. Compared with values in the control the drop in LH pulses in the treatments was higher in Blackface than in Finns indicating a higher sensitivity to negative

feed-back in Blackface.

4.5.1 Mean LH levels

In the analysis where overall mean LH levels were tested for differences there were significant ($P<0.01$) effect due to breed, occasion, treatment and animals within group within breed. The analysis of variance table is given in Table 4.4. Individual animal mean LH values (untransformed) over the four occasions in the two breeds are given in Table 4.5. Figure 4.1 shows the graph drawn for the fitted values for each of the breeds, treatments and occasions. As all the treatments did not behave in the same manner in the four occasions in the two breeds, statistically significant breed x treatment, treatment x occasion and breed x treatment x occasion interaction terms are explainable. In general mean LH levels tend to drop after the treatment but the effect is more in the Blackface ewes than in Finn ewes indicating that Blackface breed is more sensitive to negative feed back of oestradiol 17β.

4.5.2 Mean FSH values

When the first 8 samples of FSH values were subjected to analysis of variance there were no significant group or breed differences but the variation due to animals within treatment groups within breeds were significant. Although with high animal variation there were no chance differences between the groups.

In the analysis where all 38 samples were considered there were significant ($P<0.01$) animal and occasion effects. The breed x occasion breed x treatment and treatment x occasion two way interaction terms and

Figure 4.1. Fitted LH values over the four occasions for each of the treatment groups and breeds

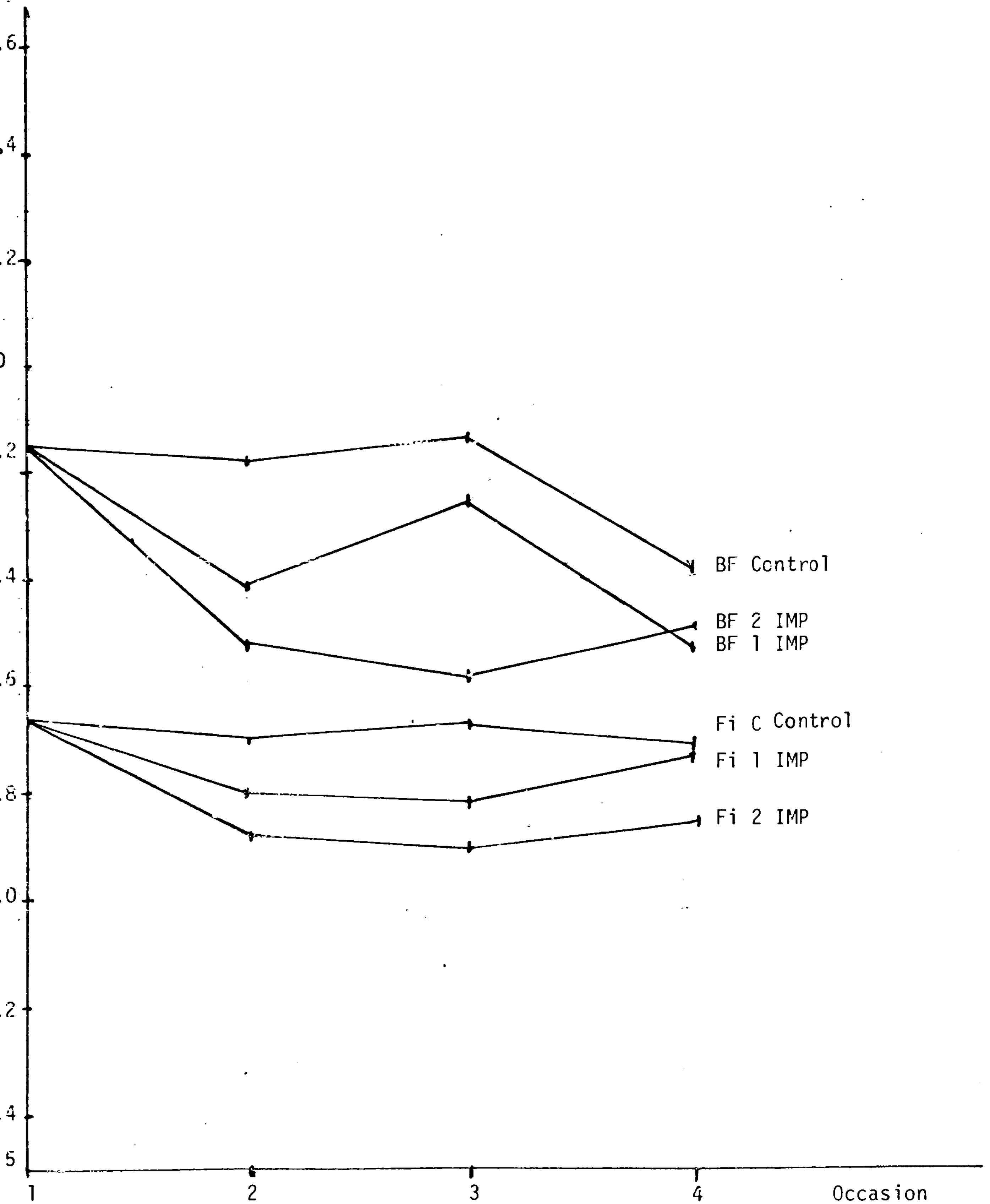


Table 4.4: Analysis of variance for LH levels throughout the sampling period

Effect	DF	M.S.	F. ratio
Breed	1	57.531	21.866**
Animals within groups within breeds	43	2.631	14.377**
Occasion	3	0.763	4.169**
Treatment	2	0.725	3.961**
Breed x occasion	3	0.166	0.907
Breed x treatment	2	0.673	3.677**
Treatment x occasion	4	0.675	3.688**
Breed x occasion x treatment	4	0.625	3.415**
Error	864	0.183	

Breeds were tested against the animal mean square and the other terms were tested against error.

Table 4.5 Individual animal mean LH (ng/ml) levels over the four occasions in Scottish Blackface and Finnish Landrace

Treatment and animal no	Occasion				Treatment and animal no	Occasion			
	1	2	3	4		1	2	3	4
Control					Control	0.977	0.648	0.596	0.704
BF1	0.833	0.873	0.719	1.170	Fi1	0.954	0.413	0.390	0.289
BF2	1.319	1.336	1.314	1.240	Fi2	0.475	0.413	0.403	0.385
BF3	0.867	0.679	0.871	0.998	Fi3	0.469	0.408	0.408	0.730
BF4	0.517	0.452	2.675	0.598	Fi4	0.569	0.593	0.508	0.455
BF5	0.585	0.316	0.606	0.295	Fi5	0.657	0.344	0.544	0.360
BF6	0.727	0.731	0.680	0.498	Fi6	0.318	0.346	0.417	0.355
BF7	0.720	1.190	0.635	0.524	Fi7	1.172	1.071	0.949	0.919
BF8	1.173	0.492	0.269	0.351	Fi8	0.490	0.440	0.429	0.321
One implant					One implant				
BF9	2.381	1.933	1.741	1.558	Fi9	0.757	0.480	0.507	0.364
BF10	1.316	0.816	0.764	0.664	Fi10	0.682	0.798	0.716	0.670
BF11	1.646	1.618	1.469	2.393	Fi11	0.306	0.239	0.225	0.279
BF12	0.898	0.536	1.659	0.513	Fi12	0.878	0.563	0.412	0.415
BF13	0.938	0.575	0.542	0.629	Fi13	0.423	0.358	0.293	0.340
BF14	1.156	0.971	0.609	0.559	Fi14	0.592	0.292	0.403	0.422
BF15	1.101	0.774	1.156	0.633	Fi15	0.490	0.359	0.337	0.281
BF16	1.786	1.547	1.424	1.209	Fi16	0.812	0.574	0.540	0.579
Two implant					Two impant				
BF17	0.937	0.901	0.764	0.807	Fi17	0.552	0.417	0.489	0.510
BF18	0.593	0.535	0.498	0.614	Fi18	0.396	0.305	0.369	0.278
BF19	1.113	1.111	0.948	0.876	Fi19	0.502	0.496	0.552	0.463
BF20	1.094	0.826	0.491	1.007	Fi20	0.484	0.456	0.447	0.616
BF21	0.681	0.399	0.443	0.421	Fi21	0.604	0.591	0.602	0.620
BF22	1.259	0.891	0.966	0.739	Fi22	0.404	1.358	0.423	0.709
BF23	0.676	0.439	0.493	0.439	Fi23	0.594	0.265	0.247	0.237
BF24	1.373	0.490	0.364	0.695	Fi23	0.542	0.042	0.471	0.511
BF25	0.932	0.309	0.458	0.369	Fi24	0.593	0.519	0.446	0.432

Table 4.5 Continued

Treatment and animal no	Occasion				Treatment and animal no	Occasion			
	1	2	3	4		1	2	3	4
Treatment mean					Treatment mean				
Control	0.842	0.758	0.971	0.710	Control	0.760	0.584	0.580	0.564
1 implant	1.402	1.096	1.170	1.019	1 implant	0.617	0.457	0.429	0.418
2 implant	0.966	0.655	0.602	0.659	2 implant	0.519	0.505	0.449	0.486

BF = Blackface Fi = Finn

breed x occasion x treatment three way interaction were significant ($P<0.01$). The analysis of variance table is given in Table 4.6.

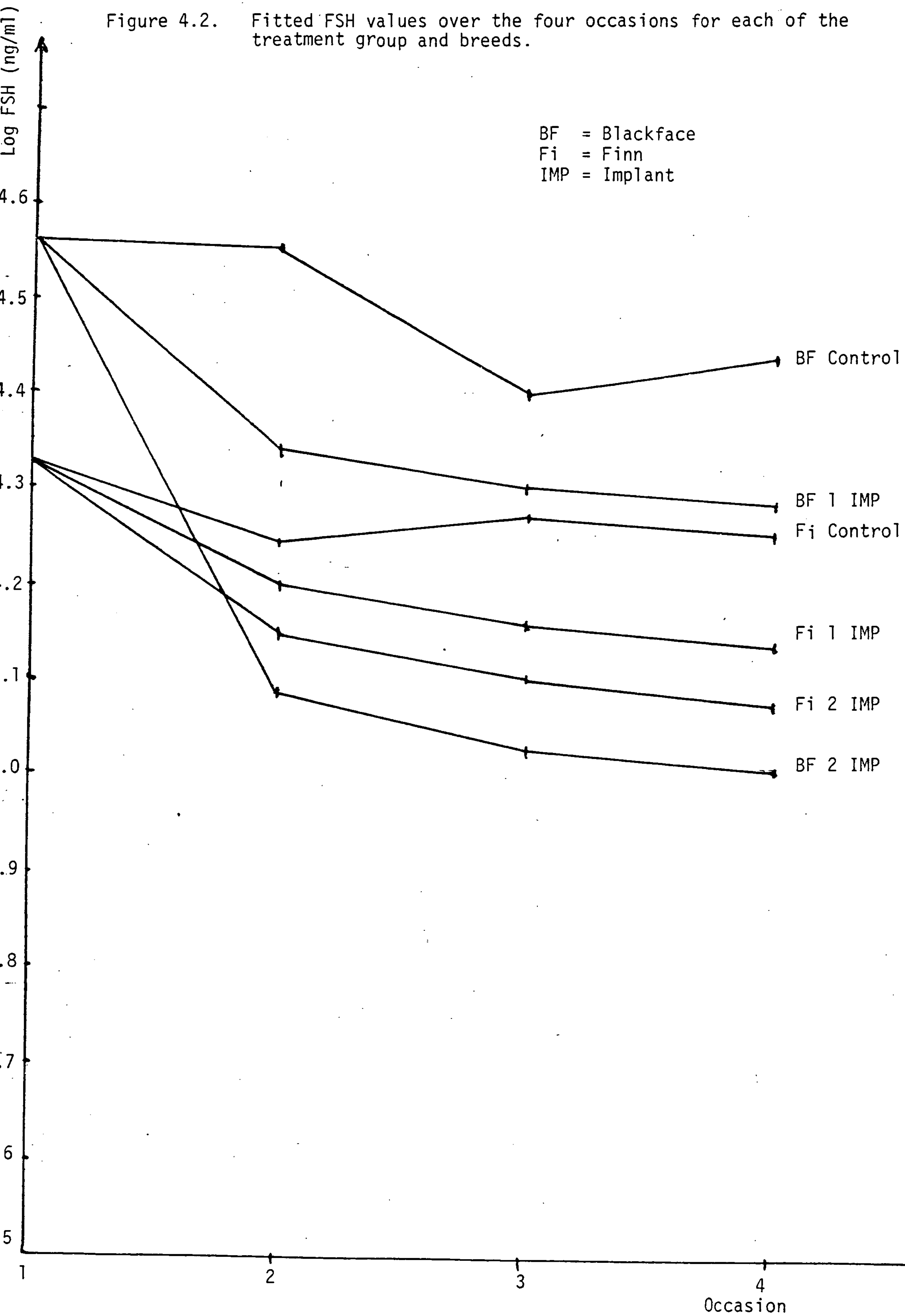
Table 4.6: Analysis of variance for FSH data

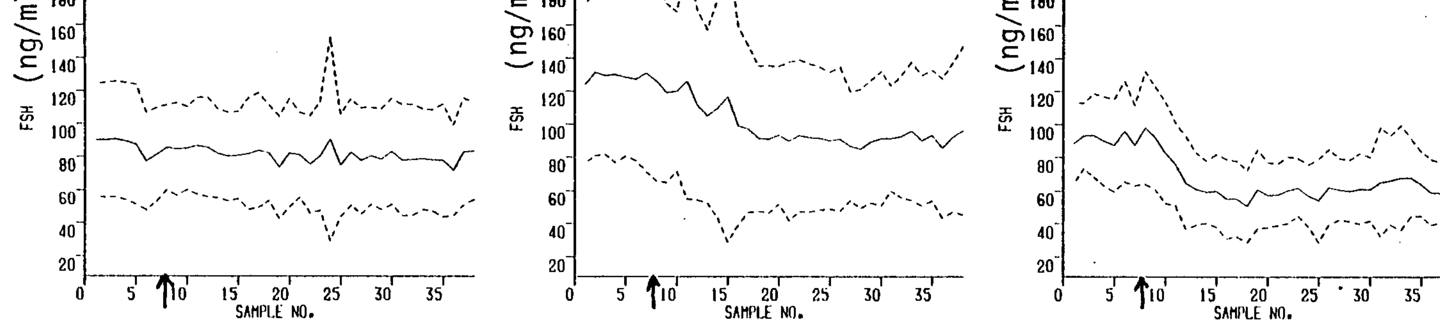
Effect	DF	M.S.	F. ratio
Breed	1	7.831	2.334
Animals within group within breed	43	3.354	104.812**
Occasion	3	0.413	12.906**
Treatment	2	1.817	56.781**
Breed x occasion	3	0.134	4.187**
Breed x treatment	2	0.276	8.625**
Treatment x occasion	4	0.128	4.000**
Breed x treatment x occasion	4	0.217	6.781**
Error	1832	0.032	

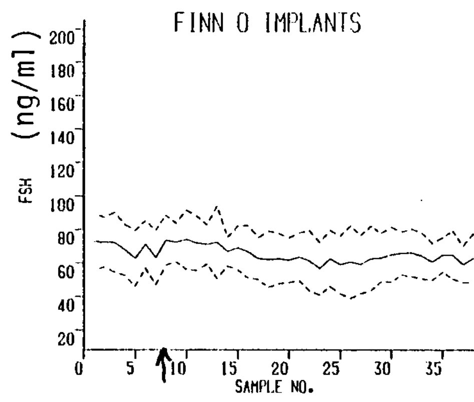
Breed was tested against animals and the other terms were tested against error.

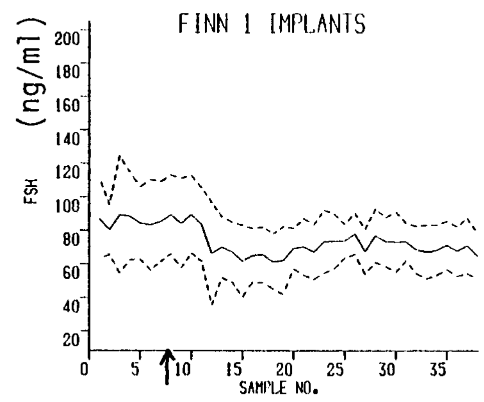
Individual animal mean FSH levels over the four occasions in the two breeds are given in table 4.7. The fitted values for each of the groups and occasions within breed are graphically represented in figure 4.2. The observed statistically significant interactions are explainable from the graph drawn with the fitted values. In general FSH values tend to drop after the treatment but the effect of treatment is more marked in Blackface breed showing a higher sensitivity to negative feed-back to oestradiol 17β. The changes seen in absolute concentrations in each treatment is shown in Figure 4.3 and 4.4.

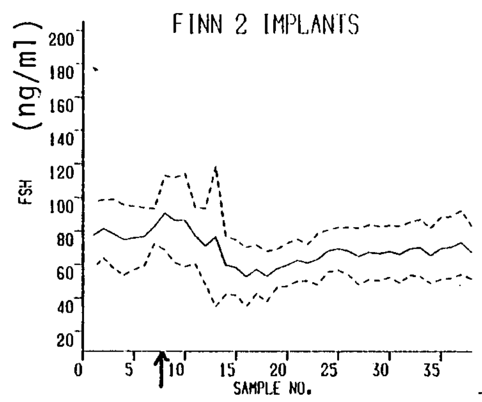
Figure 4.2. Fitted FSH values over the four occasions for each of the treatment group and breeds.











Arrow indicate the time of treatment

OBSERVED FSH MEAN LEVELS+/- 1 STANDARD DEVIATION.

Figure 4.4. Untransformed FSH values in the treatment groups in the two breeds.

dotted line indicate one standard deviation

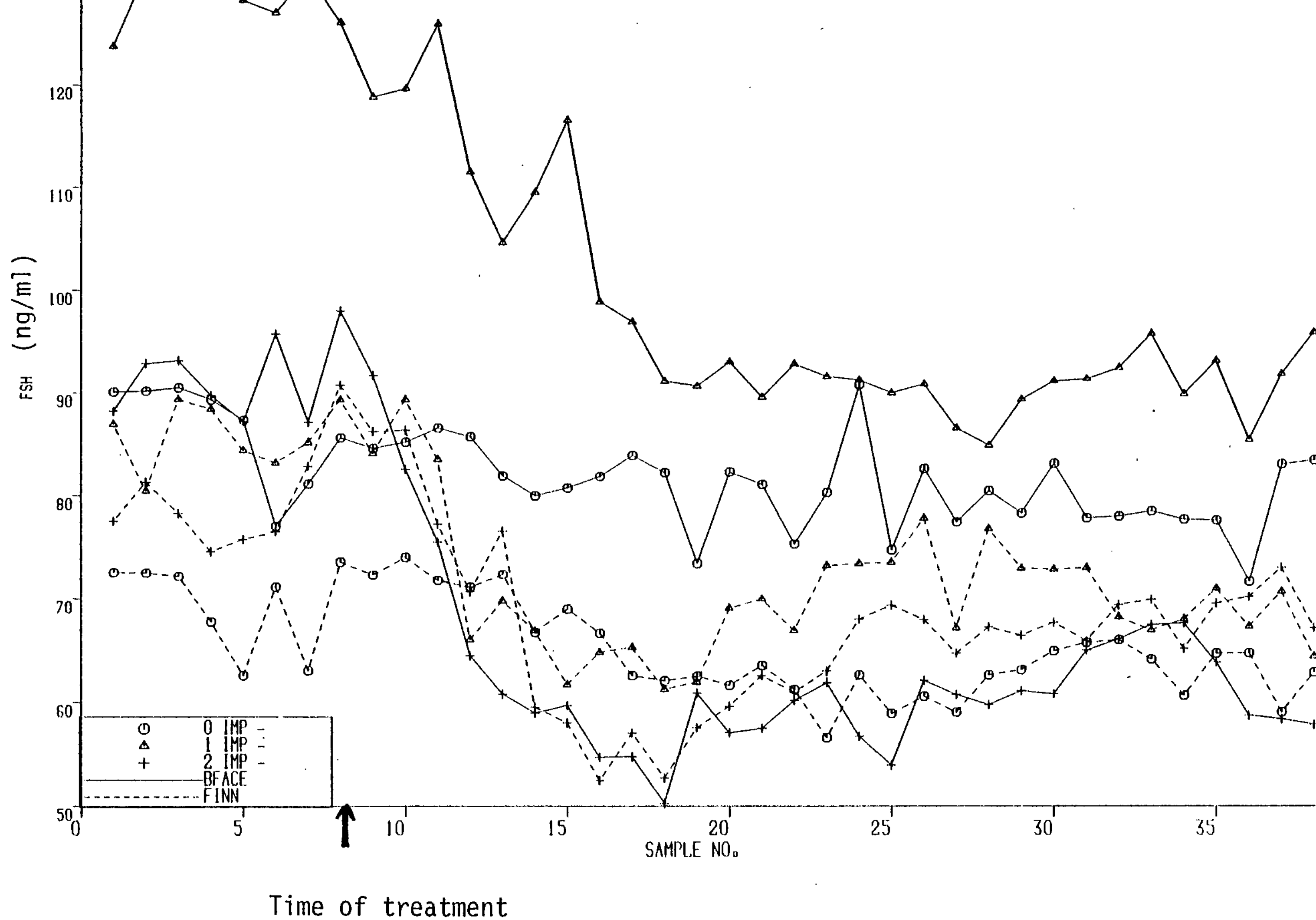


Figure 4.3. Graph of untransformed FSH values over the 38 occasions.

Table 4.7: Individual animal mean FSH (ng/ml) levels over the four occasions in Scottish Blackface and Finnish Landrace

Treatment and animal no.	Occasion				Treatment and animal no.	Occasion			
	1	2	3	4		1	2	3	4
Control					Control				
BF1	78.56	94.68	99.37	105.1	Fi1	69.83	72.18	71.38	67.09
BF2	63.63	66.18	64.00	59.68	Fi2	68.16	65.39	50.37	49.58
BF3	80.13	96.47	92.29	83.09	Fi3	92.24	98.64	95.51	88.66
BF4	101.4	87.54	60.65	76.47	Fi4	54.34	61.22	62.80	70.37
BF5	98.41	113.9	116.8	102.8	Fi5	64.82	63.06	56.32	57.42
BF6	68.35	38.95	36.94	43.39	Fi6	89.66	69.68	54.37	56.33
BF7	151.8	112.1	123.8	119.9	Fi7	56.51	54.00	52.71	55.72
BF8	48.88	49.01	49.42	37.49	Fi8	59.80	57.65	47.06	62.91
One implant					One implant				
BF9	209.8	196.9	144.1	123.0	Fi9	77.02	76.11	69.85	67.30
BF10	148.1	86.74	72.88	62.09	Fi10	82.33	59.56	86.07	75.04
BF11	116.5	79.89	75.51	69.71	Fi11	127.0	86.99	79.04	67.53
BF12	109.4	101.7	96.13	105.4	Fi12	74.29	50.78	50.89	47.44
BF13	104.2	78.98	61.65	65.63	Fi13	104.5	98.95	92.94	92.64
BF14	73.34	52.57	59.59	67.55	Fi14	69.14	56.45	65.35	62.07
BF15	107.5	98.46	88.36	84.37	Fi15	66.28	63.55	63.18	65.71
BF16	210.5	200.9	166.5	180.7	Fi16	76.03	75.13	73.17	72.32

Table 4.7 (continued)

Treatment and animal no.	Occasion				Treatment and animal no.	Occasion			
	1	2	3	4		1	2	3	4
Two implant					Two implant				
BF17	56.15	40.00	45.26	43.92	Fi16	64.33	61.59	47.87	41.96
BF18	84.39	59.13	56.58	66.27	Fi17	74.11	60.58	86.41	83.72
BF19	114.5	89.36	82.41	87.92	Fi18	52.66	40.70	47.79	52.00
BF20	85.84	57.99	56.69	55.86	Fi19	81.70	69.71	64.52	64.02
BF21	70.27	37.90	30.74	32.55	Fi20	100.2	86.86	75.97	82.24
BF22	109.8	64.36	65.83	59.32	Fi21	90.11	71.92	61.27	57.11
BF23	61.11	44.19	44.91	50.96	Fi22	103.1	86.54	73.85	80.96
BF24	114.8	77.06	59.03	65.52	Fi23	83.60	59.12	65.98	87.38
BF25	122.0	105.3	93.98	104.4	Fi24	67.73	58.73	68.80	70.11
Treatment mean					Treatment mean				
Control	86.39	82.35	80.40	78.49	Control	69.42	67.72	61.31	63.51
1 Implant	134.9	111.9	95.59	94.80	1 Implant	84.68	70.93	72.57	68.76
2 Implant	90.98	64.25	59.41	62.96	2 Implant	79.72	66.17	65.82	68.83

BF = Blackface Fi = Finn

4.5.3 Ovulation rate

In the ovulation rate data there were significant (P<0.01) breed and treatment effects, the breed x treatment interaction was not significant. The analysis of variance table is given in Table 4.8.

Table 4.8: Analysis of variance for ovulation rate

Effect	DF	M.S.	F. ratio
Breed	1	18.991	33.553**
Treatment	2	3.763	6.648**
Breed x treatment	2	0.836	1.477
Error	48	0.566	

The fitted values for ovulation rate shows a much higher drop in ovulation rate between one and two implants in the Blackface breed than in the Finn breed. The use of a reduction in mean number of eggs shed by a group of animals as a measure of the effect of treatment may underestimate those effects as some animals fail to ovulate and an animal cannot score less than nought. The fitted values for ovulation rate are given in Table 4.9.

As the reduction in ovulation rate is much higher in the Blackfaces than in Finns, it shows that Finns could tolerate more oestradiol 17β in the circulation without stopping the ovulation rate than the Blackfaces. Table 4.10 shows the number of animals ovulating in each treatment.

Table 4.9: Fitted values of ovulation rate for the three treatment groups in Blackface and Finn

	Blackface	Finn
0 Implant	1.01	3.11
1 Implant	0.66	1.78
2 Implant	0.33	1.63

Table 4.10: Number of animals ovulating in each treatment

	Blackface ovulate	Finn ovulate
0 Implant	9/9	9/9
1 Implant	6/9	8/9
2 Implant	3/9	9/9

With increasing oestradiol implants significantly ($P<0.05$) less number of animals ovulate in Blackface but in Finns there is no significant reduction in the number of animals ovulating. Even with high oestradiol levels (2 implants) all the animals ovulate in Finns but in Blackface only 3 animals ovulate out of 9 animals. Although the mean ovulation rate was reduced to one half that of the controls in Finns it did not have an effect on the proportion of ewes which ovulated. The ewes in the Finn breed were therefore less sensitive to the additional oestradiol as measured by the ability to ovulate.

4.6 Discussion

There were no significant differences between Blackface and Finnish Landrace in the number of LH pulses or mean LH levels in the pretreatment period. This result is in agreement with the previous finding of Land et al., (1973) and Cahill (1979) that the concentration of LH in peripheral plasma during the oestrous cycle were similar in breeds of sheep differing three fold in ovulation rate. When the oestradiol implants were given to Scottish Blackface and Finnish Landrace during the luteal phase (day 10-12 of the oestrous cycle) the number of LH pulses occurring decreased in both breeds, but the decrease was more marked in Blackface ewes than in Finn ewes indicating that the Finns are less sensitive to negative feed-back of oestradiol 17β in releasing LH from hypothalamus/pituitary. It shows that the Finn ewes could tolerate higher levels of oestradiol 17β.

Oestradiol 17β implant also decreased the mean LH levels to a greater extent in Blackface ewes than in the Finn ewes and indicated that mean LH levels are reduced in the two breeds to different levels. Therefore it shows that there is breed variation in sensitivity of the hypothalamus/pituitary to negative feed-back of oestradiol-17β in LH release both in LH pulse frequency and mean LH levels. Similar observation has been made in ovariectomised sheep (Land, Wheeler and Carr, 1976) in relation to mean LH levels.

There were no breed differences in the peripheral FSH levels in the control animals and pre-treatment blood samples. This results also confirms the finding of Bindon et al., (1978) and Cahill (1979) that the between breed variation in ovulation rate is not explained by the peripheral FSH levels. In Blackface ewes FSH levels were reduced to a

greater extent than in Finns after treatment with oestradiol 17β implants. It indicated that the two breeds differ in sensitivity to negative feed-back of oestradiol 17β. Finn being a high prolific breed can tolerate more oestradiol 17β in the circulation without reducing the FSH secretion. Blackface reduce the FSH releasing pattern to a greater extent showing that they are more sensitive to oestradiol negative feed-back in releasing FSH. There are no other between breed studies reported on the exogenous oestadiol on the FSH releasing patterns in the ewe. However this finding also confirms that oestradiol 17β is also involved in regulating FSH secretion and that breeds differ in their sensitivity to oestradiol negative feed-back on FSH release.

With oestradiol implants Blackface ewes reduce the ovulation rate to a greater extent than in the Finn ewes. Land (1976) also found with exogenous oestradiol injections that Finn ewes could tolerate more oestradiol 17β than Blackface ewes without stopping ovulation. This finding was more obvious when the number of animals ovulating was taken into account. In the Finn breed all the animals ovulated with two implants, but in Blackface only 3 animals ovulated out of 9. This confirms that there is between breed variation in sensitivity of the hypothalamus/pituitary to negative feed-back of gonadal steroids and that the sensitivity relates to the ovulation rate of the breed.

It is now accepted that the gonadotrophin levels found across breeds (Bindon et al., 1979) or between strains within the same breed (Bindon and Piper, 1980) are similar. However, the oestradiol levels found between breeds are different. Cahill, Saumande, Revault, Blanch, Thimonier, Mariana and Mauleon (1981) with sensitive

oestradiol assay systems found that the high prolific breeds to have a higher concentration of oestradiol 17β throughout the oestrous cycle than the low prolific breeds. With similar gonadotrophin levels and different oestradiol levels found between breeds it can be argued that the prolific breeds are less sensitive to negative feed-back from the ovarian steroids to the gonadotrophic release so that the high prolific breeds could produce more ovarian follicles and ovulate more.

The mechanism of feed-back action of oestradiol 17β on the hypothalamus/pituitary is not clear. However feed-back action of oestrogen is probably mediated via specific receptors in the hypothalamus/pituitary. Differences in total cytoplasmic oestrogen receptors in pituitary have been reported in Merino and Merino x Border Leicester (crossbred) ewes (Clarke, Burnman, Funder and Findlay, 1981). High prolific breeds have a higher number of total cytoplasmic oestradiol receptors in the pituitary than the low prolific breeds. It may be possible that the high prolificacy breeds have a higher number of oestradiol receptors in the hypothalamus/pituitary so that a greater concentration of oestradiol is required to saturate all the receptor sites than for less prolific breeds and hence highly prolific breeds could tolerate more oestradiol in the circulation without reducing the gonadotrophic release. In this way high prolificacy breeds could produce more ovarian follicles and ovulate more eggs.

Chapter 5: Genetic variation in the sensitivity of the hypothalamic/ pituitary to negative feed-back of oestradiol 17β

5.1 Introduction

The number of young born (litter size) is a complex character determined by ovulation rate and embryonic losses. Several workers have considered ovulation rate as a selection cirterion for increasing litter size in sheep (Hanrahan, 1974; Land, 1974). Genetic variation in ovulation rate has been demonstrated both in farm and laboratory animal species. In mice ovulation rate has been shown to respond to direct selection (Land and Falconer, 1969; Bradford, 1969) and to change as a correlated response to selection for other traits (Bradford, 1971). Zimmerman and Cunningham (1975) obtained a realised heritability estimate of 45% from selection in pigs. In sheep heritability estimates of $0.57 \pm .28$ and $0.35 \pm .19$ have been reported for ovulation rate in Finnish Landrace and Galway breeds respectively (Hanrahan, 1980). Furthermore a number of high repeatability estimates available from different breeds suggest a considerable amount of genetic variation in ovulation rate in sheep.

An understanding of the underlying physiological variation in ovulation rate would enable development of improved selection criteria for increasing ovulation rate. Such knowledge is also necessary for development of reliable methods of phenotypic modification of gonadal function in the female (i.e. superovulation). There have been two studies reported on the underlying physiological mechanism of genetic variation in ovulation rate in mice. Bradford (1969) and Land and Falconer (1969) selected mice on their response to exogenous gonadotrophins.

However the genetic correlation reported for the natural and induced ovulation rate was low (Land and Falconer, 1969). Most of the other work involved a study of between breed variation in physiology of ovulation and reproduction (Cahill, 1979, Land, 1974). Some of the physiological criteria such as the time interval from the onset of oestrus up to the preovulatory LH peak have been shown to differ between breeds (Cahill, 1969; Wheatton, Raabound and Burril, 1977) but none of such characters have been shown to be genetic in origin.

Variation in the sensitivity of the hypothalamus/pituitary to the negative feed-back effects of gonadal steroids on the gonadotrophin release has been postulated to underly genetic variation in ovulation rate among some breeds of sheep i.e. it appears to be the cause of natural variation (Land, 1976; Land, Wheeler and Carr, 1976). The evidence for such an argument has been reviewed (Land and Carr, 1979). Increasing ovulation rate has been reported after removing the potential feed-back hormones by active immunization (Scaramuzzi, Davidson and Van Look, 1978). The evaluation of the above hypothesis is complicated by the role of a number of steroids, oestradiol 17β, oestrone, progesterone, androgens and possibly inhibin in the feed-back control of gonadotrophin release.

This experiment was undertaken to investigate the genetic variation in sensitivity of the hypothalamus/pituitary to the negative feed-back of oestradiol 17β as oestradiol 17β is an important feed-back hormone in the female. The direct effect of antibodies against oestradiol 17β on the ovulation rate of mice was also studied. The sensitivity of the hypothalamus/pituitary to negative feed-back was measured indirectly by recording the ovulation rate after using an antiserum against oestradiol 17β to reduce feed-back. The ovulation rate of the

offspring and parent are recorded after modifying the negative feed-back with same amount of antiserum against oestradiol 17β. The heritability of the trait is determined by the offspring parent regression of the treated ovulation rate.

5.2 Materials and Methods

5.2.1 Genetic stock

The mice used in this experiment were the parents and their progeny of generation zero of the "G" line developed by Miss G. Sharp. Line G was developed as follows. Two inbred lines JU and CBA were crossed to form a crossbred called JUCBA. JUCBA animals were crossed to an outbred line (CFLP) to form the JCC progeny. Generation zero of the "G" line came after one generation of inter se breeding of JCC animals.

5.2.2 Feeding and management

This experiment was carried out at the mouse house of the Institute of Animal Genetics, Edinburgh. Feeding and management were as described by Land and Falconer (1969).

5.2.3 Antiserum

The antiserum used in this experiment was raised against conjugate of oestradiol 17β-BSA in castrate male sheep. The titre of the antiserum and the cross reactivity was determined by the methods described in chapter 2. The titre of the antiserum was 1:56,000. The cross reactivity of the antiserum is given in Table 5.1. Antiserum

was stored at -20°C in 20ml and 5ml screw capped bottles. Before injection antiserum was thawed in a water bath at 38°C. Antiserum against oestradiol 17β was injected intraperitonially by using a hypodermic needle (26 G 5/8') fitted to a 1 ml disposable syringe (Becton-Dickson). All the antiserum injection were done between 1300 and 1600 hours.

Table 5.1 Cross reactivity of the oestradiol 17β antibody

Compound tested	Percentage cross reaction (oestradiol 17β = 100 percent)
Oestradiol 17β	100
Oestradiol 17α	1.2
Oestrone	4.2
Androstenedione	<0.001
Testosterone	<0.002
Progesterone	<0.002

5.2.4 Bleeding for antibody titre

Bleeding by Cardiac puncture was performed after anaesthatising the mouse with ether and opening the thoracic cavity at the time of recording the ovulation rate. The volume of blood withdrawn per mouse varied from 600-900 µl. Plasma was separated within two hours of bleeding by centrifugation and stored in 0.5ml polystyrene cups at -20°C until titrated for oestradiol -17β antibodies.

5.2.5 Recording of ovulation rate

Males were introduced to the females in cages on each occasion between 1300 and 1500 hours. Naturally ovulation occurs close to the time of mating. After introducing the male each female was examined every morning between 0800 and 1000 hrs and the occurrance of ovulation was identified by the presence of the copulatory plug in the vagina. Recording of ovulation was done by a method similar to that described by Land and Falconer (1969). After detecting the vaginal plug the female was killed and the abdominal cavity was dissected. Left and right fallopian tubes were carefully removed and placed in a drop of saline in two separate watch glasses. Under a dissecting microscope the presence of ova in the fallopian tube could be identified as a discrete swelling. The swollen wall of the fallopian tube was nicked, and the clump of ova embedded in the cumulus cells oozed out. The clump of ova with cumulus cells was drawn to the edge of the saline drop and flattened and the ova identified and counted. Females with vaginal plugs were always dissected before 12.00 hrs at which time the cumulus was still in good condition.

5.2.6 Preliminary experiment

Before starting the main experiment a preliminary experiment was set up in order to find the effect of the oestradiol 17β antiserum on the ovulation rate and the correct dose to administer to obtain an increase in ovulation rate. Sixty first parity (after the first litter) female mice were randomly divided into four groups of equal size.
One group was kept as control and the other groups were injected either with 0.1ml, 0.3ml or 0.5ml of oestradiol 17β antibody intraperitonially. After

antiserum treatment animals were mixed and kept in groups of four per cage. Two days after the treatment a mature fertile male was introduced to each of the cages. After the introduction of the male every morning females were examined for vaginal copulatory plugs and their ovulation rate was recorded. Twelve days after the introduction of the males the experiment was terminated.

The results of this preliminary experiment are summarised in table 5.2. An analysis of variance showed there to be significant (P<0.01) group effects. The group that was treated with 0.3ml of

Table 5.2 Preliminary experiment results

NUMBER OF ANIMALS INJECTED	TREATMENT ANTIBODY INJECTED (ml)	NUMBER OF ANIMALS OVULATION	MEAN OVULATION±SE	MEAN DAYS TAKEN TO OVULATE
15	0.0	15	12.33±0.22	3.26±.26
15	0.1	15	12.46±0.31	4.46±.31
15	0.3	15	15.52±0.31	5.50±.37
15	0.5	9	13.88±0.32	6.22±.39

oestradiol 17β antibody gave the highest response in ovulation rate. From this preliminary experiment it was decided to treat the animals with 0.3ml of antibody to increase the ovulation rate in the experimental animals.

5.2.7 Main experiment

All the females of generation zero of the "G" line were

collected after weaning their first litters. At this stage their approximate age was about 98 days. There had not been any selection practised on this population of animals at this stage. After collecting the females they were mixed and kept in groups of four for 3-4 days. After recording their weight each female was injected with 0.3ml of oestradiol 17β antiserum intraperitoneally and kept in cages. Two days later a fertile male was introduced to each of the cages and females were examined daily for vaginal plugs and their ovulation rate was recorded. The time taken from the introduction of the males up to the time of ovulation was also recorded. All these animals were considered as parents for this experiment. Fourty of these were kept untreated as control to the main experiment and their ovulation was recorded. The offspring obtained from the first litter of these parents were all collected after weaning their first litters. The families that had more than one female were divided into two groups of equal size or to that of nearest possible equal size. When the families were divided into two equal groups, one half of the animals were treated and the other half kept untreated. When the families were divided into two unequal groups the larger group was treated with antiserum and the other group was untreated. In the families with only one member it was always treated. Treated and untreated animals were kept in cages as mixed groups. Two days after treatment with antiserum a fertile male was introduced and the ovulation rate was recorded both in the treated and untreated animals. At the time of recording of ovulation rate blood samples were collected from a random sample of the animals treated with antiserum.

The offspring of the control parents were also collected after weaning their first litters. Progeny families from the control

parents were also divided into two groups as equally as possible as in the case of main experiment progeny families. In the control progeny when the families had two equal groups one group was treated and the other group was untreated. In the families with two unequal number of progeny groups, always the larger group was kept untreated and the smaller group was treated. Families that had only one individual were always treated.

For all these parents and progeny animals their three week body weight, six week body weight, first litter size and pedigree information were supplied by Miss G. Sharp.

5.3 Statistical analysis

Before analysing the total ovulation rate results a test was made to find out whether there were significant differences in ovulation rate in right and left side ovaries, both in parents and offspring, by using a 't' test. There were no significant differences in ovulation rate between the right and left ovary and it was decided to analyse the total ovulation rate. To check for any time trend in ovulation rate, monthly mean ovulation rates were plotted against months and a significant ($P<0.01$) time trend was shown. Although all the animals in this experiment were first parity animals, at the time of treatment (antibody injection) there was variation in weigh and age. The ovulation rate was subjected to regression analysis taking age at ovulation, time taken to ovulate from the injection, body weight at 3 weeks 6 weeks and at the time of treatment as independent variables.

The heritability of the treated ovulation rate was estimated by daughter-dam regression. Two methods were used. The

first method is to repeat the dam for each daughter in the family and the second method is to substitute the mean of the daughters for the daughter value. When the family size varies the second method can be improved by using a weighted regression (Kempthorne and Tandon, 1953). In calculating the regression, sums of squares and cross products were calculated within months as the effect of month was significant.

In order to calculate the weighted regression, the unweighted regression coefficient and intraclass correlation was estimated from an unweighted regression analysis and from full-sib family analysis of variance respectively.

$$\text{Variance (family mean)} = (t - r^2 + \frac{1-t}{n})\sigma_p^2$$

where t is intraclass correlation, r is offspring parent correlation, σ_p^2 is phenotypic variance and n is family size. The estimated offspring parent correlation and intraclass correlation was not significantly different from zero so that when t and r equal zero in the above formula the variance of the family mean equal to $(1/n)\sigma_p^2$ and therefore no weighting was done. Heritability was calculated as double the regression coefficient.

The regression of the daughters first litter size and daughters' untreated ovulation rate on the mothers' treated ovulation rate was estimated in the families that had more than one individual. As the intraclass correlation for the untreated ovulations and the unweighted regression of untreated ovulation on the treated mothers' ovulation were not significantly different from zero, no weighting was done. For the litter size data a weighted genetic regression on the mothers'

treated ovulation was estimated by using Falconer's (1963) modification of the optimum estimation procedure of Kempthorne and Tandon (1953), weighting the sums of squares and products from each dam family according to the family size.

The standard error of the regression coefficient was calculated from the following formula given by Falconer (1963).

$$V(b) = \frac{1}{\Sigma W-2}\left[\frac{\Sigma(wy^2)}{\Sigma(wx^2)} - b^2\right]$$

where Y is the character in the offspring, x is the character in the dam, w is the weight and b is the regression coefficient. In the families that had more than one offspring sib covariance was calculated between the mean treated ovulation rate and mean untreated ovulation rate. The genetic correlation was estimated by using the sib covariance (Falconer, 1960). The genetic correlation between the two characters (x and y) is given by the formula:

$$V_G = \frac{\text{Cov (full sib)}}{\sqrt{VB_X \quad VB_Y}}$$

where VB_X and VB_Y are full sib variance components. As the number varied within a family for treated and untreated individuals the family means for each of the characters were weighted according to the following weighting factor, inverse of the variance of the covariance

$$W_t \; \alpha \frac{1}{\text{Cov}B^2 + (VB_X + \frac{VW_X}{N_X})(VB_Y + \frac{VW_Y}{N_y})}$$

where VB_X and VB_Y between family variance for the two characters, VW_X

and VW_Y are within family variances for the two characters, N_X and N_Y are the number of individuals recorded for each character within a family.

Control animal ovulation rate data were analysed to find out the mean and variances of the treated and untreated ovulation rates. Means were tested for differences between control and treated animals. Antibody titres estimated in the plasma samples collected from the animals in different days after the injection of antibody were used to calculate the half-life of the antibody by using the method described in Chapter 3.

5.4 Results

5.1.2 Preliminary experiment

From the preliminary experiment results (see Table 5.2) it was shown that there were significant ($P<0.1$) differences between the antiserum treatment groups. It also showed that the group which had 0.5 ml antibody ovulation was blocked. Out of the 15 animals which had 0.5 ml antibody 6 animals did not ovulate.

The mean and variance of the ovulation rate in parent and progeny generations of the experimental and control animals are given in Table 5.3. The mean ovulation of the treated individuals in the offspring and parent generations was significantly increased ($P<0.01$) when compared with the mean untreated progeny. The variance of the treated ovulation was significantly increased from that of untreated ovulation rate. The variance of the untreated ovulation rate was 4.19 and the variance of the treated ovulation rate was 6.12.

Table 5.3: Mean ovulation rate and variance in parent and progeny generations of the control and experimental animals

Group	No. recorded	Mean	Variance
Control			
Parent untreated	40	12.97	3.99
Offspring untreated	129	13.03	4.03
Offspring treated	83	16.73	5.97
Experimental			
Parent treated	343	17.05	6.12
Offspring treated	724	16.88	6.07
Offspring untreated	422	12.88	4.19

5.4.2 Regression analysis

The regressions on ovulation rate on body weight are given in Table 5.4. There were significant regressions on body weight of animals at various stages on the treated and untreated ovulation rate. The regression analysis showed that the age of the animal did not have any significant effect on the ovulation rate. The mean age of the animals at the time of ovulation was 114 days. There was no significant regression for the treated ovulation rate on the number of days taken to ovulate from the time of introduction of the males. The mean number of days taken to ovulate from the time of introduction of the male was 5.52 days.

Table 5.4: Regression of ovulation rate on body weight

	Mean body weight (gr)	No. of animals	Regression
Mother (treated)			
(1) Body wt. at 3 week	10.72±0.13	343	0.16±0.09
(2) Body wt. at 6 week	21.82±0.27	343	0.24±0.03**
(3) Body wt. at injection	35.82±0.33	343	0.18±0.06**
Offspring (Treated)			
(1) Body wt. at 6 week	20.12±0.19	724	0.23±0.02**
(2) Body wt. at injection	32.74±0.37	724	0.18±0.03**
Offspring (untreated)			
(1) Body wt. at 6 week	21.73±0.21	422	0.12±04**
(2) Body wt. at injection	33.73±0.41	422	0.18±.04**

5.4.2A. Heritability

The regression coefficient of the treated ovulation rate of the offspring on the treated ovulation of the mother are given in Table 5.5.

Table 5.5: Regression of treated ovulation rate of the offspring on the treated ovulation rate of the mother

Method of estimation	Regression, offspring on parent
(1) Each individual repeated on parent	0.018±.033
(2) Family mean on the parent	0.003±.042

The number of families involved in the estimation of the regression were

343. Table 5.6 give the distribution of the family size for the treated and untreated animals.

Table 5.6: Distribution of family size in treated and untreated offspring

No. of females	No. of families treated	No. of families treated
1	128	103
2	101	104
3	72	37
4	33	
5	8	
6	1	

Both the regressions were not significantly different from zero. The heritability estimates from the above two methods were $0.037 \pm .066$ and $0.006 \pm .082$ respectively. The intraclass correlation obtained by analysis of full sib families of the treated families were $0.003 \pm .046$ and the estimate was not significantly different from zero.

5.4.3 Genetic regression

The genetic regression for the untreated daughter ovulation rate and daughter litter size on the mothers' treated ovulation rate are given in Table 5.7.

Table 5.7: Genetic regression of the daughter untreated ovulation rate and litter size on mothers' treated ovulation rate

Method of estimation	Genetic regression	Estimate
Unweighted	Daughters' untreated ovulation rate on mothers' treated ovulation	0.07±.04
Weighted	Daughters' first litter size on mothers' treated ovulation rate	0.01±.02

Both the estimates were not significantly different from zero. The distribution of the untreated daughter family size is given in Table 5.6. The intraclass correlation obtained for untreated ovulation rate by the full sib analysis was 0.0230±.1174 and it was not significantly different from zero. The partial genetic regression calculated for the offspring untreated ovulation rate on the mothers' treated ovulation rate by holding the body weight at various stages constant are given in Table 5.8.

The estimates obtained were not significantly different from zero. The weighted genetic regression of the daughters' first litter size on the mothers' treated ovulation rate fell between the two unweighted regressions.

Table 5.8: Partial genetic regressions of offspring untreated ovulation rate on the mothers' treated ovulation when the weight of the offspring at various stages kept constant

Method of estimation	Partial genetic regression estimate
(1) Weight at 3 week kept constant	0.028±.038
(2) Weight at 6 week kept constant	0.125±.052
(3) Weight at injection kept constant	0.049±.037

5.4.4 Genetic correlation

The genetic correlation calculated between treated and untreated ovulation rate from the sib covariance was meaningless. The numerator of the formula used to calculate the genetic correlation attained a higher value than the denominator and the calculation was not further proceded. The experiment was not designed to estimate genetic correlation from the sib covariance as the family sizes were small.

5.4.5 Phenotypic correlation

The phenotypic correlation calculated between the first litter size and subsequent treated ovulation rate of the daughters' and mothers' was 0.01±.02 and it was not significantly different from zero.

5.4.6 Half-life of oestradiol 17β antiserum

The half-life estimated for the oestradiol 17β antibody in the

blood circulation of the mice was 10.53±.23 days.

5.5 Discussion

As in the case of sheep, the ovulation rate in mice can be significantly increased by modification of the negative feed-back system through passive immunization against oestradiol 17β. The results indicate that similar physiological mechanism of negative feed-back of gonadal steroids operate in the sheep and mice. As similar physiological mechanism govern the ovulation mechanism both in the sheep and mice with respect to negative feed-back, the use of mouse as a model to study the genetic variation in negative feed-back system is justifiable. When the antibody is given in excess animals stop ovulation completely possibly because the oestradiol positive feed-back is blocked. It may also be possible that with excess of oestradiol antibodies, oestrous behaviour is stopped and the oestrous cycle is disrupted. A similar phenomenon has been observed in sheep with excess of oestradiol antibodies (Fairclough et al., 1976). It is clear from these results that by giving the appropriate dose of antibody the ovulation rate in mice can be significantly increased.

The variation in ovulation rate after treatment with PMSG was much higher than the natural ovulation (variance of the egg number was 49.0 with a mean ovulation rate of 14.0) even when the mean was taken into account (Falconer, Edwards, Fowler and Roberts, 1961). R.B. Land (unpublished data) observed a change in ovulation rate after modification of the negative feed-back system in sheep without a change in variance. It is interesting to note in this experiment also the change in mean ovulation rate in the treated animals

was not accompanied by a large change in variance. Since the variation is not changed very much as in the case of PMSG induced ovulation rate, it can be argued that the negative feed-back system control at least part of the natural phenotypic variation in ovulation rate. There is no significant effect of age on the treated ovulation rate. Roberts (1956) also reported that the age of animal did not have a significant effect on natural ovulation rate.

There is no significant regression on the number of days taken to ovulate on the induced ovulation rate. This finding can be explained by the longer half-life of oestradiol 17β antibody. In the chapter 3 it was shown that the effect of antibody on the ovulation rate is dose dependant. As the mean number of days taken to ovulate after antibody injection is nearly half that of the half-life, most of the animals at the time of ovulation have a similar antibody titre and therefore the effect of time taken to ovulate was not significant. Almost all the animals at the time of ovulation had more than half of the antibodies injected in the circulation.

The regression of treated ovulation rate on the body weight at various stages were significant. The phenotypic regressions obtained for ovulation rate on the body weight are comparable to those for natural ovulation reported by Land (1970). The increased ovulation rate obtained after passive immunization against oestradiol 17β reflects the phenotypic expression of the sensitivity of the hypothalamus/ pituitary to negative feed-back. The heritability estimated for the sensitivity of the hypothalamus/pituitary to negative feed-back was not significantly different from zero. The results indicate that the ovulation rate obtained after modification of the negative feed-back

system has a very low heritability and that if animals are selected for treated ovulation rate there will not be any response in the treated ovulation rate. When the partial regression was calculated by holding the body weight at various stages constant the offspring parent regression did not change and indicated that there are no maternal effects affected through the body weight for the first parity females. Even if there are maternal effects for the sensitivity to the negative feed-back they may have been reduced as the treated ovulation rate is recorded after the first parity. Vangen (1980) showed that for the litter traits maternal effects are less in pigs when they were recorded after the first parity.

Genetic regression of the daughters' untreated ovulation rate and first litter size on the mothers' treated ovulation rate was not significantly different from zero. It indicated that if the mothers are selected on the treated ovulation rate there will not be any correlated response in natural ovulation rate or litter size. Insignificant phenotypic correlation between the litter size and the treated ovulation rate showed that the treated ovulation rate does not give any indication of the phenotypic expression of the litter size.

The hypothesis of the between breed variation in sensitivity of the hypothalamus/pituitary to negative feed-back of gonadal steroids is that breeds with higher ovulation rate have a lesser sensitivity to negative feed-back hormones and therefore can tolerate more negative feed-back hormones in the circulation (i.e. oestradiol 17β). It also explains that if oestradiol is taken out of the circulation less sensitive breeds will ovulate more. Although it explains the physiological differences between the high and low ovulating breeds and also possibly

between the high and low ovulating individuals the trait has very little additive genetic variation within a breed.

Although sensitivity to negative feed-back explains part of the between breed variation in ovulation rate, within a breed the character is very highly sensitive to environmental variation. Seasonal breeding (Legan Karsch and Foster, 1977) and variation in ovulation rate due to nutrition (Findlay, Helen, Jonas and Cumming, 1978) in sheep has been explained through the variation in the sensitivity of the hypothalamus/pituitary axis, to negative feed-back. As the trait is very much sensitive to the environmental variation low heritability can be expected. Similarly between breed physiological differences in milk production do not explain the within breed variation in milk production (Osmond, 1980). Although part of the variation in ovulation rate observed between breed can be explained by the variation in hypothalamus/pituitary sensitivity to negative feed-back, the hypothalamic/pituitary sensitivity either determined by oestradiol receptors in the pituitary or any other form do not show substantial amount of genetic variation.

It is known that part of the natural variation in ovulation rate is determined by the growth of the animal (Bradford, 1971). When the mice were selected for growth there was a correlated response in litter size probably through increased ovulation rate (Bradford, 1971). Phenotypically heavy animals ovulate more eggs. In this study also a high positive correlation was observed between the body weight and the treated ovulation rate. Such association is possible through the natural variation in ovulation. Heavier animal ovulate more eggs and thereby when their negative feed-back system is modified they

ovulate more eggs in accordance with their body weight.

Results of this experiment suggest that the trait does not have substantial amount of additive genetic variability that can be exploited by mass selection. As this experiment was not designed to estimate very low heritability it is not possible to comment on the magnitude of the heritability. However, the possibility of increasing ovulation rate through the modification of negative feed-back is interesting. This technique could be used to superovulate animals without much change in variance in the ovulation rate, so that the ovulation rate could be increased predictably. Such techniques are especially important in sheep in which the ovulation rate is the limiting factor for increasing prolificacy. If the technique is refined it could be used commercially to increase the ovulation rate in animals especially in sheep and thereby to increase the litter size.

Chapter 6: General discussion and conclusions

6.1 Passive immunization

Mean LH levels and LH pulse frequently were increased after passive immunization again all four steroids. Mean FSH levels were increased after passive immunization against E_1 and E_2 but not against A and T. When the existing buffering systems between gonadotrophin and feed-back equilibria are changed through modification of the negative feed-back while maintaining them, rather than overriding them, the changes seen in gonadotrophic levels are only transcient and the system tend to equilibrate. Passive immunization against gonadal steroids can change the existing gonadotrophic and negative feed-back equilibria within physiological limits.

The ovulation rate was significantly increased in four groups of animals after passive immunization against E_1, E_2, A and T. It can be concluded that ovulation rate in sheep could be increased significantly by reducing the negative feed-back through immunization against gonadal steroids without reducing the positive feed-back. Although the length of the oestrous cycle is slightly changed after the antiserum treatment there is no general pattern in the change of oestrous cycle length in the four treatment groups. Increased antibody titre also had an effect on the number of animals showing oestrus. Such effects were minimal in the group immunized against testosterone. Passive immunization against all four steroids increased the ovulation rate but the lambing percentage was significantly increased only in the group imminized against testosterone.

As the ovulation rate in sheep can be increased by immunization against gonadal steroids without much change in variance the possibility

of using such techniques as a practical method of superovulation can be considered. An advantage of passive immunization over active immunization is that the antibody titre achieved in the circulation in passive immunization can be regulated. If this technique is to be used as a practical method of superovulation production of antisera with high titres is essential in order to reduce the dose to be administered. Furthermore the cross reactivity of an antibody may change response observed and therefore production of specific antibodies would be essential in order to standardise the method. Because of the curvilinear response in ovulation rate in sheep within a wide range of antibody titres it may not be possible to achieve high ovulation rates such as in superovulation with PMSG. However such a relationship within a wide range gives an advantage that for a given ovulation rate the titre achieved in the circulation is not very specific. Even if the titre is slightly out of range the response observed is not changed to a great extent.

Modification of gonadal feed-back through other methods may be considered to improve gonadal function. An alternative is to reduce the feed-back effect by use of anti or weak oestrogen which would reduce the inhibitory effects of endogenous oestrogens. Snook and Hansel (1971) reported a rise in ovulation rate of sheep following treatment with anti-oestrogen clomphene. The use of 'anti-oestrogens' to increase gonadal function through modification of negative feed-back is now being considered at ABRO. Increased ovulation rate obtained after immunisation against androstenedione and testosterone could be explained through the effects of androgens on the production of inhibin in the granulosa cells. Androgens stimulate inhibin production in the granulosa cells (Franchimont, 1981) which then

feed-back on the hypothalamus/pituitary and reduce FSH production and hence the ovulation rate is reduced. When the androgens are bound by antibodies the inhibin production is decreased and the ovulation rate is thereby increased. With the understanding of such mechanisms inhibin may be considered as a candidate for immunization to modify the gonadal function.

In this experiment when an antiserum was made with 1:1 ratio with all the four antisera its effect on the ovulation rate was not significant. As the mix antiserum was used only on one occasion with few animals no conclusions can be made on the effect of mix antiserum on the ovulation rate. Various other combinations of 2, 3 or 4 antisera with different ratios may be tried out to reduce the negative feed-back in order to improve gonadal function. The collective effect of small change in such a group of hormones could be sufficient to achieve the desired change in ovulation rate but the equilibria of none of the hormones would be disrupted to such an extent that other aspects of reproduction were adversely affected.

Between breed variation in sensitivity to hypothalamus/ pituitary to negative feed-back has been reported in males (Land and Carr, 1975). The testis growth of Merino lambs has been increased through modification of the negative feed-back by using passive immunization against oestrogens (Land, Baird and Carr, 1981). If the final size of the testis could be increased it may be a route to increase the gonadal function of males (i.e. sperm production) of particular merit. In the past there had been lot of interest in increasing litter size in sheep both by genetic and environmental means. Direct selection methods have not been successful due to a

number of reasons as outlined in Chapter 1. There are no reliable methods of increasing litter size by environmental means. Although passive immunization against A, T and mix antiserum increases the lambing percentage the results were only significant in the T group. But it is difficult to conclude on the A and mix groups because of the small number of animals used and also the experiment was conducted towards the end of the breeding season. However, subsequently by using large number of animals treated at different stages of the oestrous cycle the lambing percentage was significantly increased after passive immunization against A, T and mix antiserum (R.B. Land, personal communication). A group of sheep could be immunized with A, T and mix antiserum irrespective of the stage of oestrous cycle to increase ovulation rate and litter size as the time of treatment has no significant effect on the ovulation rate or lambing percentage. These effects may be explained through the relatively long half-life of antibody in relation to the oestrous cycle length in sheep. It can be concluded that this method could be improved to increase litter size in sheep.

6.2: Oestrogen implants and gonadotrophins in Scottish Blackface and Finnish Landrace ewes

The mean pulse frequency of LH and mean FSH levels were reduced to a greater extent in the Blackface ewes than in the Finn ewes. Ovulation rate and the number of animals ovulating were also more markedly reduced in Blackface than in Finns. The results of this experiment confirm the finding of Land and Wheeler and Carr (1976) and Land (1976) and extends the findings to LH pulse frequency, mean FSH levels and to intact animals that the variation in sensitivity of

the hypothalamus/pituitary to negative feed-back of gonadal steroids is related to the between breed variation in ovulation rate.

Mouse studies

A mouse model was used to estimate the heritability of the sensitivity of the hypothalamus/pituitary to negative feed-back of oestradiol 17β by offspring parent regression. As with sheep, immunization against oestradiol 17β in mice increases the ovulation rate significantly. It may be concluded that both in sheep and mice similar physiological mechanism exists for the negative feed-back control of ovulation rate. The heritability estimated was not significantly different from zero. The genetic regression of the natural ovulation rate and litter size on the treated ovulation rate was not significantly different from zero. Although between breed variation in ovulation rate in some sheep breeds can be explained through the sensitivity of the hypothalamus/pituitary to negative feed-back of gonadal steroids, within the mouse population studied the trait show very little genetic variation.

Spearow (1980) studied the physiological basis of genetic differences in ovulation rate in mice selected from the same base population for litter size, postweaning body weight and in unselected control, and reported that the strains differed in sensitivity of the hypothalamus/pituitary to negative feed-back effects. He concluded that the selection for postweaning gain increased the natural ovulation also through decreasing the negative feed-back products from a large number of follicles to a large blood and fat pool. However,

his study also showed that selection for litter size and gain increase the natural ovulation rate through different mechanisms. Selection for litter size increased the natural ovulation rate by increasing the number of follicles at many stages of development, by changing the follicular growth rate at specific stages of development, and selection for gain increased the natural ovulation rate through a dramatically increased rate of induction of LH receptors and steroidogenesis by FSH and oestrogen. Even within a strain the increased ovulation achieved by different selection methods has a different physiological basis. It may well be possible that within breed differences in ovulation rate have a different physiological basis from that between breeds. Similarly the between breed differences in milk production explained by insulin responses do not explain the within breed variation in milk production (Osmond, 1980). So far most of the work done on physiological basis of genetic differences in ovulation rate on sheep has been concentrated on between breed studies. However, Booroola sheep and control Merino strains present an appropriate model for studying the within breed physiological variation in ovulation rate. Such studies are now being undertaken by CSIRO research workers in Australia.

The main objective of the study of the physiological basis of genetic differences in ovulation rate is to identify indirect selection criteria for increasing ovulation rate in sheep as the measurement of ovulation rate is technically difficult. The merits of indirect selection are dealt in general by Falconer (1960), and, accordingly are dependant upon the selection intensity per unit time (i/1) applicable to predictive and the desired trait, the respective heritabilities (h_p^2 and h_d^2) and the genetic correlation between the

two characters. It can be shown that the indirect selection cannot be expected to be superior to direct selection unless the predictive character has a substantially higher heritability than the desired character and the genetic correlation between the two characters is high or unless a substantially higher intensity of selection can be applied to the secondary character. Ovulation rate is sex limited but the underlying physiological character could be measured in both sexes so that a higher selection intensity would be possible by indirect selection. In such a situation even if the heritability of the physiological trait is not substantially high, sufficient extra response may be achieved in ovulation rate by indirect selection if the genetic correlation between the two characters is high. Walkley and Smith (1980) considered physiological traits in genetic selection for litter size in sheep. They considered indirect selection not as an alternative method but as complementing each other in combined selection with litter size. It also included use of repeated measurements on indirect physiological traits and the use of paternal half-sib records which are usually available. By considering a large range of possible situations they showed that with low genetic correlations (under 3.0) the gain in response is likely to be small. But as the genetic correlation rises the gain in response can become as large as 50-100%. Therefore a search should be made for physiological traits (or combination of traits) which are intrinsically related to litter size and ovulation rate. As an example of extra response, suppose that sex limited indirect physiological trait has a heritability of 30% and a genetic correlation of 0.5 with litter size. The gain over the direct selection on litter size would be about 15% for indirect selection and about 50% for combined selection. Thus if such parameters in males or females

can be identified it could provide a useful tool in improving litter size in sheep.

Ovulation is physiologically a complex character. It involves hormonal inter-relationships between the ovary and hypothalamus/pituitary so that the trait may be physiologically broken down into number of stages. Although heritability of ovulation rate is high enough to respond to direct selection it may be possible that there is small amount of genetic variation in number of stages and that it may be difficult to find out one physiological component with high genetic variation. However the three fold difference in ovulation rate found between Booroola and control Merino has been attributed to single gene effects (Piper and Bindon, 1980).

Finally the work reported in this thesis show that there is very little genetic variation in sensitivity of the hypothalamus/pituitary to negative feed-back of oestradiol 17β in the mouse population studied. However, the variation in that system could be used to modify gonadal function at the phenotypic level. More investigations are needed before using passive immunisation technique as a practical method of improving gonadal function. It is suggested that more within breed or strain physiological studies are required in relation to ovarian function and it's feed-back control to understand the physiological basis of genetic variation in ovulation rate.

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