A STUDY OF THE EFFECTS OF NUTRITIONALLY-INDUCED BODYWEIGHT DIFFERENCES ON OVARIAN FUNCTION IN THE EWE

A thesis presented in partial fulfilment
of the requirements for the degree
of Master of Agricultural Science
in Animal Science
at Massey University

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1987

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ABSTRACT

The aim of this study was to investigate the mechanisms by which nutritionally-induced bodyweight differences(i.e.the so-called "static bodyweight effect") influence ovulation rate in sheep.

Seventy Romney ewes (5-7 years old) were randomly divided into 2 groups of 35 each and differentially grazed on mixed ryegrass/white clover pastures for 12-16 weeks to generate two treatment groups differing in mean bodyweight by 14.9±1.8kg (mean ± s.e.d.) early in the breeding season. Within each bodyweight (BWT) group, ewes were further divided into 3 blocks. Blocks 1 and 2 were used for the main experiment and only ovulation rate data were collected from ewes in block 3.

The oestrous cycles of all ewes were synchronized by treatment for 14 days with progesterone-impregnated intravaginal sponges. Two weeks after sponge withdrawal, ewes in block 3 were subjected to laparoscopy to record their ovulation rate.

After returning to oestrus following sponge withdrawal, ewes in block 1 and 2 were housed indoors and fed a maintenance diet of lucerne chaff until the end of the experiment about 16 days later. On day 12 of the synchronized oestrous cycle, ewes were injected with 150µg cloprostenol to induce luteolysis. Laparotomies were performed at 0, 24, 48 (block 1 only), and 76h after the prostaglandin treatment to study the patterns of preovulatory follicular development. The number of corpora lutea present on the ovaries at the time of the first laparotomy were also recorded. Ewes were blood-sampled by jugular venipuncture during the late luteal and follicular phases of the cycle and the plasma concentrations of FSH and LH were measured. After the laparotomy study, ovaries of the ewes were removed, fixed in Bouin's fluid and the left ovaries serially sectioned at 10µm thickness. Every 5th section was mounted and observed under a light microscope to study the populations of follicles 0.2mm or greater in diameter.

Ewes in the high BWT group (H) had significantly higher ovulation rates than those in the low BWT group (L) (H=1.73±0.20, L=1.18±0.13, P<0.001). On average, ovulation rate increased by 3.1% for each kilogram increase in bodyweight. Significant relationships between bodyweight and ovulation rate also existed within treatment groups (P<0.05). Compared with ewes in the low BWT group, ewes in the high BWT group had more follicles ≥2mm in diameter present on the ovarian surface at the time of the first laparotomy(H=10.70±1.19, L=7.66±0.75, P<0.001); a greater number of follicles being recruited early in the follicular phase (H=3.79±0.19, L=2.80±0.30, P<0.05); and a lower intensity of selection through atresia late in the follicular phase (H=43.7±4.2%, L=53.9±5.5%, P<0.10). There were no differences between BWT groups in the total number of follicles 0.2mm or greater in diameter, their size distribution or rate of atresia, but ovaries of ewes in the high BWT group had significantly more healthy follicles greater than 2mm in diameter than those of ewes in the low BWT group. Within treatment, bodyweight was significantly and positively correlated with the numbers of healthy follicles of 0.5-1 and 1-2mm diameter. Plasma FSH levels decreased during the follicular phase, but there was no effect of treatment on mean FSH concentrations during the late luteal and follicular phases. For ewes in block 2, preovulatory LH surges had occurred in most animals by about 72h after prostaglandin injection, with no difference in the time interval from prostaglandin injection to the onset of the LH surge (H=60.8±3.8h, L=60.9±3.1h, P>0.10).

It is concluded that variation in ovulation rate due to nutritionally-induced bodyweight differences is associated with changes in the number of follicles being recruited into the actively growing pool shortly after luteolysis and the proportion of the recruited follicles that become atretic at the time of selection late in the follicular phase of the oestrous cycle. However, large differences in bodyweight do not appear to influence the antral follicle populations in the ovary. FSH, which plays many important roles during follicular development, may not be involved in the control of bodyweight-induced variation in ovulation rate.

ACKNOWLEDGEMENTS

To start my long list of acknowledgements, I want to express my special appreciation and gratefulness to my supervisors, Dr M.F. McDonald and Dr S.N. McCutcheon, for their continued interest, guidance, help and advice during the experimental work and the preparation of this manuscript. Thank you very much for sharing your knowledge and experience, both scientific and philosophical, with me and for the encouragement given to me when things were tough.

I want to thank Miss Maria Dattena and Mr Sam Peterson for help with the surgery. The excellent technical help given by Mr M.A. Wycherley, Mr A.M.D.May, Mrs J.A.Rumbal and Miss M.F.Scott at various stages of the experiment is gratefully acknowledged.

For the skilled help with blood sampling, I want to thank all the members of the experienced blood-sampling team of the Animal Science Department: Mark Carter, Carolyn Clark, Machteld van Maanen, Peter Morgan, Leon Pijls, Christine Roberts, Margaret Scott and Guo Qiang Xing.

Gratitude is also extended to Mr W.B.Parlane, Technician-in-Charge of the Animal Physiology Unit, for providing the facilities and for helping with the experiment.

For help with histological study, supply of sectioning equipment and staining agents and for the smiling face, I want to thank Mr M.J. Birtles of the Department of Physiology and Anatomy.

I appreciate very much the invaluable help given to me by Mark Carter during the experimental work and data analysis. Thank you very much, Mark.

Special thanks are due to Prof A.L. Rae and Mr J.M. Rendel for their help with statistical analysis; Dr K.R.Lapwood and Mr H.J.Elgar for help with hormone assays; Mr P.H.Whitehead for providing the experimental animals and Mr L.Williams for caring for the animals.

I also want to thank all the postgraduate students and staff in the Department of Animal Science for friendship and support which made my stay in New Zealand an enjoyable and memorable one.

The financial support from the Educational Commission of the People's Republic of China is gratefully acknowledged.

Finally, special thanks are due to my family for love and encouragement without which I could not have achieved so far.

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A	Advanced atretic follicles
B	Block (effect)
BXT	Block X Treatment (interaction)
BWT	Bodyweight (or bodyweight effect)
°C	Degree Celcius
c.v.	Coefficient of variation
E	Early atretic follicles
FSH	Follicle-stimulating hormone
g	Gram
GnRH	Gonadotrophin releasing hormone
h	Hour
H	High bodyweight group
He	Healthy follicles
kg	Kilogram
L	Low bodyweight group
La	Late atretic follicles
LH	Luteinizing hormone
ME	Metabolisable energy
MJ	Megajoules
ml	Milliliter
mm	Millimeter
ng	Nanogram
O.R.	Ovulation rate
PMSG	Pregnant mare serum gonadotrophin
s.e.	Standard error
T	Treatment (effect)

CHAPTER I: INTRODUCTION

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1 The importance of high reproductive performance in sheep

The number of lambs raised per ewe mated has a marked effect on both the biological and economic efficiency of sheep production systems. Except under harsh environmental conditions, in which multiple births may lead to high lamb mortality or place the life of the ewe at risk, increased reproductive rate will contribute substantially to the efficiency and profitability of sheep production systems (Dickerson, 1970; Rae, 1986).

In most sheep production systems, one of the major costs is that of maintaining the breeding ewe (McGuirk, 1976). Therefore, increased reproductive rate allows this fixed cost to be spread over more lambs. Robinson et al (1977) have calculated that the cost of producing a unit of carcass meat can be markedly reduced by increasing the lambing rate of the breeding flock. The improved profitability which results from increasing reproductive rate must be balanced against factors such as the high cost of feed during pregnancy and lactation, reduced wool production from the ewe carrying and suckling twins in comparison with a single lamb, and the increased post-weaning costs per offspring resulting from their smaller size at weaning (Dickerson, 1970;McGuirk, 1976). Nevertheless, because of likely improved profitability, there exists much interests in finding ways of improving the reproductive performance of the breeding ewe (De Lange, 1984; Rae, 1986).

2 Determinants of sheep reproductive performance

Reproductive rate in sheep is usually defined as the number of lambs weaned per ewe joined. It is a complex trait involving several components - including fertility, prolificacy and lamb survival - each of which is influenced by many factors.

Fertility is defined as the proportion of ewes mated that give birth to lambs. It has an upper limit of 100 percent and the

fertility of adult ewes joined in the autumn generally approaches this limit provided nutrition is good (Knight, 1980a; Fogarty, 1984).

Prolificacy, or litter size, is defined as the number of lambs born per ewe lambing. It is determined by the ewe's ovulation rate (O.R.), fertilization rate and prenatal mortality. Under normal conditions, the fertilization rate of healthy adult ewes is generally high and the fertilization of multiple ovulations is largely an all-or-none event rather than dependent on the number of ova shed from the ovaries (Restall et al., 1976; Kelly & Allison, 1979). Thus fertilization rate often does not contribute greatly to variation in litter size (Kelly, 1984). An exception may be in animals with high O.R., such as the Booroola Merino ewes (Trounson & Moore, 1974; Kelly, 1984) and ewes which have been superovulated (McKelvey & Robinson, 1986), where partial failure of fertilization may occur. Therefore, litter size in sheep is mainly determined by O.R. and embryonic mortality.

Lamb survival rate is the proportion of lambs born that survive to weaning. It is determined by the lamb viability, the maternal rearing ability and by climatic factors. However, efforts to increase lamb survival rate through approaches other than improving management are likely to yield only limited results (Alexander, 1984).

Although fertility and lamb survival can, in some situations, contribute greatly to variation in the reproductive performance of ewes, litter size is generally considered to be the major determinant of reproductive performance (Fogarty, 1984; Bradford, 1985). Of the factors that influence litter size in sheep, O.R. and embryonic mortality are the two most important.

2.1 Ovulation rate as a determinant of litter size

Both O.R. and litter size vary greatly between and within breeds (Hanrahan, 1982). Based on a survey of a large number of flocks, Knight (1980a) reported that about 80% of the variation among flocks in the number of lambs born was associated with variation in O.R..

However, as the number of embryos entering the uterus increases, the probability of embryo survival declines in a linear manner (Kelly, 1984), resulting in a curvilinear relationship between O.R. and litter size (Hanrahan, 1982).

2.2 Embryo survival as a determinant of litter size

In sheep, a considerable proportion of ova shed are not represented by live lambs born. When considered over a wide range of environmental conditions and across many different breeds, the rate of embryo mortality not associated with any identifiable factors usually falls within the range of 20-30%. Most of this occurs before implantation (Edey, 1969, 1979; Bolet, 1986). In addition, several factors have been found to increase embryo mortality beyond this basal rate. These factors include nutrition, high ambient temperature, ovulation rate, site of ovulation, age of the ewe, stage of the breeding season and lactational status (for reviews see Edey, 1969, 1979; Kelly, 1984; Wilmut et al., 1985; Robinson, 1986).

Despite its magnitude, both the causes of the basal mortality and the mechanisms by which each of these factors induces embryo mortality are still not clear, although genetic aberrations of the embryos (Bishop, 1964; Bolet, 1986) and unfavourable uterine environments (Wilmut et al., 1985; Fisher & Beier, 1986) have been implicated. The lack of understanding of the mechanisms of both the basal and induced mortality has greatly limited attempts to increase reproductive rate by improving embryo survival.

It has also been shown that there is little genetic variation in embryo survival between or within breeds. Interbreed embryo transfer experiments involving a wide range of breeds have failed to show any significant effects of the genotypes of either the dam or the embryo on embryo survival (Bradford, 1972; Hanrahan, 1982; Hanrahan & Quirke, 1985). However, there have been reports of breed differences in embryo survival (Bradford, 1985; Meyer, 1985). Little information is available on within-breed variation in embryo survival. Limited evidence suggests that it is likely to be small. Within-breed

selection for the number of lambs born, which resulted in an increase in litter size, did not improve embryo survival significantly and the increase in litter size could be fully accounted for by the resulting increase in O.R. (Hanrahan, 1982; Meyer & Clarke, 1982; Hanrahan & Quirke, 1985). Hanrahan and Quirke (1982) found significant variation among donor ewes in the survival rate of their embryos following transfer but the repeatability of embryo survival between years appeared to be low (Hanrahan, 1982; Hanrahan & Quirke, 1985).

Under most situations, therefore, reproductive rate in sheep is mainly determined by litter size. Both O.R. and embryo survival are major factors influencing litter size. However, insufficient understanding of the causes of embryo mortality, and the small variation in embryo survival between ewes, have greatly reduced the potential to increase litter size by decreasing embryo mortality,except by avoiding some of those identifiable environmental factors. Therefore, O.R. is the single most important factor determining litter size in the ewe. The importance of O.R. as a determinant of litter size is further enhanced because it can be readily manipulated with currently available techniques.

3 Factors influencing ovulation rate in the ewe

Several factors including season, age, genetics, nutrition and hormonal therapy have been found to influence ovulate rate of the ewe. The extent to which each of these factors is understood varies. In this section, the effects of season, age, genetics and nutrition on O.R. will be discussed, leaving the effects of hormonal therapy to be considered later.

3.1 Season

Seasonal changes in O.R. have been extensively documented by many studies involving several breeds and their crosses. Early studies used data obtained by joining flocks at different times of the year (Allden, 1956; Watson & Radford, 1966; Fogarty, 1978). More precise estimates of the seasonality of O.R. have been obtained by repeated observations on the same animals throughout the year at regular intervals (Lamberson & Thomas, 1982; Scaramuzzi & Radford, 1983; Phillips et al., 1984; Thimonier et al., 1985). Generally the O.R. is low at the beginning of the breeding season, rises rapidly to a maximum and then declines gradually to reach a minimum just before the ewes become anoestrus.

Since most of these studies were carried out on pasture-fed animals, the data may be confounded with the effects of nutrition and bodyweight (Gherardi & Lindsay, 1982; Scaramuzzi & Radford, 1983). However, variation in O.R. with time of the year has been reported in pen-fed animals on a constant level of nutrition (Radford, 1959; Dunn et al., 1960).

3.2 Age

The O.R. of the ewe varies with her age. It is low in hogget ewes, increases to reach a peak at about three to five years of age and then decreases slowly as the animal becomes old (McKenzie & Terrill, 1937; Turner & Dolling, 1965; Lindsay et al., 1975; Williams et al., 1978; Ricordean et al., 1982; Cahill, 1984; Davis & Howarth, 1984; Gunn et al., 1986).

The mechanisms by which age influences O.R. are not clear. The low O.R. in hogget and maiden ewes may be due to the low bodyweight of these animals (Scaramuzzi & Radford, 1983). Comparison of the reproductive performance of groups of ewes differing in age but of similar body size and condition has revealed only a very small age effect (Gunn et al., 1986). Although it has been reported that the increase in O.R. with age can be explained by the increased number of growing follicles in older compared to younger animals (Cahill, 1984), this has not always been supported (Driancourt et al., 1985a).

3.3 Genetics

Genetic variation in the O.R. of sheep has been well documented. There is considerable information on breed differences in ovulation rate (see reviews by Hanrahan, 1982; Meyer, 1985). Although the vast majority of the domesticated sheep breeds have O.R. between 1 and 2, there are several breeds known to have ovulation rates approaching or exceeding three (Scaramuzzi & Radford, 1983; Haresign, 1985; Bindon & Piper,1986; Table 1). These highly prolific breeds are a valuable genetic resource which can be used to improve the reproductive performance of low-prolificacy breeds by crossbreeding. Furthermore, they provide a powerful tool for the study of the physiological mechanisms which control O.R. (see later sections).

In addition to between-breed variation in O.R., there is also large variation in O.R. within breed which readily responds to selection (Hanrahan & Quirke, 1982, 1985). Estimates of the heritability of O.R. have yielded values as high as 0.5 (Hanrahan, 1980, 1982; Hanrahan & Quirke, 1985). This estimate has been confirmed by a realized heritability of 0.50 in a selection experiment on Finnish Landrace ewes (Hanrahan & Quirke, 1982). Such values are much higher than the 0.10 reported for the heritability of litter size (Land et al., 1983). Since substantial evidence points to O.R. being the main factor limiting litter size, with small (if any) variation in embryo mortality within and between breeds (see section 2), selection for litter size should be more efficient if O.R. instead of litter size was used as the selection criterion (Hanrahan, 1980, 1982;Land et al.,

1983). However, further studies on O.R. as a selection criterion are needed before it can be used in sheep breeding (Hanrahan & Quirke, 1982; Bradford, 1985).

Table 1: Estimates of ovulation rates of prolific sheep breeds

Breed	Origin	O.R.		References
		Mean	Range	
Booroola	Australia	4.2	1-11	Piper et al.1985
Romanov	Russia	3.4	1-7	Ricordean et al., 1982
Finnsheep	Finland/Russia	3.5	1-7	Hanrahan & Quirke,1975
D'Man	Morocco	2.8	1-8	Lahlou-Kassi et al.1985
Hu Yang	China	3.1		Guo et al., 1981
Cambridge	Britain	4.0	2-13	Hanrahan & Owen, 1985

3.4 Nutrition

The effects of nutrition on reproductive performance have been recognized for many years (see review by Coop, 1966a). However, most of these early studies were based on general observations and it was not until the 1960's that well planned experiments were carried out to study the precise relationship between nutrition and reproductive performance (Allen & lamming, 1961; Coop, 1966b; Killeen, 1967; Allison, 1968; Edey, 1968; Fletcher, 1971). These studies demonstrated that both the absolute bodyweight and the bodyweight change of the ewe at the time of mating could influence her O.R. and lambing performance. The terms "static" and "dynamic" were introduced to describe these bodyweight effects.

3.4.1 The static effects of bodyweight

It has been demonstrated in many studies that heavy ewes have higher O.R. than light ewes (Allen & lamming, 1961; Wallace, 1961; Killeen, 1967; Allison, 1968, 1975; Edey, 1968; Fry et al., 1986; Rhind et al., 1986; Rhind & McNeilly, 1986). Analysis of the relationship between bodyweight and O.R. has indicated that O.R. increases by approximately 2-3 percent for each kilogram increase in mean flock bodyweight (Cumming, 1977; Fletcher, 1971; Morley et al., 1978; Kelly & Johnstone, 1982; Smith et al., 1983a). This relationship appears to be similar over many breeds and their crosses (Morley et al., 1978).The magnitude of response reported for O.R. is somewhat higher than that reported for lambing performance (1.3% per kilogram increase in bodyweight, Coop, 1966b), suggesting a higher embryo loss with increased O.R. (Kelly, 1984; Wilmut et al., 1985). The relationship between O.R. and bodyweight within flocks is not as strong as that between flocks (Coop & Hayman, 1962; Kelly & Johnstone, 1982). This is undoubtedly caused by the dissimilarities in the way ewes within flocks achieve bodyweight differences as compared with the between-flock situation (Morley et al., 1978).

The relationship between bodyweight and O.R. is not always apparent (Allison, 1977; Cumming, 1977) and may depend on whether the relationship is measured within flocks of similar genetic composition or between flocks or breeds (Morley et al., 1978). There is some evidence that the increase in O.R. with bodyweight may level off at high bodyweights and body condition. Ovulation rate and lambing performance may even be depressed in extremely fat ewes (Rattray, 1986).

Two factors contribute to the variation in bodyweight: body size and body condition. However, reports on their ralative contribution to variation in O.R. are inconsistent. Knight and Hockey (1982) reported that body size and condition were independent of each other. They contributed about equally to the variation in bodyweight and both accounted for significant variation in O.R.. However, in Greyface ewes selected for large body size, ewes with similar body condition

had similar O.R. despite differences in body size and bodyweight (Ducker & Boyd, 1977). In contrast, Gunn et al (1972) found that, in Scottish Blackface ewes, high bodyweight groups had higher O.R. despite similar condition score to the low bodyweight groups. These situations are difficult to explain and may be related to whether differences in body size arise from genetic or environmental effects. Bodyweight remains to be the best indicator of O.R. (Knight & Hockey, 1982).

3.4.2 The dynamic effects of bodyweight

The flushing of ewes by placing them on a high plane of nutrition some time before mating has long been adopted as an important farm management practice (Coop, 1966a). Ewes that are flushed at the time of mating will have higher O.R. and lambing performance than unflushed ewes of similar bodyweight, an effect which is independent of bodyweight itself (Coop, 1966b; Killeen, 1967). This effect of flushing has been confirmed by many later studies (Haresign, 1981; Smith et al., 1983a; Gunn et al., 1984a,b; Rhind et al., 1985). However, Morley et al (1978) summarized and analysed data from several studies and concluded that, while the existence of such a dynamic effect is without question, its expression varies with experimental conditions. The conditions required to maximise the flushing response are yet to be determined.

Many factors have been found to influence the ewe's ovulatory responses to flushing. It has been shown that light ewes are more responsive to flushing than heavy ewes (Knight, 1980b, Rattray et al., 1980, 1983; Rhind et al., 1984b). Ewes in good body condition may not respond to premating flushing (Gunn et al., 1984b). In contrast, some studies have failed to find such an effect (Wallace, 1961; Coop, 1966b; Kelly & Johnstone, 1982). There is no indication that ewes which are deliberately kept in poor condition and then flushed will have higher O.R. than ewes kept in good condition.

Within certain limits, the flushing response appears to be roughly proportional to the duration of flushing (Wallace, 1951;

Allen & Lamming, 1961; Coop, 1966b; Rattray et al., 1980; Smith et al., 1983a). However, with extended periods of flushing, part of the response may be accounted for by bodyweight gain (Rattray et al., 1980; Smith et al., 1983a). A minimum flushing period of 3 weeks (about one oestrous cycle) seems to be needed to obtain significant increase in O.R., with small or no effects apparent for flushing periods of less than 3 weeks (Allen & Lamming, 1961; Coop, 1966b; Smith et al., 1983a). Although O.R. was increased when ewes were flushed for 6 weeks compared with those flushed for only 3 weeks, no additional increase in lambing rate was obtained (Smith et al.,1983a).

Flushing is a relative term and it does not necessarily require a certain level of feeding. Thus flushed ewes losing weight can still give responses so long as they receive better nutrition prior to and during joining than unflushed ewes (Coop, 1966b). Most of the studies have given no quantitative characterization of the amount and type of feed offered to and consumed by the ewe. However, some recent studies have demonstrated that both the quality and quantity of the feed are important factors determining the ewe's responses to flushing (Rattray et al., 1980, 1983).

While a large number of breeds and crossbreeds bave been shown to respond to flushing, little information is available on the comparative responses of different breeds. There have been conflicting reports concerning the sensitivity of Booroola Merino crosses to flushing (Allison & Kelly, 1979; Bray et al., 1980). It has been found that Coopworth ewes may be more responsive to flushing than Romney ewes (Rattray et al., 1981, 1983; Smith et al., 1982). The conditions under which the breeds have originated may have modified their sensitivity to nutritional changes.

The age of the ewe may also influence her response to flushing (Rattray, 1977). Flushing appears to be less effective in young compared to mature ewes and may have no effect in ewe lambs (Allen & Lamming, 1961; Southam et al., 1971; Dyrmundsson, 1973; Smeaton et al., 1982). No stage of the oestrous cycle has been shown to be particularly critical (Rattray, 1977), but stage of the breeding

season can influence the bodyweight-O.R. relationship. Response to flushing is greatest early or late in the breeding season and least in mid-season (Rattray, 1977).

3.4.3 The effects of protein intake

The effects of protein intake on O.R. have been extensively studied in Australia in association with lupin supplementation. Numerous studies have demonstrated that protein supplements (e.g. in the form of lupin) can significantly increase O.R. (Lightfoot & Marshall, 1974; Knight et al., 1975; Brien et al., 1976; Lindsay, 1976, 1983; Davis et al., 1981; Fletcher, 1981; Gherardi & Lindsay, 1982; Scaramuzzi & Radford, 1983; Oldham & Lindsay, 1984; Smith, 1985b). The response of ewes to lupin grain supplementation can take place within one week with no further effects of supplementation beyond one cycle (Knight et al., 1975; Fletcher, 1981; Oldham & Lindsay, 1984). Such rapid responses cannot be accompanied by measurable changes in bodyweight, suggesting a response to nutrient supply independent of bodyweight or bodyweight change (Lindsay,1976). Within certain limits, the response is quantitatively related to the rate of lupin grain supplementation (Lightfoot & Marshall, 1974). However, despite these reports of positive response, large-scale field trials in Western Australia have obtained responses to supplementation, in terms of lambs born per ewe, ranging from -14 to +21% (Croker et al., 1985; Croker, 1986). This degree of variability could not be reduced by increasing the rate of supplementation.

There appear to be some interactions between energy and protein contents of the feed. Fletcher (1981) found an ovulatory response to increased protein intake only at low energy level, with no effect of increased protein intake at a moderate digestible energy intake. In contrast, Davis et al., (1981) showed that the percentage of multiple ovulations was increased when protein intake was increased at all but the low levels of energy intake.

The effective component in lupin appears to be protein because similar responses could not be achieved with supplementation of cereal

grains which increased the bodyweight of supplemented ewes (Lindsay, 1976). Some studies have demonstrated the superiority of lupin protein over protein from other sources (Davis & Cumming, 1976; Fletcher, 1981). However, lupin grain has no advantage over soybean and protected casein when fed at equal levels of crude protein intake (Davis et al., 1981). The superiority of lupin and soybean over other protein sources seems to be due to their high protein content and low protein degradability in the rumen (Hume, 1974).

There is also recent evidence suggesting that the main effect of lupin grain is to provide energy substrates, especially glucose (Teleni et al., 1984; Teleni & Rowe, 1986). These results indicate the difficulties in the study of protein metabolism in ruminants. One problem is that the nutrients in the feed are considerably modified before they become available to the animal. The protein requirement is influenced by energy intake and that part of the protein intake which cannot be utilized for protein synthesis by the animal at the current level of energy intake will be used to provide energy substrates. This may help to explain the discrepancies encountered in protein supplementation experiments. When the protein content of the basal diet is low, supplementation of protein may increase O.R. while energy supplementation may be without effect. On the other hand, if the basal diet contains an adequate amount of protein and is low in energy content, then energy rather than protein supplementation is expected to result in improved O.R..

In general, each of the four factors discussed above can significantly influence the O.R. of the ewe. In situations where early lambing or breeding of sheep at a young age is desirable, the lower O.R. of ewe lambs or ewes early in the breeding season may become a major disadvantage if a high lambing rate is sought. Utilization of breed differences in O.R. by crossbreeding provides a fast, effective way to improve the reproductive rate in sheep. However, breed differences in other production characters have greatly limited the potential use of this approach. Within-breed selection is effective

in increasing the reproductive performance in sheep with an annual rate of response of about 1.5% being obtained by several selection experiments (Land et al., 1983). Nutrition, which is the basis of any production process, is a very important factor influencing the O.R. of ewes. No matter how large the reproductive potential, its full expression depends on the supply of adequate nutrition. What is more, good nutrition prior to and during mating also increases the reproductive rate by increasing fertility and may have carryover effects on mortality, both prenatal (through effects on embryo development, McKelvey & Robinson, 1986) and postnatal (through effects on lamb birth weight, Alexander, 1984).

4 Physiological basis for the nutritional effects on ovulation rate

Although the effects of nutrition on O.R. have been well-known for some time, the mechanisms by which nutrition achieves these effects are still not clear. This is partly due to a lack of information on the mechanisms that normally regulate O.R. during the oestrous cycle. Recent studies have concentrated on determining the correlations between ovarian follicular development, plasma levels of gonadotrophins and O.R., but it has not been possible to establish the cause-effect relationships between these characteristics.

4.1 Effects of nutrition on follicular growth

Whatever the mechanisms controlling O.R., their effects must finally be seen in the patterns of follicular development and maturation (Scaramuzzi & Radford, 1983). Follicular growth and development is a very complex process which starts from the initiation of growth of primordial follicles, passes through the preantral and antral phases, and culminates usually in atresia and less commonly in ovulation (Cahill, 1984). The initiation of growth of primordial follicles is a continuous process irrespective of the reproductive status of the animal until the reserve of primordial follicles is exhausted (Mauleon & Mariana, 1977). Once a follicle enters the growing phase, it will continue to develop without interruption until it either ovulates or becomes atretic (Richards, 1978; Peters & McNatty, 1980).

4.1.1 Effects of nutrition on follicular population

The ovary of the adult ewe contains between 12,000 and 86,000 small follicles (with ≤ 2 layers of granulosa cells) and between 100 and 400 larger follicles (with >2 layers of granulosa cells) at various stages of development. Of these, only 10-40 follicles have a diameter of ≥ 1mm (Brand & de Jong, 1973; Cahill *et al.*, 1979; McNatty *et al.*, 1982). At any given time, the number of follicles in the ovary at a particular stage of development is dependent on the number entering that stage, the speed with which the follicles pass through the stage and the number disappearing through atresia.

When a primordial follicle begins to grow, it first enters the preantral phase. The growth of preantral follicles is extremely slow. It has been estimated in sheep that, of the 6 months required for a follicle to grow from the stage at which it consists of 3 layers of granulosa cells (diameter 0.06 mm) to the preovulatory size (6-8mm), 4.3 months are spent in the preantral stage (Turnbull et al., 1977; Cahill and Mauléon, 1980). The transition from a preantral to an antral follicle is a gradual process. The antrum first appears in ovine follicles of 0.15-0.3mm diameter. After antral formation, the increase in follicle diameter is due to both an increase in granulosa cell number and an increase in antral fluid accumulation. Follicular growth rate is slow in antral follicles of up to 0.4 mm in diameter. Thereafter, follicles enter a phase of rapid growth, with the rate of granulosa cell proliferation reaching a maximum in follicles 0.7-1.5 mm in diameter. Next there is a progressive decrease in granulosa cell multiplication as follicles continue to increase in size until the mitotic index of granulosa cells is almost zero in preovulatory follicles (Turnbull et al. 1977; Cahill & Mauléon, 1980). This general pattern of follicular growth in sheep persists at all stages of the oestrous cycle and after PMSG treatment (Turnbull et al., 1977).

Most follicles entering the growing phase terminate their growth in the antral phase through atresia, while only a few selected ones achieve full maturation and ovulate (Byskov, 1978). Atresia is rarely seen in preantral and small antral follicles, but the proportion of atretic follicles increases with increasing follicular diameter (Brand & de Jong, 1973; Turnbull et al., 1977). In sheep, about two thirds of the large follicles in the ovary are atretic (Brand & de Jong, 1973).

Changes in numbers of follicles and their growth and atretic rates have been found to be associated with nutritionally-induced variation in O.R.. Flushed ewes or ewes of high bodyweight or body condition have significantly more large follicles on their ovaries than unflushed ewes or ewes of low bodyweight or body condition (Allen & Lamming, 1961; Allison, 1977; Knight, 1980b; Haresign, 1981a;Fry

et al., 1986; Rhind & McNeilly, 1986). At least in one study (Allen & Lamming, 1961), the reduction in the number of large antral follicles appeared to be caused by a lack of gonadotrophin stimulation because, while injection of PMSG did not significantly alter the size-distribution of follicles in flushed ewes, the same treatment resulted in the appearance of large antral follicles in unflushed ewes. The increased number of large follicles in ewes of high body condition is associated with a higher proportion of healthy follicles (as assessed by follicular fluid oestradiol concentrations) than in ewes of low body condition (McNeilly et al., 1987). No information is available on the effect of nutrition on the total number of ovarian follicles, their distribution and growth rate.

Changes in ovarian follicle populations have also been shown to be associated with variation in O.R. in several other situations. Quantitative histological examinations of ovaries from mature Romanov (O.R.=3.1) and Ile-de-France (O.R.=1.5) ewes have shown that Romanov ewes had significantly more growing follicles at all stages of growth, and fewer primordial follicles, on their ovaries (Cahill et al., 1979). However, this has not been supported by a recent study which found similar numbers of antral follicles in the two breeds (Driancourt et al., 1986). When compared with control Merino ewes, Booroola Merino ewes have fewer primordial follicles, more preantral follicles, similar total numbers of antral follicles and a higher proportion of atretic follicles (Driancourt et al., 1985a). The consistent inverse relationship between O.R. and the number of primordial follicles seems to suggest a higher rate of initiation of primordial follicle growth in prolific than non-prolific sheep breeds (Cahill et al., 1979; Driancourt et al., 1985a). However, this may also be caused by a genetic effect on the total number of primordial follicles or may be simply due to difference in litter size, which has been found to influence the number of primordial follicles present on the ovary of new born lambs (Tassell et al., 1983). Within Merino and Ile-de-France breeds, ewes that had twin ovulations also had more antral follicles than those having only a single ovulation (Cahill et al., 1979; Driancourt et al., 1985a).

Follicular growth rate, measured by granulosa cell mitotic index, has been compared between groups of ewes with different ovulation potentials due to breed effect or steroid immunization. Increase in O.R. in these situations is not associated with increase in follicular growth rate (Cahill et al., 1979; Cahill & Mauleon, 1980; Scaramuzzi & Hopkinson, 1984; Driancourt et al., 1985a). Studies with Booroola Merino ewes have shown that, while the overall pattern of granulosa cell multiplication is similar between Booroola and control Merino ewes, follicles from the Booroola ewes enter the rapid growth phase earlier than those from control Merino ewes (Driancourt et al., 1985a). It has been estimated that, in Merino ewes, about 3-4 follicles enter the rapid growth phase (follicle diameter of about 0.5mm) per day (Turnbull et al., 1977), with more follicles entering this phase each day in twin-ovulating ewes than in single-ovulating ewes (Turnbull et al., 1978).

Treatment of ewes with exogenous pregnant mare serum gonadotrophin or FSH, while resulting in increased O.R., does not increase the number of large antral follicles (Bindon & Piper, 1982a; McNatty et al., 1982, 1985; Monniaux et al., 1983; Moor et al., 1984). The superovulatory effects of these exogenous gonadotrophins are achieved via their ability to prevent atresia of large antral follicles (Hay & Moor, 1978; McNatty et al., 1982, 1985; Moor et al., 1984) and their ability to increase the growth rate of medium-sized antral follicles (Turnbull et al., 1977; Dott et al., 1979). As a result, the number of healthy antral follicles increases following gonadotrophin stimulation.

Increase in O.R.is not always associated with changes in ovarian follicle populations.The prolific D'Man ewes do not have significantly more growing follicles on their ovaries than comparable low prolificacy breeds (Lahlou-Kassi & Mariana, 1984). Histological studies of the follicular population in ovaries of ewes immunized against androstenedione have failed to reveal any effect of immunization on the number of antral follicles, their size distribution or atretic rate (Scaramuzzi, 1984; Scaramuzzi & Hopkinson, 1984; Driancourt et al., 1985c).The main feature of the ovaries of immunized ewes is the pres-

ence in the ovary of large, nonatretic follicles in the final follicle classes.

Therefore, while increases in the number of healthy growing follicles and their growth rate could be one approach by which high O.R. is achieved in some situations, such as the nutrition and breed effects, the fact that this is not a general feature casts some doubts on its importance in the control of O.R.. Convincing evidence opposing follicular number as a determinant of O.R. comes from the observation that unilateral ovariectomy as late as day 14 of the oestrous cycle does not reduce the ovulation rate at the following oestrus despite the 50% reduction in follicular population (Land, 1973; Findlay & Cumming, 1977; Fry et al., 1987). It would, therefore, appear that follicle number, a measure of follicular turnover within the ovary, is not a determinant of O.R. in so far as other mechanisms can compensate for a very slow rate of turnover in the population of growing follicles (Scaramuzzi & Radford, 1983). Such compensatory mechanisms must involve a reduced rate of atresia and/or an increased rate of follicular growth. Thus it may be the mechanisms which cause the increase in the number and viability of large growing follicles that are responsible for the increase in O.R..

4.1.2 Effects of nutrition on preovulatory follicular development

In sheep, follicular development continues unaltered during the luteal phase with follicles reaching a diameter of 4-6mm before becoming atretic (Baird et al., 1975; Turnbull et al., 1977; Peters & McNatty, 1980; McNatty et al., 1981b). These follicles are capable of responding to increasing LH stimulation and undergoing the final stages of follicular maturation and ovulation. Early studies have indicated that growth of large follicles occurs in waves during the oestrous cycle with one (Hutchinson & Robertson, 1966), two (Brand & de Jong, 1973), or 3-4 (Smeaton & Robertson, 1971) waves being reported. These results are in contrast with other experimental evidence showing a lack of follicular waves at regular intervals (Turnbull et al. 1977; Driancourt et al., 1985b). Treatment of ewes with PMSG on successive days during the middle and late luteal phase

has failed to show any significant differences between days in O.R. (Cumming & McDonald, 1967; Hay & Moor, 1975).

Development of follicles is intensified during the 2-3 days from luteal regression to ovulation. Following luteolysis, large follicles on the ovary are stimulated to undergo rapid development in response to the increaing LH secretion at this time. This leads to the differentiation and ovulation of usually one, or occasionally two, follicles in most sheep breeds (McNatty, 1982; Baird, 1983; Driancourt et al., 1985b). The differentiation of the ovulatory follicle is a two-step event involving recruitment and selection (Di Zerega & Hodgen, 1981).

Recruitment of follicles is defined as the mechanism whereby the final group of follicles, which have the potential to become the ovulatory follicle(s), are brought forth for further development under increasing gonadotrophin stimulation (Cahill, 1984). In sheep, recruitment occurs around 48h before the LH peak and possibly coincides with luteolysis (Acritopoulou et al., 1977; McNatty et al., 1981a; Driancourt et al., 1985b). Soon after luteal regression, the atresia among follicles more than 2mm in diameter results in the formation of a pool of recruited follicles (Driancourt & Cahill, 1984). The recruited follicles are possibly the healthy follicles with a diameter of more than 2mm at the time of luteolysis (Driancourt & Cahill, 1984; Tsonis, et al., 1984; Driancourt et al., 1985b). Follicles of less than 2mm in diameter are unable to grow to the preovulatory size within the 2-3 days prior to the LH surge (Tsonis et al. 1984).

In sheep the number of follicles recruited is greater than the number which will ovulate (McNatty, 1982; Driancourt & Cahill, 1984). As the follicular phase progresses, (usually) one follicle is selected to undergo privileged development and ovulate, a process termed selection (Cahill, 1984). The selection of the ovulatory follicle(s) is thought to occur late in the follicular phase (Driancourt et al., 1985b). Thereafter, the selected follicle will achieve dominance over other follicles and suppress the development of all non-ovulatory

large follicles (Baird, 1983). As a result, the growth of follicles into the recruitable range is halted and all the unselected large follicles become atretic (Driancourt & Cahill, 1984).

If these proposed mechanisms for the differentiation and maturation of the ovulatory follicle(s) in sheep are true, then increased ovulation rates could be achieved by two mechanisms: more follicles available for recruitment and lower intensity of selection through atresia.

It has been possible, by ink-labelling and repeated laparotomy, to monitor the patterns of development of large antral follicles during the period from luteal regression to ovulation (Driancourt & Cahill, 1984). With this technique, it has been shown that the high O.R. in ewes of heavy bodyweight is the result of both an increased number of follicles being recruited and a reduced proportion of follicles becoming atretic at the time of selection (Fry et al., 1986). The increase in O.R. in response to short-term lupin feeding is due to a reduction in the number of follicles recruited that become atretic at selection (Nottle et al., 1985).

Changes in preovulatory follicular dynamics with increasing O.R. have also been demonstrated in other situations. In Booroola Merino ewes, the high O.R. is achieved through an extended period of time during which recruitment of ovulatory follicles takes place, together with a low intensity of selection and the ability of fully grown follicles to maintain their viability over an extended period (Driancourt et al., 1985a, 1986). A greater number of recruitable follicles, together with a similar intensity of selection through atresia, are the features associated with the high O.R. of Romanov compared with Ile-de-France ewes (Driancourt et al., 1986). The high O.R. of Finnish and steroid-immunized ewes results from a reduced intensity of selection through atresia late in the follicular phase with similar numbers of follicles being recruited (Driancourt et al., 1985c; Driancourt et al., 1986).

In general, increases in the number or follicles being recruited and/or reduction in the intensity of selection through atresia late in the follicular phase are possible mechanisms by which nutrition and other factors might influence O.R.. Different factors which influence O.R. may affect preovulatory follicular development through different combinations of these mechanisms.

4.2 Effects of nutrition on hormone action

All the factors that affect O.R. are thought to act through the effects of the hypothalamo-pituitary axis on the growth and maturation of ovarian follicles (Scaramuzzi & Radford, 1983). Changes in the patterns of follicular growth are, therefore, secondary to the more fundamental changes in the mechanisms which control this process.

Follicular development depends on an intimate interaction between the hypothalamo-pituitary axis and the ovary. Gonadotrophins secreted by the pituitary gland stimulate ovarian follicular development and production of steroid hormones (and possibly inhibin) from developing follicles (Baird & McNeilly, 1981). The steroids and inhibin thus produced then feed back to the hypothalamo-pituitary axis to control gonadotrophin secretion (Karsch et al., 1978). These interactions form a closed loop of negative feedback mechanisms. Therefore, the number of follicles that develop and eventually ovulate at any time will depend upon the level of gonadotrophins reaching the ovary and the ovarian responsiveness to gonadotrophins. Variation in O.R. can be brought about by changes at any point in this closed loop which lead to increased gonadotrophin secretion,including: reduction in the production of ovarian steroids and inhibin per developing follicle; increased clearance rate of ovarian steroids and inhibin; increased gonadotrophin secretion in response to a fixed level of GnRH; or reduced sensitivity of the hypothalamo-pituitary axis to the negative feedback inhibition of ovarian steroids and inhibin. However, large changes in O.R. can only be expected when the negative feedback control of the ovary on gonadotrophin secretion is bypassed.

4.2.1 Effects of nutrition on circulating levels of gonadotrophins

4.2.1.1 Luteinizing hormone

In the ewe, the pattern of endogenous LH secretion during both the luteal and follicular phases of the oestrous cycle is pulsatile in nature. Basal levels of 0.1-2.0 ng/ml are interspersed with small, short-lived episodes, each of 5-15 ng/ml and of about 30-45 minutes duration (Haresign et al., 1983). During the luteal phase of the oestrous cycle, the high level of progesterone secreted by the corpus luteum, acting in synergism with the basal oestradiol level, effectively suppresses LH secretion (Hauger et al., 1977; Karsch et al., 1980; Goodman & Karsch, 1980). As a result, LH secretion during the luteal phase is characterized by both a low basal level and a low pulse frequency (Baird et al., 1976; Hauger et al., 1977). Following the onset of luteolysis, plasma progesterone levels decline. This frees the pulse generator from the negative feedback control of progesterone and, as a result, the frequency of pulsatile LH secretion begins to accelerate (Baird, 1978; Karsch et al., 1979, 1983). Without the synergistic action of progesterone, oestradiol itself further enhances LH pulse frequency (Karsch et al. 1983). The basal LH levels also increase during the follicular phase until the preovulatory surge occurs about 48-60h after the onset of luteolysis (Baird & Scaramuzzi, 1976; Hauger et al., 1977; Baird et al., 1981). However, despite the increase in LH pulse frequency, the pulse amplitude decreases during the follicular phase possibly due to the increased oestradiol feedback inhibition (Haresign et al., 1983) and/or increased frequency of GnRH secretion (Clarke & Cummins, 1985).

LH is essential for successful follicular development. It is involved in the stimulation of steroid production from developing follicles (Baird et al., 1976; Baird, 1978; Scaramuzzi & Baird, 1979) and the marked increase in tonic LH secretion during the follicular phase is responsible for driving large antral follicles through their final stages of maturation (Baird & McNeilly, 1981; McNatty et al., 1981a; Driancourt et al., 1985b). Therefore, the concentration and/or the pulse frequency of LH secretion may determine

the number of antral follicles which proceed through their final stages of maturation and ovulate.

Studies comparing the patterns of LH secretion between groups of ewes differing in O.R. due to nutritional treatments have obtained inconsistent results. While some studies have failed to find any significant differences in the LH profiles (Radford et al., 1980; Scaramuzzi & Radford, 1983; Rhind et al., 1984; Rhind & McNeilly, 1986), differences in LH secretion have been reported for ewes of different bodyweights or on different levels of nutrition (Haresign, 1981b; Rhind et al., 1985, 1986). However, no difference in LH secretion between ewes with different O.R. within treatment groups has been found (Rhind et al., 1985).

The correlation between LH secretion and O.R. has also been studied for other situations. Attempts to demonstrate greater LH secretion in prolific breeds have generally not been successful (Cahill et al., 1981; Bindon & Piper, 1982b; Wedd & England, 1982a; Scaramuzzi & Radford, 1983; Lahlou-Kassi et al., 1984; Bindon et al., 1985). Indeed, mean LH concentrations in the preovulatory period have been found to be lower in the prolific Finnsheep and D'Man ewes than in comparable breeds of low prolificacy (Webb & England, 1982b; Lahlou-Kassi & Marie, 1985), possibly due to greater negative feedback inhibition associated with the high O.R.. However, ewes consistently having twin ovulations had significantly higher LH pulse frequency during the luteal phase of the oestrous cycle than their flockmates consistently having a single ovulation (Thomas et al., 1984). A similar trend has also been reported by Rhind & McNeilly (1986).

Ovulation is caused by the preovulatory LH surge. Studies of this LH surge have not found consistent relationships between O.R. and the characteristics of the preovulatory LH peak (Quirke et al., 1979; Haresign, 1981a, 1985; Bindon & Piper, 1982b; Rhind et al., 1985; Rhind & McNeilly, 1986), except that the interval from the onset of oestrus to the start of the LH surge is significantly longer in prolific than in non-prolific breeds (see reviews by Bindon & Piper, 1982b; Haresign, 1985). The significance of this longer

interval in the determination of O.R. is not known. It may be only associated with, rather than a determinant of, the high O.R. (Cahill et al., 1981).

4.2.1.2 Follicle-stimulating hormone

In contrast to LH, the pattern of FSH secretion during the oestrous cycle is less clearly defined due mainly to the difficulties in developing specific and sensitive assay methods for this hormone (McNeilly et al., 1976). During the luteal phase of the oestrous cycle, the levels of FSH fluctuate without any defined patterns (Salamonsen et al., 1973; Pant et al., 1977; Goodman et al., 1981). Small pulses of LH are not accompanied by increases in FSH concentrations (Baird & McNeilly, 1981). Attempts to find endogenous rhythms for FSH secretion have been unsuccessful (Miller et al., 1981; Bister & Paquay, 1983). Following the onset of luteolysis, the concentration of FSH remains unchanged during the early part of the follicular phase and is significantly depressed late in this phase (Baird & McNeilly, 1981; Baird et al., 1981). This decrease in FSH concentration late in the follicular phase is thought to be due to the increased negative feedback inhibition by oestradiol and inhibin, which at this time are being secreted in large amounts by the dominant follicle. Two peaks of FSH secretion have been reported to occur during the oestrous cycle, the first coincident with the preovulatory LH peak at oestrus and the second one day later (McNeilly et al., 1976; Pant et al., 1977; Haresign et al., 1983). However, Goodman et al. (1981) have reported the presence of only a single FSH peak coincident with the preovulatory LH peak.

FSH plays many important roles in folliculogenesis. Studies (particularly in the rat) have shown that FSH is the most important hormone responsible for stimulating the early stages of follicular development, including follicular cell proliferation (Rao et al., 1978), antral formation (Richards et al., 1976; Cahill, 1981) and stimulation of the granulosa cell aromatase system (Erickson & Hseuh, 1978; Dorrington & Armstrong, 1979; McNatty, 1982; McNatty et al., 1985). The acquisition of granulosa cell aromatase activity, which is

essential for the biosynthesis of oestradiol from androgens, is important for continued development of the follicle because oestradiol has been found to play several key roles during follicular development. The most important include stimulation of follicular cell proliferation and modification of gonadotrophin actions on follicular cells (Rao et al., 1978; Richards, 1980). Development of ovarian follicles is accompanied by the appearance of LH receptors on granulosa cells (Carson et al., 1979; Webb & England, 1982a,b). FSH, acting in synergism with oestradiol, has been found to be responsible for stimulating this process (see reviews by Richards, 1979, 1980). Development of abundant LH receptors on the granulosa cells of a follicle renders it capable of responding to increased LH stimulation, (e.g. during the follicular phase), to rapidly undergo the final stages of follicular growth and maturation (Webb & Gauld, 1985a,b).

Although LH has been implicated as the major hormone responsible for stimulating the development of large antral follicles during the follicular phase, FSH is also required for the late stages of follicular development. FSH deprivation during the follicular phase results in rapid loss of granulosa cell receptors for FSH, LH and oestradiol, and an inability of the large follicles to ovulate and luteinize (Richards, 1980). Decreases in FSH levels produced by injection of oestradiol or follicular fluid induce atresia of the dominant follicle in monkeys (Zeleznik, 1981) and delay ovulation in sheep (McNeilly, 1984). Therefore, it appears most likely that a minimum concentration of FSH as well as LH is required for the development of large antral follicles. In fact, FSH has been implicated as a possible mediator involved in selection of the ovulatory follicle(s) late in the follicular phase and hence in the determination of O.R. (McNatty, 1982; Zeleznik, 1982; Baird, 1983).

Changes in FSH concentrations during the oestrous cycle have been found to be associated with nutritionally-induced variation in O.R.. The higher O.R. of ewes in high body condition is associated with elevated FSH concentrations during both the luteal and follicular phases of the oestrous cycle compared with ewes in low body condition (Rhind & McNeilly, 1986). It has also been found that high feed

intake reduces the rate at which FSH level decreases during the follicular phase (Rhind et al., 1985). However, some studies have reported no effect of high level of nutrition on FSH concentrations despite a significant increase in O.R. (Findlay and Cumming, 1976; Rhind et al., 1984, 1986). Although changes in FSH concentrations have been reported in ewes supplemented with lupin grain (Brien et al., 1976), this has not been supported by other studies (Findlay & Cumming, 1976; Davis et al, 1981). As with LH, there is no significant correlation between FSH concentration and O.R. within treatment groups (Rhind et al., 1985; Rhind & McNeilly, 1986).

Changes in FSH concentrations associated with differences in O.R. have also been reported for other situations. FSH levels were higher during days 8-3 prior to ovulation in ewes which were to have twin ovulations compared to those having single ovulations (Davis et al., 1981). A similar result has been reported by McNatty et al., (1985). Comparisons of FSH concentrations between breeds differing widely in O.R. have yielded inconsistent results. It has been reported that the prolific Booroola and D'Man ewes have higher concentrations of FSH between luteal regression and the onset of the preovulatory gonadotrophin surges than comparable breeds of low prolificacy (Lahlou-Kassi et al., 1984; Bindon et al., 1985). In both cases, the FSH levels declined over the preovulatory period, but the rate of decline was slower in the prolific than in the non-prolific breeds. However, Booroola Merino ewes with higher O.R. do not exhibit higher plasma FSH levels than those with lower O.R. (Bindon, 1984). The same situation has not been examined in D'Man ewes. Studies in the prolific Finnish Landrace (Webb & England 1982a) and Romanov ewes (Cahill et al., 1981; Bindon et al., 1979) have failed to find any significant difference in FSH concentrations between these breeds and breeds of low prolificacy.

Cahill et al., (1981) found that the second FSH peak was higher in Romanov than Ile-de-France ewes and was significantly correlated with the number of antral follicles present on the ovary 17 day later. This has led the authors to suggest that the periovulatory FSH concentrations may influence the O.R. at the next cycle by altering

the number of follicles entering the antral phase. However, depression of the second FSH peak by treatment with ovine follicular fluid did not have any significant effect on subsequent O.R.and cycle length (Bindon et al., 1985).

4.2.1.3 Manipulation of gonadotrophin status and its effects on O.R.

The importance of gonadotrophins in the control of O.R. can be demonstrated in situations where gonadotrophin secretion is artificially altered. Attempts to manipulate the pattern of LH secretion during the follicular phase have obtained equivocal responses. McLeod et al., (1983) noted a positive correlation between O.R. and tonic LH secretion up to the time of the preovulatory LH surge in seasonally anoestrous ewes continuously infused with low doses of GnRH. However, administration of LH at high frequency during the follicular phase failed to increase O.R. significantly (Scaramuzzi & Radford, 1983). In contrast, O.R. has been reported to be increased by hCG injection around luteolysis (Radford et al., 1984). McNatty et al., (1981a) showed in anoestrous ewes that ovulation and normal luteal function could be induced with LH levels (in the follicular phase) ranging from 0.7- to 4-fold that seen in the normal oestrous cycle. Similar ovarian function could also be stimulated with as few as 16 or as many as 70 LH pulses of comparable size.

In contrast to LH, increase in FSH concentrations has been consistently shown to result in increased O.R.. Ovulation rate is increased by administration of exogenous gonadotrophin preparations such as pregnant mare serum gonadotrophin (PMSG) (Robinson, 1951; Gherardi & Lindsay, 1980; Gordon, 1983), crude extracts of pituitary gland (Moore & Shelton, 1962,1964; Boland & Gordon, 1977, 1982) and follicle-stimulating hormone (Wright et al., 1981; McNatty et al., 1985). Although PMSG and pituitary extracts possess both FSH and LH activities (Bindon & Piper, 1982a), the superovulatory effects of these preparations have been shown to be due to their FSH activity (Murphy et al., 1984).

The high O.R.of steroid-immunised ewes is associated with increased LH secretion at all times of the oestrous cycle, but FSH concentrations are only increased in ewes immunized against oestrogens. In ewes immunized against androgens, FSH concentrations either remain unchanged (testosterone) or are slightly depressed (androstenedione) (Scaramuzzi & Hopkinson, 1984; Webb et al., 1984; Cox et al., 1985; Smith, 1985a; Campbell et al., 1987). Nevertheless, immunization against both types of steroids is equally effective in increasing O.R.. Whether the differences in the effects of immunization against androgens and oestrogens on gonadotrophin secretion mean that they operate via different mechanisms is not yet clear. However, it has recently been reported that increased O.R. after active immunization against androstenedione was associated with increased FSH concentration during the luteal but not the follicular phase of the oestrous cycle (McNatty et al., 1987).

Injection of charcoal-treated bovine follicular fluid rich in inhibin from days 1 to 11 of the luteal phase has been found to increase O.R. following cloprostenol-induced luteolysis on day 12 (Wallace & McNeilly, 1985). Although FSH secretion was reduced during the treatment period, there was a rebound increase in plasma FSH levels after the induced luteolysis at the end of the treatment. Since LH concentrations were also increased during the entire period of treatment, and during the first day after prostaglandin injection, it is not possible to conclude whether the increase in O.R. was due to the increased FSH or LH secretion, or both. Nevertheless, these results suggest that the increase in O.R. after treatment of ewes with bovine follicular fluid in the luteal phase of the oestrous cycle is associated with changes in the patterns of gonadotrophin secretion. The low FSH concentrations up to the time of luteolysis are not essential for obtaining high O.R..

The ovarian follicles of Booroola Merino ewes contain less inhibin than those of control Merino ewes (Cummins et al., 1983). Since inhibin acts on the pituitary gland to selectively inhibit FSH secretion (Channing et al., 1982), this result suggests that the high O.R. of Booroola Merino ewes may be due to less negative feedback

inhibition by inhibin on FSH secretion. Immunization of ewes against partially purified follicular fluid has been shown to increase O.R. (O'Shea et al., 1984; Henderson et al., 1984). However, the high O.R. of ewes actively immunized against an inhibin-rich fraction of follicular fluid was not associated with any increase in FSH secretion (Bindon et al., 1985).

In view of the present evidence, it is not possible to draw definite conclusions about whether, or which, gonadotrophins are involved in the control of O.R.. Although LH is required for follicular steroid production and preovulatory follicular development, the LH concentrations and pulse frequency may not be involved in the control of O.R.. Land et al., (1982) argued that low LH levels might favour high O.R. because LH stimulates steroid production from the ovary. Lower LH levels might therefore results in lower steroidogenic stimulus to the developing follicles and hence reduced production of ovarian steroids per developing follicle. This situation also favours the maintenance of FSH concentration (Land et al., 1982).

FSH plays a number of very important roles in folliculogenesis. It is believed to act specifically on granulosa cells to influence the viability of ovarian follicles and ultimately their ability to ovulate (Richards, 1980; Monniaux et al., 1983; McNatty et al., 1985). There is evidence indicating that the increase in FSH concentrations required to bring about changes in O.R. may be small (Brown, 1978; Zeleznik, 1982; Baird, 1983). With the currently available assay method for FSH, a statistically insignificant change in FSH concentrations may have significant biological effects at the ovarian level. Therefore, it seems reasonable to suggest that multiple ovulations in sheep result from increased FSH stimulation during the late luteal and the follicular phase of the oestrous cycle. However, more critical evidence relating FSH concentration to O.R. is needed. While increasing FSH levels by administration of exogenous FSH has been found to increase O.R., there may be other situations in which a high O.R. is not associated with elevation in circulating FSH levels because of the likely increased negative feedback control by ovarian steroids and inhibin on gonadotrophin secretion.

4.2.1.4 Interactions between nutrition, circulating steroid levels and gonadotrophin secretion

If gonadotrophins are important determinants of O.R., then the question is, how does the ewe maintain sufficient gonadotrophin production in the face of a high O.R.? The secretion of gonadotrophins is stimulated by gonadotrophin releasing hormone (GnRH) secreted in the hypothalamus (Haresign et al., 1983). Gonadotrophin secretion by the pituitary gland is dependent on the amount of GnRH reaching the gland and the pituitary sensitivity to GnRH, both of which are controlled by ovarian steroids and inhibin (Karsch et al., 1978). Therefore, changes in gonadotrophin secretion can be brought about by: changes in the circulating steroid and inhibin concentrations; the sensitivity of the hypothalamo-pituitary unit to the negative feedback effects of ovarian steroids and inhibin; the amount of GnRH reaching the pituitary gland; and the pituitary sensitivity to GnRH.

Little information is available about the effects of nutrition on circulating levels of steroid hormones except that high nutrition tends to result in reduced progesterone concentration (Parr et al., 1982; Williams & Cumming, 1982; Rhind et al., 1985). Although Knight et al (1981) have reported elevated oestrogen levels with lupin feeding, there is generally a lack of information on the changes in oestrogen secretion caused by nutrition.

Steroid hormones are mainly metabolized in the liver, a process catalysed by several enzymes (Chatterton, 1982). Studies of the effects of nutrition on the rate of steroid hormone metabolism have shown that good nutrition increases the clearance rate of progesterone (Parr et al., 1982) and possibly oestradiol (Payne et al., 1987). This effect of nutrition is more likely to be realized through the effects of nutrition on liver size, and hence the total liver capacity to metabolise steroids, rather than on specific enzymes responsible for steroid metabolism (Smith et al., 1986; Payne et al., 1987).

No studies have been reported concerning the effects of nutrition on the sensitivity of the hypothalamo-pituitary axis to the negative feedback effects of ovarian steroid hormones in sheep. Studies in cattle have demonstrated that energy restriction potentiates the oestradiol suppression of LH secretion in ovariectomized heifers (Imakawa et al., 1984).

Few experiments have been carried out to specifically study the effects of nutrition on GnRH secretion and the pituitary sensitivity to GnRH. Several studies have shown that nutrition influences the LH pulse frequency (Jones et al., 1983; Rhind et al., 1985, 1986). Since each LH pulse is thought to be caused by a pulse release of GnRH from the hypothalamus (Clarke & Cummins, 1982; Levine et al., 1982), this indicates that GnRH secretion is affected by nutrition. These results may be confounded with the effects of nutrition on the hypothalamic sensitivity to the negative feedback effects of ovarian steroid hormones on GnRH secretion. Nevertheless, reduction in LH frequency due to energy restriction has been demonstrated in ovariectomized gilts (Armstrong & Britt, 1985), suggesting that nutrition influences the pulsatile LH secretion by acting, at least in part, on the hypothalamus to affect the frequency of GnRH secretion.

Comparison of the LH profiles in groups of ewes subjected to different nutritional treatments has failed to show significant differences in the LH pulse amplitude or the peak value of the preovulatory LH surge, suggesting that nutrition has no effects on the amount of GnRH released in each pulse and/or the pituitary sensitivity to GnRH (Haresign, 1981a; Rhind et al., 1984, 1985, 1986; Rhind & McNeilly, 1986). Studies on the pituitary responsiveness to GnRH challenge have found that undernutrition results in a significantly greater LH response to exogeneous GnRH stimulation compared with good nutrition (Haresign, 1981b). The same situation has also been reported in ovariectomized gilts (Armstrong & Britt, 1985). While these results are contradictory to reports that undernutrition reduces circulating LH concentrations, they can be reconciled by suggesting that undernutrition may affect the release, rather than the synthesis, of LH from the pituitary gland. This is in agreement with an early

study which showed that the biopotency of gonadotrophins in the anterior pituitary gland was higher in ewes fed submaintenance diet than in flushed ewes (Allen & Lamming, 1961).

Given that nutrition influences O.R. through the effects of the hypothalamo-pituitary unit on the growth and development of ovarian follicles, one problem remaining is how nutrition exerts its influences on the reproductive system. As discussed earlier in this section, an increased clearance rate of ovarian steroid hormones due to increased liver size could be one way by which nutrition increases O.R.. However, that this may not be the only mechanism has been suggested by the effects of nutrition on gonadotrophin secretion in ovariectomized animals (see above). At present, it is not known whether nutrition influences the reproductive system through its effects on the general nutritional status of the animal or through its effects on the circulating levels of some specific metabolites or non-reproductive hormones, such as insulin. The latter mechanism is possible because lupin grain supplementation results in elevated O.R. within one week (Oldham & Lindsay, 1984) and infusion of glucose and acetate for nine days late in the oestrous cycle has also been found to increase O.R. (Teleni et al., 1984; Teleni & Rowe, 1986). Responses occurring this rapidly are not accompanied by noticeable changes in the bodyweight of the animal.

In several species, it has been shown that insulin may have direct effects on ovarian follicular development and hypothalamo-pituitary axis function (May et al., 1980; Adashi et al., 1981; May & Schomberg, 1981; Jones et al., 1983). Administration of exogenous insulin has been reported to increase O.R. in gilts and heifers (Jones et al., 1983; Harrison & Randel, 1986). The large amount of insulin administered in these studies may induce pharmacological, rather than physiological, responses. Nevertheless, these results demonstrate a possible mechanism by which nutrition influences O.R. because high energy intake and body condition have been found to increase circulating concentrations of insulin (McCann et al., 1985; Procknor et al., 1985; Harrison & Randel, 1986).

4.2.2 Ovarian sensitivity to gonadotrophins

Comparison of gonadotrophin levels is meaningless if the ovarian sensitivity to gonadotrophins differs. Heavy ewes or ewes on a high plane of nutrition at the time of treatment are more responsive to exogenous gonadotrophins, as demonstrated by a greater ovulatory response to a standard dose of PMSG, than low bodyweight ewes or ewes on a low level of nutrition (Guerra et al., 1971; Allison, 1975; Fry et al., 1986). The greater sensitivity to gonadotrophins may partly account for the high O.R. of heavy or flushed ewes.

Increase in the superovulatory response to exogenous gonadotrophins has also been reported in other situations. Prolific sheep breeds show a greater ovulatory response to PMSG than comparable non-prolific breeds. This has also been confirmed in Romney ewes selected for prolificacy (see reviews by Bindon & Piper, 1986;Bindon et al., 1986). In contrast, steroid immunization, which causes increased O.R., does not result in the ovary being more sensitive to gonadotrophins because the ovulatory responses to a standard dose of PMSG are similar in immunized and non-immunized ewes (Smith et al., 1983b; Boland et al., 1985). However, there is some evidence indicating that immunized ewes are more responsive to PMSG at a low dose level than control animals (Scaramuzzi & Hopkinson, 1984).

It may be argued that the greater ovulatory response after PMSG treatment is due to the greater number of large antral follicles which already existed on the ovary of those highly responsive animals at the time of PMSG injection. However, when all follicles >2mm in diameter were cauterized before treatment, heavy ewes still had a greater number of large follicles (>4mm) on their ovaries 48h after PMSG injection compared to light ewes (Fry et al., 1986).

Increased sensitivity of individual follicles to gonadotrophins will result in increased O.R. only if there is no corresponding increase in the production of ovarian feedback products per follicle and/or in the hypothalamo-pituitary sensitivity to the negative feedback effects of oestrogens and inhibin (Spearow, 1985). Increased

follicular sensitivity to gonadotrophins is expected to be associated with changes in the number of receptors for gonadotrophins and/or the post-receptor events. In mice, differences in the ovulatory response between selected lines have been shown to be due to line differences in the number and affinity of LH receptors after PMSG treatment and cAMP production following hCG stimulation (Spearow, 1985). However, it is not known whether the same situation exists in domestic animals (McNeilly et al., 1987).

In summary, nutritionally-induced variation in O.R.is associated with changes in ovarian antral follicle populations, patterns of pre-ovulatory follicular development, circulating levels of gonadotrophins and ovarian sensitivity to gonadotrophins. However, it has not been possible to establish the cause-effect relationships between these changes and ovulation rate. More fundamentally, it is not known how nutrition exerts its influence on the reproductive system of the ewe.

5 Purpose and scope of the investigation

The efficiency of most sheep production systems can be markedly improved by increasing litter size. Variation in litter size is mainly determined by variation in ovulation rate. Of the many factors that have been found to influence ovulation rate in the ewe, nutrition is one of the most important. However, there has been a lack of detailed information on how the nutritional effect on ovulation rate is achieved. An understanding of this would allow ovulation rate to be controlled more effectively through nutrition, and would also help to elucidate the physiological mechanisms by which other factors influence ovulation rate in sheep.

The present trial was conducted to examine the mechanisms by which nutritionally-induced bodyweight differences affect O.R. of the ewe. Studies of the effects of breed and other factors on O.R. have shown that, in these situations, variation in O.R. is associated with changes in the antral follicle populations and the patterns of preovulatory follicular development. Accordingly, one of the aims of the present trial was to examine the changes in ovarian follicle populations and preovulatory follicular development associated with bodyweight-induced variation in O.R.. FSH is a very important hormone involved in the control of follicular development and is considered to be a possible determinant of O.R. in sheep. Therefore, a further objective of the trial was to investigate whether there was any difference in circulating FSH concentrations between ewes of high vs low bodyweights.

Two groups of mature ewes of similar initial mean bodyweights were differentially fed over the summer to generate differences in bodyweight early in the breeding season. After synchronization of oestrus, the dynamics of preovulatory follicular development were studied by ink-labelling selected large follicles and following their progress at repeated laparotomies during the follicular phase of the oestrous cycle. After the laparotomy study, the ovaries were removed, fixed and the left ones serially sectioned. All follicles $\geq$0.2mm in diameter in the sections were counted and measured, and their health

status observed. Plasma FSH levels were determined in blood samples taken prior to and during the follicular phase of the synchronized oestrous cycle. These studies therefore address the following questions:

(1) Is the difference in O.R. between ewes of high vs low bodyweights associated with changes in the patterns of preovulatory follicular development;

(2) Are there differences in the total number of antral follicles, their size distribution and rate of atresia between ewes of high vs low bodyweights.

(3) To what extent is circulating FSH involved in the control of bodyweight-induced variation in O.R..

CHAPTER II: MATERIALS AND METHODS

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1 Animals and treatments

Seventy Romney ewes, between five and seven years old, were selected from a single flock in December 1986. These animals were randomly divided into two groups of 35 each and assigned to either a high or a low nutrition treatment during the following 12-16 weeks. The ewes were weighed 24h after fasting at the beginning of the nutritional treatment and every two weeks thereafter (except at week 6, at which ewes were weighed after 48h fasting due to management difficulties). The pasture allowance provided to each group was adjusted regularly according to the previous weight data in order to achieve a weight difference of at least 10kg at the start of the experiment. One harnessed teaser ram was running with each group of ewes during the entire period and tupping marks were recorded at the time of weighing.

Within each treatment group, ewes were further divided randomly into 3 blocks (Table 2). Blocks 1 and 2 were used for the main experiment, while block 3 was used only for the collection of ovulation rate data (by laparoscopy).

The oestrous activity of all ewes was synchronized by treatment for 14 days with progesterone-impregnated intravaginal sponges. At the time of sponge insertion for animals in the first block, more than 50% of the ewes in each treatment group had been marked by the teaser ram. Dates of sponge withdrawal for each block are shown in Table 2. Before sponge withdrawal, the ewes in block 1 and 2 were weighed and transferred to a small paddock at the Animal Physiology Unit together with two teaser rams. The oestrous activity was checked regularly during the period 30-76h after sponge withdrawal. As far as possible, only ewes coming into oestrus within the period of 36-72h were selected for the experiment. The unselected ewes in block 1 and 2 were discarded.

Table 2: Assignment of animals and the dates of treatment

Block	Treatment[a]	Number of ewes assigned	Number of ewes selected	Date of sponge withdrawal
1	H	10[b]	7	17 March
	L	10	7	17 March
2	H	12	8	14 April
	L	12	8	14 April
3	H	13	14	30 March
	L	13	13	30 March

a: Treatment groups: H=high bodyweight; L=low bodyweight.

b: One ewe in this group lost her progesterone sponge and is included in block 3.

The selected animals were housed in the Animal Physiology Unit in wire pens on steel grating and fed a maintenance diet of lucerne chaff supplemented with minerals. This feeding regimen was adopted to minimize confounding of the dynamic and static effects of bodyweight on O.R.. The metabolisable energy (ME) requirement for maintenance was calculated as 0.53MJ ME per kg bodyweight$^{0.75}$ (Rattray, 1986). The lucerne chaff was assumed to contain 85% dry matter (DM) and 8.5 MJ ME/Kg DM (Coop, 1986). The mineral supplement (59% sodium chloride, 37% sodium sulphate, 4% sodium molybdate) was fed with the lucerne chaff at 2g/head every second day to counteract possible copper toxicity.

2 Laparotomy and prostaglandin treatment

Ewes in block 1 were treated with 150µg cloprostenol (Estrumate, Coopers Tierarzneimittel, GmbH, West Germany) to induce luteolysis 12 days after the detection of oestrus. The prostaglandin (PG) was injected intramuscularly on completion of the first laparotomy on each animal with further laparotomies at 24, 48 and 76h after PG injection.

Laparotomies were performed alternately on ewes from each treatment. On each occasion, ewes were laparotomized in a fixed sequence so that the time interval from PG treatment to each laparotomy was consistent for all animals.

It was subsequently found that a high proportion of ewes from the first block failed to ovulate. This might have been due to the anaesthesia and/or stress associated with the 48h laparotomy coinciding with, and inhibiting, the preovulatory LH surge which has been found to occur around 48h after prostaglandin injection (Acritopoulou et al., 1977;Acritopoulou & Haresign, 1980). Hence the 48h laparotomy was not performed in block 2 animals. All the other procedures for block 2 were the same as for block 1.

Prior to laparotomy, ewes were anaesthetized by an intravenous injection of the minimum amount (approximately 7-8ml) of 5% (W/V) thiopentone sodium (Intraval, May and Baker, New Zealand) required to induce anaesthesia for the whole period of surgery (about 20 minutes). A mid-ventral incision was made in the abdominal wall and the uterus and ovaries were exteriorized. The number of corpora lutea (CL) and the ovary in which they were present were recorded. All follicles $\geq$2mm in diameter were measured to the nearest 0.5mm using a divider and a ruler graduated to 0.5mm division. The size and position of the 3 largest follicles on each ovary were recorded and ink-labelled by injecting several dots of sterilized Indian ink into the ovarian stroma tissue surrouding the follicles with a thin-pointed glass pipette .

At subsequent laparotomies, the diameters of previously-labelled follicles were again measured. If any unlabelled follicle had grown into one of the 3 largest, it was also measured and labelled. A follicle was considered to be healthy when it increased in size by at least 0.5mm between two successive laparotomies or, in the case of very large follicles (>6mm), maintained its size between the third and fourth laparotomies.

3 Blood sampling

Ewes in block 1 were blood-sampled by jugular venipuncture to measure the changes in plasma FSH concentrations around and during the follicular phase. Blood sampling commenced on day 11 after the detection of oestrus, (i.e. 1 day before the injection of prostaglandin), and continued on the following three days.

Five samples were collected each day at 1.5h interval, commencing at 10.00h. All the blood samples were collected into heparinized vacutainers and centrifuged for 15 minutes at 2700g. The plasma was then transferred into duplicate vials and stored at -12°C.

Blood sampling in block 2 was similar to that in block 1 except that ewes were sampled every 3h during the period 24-72h after PG injection. This was done to determine whether the failure of block 1 ewes to ovulate might have been due to absence of the preovulatory LH surge.

4 Histological procedures

4.1 General

After the 4th laparotomy, ewes in block 1 continued to receive the maintenance level of nutrition and their ovaries were removed 5 days after the last laparotomy. For ewes in block 2, the ovaries were removed at the last laparotomy so that the health status of the preovulatory follicles could be determined. After removal, the ovaries were cut in half and fixed in Bouin's solution for 23-24h. The left ovaries of ewes in both blocks were serially sectioned at 10μm and every fifth section was mounted and stained with haematoxylin and eosin. Limited numbers of sections were also made from the right ovaries of ewes in block 2 for assessment and measurement of the preovulatory follicles.

Tracing and measurement of all follicles $\geq$0.2mm in diameter was made with a projection microscope. Follicle diameter was taken as the

mean of two measurements at right angles to each other on the section where the area of the follicle was maximal. The basal membrane of the follicle was taken as its outer limit. All the follicles $\geq$0.2mm in diameter were then observed under a light microscope at 400X magnification for the assessment of health status.

4.2 Classification of follicles

All follicles were arbitrarily classified into 4 groups according to follicle diameter(D): $D \leq 0.5$, $0.5 < D \leq 1$, $1 < D \leq 2$, $D > 2$mm. Follicles were also divided into four health status classes. The criteria used in this classification were based on published studies (Brand & de Jong, 1973; Turnbull et al., 1977):

a) Healthy follicles were defined as follicles with no more than three degenerating cells with pycnotic nuclei. No structural abnormalities as described below were present in healthy follicles.

b) Early atretic follicles were defined as follicles with a moderate number of pycnotic nuclei or atretic bodies present on the inner edge of, or within, the membrana granulosa. In addition, local destruction of the basal membrane and loss of orientation of the basal layer of granulosa cells were used as indications of early atresia.

c) Advanced atretic follicles were defined as follicles with a large number of atretic bodies both along the edge of, and within, the membrana granulosa. In follicles classified as advanced atretic, the basement membrane was either partially or completely lacking and the organization of the membrana granulosa showed signs of disintegration, such as loosening and sloughing of granulosa cells. Mitosis of granulosa cells was rarely seen in follicles undergoing advanced atresia.

d) Late atretic follicles were defined as follicles with widespread destructions of the follicle structure, such as loss of granulosa cells, decrease in follicle size, fibrous changes in the antrum and degeneration of the theca tissue.

5 Hormone assays

5.1 Follicle-stimulating hormone

Plasma FSH levels were measured by a double antibody radioimmunoassay. Ovine FSH (NIAMDD-oFSH-I-1/AFP-5679C) was used for iodination by the chloramine-T method (Greenwood et al., 1963) and ovine FSH (NIAMDD-oFSH-RP-1) as a reference standard. The antiserum was rabbit anti-ovine FSH (NIAMDD-anti-oFSH-1/AFP-C5288113) used at a final tube dilution of 1:80,000. (All the above materials were courtesy of N.I.A.M.D.D., U.S.A.). The assay had a linear range of 0.5-80 ng/ml. Assay sensitivity was 0.35 ng/ml. The intra-assay coefficients of variation (c.v.) were 8.2, 2.9 and 2.8%, and inter-assay c.v. 9.1, 7.5 and 4.9%, at mean FSH concentrations of 2.41, 10.41 and 20.28 ng/ml respectively. Crossreactions with oLH, oPRL, oGH, hACTH were all below 0.2% (Technical report number 141 of the Pituitary Hormones and Antisera Center, Harbor-UCLA Medical Center, California, U.S.A.).

5.2 Luteinizing hormone

Plasma LH levels were analysed by a double antibody radioimmunoassay based on that described by Niswender et al. (1969), as modified by Barrell and Lapwood (1979). The following materials were used in this assay: ovine LH (NIH-LH-S18, courtesy of N.I.A.M.D.D., N.I.H., U.S.A.) as standards; rabbit anti-ovine LH serum (pool #15, courtesy of Dr G.D. Niswender, Colorado State University, U.S.A.); and highly purified ovine LH (LER-1374A, courtesy of N.I.A.M.D.D., N.I.H., U.S.A) for radioiodination. The assay had a linear range of 0.07-8 ng/ml. Assay sensitivity was 0.06 ng/ml. The intra-assay c.v. were 10.0 and 4.6%, and the inter-assay c.v. 1.3 and 5.4% at mean LH concentrations of 0.45 and 4.43ng/ml respectively.

6 Statistical analysis

6.1 Oestrus data

The G-test (Sokal & Rohlf, 1969) was used to test the difference between ewes in the high and low bodyweight groups in the interval from sponge withdrawal to the onset of oestrus.

6.2 Ovulation rate data

The O.R. data were converted into binomial data depending on whether the O.R. was single or multiple (all ewes ovulated and only one ewe had an O.R. of greater than 2), and the logit transformation was applied. The transformed data were then analysed by an iterative weighted least square procedure (Gilmour, 1985), which performed maximum likelihood estimates on Logit(P) ($\text{Logit}(P)=\ln\frac{P}{1-P}$, where P is the probablity of multiple ovulations). The most general model fitted was:

$$Y_{ijk} = \mu + B_i + T_j + (BT)_{ij} + \beta_1 WT_{ijk} + \beta_2 WD_{ijk} + e_{ijk}.$$

Where:

Y_{ijk} = an observation on the kth ewe in the ith block and jth treatment.

μ = population mean.

B_i = the ith block effect (i=1...3).

T_j = the jth treatment effect (j=1...2).

$(BT)_{ij}$ = the interaction of the ith block with the jth treatment.

WT_{ijk} = the bodyweight of the kth ewe in the ith block and jth treatment at the time of ovulation.

β_1 = regression coefficient for bodyweight.

WD_{ijk} = the change in bodyweight for the kth ewe in the ith block and jth treatment during the cycle for which ovulation rate was recorded.

β_2 = regression coefficient for bodyweight change.

e_{ijk} = the error associated with individual ewe.

6.3 Laparotomy data

The total number of follicles ≥2mm in diameter present on the ovary at the time of the first laparotomy was analysed using analysis of variance (ANOVA). Due to the lack of normal distribution, the remaining data in this study were analysed using the nonparametric rank-sum test (Lehmann & D'Abrera, 1975). The observations were ranked within blocks and the normal approximation was used to calculate the test statistics for the treatment effect.

6.4 Histological data

The mean numbers of follicles in various classes were analysed by ANOVA after square root transformation of the data. However, in several cases, the lack of normality in distribution and/or the lack of homogeneity in variances could not be corrected by the square root or other transformations. In these cases, an ANOVA was performed first and the nonparametric test was then used to test the treatment effect. The distributions of follicles among different classes were analysed using the SAS "CATMOD" package which performs maximum-likelihood estimations of parameters for the log-linear models of functions of response frequencies and analysis of generalized logits.

6.5 Hormonal profiles

The effects of Block and Treatment on the mean concentrations of FSH on different days were analysed using ANOVA and the multivariate analysis of variance was used to compare the FSH concentrations between days.

Detection of the preovulatory LH surge was carried out using the method described by Christian et al (1978). Briefly, the LH values for each ewe were classified into an inlier subgroup and an outlier subgroup using the skewness coefficient test. Since, in most animals, the occurrence of the LH surge was very obvious with LH levels increasing from baseline values of less than 1.5ng/ml to at least 25ng/ml within one or two sample times, the original values were used

in the skewness test without correction for serial correlation (Christian et al., 1978). A preovulatory LH surge was considered to consist of at least 2 time adjacent outliers. Since, in the present trial, the peak values for the LH surges as defined above were all greater than 25ng/ml, the probability that the 2 outliers represented 2 LH spikes was very small.

6.6 Levels of statistical significance

The following symbols have been used throughout the text to indicate various levels of significance:

SYMBOL	LEVEL OF SIGNIFICANCE
***	$P<0.001$
**	$0.001<P<0.01$
*	$0.01<P<0.05$
+	$0.05<P<0.10$
NS	$0.10<P$

CHAPTER III: RESULTS

CHAPTER III: RESULTS

1 Bodyweight changes

The mean bodyweights at the beginning and end of the nutritional treatments and the bodyweight changes induced by the treatments for ewes in each of the block X treatment groups are presented in Fig.1 and Table 3. The nutritional treatments induced significant differences in bodyweights between ewes in the high and low feeding regimens, but there were no block or block X treatment effects. At the time of sponge withdrawal, the differences in the mean bodyweights between ewes on the high and low feeding regimens were 12.2, 16.1 and 15.6kg for ewes in block 1, 2 and 3 respectively (Table 3).

Weight changes during the indoor feeding period were also of interest since ewes had been fed at calculated maintenance with the objective of holding weight constant. To confirm the effectiveness of the maintenance feeding regimen during this period, ewes in block 1 were again weighed at the end of the experiment. After 2 weeks of feeding on this regimen, the changes in bodyweights were $+0.7\pm0.5$ and $+3.2\pm1.0$kg for ewes in the high and low bodyweight (BWT) groups respectively.

2 Onset of Oestrus

The pattern of onset of oestrus after the withdrawal of intravaginal sponges is shown in Fig 2. The data were pooled across blocks because of small sample sizes. Four ewes in the low BWT group failed to show oestrus within 96h following sponge withdrawal. Treatments significantly influenced the time interval from sponge withdrawal to the onset of oestrus, ewes in the high BWT group coming into oestrus earlier(43.9 ± 2.6h) than ewes in the low BWT group (56.6 ± 3.1h, $P<0.05$).

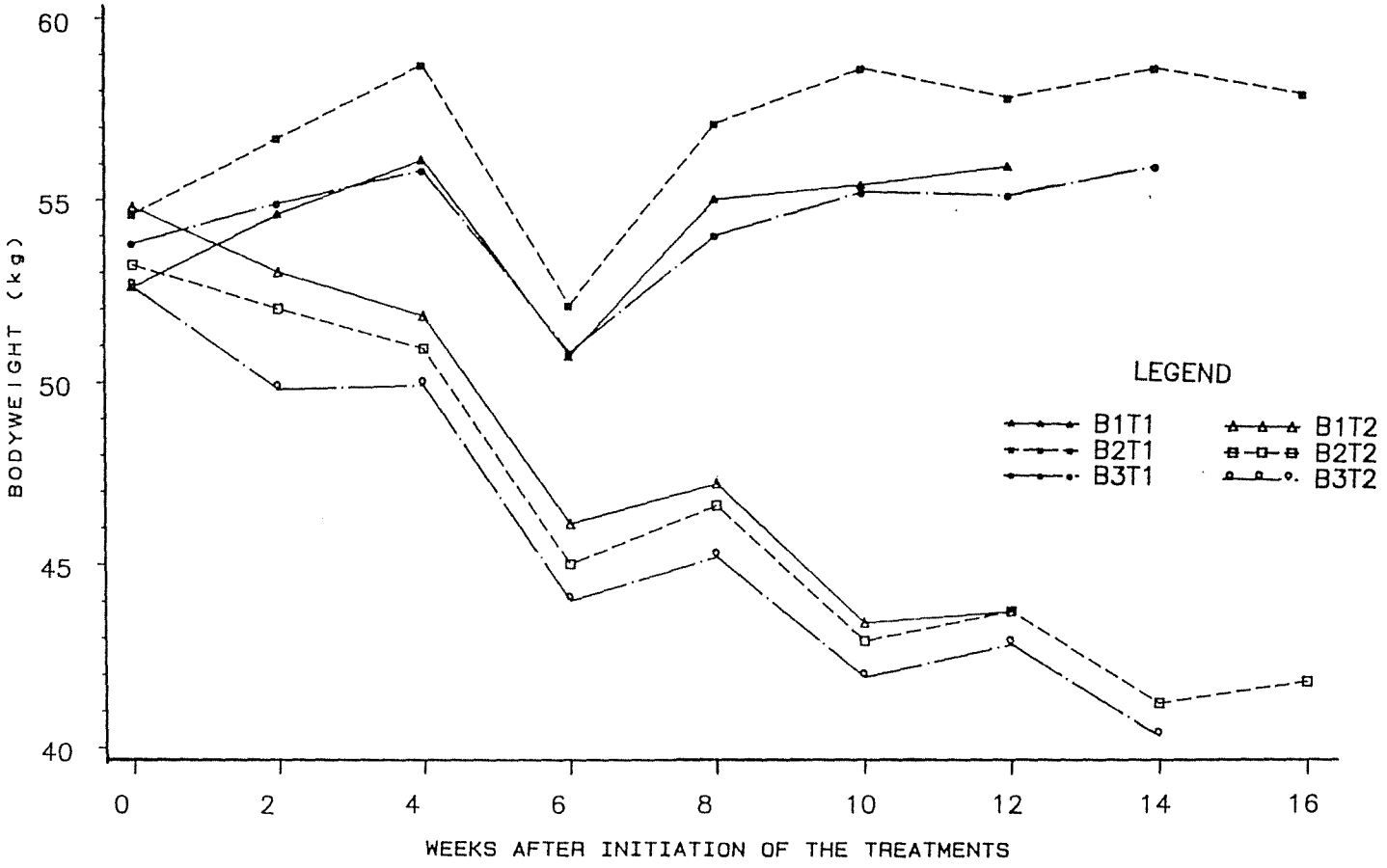

Fig 1: The mean bodyweights of ewes in the six block(B) by treatment(T) groups (see the legend) at different times (weeks) after the initiation of the nutritional treatments

Table 3: Effects of block(B) and treatment(T) on bodyweight (BWT) and bodyweight changes

Block	1		2		3		Significance		
Treatment[a]	H	L	H	L	H	L	B	T	BXT
No of Ewes	7	7	8	8	14	13			
Initial BWT(kg)	52.6±2.0	54.8±3.0	54.6±2.3	53.2±1.8	53.8±2.0	52.6±1.9	NS	NS	NS
Final BWT(kg)	55.9±2.5	43.7±4.0	57.9±2.0	41.8±1.6	55.9±2.1	40.3±1.7	NS	***	NS
BWT Change (kg)	3.3±1.1	-11.1±2.0	3.3±1.0	-11.4±1.3	2.1±1.1	-12.3±0.7	NS	***	NS

a: Treatment groups: H=high bodyweight; L=low bodyweight.

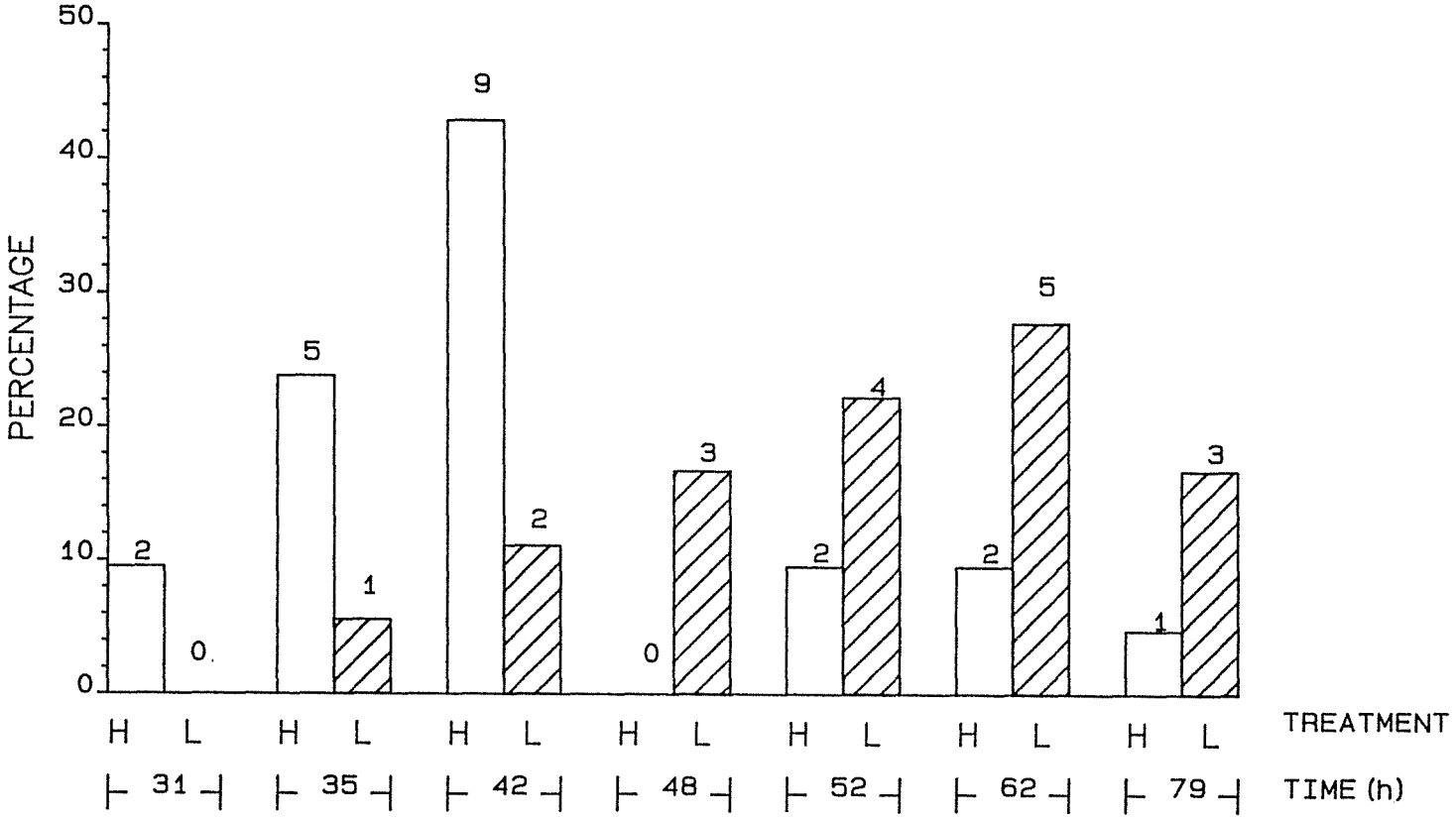

Fig 2: Percentage of ewes showing oestrus at different times (time values represent midpoints between consecutive observations) following sponge withdrawal for animals in the high (open bars) and low (hatched bars) bodyweight groups. Numbers of animals are shown above the bars.

3 Ovulation rate

The ovulation rates, as determined by counting the number of corpora lutea present on the ovaries at the time of the first laparotomy (blocks 1 and 2) or through laparoscopy (block 3) for the cycle following sponge withdrawal, are presented in Table 4. The ovulation rate was significantly ($P<0.001$) higher for ewes in the high BWT group (1.73 ± 0.20) than for ewes in the low BWT group (1.19 ± 0.13). There were no effects of block or block X treatment interaction ($P>0.10$). The ewe bodyweight at the time of ovulation had a highly significant ($P<0.001$) effect on ovulation rate when fitted first in the model. On average, O.R. increased by 3.1% for each kilogram increase in bodyweight. When fitted after adjustment for the bodyweight effect, the treatment effect was no longer significant. That is, variation in O.R. due to treatment could be largely accounted for by the treatment effect on bodyweight. Conversely, a significant ($P<0.05$) bodyweight effect persisted when it was fitted after treatment, suggesting that bodyweight influenced O.R. within treatment groups. The effect of bodyweight change during the cycle prior to ovulation was significant ($P<0.05$) when fitted after treatment. This effect was only partially removed by prior correction for bodyweight ($P<0.10$), suggesting that, to some extent, bodyweight and bodyweight change exerted independent effects on O.R..

Table 4: Effects of block(B), treatment(T) and bodyweight(BWT) on the ovulation rate during the cycle following sponge withdrawal

Block	1		2		3		Significance			
Treatment[a]	H	L	H	L	H	L	B	T	BXT	BWT[b]
No of Ewes	7	7	8	8	14	13				
Ovulation rate	1.86±0.14	1.29±0.18	1.50±0.19	1.13±0.13	1.79±0.24	1.17±0.11	NS	***	NS	*

a: Treatment groups: H=high bodyweight; L=low bodyweight.
b: Regression of ovulation rate on bodyweight at the time of ovulation (after correction for other factors).

4 Follicle development during the follicular phase

4.1 Dynamics of preovulatory follicular development

Data from the repeated laparotomy study are summarized in Table 5. Compared with ewes in the low BWT group, the high O.R. of ewes in the high BWT group was associated with:

a) More follicles $\geq$2mm in diameter present on the ovary at the time of the first laparotomy ($P<0.001$);
b) More follicles being labelled at the first laparotomy ($P<0.05$);
c) A marginally higher proportion of labelled follicles continuing to grow between the first and second laparotomy ($P<0.10$);
d) Similar number of unlabelled follicles growing between the first and second laparotomy ($P>0.10$);
e) More follicles being recruited,as measured by the total number of follicles of $\geq$2mm diameter that grew between the first and second laparotomy ($P<0.05$);
f) More follicles continuing to grow between the second and the last laparotomy ($P<0.001$);
g) A marginally lower intensity of selection through atresia, as measured by the ratio of the number of regressing follicles to the total number of follicles followed between the second and last laparotomy ($P<0.10$).

4.2 Ovulatory activity following prostaglandin injection

At the time of the last laparotomy about 76h after prostaglandin injection, no ewes in block 1 had ovulated. When these ewes were again checked by laparoscopy 24h later, only 2 ewes from the high BWT group had apparently ovulated. However, adhesion and bleeding caused by the repeated laparotomies made successful use of the laparoscopy technique difficult and possibly inaccurate. At the time of ovariectomy 5 days after the last laparotomy, 5 of 7 ewes in the high BWT group and 3 of 7 ewes in the low BWT group had ovulated. The O.R. per ewe ovulating was 1.60 ± 0.24 and 1.00 ± 0.00 for ewes in the high and low BWT groups respectively. It was at this stage that a decision was made to determine why ewes in block 1 had failed to ovulate at the expected time. For ewes in block 2, only 2 from the high BWT group had ovulated by the time of the last laparotomy.

Table 5: Numeric features of preovulatory follicle development for ewes in the high and low BWT groups

Block	1		2		Pooled		Significance
Treatment[a]	H	L	H	L	H	L	of treatment
No of ewes	7	7	7[b]	8	14	15	effects
Total number of foll. ≥2mm in diameter	12.14±1.94	8.71±0.92	9.28±1.39	6.75±1.16	10.71±1.19	7.66±0.75	***
Number of follicles labelled at 1st lap.	6.00±0.00	5.14±0.40	5.43±0.37	4.63±0.60	5.71±0.29	4.87±0.51	*
Labelled at 1st lap. growing until the 2nd laparotomy	3.43±0.21	2.29±0.42	3.14±0.26	2.38±0.50	3.29±0.17	2.34±0.33	*
% labelled follicles growing until the 2nd laparotomy	57.14±3.37	46.43±9.42	58.33±4.07	44.87±8.12	57.74±2.54	45.60±6.17	+
Not labelled initially but growing to be labelled at 2nd lap.	0.43±0.20	0.57±0.20	0.57±0.20	0.38±0.26	0.50±0.14	0.47±0.17	NS
Total growing between 1st & 2nd laparotomies	3.86±0.26	2.86±0.40	3.71±0.29	2.75±0.45	3.79±0.19	2.80±0.30	*
Labelled at 2nd lap. growing until 3rd lap.	2.86±0.26	2.00±0.44	N/A[c]	N/A	N/A	N/A	

Labelled at 2nd or 3rd lap growing until last laparotomy	2.14±0.26	1.29±0.18	2.00±0.00[d]	1.25±0.25	2.07±0.18	1.27±0.22	***
% follicles regressing between 2nd & last lap.	43.09±7.23	53.57±3.57	44.29±4.10	54.25±9.85	43.68±4.16	53.93±5.53	+

a: Treatment groups: H=high bodyweight; L=low bodyweight.
b: One ewe in this group was excluded from analysis due to haemorrhage caused by rupture of a big follice at the time of the first laparotomy.
c: N/A - not applicable.
d: Two ewes had already ovulated at the time of this laparotomy and the ovulations were considered to be growing follicles for the purpose of statistical analysis

5 Histological studies

5.1 Total number of follicles

The total numbers of follicles ≥0.2mm in diameter observed on serial sections of the left ovaries of ewes in each treatment by block are presented in Table 6.Treatment had a marginally significant ($P<0.10$) effect on the total number of follicles, ewes in the high BWT group having more follicles than those in the low BWT group. Across treatments and blocks, the total number of follicles was significantly correlated with the ovulation rate of the previous cycle ($r=0.40$, $P<0.05$).

5.2 Numbers of follicles in different size classes

The mean numbers of follicles per ewe in each of the 4 size classes are presented in Table 6. Generally, the numbers of follicles in different size classes were not affected by treatment or bodyweight, except for a marginally significant ($P<0.10$) effect of treatment on the number of follicles ≤0.5mm in diameter. There were small but significant correlations between the O.R. of the previous cycle and the number of follicles of 0.5-1 ($r=0.33$, $P<0.10$) and 1-2mm ($r=0.43$, $P<0.05$) diameter.

When the effects of block, treatment and bodyweight on the distribution of follicles within the size classes were analyzed, the only significant effect was the block by treatment interaction. This effect of interaction occurred only in follicles of ≤0.5mm in diameter.

5.3 Numbers of follicles in different health status classes

The mean numbers of follicles in each of the 4 health status classes are presented in Table 6. There were significant block and treatment effects on the numbers of late atretic follicles. Ovaries of ewes in block 2 contained more late atretic follicles than those of ewes in block 1 ($P<0.10$). High BWT treatment significantly ($P<0.05$)

Table 6: Effects of block(B), treatment (T) and bodyweight(BWT) on the total and mean numbers of follicles in different Size or health status classes

Block	1		2		Significance			
Treatment[a]	H	L	H	L	B	T	BXT	BWT[b]
No. of ewe	7	7	8	8				
Total No. of foll.	78.0±15.2	50.1±10.0	74.1±12.7	70.6±14.4	NS	+	NS	NS
No foll. in size class								
≤0.5mm	53.0±13.2	29.4±9.1	46.1±10.9	45.3±13.1	NS	+	+	NS
0.5-1mm	10.3±2.4	9.1±2.1	11.9±2.5	10.8±1.9	NS	NS	NS	NS
1-2mm	11.4±1.5	8.6±1.3	12.8±2.1	12.6±1.7	NS	NS	NS	NS
>2mm	3.3±0.7	3.0±0.6	3.4±0.6	2.0±0.5	NS	NS	NS	NS
No. foll. in status class[c]								
He	49.3±13.2	32.3±8.8	42.1±10.7	44.1±12.9	NS	NS	NS	NS
E	5.6±1.8	4.1±1.2	5.6±1.9	6.4±1.5	NS	NS	NS	NS
A	6.7±1.4	4.1±1.1	5.9±1.6	4.0±1.5	NS	NS	NS	NS
La	16.4±3.8	9.6±2.1	20.5±3.8	16.1±2.8	+	*	NS	NS

a: Treatment groups: H=high bodyweight; L=low bodyweight.

b: Regressions of follicle number on bodyweight (fitted after block and treatment).

c: The symbols used for the status classes are: He=healthy follicles; E=early atretic follicles; A=advanced atretic follicles; La=late atretic follicles.

increased the number of late atretic follicles. The number of healthy follicles was significantly correlated with the O.R. of the previous cycle ($r=0.38$, $P<0.05$).

Block ($P<0.05$), treatment ($P<0.05$) and bodyweight ($P<0.05$) all had significant effects on the distribution of follicles within the 4 health-status classes. The proportions of follicles in the healthy ($P<0.01$) and advanced atretic ($P<0.05$) classes were significantly higher in block 1 than in block 2. High BWT treatment significantly ($P<0.01$) increased the proportion of advanced and late atretic follicles (i.e. high BWT treatment significantly reduced the proportion of healthy follicles). There was a significant ($P<0.01$), positive association between BWT and the proportion of healthy follicles.

5.4 Numbers of follicles in different size by status classes

The mean numbers of follicles in each of the size by status classes are presented in Table 7. High BWT treatment significantly increased the numbers of advanced ($P<0.01$) and late atretic ($P<0.05$) follicles $\leq$0.5mm in diameter and marginally increased the number of advanced atretic follicles of 0.5-1mm in diameter ($P<0.10$). Ewes in the high BWT group had a significantly ($P<0.05$) higher number of healthy follicles >2mm in diameter than ewes in the low BWT group. There was a significant ($P<0.05$), positive association between the numbers of healthy follicles of 0.5-1 and 1-2 mm diameter and bodyweight (fitted after effects of block and treatment). Significant correlations existed between the O.R. of the previous cycle and the number of healthy follicles of 0.5-1 ($r=0.49$, $P<0.01$) and 1-2mm ($r=0.39$, $P<0.05$) in diameter.

When the effects of block, treatment and bodyweight on the distribution of follicles within the size X status classes were analyzed, it was found that ewes in the high BWT group had a higher ($P<0.10$) proportion of early atretic follicles $\leq$0.5mm in diameter than ewes in the low BWT group. Within treatment groups, bodyweight was significantly ($P<0.05$) and positively associated with the proportions of healthy follicles of 0.5-1 & 1-2mm diameter.

Table 7: Effects of block(B), treatment(T) and bodyweight (BWT) on the numbers of follicles in different size by status classes

	Block	1		2		POOLED		Significance of effects			
	Treatment[a]	H	L	H	L	H	L	B	T	BXT	BWT[b]
	No of ewes	7	7	8	8	15	15				
Size class	Status class[c]										
Diam≤0.5mm	He	39.4±5.2	25.1±4.4	34.2±4.3	36.9±7.5	36.7±3.3	31.4±4.6	NS	NS	NS	NS
	E	1.7±1.4	0.1±0.1	0.9±0.3	0.9±0.4	1.3±0.7	0.5±0.2	NS	NS	NS	NS
	A	1.7±1.1	0.1±0.1	0.1±0.1	0	0.9±0.5	0.1±0.1	*	**	NS	NS
	La	10.1±2.3	4.0±1.1	10.9±2.0	7.5±2.0	10.5±1.5	5.9±1.2	NS	*	NS	NS
0.5<Diam≤1mm	He	6.0±1.2	4.4±0.8	3.7±0.5	4.5±0.6	4.8±0.7	4.5±0.5	NS	NS	NS	*
	E	0.3±0.2	0.3±0.2	0.2±0.3	0.4±0.2	0.3±0.2	0.3±0.1	NS	NS	NS	NS
	A	1.1±0.6	0.3±0.2	1.2±0.6	0.6±0.5	1.2±0.4	0.5±0.3	NS	+	NS	NS
	La	2.9±1.3	4.1±1.3	6.6±1.7	5.2±0.9	4.9±1.2	4.7±0.8	+	NS	NS	NS
1<Diam≤2mm	He	3.0±0.9	2.1±0.5	3.0±0.7	2.4±0.9	3.0±0.6	2.3±0.5	NS	NS	NS	*
	E	3.0±0.9	3.0±0.6	4.2±1.4	4.7±0.5	3.7±0.8	3.9±0.5	NS	NS	NS	NS
	A	2.9±0.5	2.7±0.7	3.2±1.3	3.1±1.2	3.1±0.7	2.9±0.7	NS	NS	NS	NS
	La	2.6±0.8	0.7±0.5	2.2±0.5	2.4±0.6	2.4±0.4	1.6±0.5	NS	NS	+	NS
Diam>2mm	He	0.9±0.4	0.6±0.2	1.1±0.1	0.4±0.2	1.0±0.2	0.5±0.1	NS	*	NS	NS
	E	0.6±0.3	0.7±0.4	0.2±0.3	0.4±0.3	0.4±0.2	0.5±0.2	NS	NS	NS	NS
	A	1.0±0.4	1.0±0.4	1.2±0.4	0.2±0.2	1.1±0.3	0.6±0.2	NS	NS	NS	NS
	La	0.9±0.3	0.7±0.2	0.8±0.2	1.0±0.4	0.8±0.2	0.9±0.2	NS	NS	NS	NS

a: Treatment groups: H=high bodyweight; L=low bodyweight.
b: Regression of follicle number on bodyweight (fitted after correction for other factors)
c: For an explanation of the symbols used for the status classes, refer to Table 6.

5.5 Potential ovulations

The mean numbers of potential ovulations, defined here as the sum of actual ovulations and morphologically healthy follicles with a diameter of greater than 4mm after fixation, for block 2 ewes in the high and low BWT groups are presented in Table 8. The potential ovulation rate was significantly higher for ewes in the high BWT group than for ewes in the low BWT group ($P<0.05$). Although the definition for ovulatory follicles is somewhat arbitrary, these values are quite similar to the O.R. observed in the previous cycle. Accumulation of lipid droplets in the theca interna cells, a morphological change which occurs in response to the preovulatory LH surge (Bjersing, 1978), was observed in some of these follicles.

Table 8: Numbers of potential ovulatory follicles for block 2 ewes in the high and low BWT groups

Treatment[a]	No. of ewes	Mean No of follicles	Mean No of ovulations	Total potential ovulations
H	7	1.29±0.38	0.43±0.30	1.71±0.18
L	8	1.13±0.23	0	1.13±0.23

a: Treatment groups: H=high bodyweight; L=low bodyweight.

6 Hormone profiles

6.1 Follicle-stimulating hormone

The mean FSH concentrations on different days in relation to prostaglandin injection are presented in Table 9. FSH levels decreased during the follicular phase. The mean FSH concentrations on day 1 and 2 were significantly ($P<0.001$) lower than on day -1 and day 0, but there were no differences in FSH concentrations between days -1 and 0 or between days 1 and 2. There were no significant effects of block, treatment or block X treatment interaction on mean FSH concentrations on any day during the sampling period. There were also no significant interactions between day of blood-sampling and block or treatment. The data were also analysed with respect to the ovulation rate of the previous cycle and the potential ovulation rate of the present cycle, but there were no differences in mean FSH concentration between ewes with single and multiple ovulations within treatment groups.

6.2 Luteinizing hormone

Data on the occurrence of the preovulatory LH surge for ewes in block 2 are presented in Table 10. By about 72h after PG injection, preovulatory LH surges had occurred in 6 of 8 ewes in the high BWT group and 7 of 8 ewes in the low BWT group.The absence of the LH surge in one ewe from the high BWT group was probably due to a lack of preovulatory follicles on the ovary as a result of haemorrhage caused by rupture of a large follicle at the time of the first laparotomy. On average, the preovulatory LH surge occurred at 60.8 ± 3.8 and 60.9 ± 3.1h after PG injection for ewes in the high and low BWT groups respectively ($P>0.10$).

Table 9: Effects of block(B) and treatment(T) on mean FSH concentrations (ng/ml) prior to and during the follicular phase of the oestrous cycle

Block	1		2		Pooled	Significance of effects		
Treatment[a]	H	L	H	L	for all ewes	B	T	BXT
No of ewes	7	7	8	8	30			
Sampling day[b]								
Day -1	1.89±0.11	1.96±0.11	1.77±0.07	1.81±0.09	1.86±0.05[c]	NS	NS	NS
Day 0	1.80±0.13	1.86±0.14	2.13±0.08	1.85±0.11	1.94±0.06[c]	NS	NS	NS
Day 1	1.42±0.08	1.56±0.07	1.42±0.07	1.40±0.08	1.44±0.04[d]	NS	NS	NS
Day 2	1.46±0.09	1.56±0.07	1.43±0.08	1.42±0.07	1.46±0.04[d]	NS	NS	NS

a: Treatment groups: H=high bodyweight; L=low bodyweight.
b: Day 0 is the day of prostaglandin injection.
c,d: Pooled means with different superscripts differ at the P<0.001 significance level

Table 10: Onset of the preovulatory LH surge for block 2 ewes in the high and low BWT groups

Treatment[a]	No of ewes	Number of ewes with LH surge	Interval from PG injection to onset of LH surge (h)	
			Mean(±s.e.)	Range
H	8	6	60.8±3.8	48-71
L	8	7	60.9±3.1	52-72

a: Treatment groups: H=high bodyweight; L=low bodyweight.

CHAPTER IV: DISCUSSION

CHAPTER IV: DISCUSSION

The main aim of the present study was to investigate the mechanisms by which nutritionally-induced bodyweight differences influence O.R.. To achieve this, groups of ewes of similar mean initial bodyweights were differentially fed over a period of 12-16 weeks, so generating bodyweight differences of 12-16 kg between ewes in the high and low BWT groups. Weight differences of this magnitude are comparable to those used in other studies (Allison, 1975; Fry et al., 1986). To avoid confounding with the dynamic effect of bodyweight change prior to ovulation, ewes were individually fed a "maintenance" diet for the 12 days before the commencement of, and during, the indoor trial.Maintenance requirement was calculated using published data which assumes that maintenance is proportional to metabolic liveweight. At the time of weighing, about one cycle after the start of this maintenance feeding regimen, weight change was +0.7kg for ewes in the high BWT group, in contrast to a weight gain of about 3.2kg for ewes in the low BWT group during the same period. This difference in bodyweight gain between the two groups of ewes was presumably due to a difference in the maintenance requirement (per kilogram bodyweight$^{0.75}$) of heavy versus light ewes. As a result, a slight confounding of the static and dynamic bodyweight effects existed in the present study. This would have served to diminish, rather than to increase, differences due to the bodyweight effects

As expected, differences in bodyweight between ewes in the high and low bodyweight groups were associated with significant differences in O.R.for the cycle following sponge withdrawal. The 3.1% change in O.R. for each kg difference in BWT is in the upper limit of responses (2-3%) reported from previous studies (Cumming, 1977; Morley et al., 1978; Smith et al., 1983a).

Bodyweight has often been used as a measure of the effects of nutritional treatments. Two types of bodyweight effects on O.R. have been identified: the static effects of bodyweight at the time of ovulation and the dynamic effects of bodyweight change prior to

ovulation (Coop, 1966b). However, it is not known whether they operate through different mechanisms. Lindsay (1976) suggested that O.R. in ewes was related to their "net nutritional status" - a term loosely covering the sum of the endogenous catabolic sources of nutrients and uptake of exogenous nutrients from the gut. This suggests that both bodyweight and bodyweight change (which is related to the current level of feed intake) are components of the ewe's net nutritional status and are likely to influence O.R. via common mechanisms.

In all studies of this type, variation in bodyweight exists between and within treatment groups. However, the interpretation of the within-group variation in bodyweight should be different from that of between-group variation. Whereas the between-group variation in bodyweight is largely caused by the nutritional treatment applied, the within-group variation is probably due to genetic and permanent environmental factors as well as temporary variation in the nutrition and health status of the individual. Normally the relationship between bodyweight and O.R. within groups is not as strong as between groups (Coop & Hayman, 1962; Kelly & Johnstone, 1982). The same was true in this study although there existed a significant correlation between bodyweight and O.R. within groups. This may have reflected the considerable variation within groups in bodyweight and condition of the ewes associated with their being old and in some cases of poor constitution.

Following sponge withdrawal, ewes in the low BWT group came into oestrus significantly later than those in the high BWT group. This is in agreement with results from previous studies (Lamond, 1963; Allison, 1975). A likely explanation for this difference is that the clearance rate of the residual progesterone after sponge withdrawal was lower for ewes in the low BWT group than for ewes in the high BWT group as has been demonstrated previously (Williams & Cumming, 1982). This finding lends support to the suggestion that high feed intake and bodyweight may influence O.R. by increasing clearance of ovarian steroids from the circulation and hence reducing the negative ovarian feedback control on pituitary gonadotrophin secretion (Smith et al.,

1986; Payne et al., 1987). Alternatively, reduced follicular sensitivity to gonadotrophin stimulation in ewes from the low BWT group may have limited follicular growth and oestradiol secretion, so prolonging the time period required for ewes to attain the threshold oestradiol concentrations needed to induce oestrous activity.

A major component of this study involved examination of preovulatory follicular development in ewes from the two BWT groups.In sheep, follicular development is a continuous process with large follicles appearing on the ovary at all times of the oestrous cycle (Driancourt et al., 1985b). However, only one or two follicles ovulate at the end of each cycle. It has been suggested thatt he differentiation of the ovulatory follicle(s) is a two-step event involving recruitment and selection (di Zerega & Hodgen, 1981; Driancourt et al., 1985b). The process of recruitment establishes a group of follicles capable of ovulating. There is then selection from among these follicles of only one or two (in most sheep breeds) that will continue to develop and ovulate while all the other recruited follicles undergo atresia. Thus increases in ovulation rate can be achieved by two mechanisms: more follicles available for recruitment and/or a lower proportion of the recruited follicles that become atretic at the time of selection.

It has been possible to monitor the patterns of preovulatory follicle development in sheep by ink-labelling of individual large follicles and following their progress at repeated laparotomies during the follicular phase, (Driancourt & Cahill, 1984). The major limitations to the successful use of this technique are: the accuracy and repeatability of measuring follicle diameters on the ovarian surface (Spicer et al., 1987); and the interpretation of the results, because the reported time of occurrence of recruitment and selection varies greatly between animals (Driancourt & Cahill, 1984; Driancourt et al., 1985b). Thus, at a given time, recruitment or selection may have occurred in some animals but not in others. The general consensus is that recruitment occurs shortly after luteolysis and selection takes place late in the follicular phase of the oestrous cycle (Driancourt et al., 1985b). It is assumed that observation at the laparotomy performed 24h after prostaglandin injection, as in the present study,

will be able to separate the processes of recruitment and selection. However, the effects of repeated laparotomies on the physiological processes governing preovulatory follicular development and ovulation are not known. In the present study, no ewes in block 1 had ovulated at the last laparotomy (76h after the injection of prostaglandin), when most ewes are normally expected to have done so (Acritopoulou et al., 1977; Driancourt & Cahill, 1984). At the time of ovariectomy 5 days after the last laparotomy, only 5 of 7 ewes in the high BWT group and 3 of 7 ewes in the low BWT group had ovulated. The failure of ewes in block 1 to ovulate could have been caused by insufficient preovulatory LH surge and/or an inability of preovulatory follicles to respond to the LH surge. To determine which of these effects was involved, the following modifications were made to the experimental procedures for ewes in block 2: (1) the laparotomy at 48h after prostaglandin injection was not performed so as to reduce stress at a critical time of the oestrous cycle; (2) blood samples were collected every 3h during the period 30-72h after prostaglandin administration to determine the time of occurrence of the preovulatory LH surge; (3) ovariectomy was performed on completion of the last laparotomy to determine the health status of the large follicles present on the ovary.

Results from these studies on block 2 animals showed that the preovulatory LH surge had occurred in most ewes by about 72h after the PG injection. The mean time-interval of about 60h from PG injection to the onset of the LH surge is similar to that reported from some studies (Baird et al., 1981; Tsonis et al., 1984), but is longer than that reported from others (Baird & Scaramuzzi, 1976; Acritopoulou et al., 1978; Acritopoulou & Haresign, 1980). Direct comparison between these results are not possible because none of these studies were carried out using Romney ewes. The repeated anaesthetic treatments may have slightly depressed LH secretion because the anaesthetic used in this trial (thiopentone) has been shown to suppress basal LH secretion and delay the preovulatory LH surge (Clarke & Doughton, 1983; Cahill, personal communication). Nevertheless, a major feature of the occurrence of the preovulatory LH surge is the variable time-interval from PG injection to the onset of the surge among

individual animals, as has also been demonstrated by others (Bair & Scaramuzzi, 1976; Acritopoulou & Haresign, 1980). Furthermore, there were morphologically healthy preovulatory follicles present on the ovary and the numbers of such follicles were comparable to the O.R. observed in the previous cycle. These follicles were apparently capable of secreting sufficient oestradiol to induce the preovulatory LH surge. Accumulation of lipid droplets in the theca interna cells, which is a typical luteinizing change prior to ovulation (Bjersing, 1978), was observed in some of the preovulatory follicles, indicating that they were capable of responding to the preovulatory LH surge. Since ovulation usually occurs about 24h after the peak of the LH surge (Cumming et al., 1973; Acritopoulou et al., 1978), these results suggest that, given time, most ewes in block 2 would have ovulated.

From these results, the reasons for the failure of ewes in block 1 to ovulate are still not clear. The only logical, although not convincing, explanation is that the laparotomy performed 48h after PG injection might have detrimentally affected normal preovulatory events (either the preovulatory LH surge and/or follicle function.

Despite the poor ovulations in the present trial, it seems, from the above discussion, that follicular development up to the preovulatory stage during the follicular phase was not significantly altered. The high O.R. of ewes in the high BWT group was associated with an increased number of follicles being recruited and a lower proportion of the recruited follicles that become atretic at the time of selection.This is in agreement with an earlier study using Border Leicester X Merino ewes (Fry et al., 1986). The results of the present study also confirmed the observation in many studies that the nutritionally-induced variation in O.R. is associated with change in the population of preovulatory follicles during the follicular phase (Allison, 1977; Haresign, 1981a; Rhind & McNeilly, 1986).

Both FSH and LH are involved in controlling follicle recruitment (Driancourt et al., 1985b). FSH is needed for stimulating early follicular development, activating the granulosa cell aromatase system

and inducing granulosa cell receptors for LH. It thus enables follicles to respond to the increasing LH secretion during the follicular phase of the oestrous cycle. The follicles that are recruited are possibly the healthy follicles with a diameter of ≥2mm at the time of luteolysis (Driancourt & Cahill, 1984; Tsonis et al., 1984; Driancourt et al., 1985a). After the onset of luteal regression, the increasing LH secretion stimulates the production of a large amount of androgens by the theca interna cells. This increase in androgen secretion probably hastens the process of atresia of those follicles already undergoing atresia at the time of luteal regression (McNatty et al., 1982; Driancourt & Cahill, 1984). The remaining healthy follicles form the group of recruited follicles and are stimulated to undergo rapid growth in response to the increasing LH secretion. Therefore, the availability of healthy follicles ≥2mm in diameter at the time of luteolysis may be the immediate determinant of the number of follicles recruited.

The number of follicles ≥2mm in diameter present on the ovary at the time of the first laparotomy was greater for ewes in the high BWT group than for ewes in the low BWT group, as has also been reported from other studies (Allen & Lamming, 1961; Allison, 1977; Fry et al., 1986). The health status of these follicles could not be determined directly in the present study because of the time of ovariectomy. However, a higher proportion of the labelled follicles of ewes in the high BWT group went on growing between 0-24h after prostaglandin injection than that of ewes in the low BWT group. This implies that the increased number of large follicles in the high BWT group ewes was associated with an increase in the proportion of healthy follicles as compared with ewes in the low BWT group. McNeilly et al (1987) also found that high body condition was associated with an increase in the number of follicles ≥4mm in diameter during both the luteal and follicular phases, and a higher proportion of healthy follicles (as assessed by follicular fluid levels of oestradiol). Therefore, the increased number of follicles recruited by ewes in the high BWT group was probably due to an increase in the number of healthy follicles with a diameter of ≥2mm at the time of luteal regression. The reasons for the increase in the

number of healthy large follicles in ovaries of ewes in the high BWT group at the time of luteolysis are not clear. Although FSH has been shown to influence follicle viability (McNatty et al., 1985), ewes in the two BWT groups had similar FSH levels prior to luteolysis.

The mechanisms controlling the numbers of follicles selected for ovulation are not known. It has been assumed that selection of the dominant follicle and atresia of the others is due to decreasing FSH levels at that time (Baird, 1983; Driancourt et al., 1985b). Therefore, the higher the FSH concentration at the time of selection, the higher will be the number of follicles selected for ovulation. However, the present study failed to demonstrate a significant effect of treatment on FSH concentration during the follicular phase. The fact that, although all follicles are exposed to the same circulating hormone levels, only a few of the recruited follicles are protected from atresia, suggests that the mechanisms controlling selection operate at the intraovarian level. Nevertheless, a minimum level of FSH is apparently required to sustain the selected follicles (Zeleznik, 1982; McNeilly, 1984). Two hypotheses have been advanced to explain the mechanisms of selection (Driancourt et al., 1985b). The first is "developmental stability" which suggests that when a follicle has reached a given stage of maturation, it is able to maintain a state of equilibrium in the face of a decreasing FSH level due to its morphological and functional features. The second is "active elimination" which states that selection is an active process by which one follicle at a given stage of maturation actively inhibits the growth of other follicles. If either of these hypotheses is true, then the number of follicles selected for ovulation will be determined by the number of follicles attaining the required stage of maturation. Unfortunately, the mechanisms that permit the growth and development of follicles to the required stage of maturation are unknown. In fact, the mechanisms controlling O.R. in sheep are probably far more complex than proposed by either of these hypotheses because of the interconnected nature of the negative feedback mechanisms controlling gonadotrophin secretion and follicular growth.

As with other studies (Findlay & Cumming, 1976, Rhind et al., 1984, 1986), the present study has failed to demonstrate a significant effect of long-term nutritional treatment on mean FSH concentrations prior to and during the follicular phase of the oestrous cycle. This is in contrast with results from one other study which found that the high O.R. of ewes in good condition was associated with significantly higher FSH concentrations during both the luteal and follicular phases of the oestrous cycle (Rhind & McNeilly, 1986). The reasons for this discrepancy are not clear and more evidence is needed before a firm conclusion can be made.

FSH has long been considered to be involved in the control of O.R. (NcNatty, 1982; Baird, 1983; McNatty et al., 1985). Administration of exogenous FSH has been consistently shown to increase follicle growth and O.R. (Wright et al., 1981; McNatty et al., 1985). However, a number of situations exist in which differences in O.R. are not associated with differences in circulating FSH levels (Cahill et al., 1981; Webb & England, 1982a; Rhind et al., 1984, 1986). In sheep, secretion of FSH is controlled by the negative feedback effects of oestradiol and inhibin, hormones which are mainly secreted by the large healthy follicles in the ovary (Moor et al., 1975; England et al., 1981; Webb & England, 1982a). While neither oestradiol nor inhibin were measured in the present study, available evidence suggests that the secretion of these substances is likely to be proportional to the number of large healthy follicles in the ovary (England et al., 1981; McNatty et al., 1982; Tsonis et al., 1983). Therefore, the production of negative feedback factors from the ovary would likely have been higher in high BWT group ewes than in ewes from the low BWT group. If so, mechanisms must have existed which enabled ewes in the high BWT group to maintain a similar (or even a higher, Rhind & McNeilly, 1986) FSH levels as ewes in the low BWT group, despite the increase in the production of negative feedback factors from the ovary. Several mechanisms are possible: (1) ewes in the high BWT group may have a higher clearance rate for oestradiol and inhibin (Payne et al., 1987); (2) the hypothalamic-pituitary axis of ewes in the high BWT group may have a lower sensitivity to the negative feedback effects of oestradiol and inhibin (Land, 1976;

Imakawa et al., 1984; Webb et al., 1984); and/or (3) ewes in the high BWT group may have a greater ability to secrete FSH in response to a fixed amount of GnRH. The proof of these hypotheses will await future studies.

The present histological study extends our knowledge about the effects of nutritionally-induced bodyweight differences on the antral follicle population in the ovary. One important conclusion from this study is the lack of association between ovulation rate and the total number of follicles $\geq$0.2mm in diameter, their size distribution and rate of atresia. The main features of the ovaries of ewes in the high BWT group were the presence of more healthy follicles of >2mm diameter and of more follicles in the terminal stages of atresia as compared with ewes in the low BWT group.

The lack of a general association between antral follicle populations and O.R. has also been shown in situations where differences in O.R. are due to the effects of breed or steroid immunization (Lahlou-Kassi & Mariana, 1984; Scaramuzzi & Hopkinson, 1984; Driancourt et al., 1985a, 1985c, 1986). The relative independence of ovulation rate from the number of antral follicles has been convincingly demonstrated by experimental reduction of the overall follicle population. Unilateral ovariectomy as late as day 14 of the oestrous cycle does not affect the ovulation rate at the following oestrus (Land, 1973; Findlay & Cumming, 1977). It follows that follicle number, a measure of follicular turnover within the ovary, is not a determinant of ovulation rate during the natural oestrous cycle (Scaramuzzi & Radford, 1983). Rather it is the mechanisms controlling the increase in the number and viability of large antral follicles that are responsible for the differences in ovulation rate.

The ovaries of ewes in the high BWT group had a significantly higher absolute number and proportion of late atretic follicles than those of ewes in the low BWT group. This is consistent with the observation that Booroola Merino ewes have significantly higher numbers of atretic follicles in their ovaries than control Merino ewes (Driancourt et al., 1985a). The importance of this higher number of

late atretic follicles in the control of O.R. is not known and may simply reflect a faster turnover of ovarian follicles with the resultant accumulation of atretic follicles in the late stages of atresia.

The numbers of healthy follicles of 0.5-1 and 1-2mm diameter were significantly ($P<0.05$) and positively associated with bodyweight within treatment groups. The biological significance of this is difficult to explain in the absence of any significant treatment effect on these follicle characteristics. There were also significant correlations between the total number of follicles, the numbers of healthy follicles 0.5-1 and 1-2mm in diameter, and the O.R. of the previous cycle. This may be due to an effect of the number of corpora lutea on the number of growing follicles present on the ovary (Al-Gubory & Martinet, 1987). Alternatively, there may be an inherent correlation between these follicular characteristics and O.R. within individual animals.

One shortcoming of the present histological study of follicle populations is the lack of simultaneous study of follicle growth rate. The number of follicles within any class at a particular time is dependent not only on the number of follicles entering that class but also on the rate at which these follicles pass through that class. Studies have shown that the high O.R. of prolific breeds of ewes, or of ewes subjected to steroid immunization, is not associated with a significant increase in the follicular growth rate when compared with ewes of low prolificacy and non-immunized ewes respectively (Cahill & Mauleon, 1980; Scaramuzzi & Hopkinson, 1984; Driancourt et al., 1985a). However, the situation with nutritionally-induced variation in O.R. may be different. Fibroblast and epidermal growth factors (Gospodarowicz & Bialeck, 1979), insulin-like growth factors (Adashi et al., 1985), and insulin (May & Schomberg, 1981) have been found to promote mitosis in cultured granulosa cells. Since the concentrations of these factors are likely to be influenced by the nutritional status of the animal (Procknor et al., 1985; Breier et al., 1986; Harrison & Randel, 1986), it might be expected that follicle growth rate would be higher for ewes in the high BWT group than for ewes in the low BWT group.

In conclusion, the present trial confirmed that bodyweight difference caused by nutrition had a significant effect on the O.R. of the ewe. This effect of bodyweight on O.R. was associated with changes in the number of large antral follicles recruited into the actively growing growing pool shortly after luteolysis and in the proportion of the recruited follicles that become atretic at the time of selection late in the follicular phase. Few differences in the antral follicle population of the ovary were found between ewes in the high and low bodyweight groups. Although FSH plays many important roles in folliculogenesis, it may not necessarily be involved in the control of nutritionally-induced variation in O.R..

Further studies

It is apparent that more studies are needed to increase our understanding of the physiological mechanisms by which bodyweight influences O.R. in the ewe.

Studies on the changes in ovarian follicle populations and the patterns of preovulatory follicular development will provide information on the underlying physiological mechanisms controlling O.R. because follicles at different stages of development are controlled by different mechanisms. Such studies should include the measurement on follicle growth rate as it is an important factor affecting the number of follicles on the ovary at any particular time.

FSH has been considered to be one of the most important hormones involved in the control of follicular development and possibly the major determinant of O.R. in sheep. However, the present trial failed to demonstrate higher FSH concentrations in ewes of high bodyweights, a result supported by those from other studies (Findlay & Cumming, 1976; Rhind et al., 1984, 1986). Therefore, increase in O.R. in high bodyweight ewes is likely to be associated with increase in the ovarian sensitivity to FSH. Previous studies have demonstrated that the superovulatory response to a standard dose of PMSG is greater

in ewes of high BWT than in ewes of low BWT (Allison, 1975; Rhind et al., 1986). However, PMSG contains both FSH and LH activities and the large dose of PMSG administered may mean that it is the number of healthy large antral follicles present on the ovary at the time of PMSG injection, rather than the follicle sensitivity to FSH, that caused the differential responses. Studies on the ovulatory reponses to low doses of relatively pure FSH preparations should overcome some of these disadvantages. If differences between ewes of high and low bodyweight in follicle sensitivity to FSH were apparent, then studies of dissected follicles or granulosa cells cultured in vitro might allow the mechanisms responsible for these differences to be identified.

The ability of high bodyweight ewes to maintain normal FSH concentrations in the face of large number of antral follicles, and hence high production of ovarian negative feedback factors, may also be important in the regulation of O.R.. Important questions to be addressed are: 1) do the greater numbers of large antral follicles developing in ewes of high bodyweight produce more negative feedback factors as compared with ewes of low bodyweight? 2) are there differences in the circulating levels of these negative feedback substances between ewes of high vs low bodyweight. The outcomes of these studies will determine the directions of further investigations.

If it transpires that the ovaries of ewes of high bodyweight produce more negative feedback factors, but the concentrations of these factors in circulation are not higher than in ewes of low bodyweight, then there must be differences in the clearance rate between ewes of high vs low bodyweight (Payne et al., 1987). Comparison of the clearance rate of oestradiol in ovariectomized ewes of high vs low bodyweights should be able to answer this question.

If ewes of high bodyweight have higher concentrations of ovarian steroids (inhibin) than ewes of low bodyweight, then mechanisms must exist which enable ewes of high bodyweight to maintain their gonadotrophin secretion in the face of a greater production of ovarian feedback substances. Otherwise, ewes which have high production of

negative feedback factors and are sensitive to these factors will have reduced FSH concentrations. This will result in atresia in a greater proportion of antral follicles with the resultant restoration of the normal FSH concentration. Such mechanisms would involve: 1) reduced sensitivity of the hypothalamo-pituitary axis to the negative feedback effects of ovarian steroids and inhibin, and 2) increased pituitary sensitivity to GnRH. The first hypothesis could be tested by comparing the gonadotrophin levels in ovariectomized ewes treated with exogenous oestradiol or inhibin preparations. The test of the second hypothesis is difficult, not only because it is confounded with the first hypothesis, but also because the amount of gonadotrophins released in response to GnRH is dependent on both the pituitary sensitivity to GnRH and the amount of releasable gonadotrophins stored in the pituitary gland. Studies combining measurement of pituitary gonadotrophin content and the pituitary responsiveness to GnRH infusion, and studies using isolated pituitary cells, may help to clarify this point.

Finally, studies are needed to determine how the nutritional status of the ewe effects changes in the reproductive system, particularly with respect to the direct roles of specific nutrients. Since dietary constituents are largely altered before becoming available to the animal, direct infusion of specific metabolites (particularly glucose, propionate and protein) into the circulation should be used. The administration of metabolic hormones such as insulin, preferably in physiological rather than pharmacological (Jones et al., 1983; Harrison & Randel, 1986) doses, may also help to elucidate the mechanisms by which nutritional status affects O.R..

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