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THE EFFECTS OF UNDERNUTRITION ON REPRODUCTION IN GOATS

by

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ABSTRACT

It has been generally accepted that nutrition is one of the most important factors that affects reproductive performance in livestock. The impact of feed-restriction has however rarely been investigated in the goat because of the relative unimportance of the species in the developed countries, and the common assumption of its similarity to sheep and cattle. The recent increase in demand for high quality fibre in the developed countries and for animal protein in the Third World has however awakened interest in the species. The aim of this project was to provide information on the effects of undernutrition on the oestrous cycle and on pregnancy in goats.

Restriction of feed intake to only 25% of maintenance requirement from 19 days prior to oestrus reduced the number of goats that exhibited oestrus following synchronization with intravaginally inserted progestagen-impregnated sponges, reduced ovulation rate and the incidence of twin and triple ovulations and reduced the number of goats that became pregnant. A trial which examined superovulation of goats with porcine FSH suggested no effect of undernutrition on the superovulatory response and early development of goat embryo.

Feed restriction for 19 days before and 60 days after mating reduced the size and weight of foetuses and the mass of foetal fluids and tended to reduce the weight of the cotyledonary component of the placenta without affecting the number of placentomes. While length of gestation had a significant influence on foetal and placental measurements, number of foetuses had no effect. Transfer of healthy embryos to does on full or restricted rations before and/or after embryo transfer resulted in poor pregnancy rates and significant reduction in the survival of the transferred embryos irrespective of the timing of feed restriction.

In order to investigate the mediation of the observed effects of undernutrition on reproduction, plasma samples were collected at frequent intervals during the luteal, follicular and preovulatory phases of the oestrous cycle and analysed for LH and FSH concentrations. There was no significant effect of undernutrition on basal LH and FSH profiles. Study of the preovulatory surge of gonadotrophins following sponge removal and injection of $PGF_{2\alpha}$ revealed a decrease in the number of goats exhibiting a surge in LH and FSH concentrations, reduced magnitude of the surge and reduced incidence of ovulation in feed-restricted goats. Further investigation of pituitary function by injection of a large dose of GnRH demonstrated that the magnitude of the preovulatory surge of gonadotrophins was less in does on restricted feeding than in does on adequate nutrition.

In order to investigate the mechanisms by which undernutrition reduced pregnancy rates, embryo survival and growth of foetuses, plasma progesterone concentrations were studied during the 10 days immediately after oestrus and between Days 51 and 60 of gestation. Plasma progesterone concentrations were not affected by undernutrition in the first 10 days after oestrus and mating but were increased in the period between 51 and 60 days of gestation. Investigation of follicular populations in dissected ovaries and luteal sections observed under light and electron transmission microscopes and assayed for LH and prolactin binding to cells of the CL indicated that undernutrition tended to reduce the population of small follicles but had no significant effects on the luteal cell morphology and on LH and prolactin binding of the corpus luteum.

In conclusion, these results demonstrated that undernutrition delayed or suppressed the onset of oestrus, reduced ovulation and pregnancy rates and reduced foetal growth and survival in goats. The mechanisms through which these effects were mediated are complex and appear to involve both changes in the levels of

gonadotrophin, in the sensitivity of target organs to gonadotrophins and most probably in other factors produced locally in the target organs, or elsewhere, that modulate the effects of gonadotrophins or the response of the target organs.

DECLARATION

This is to declare that this thesis has been prepared by myself and has not been accepted in any previous application for a degree. The work of which it is a record has been done by myself and all sources of information have been specifically referenced. All help given by other people is indicated in the acknowledgements.

PUBLICATIONS ARISING FROM THE THESIS

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GENERAL INTRODUCTION

The goat, Capra hircus, was one of the first animals to be domesticated by man. Remains have been found in deposits that are 5 million years old while signs of domestication of the species have been found in excavations of Neolithic sites at Jericho dating from 7,000 B.C. Goats contribute to world agriculture through the provision of meat, milk, fibre and skin. The goat's adaptability to a wide variety of vegetation and climatic conditions and its ability to utilize poor quality roughage makes the species unique for efficient production in areas where other domestic ruminants cannot thrive adequately. Its low unit value, small individual size, ability to largely fend and forage for itself and ability to continue producing meat through drought periods and recover very rapidly afterwards has made the goat increasingly more acceptable to the vast majority of small family units in rural areas of the developing countries (Wilson, 1982). About 75% of the world's goat population of 470 million is concentrated in these developing countries with over two-thirds within 30^{o} of the equator (Shelton, 1978).

In the developed world however, the goat has not been appreciated or valued as a domestic animal. The relative unimportance of the species in the developed world has led to little investigative work being performed on breeding and the improvement of the species. There are very few breeds of goat that, in production or usefulness, are comparable to the most refined breeds within the other species of domesticated animals. Despite the suitable size of the goat, sheep are largely preferred for most experimental purposes.

In most text books and other publications sheep and goats are discussed as a single entity. Despite similarities to sheep however, goats exhibit significant differences in habits, physical and physiological activities, feed and water requirements,

composition of body and body products, metabolic disorders and parasites. One major difference in reproduction between the goat and the sheep is that the goat's placenta secretes large quantities of pregnanediol while in the sheep's placenta, progesterone is the major steroid produced (Heap and Flint, 1984). The outcome is that the corpus luteum in the sheep becomes no longer the most important source of progesterone and can be dispensed with by Day 50 of pregnancy without causing abortion, whereas the corpus luteum of the goat is essential throughout pregnancy.

Reproduction is a major contributing factor to efficiency of meat, milk and fibre production in domestic animals. Understanding of factors affecting reproduction is therefore essential for improving the crop of new-born and for good herd management.

It is generally accepted that nutrition is one of the most important factors affecting the reproductive performance of animals. Nutrient limitations which prevent animals from attaining their genetic potential may result from inadequate feed intake or from low quality of the diet. This may occur as a result of reduced annual nutritional provision or, more particularly, due to seasonal fluctuations in nutritional availability. Energy requirement of goats has been reported to be affected by age, body size, growth, pregnancy and lactation, hair growth, muscular activity, environmental temperature, humidity, sunshine, wind velocity, stress of any kind and shearing. A continuous source of protein in the diet is needed for cell repair and synthesis of secretions such as enzymes, hormones, mucin and milk. Below a minimum level of crude protein in the diets of goats, feed intake will be reduced, which leads to a combined deficiency of energy and protein. This deficiency further reduces rumen function and lowers efficiency of feed utilization. Mineral and vitamin requirements have not been established definitively for goats at either maintenance or production levels but are generally assumed to be similar to those in other ruminant species.

Several studies have observed that undernutrition, particularly resulting from seasonal variation in nutrient availability, has a detrimental effect on the time of onset of oestrus (Epstein and Hertz, 1964; Singh and Singh, 1974; Shelton, 1978; Smith, 1988) and on kidding rates (Singh and Sengar, 1970; Sachdeva *et al.*, 1983) in goats. Data on the specific effects of undernutrition on the different reproductive events such as folliculogenesis, ovulation, fertilization, embryo wastage, pregnancy, foetal growth and development are however lacking. Understanding of the effects of undernutrition on these events is important in order to maximize feed resources and improve kidding rate and postnatal kid survival.

The increased demand for animal protein in the third world, and for high quality fibre in the developed countries has led to increased effort at genetic improvement of goat breeds through breeding programmes. It has been noted that where nutrition is the first limiting factor, genetic improvement alone is less likely to result in real increase in productivity (Doney *et al.*, 1982). Assessment of the effects of nutritional factors on reproduction is therefore essential in any programme of planned improvement.

Objectives of the study

The objective of this study was to provide information on the effect of undernutrition on the oestrous cycle and on pregnancy in goats. Specifically, we studied the effect of undernutrition on clinical and hormonal measurements throughout the oestrous cycle with specific reference to ovulation rate, luteal function and embryo survival.

CHAPTER 1

Literature review

1.1 Reproduction in the goat

Introduction

This chapter reviews the reproductive events in the female goat and the factors that influence them. Emphasis has been given to the influence of nutrition on the oestrous cycle and early pregnancy. References have been made to sheep where relevant, and in areas where information is not available for the goat. Because of the diversity of breeds of the goat it is not possible to specifically discuss these events within each breed. Typical parameters are therefore discussed.

1.1.1 Onset of puberty

The gonads of all mammals of both sexes are quiescent for a period that starts just after birth. This period ends with the rapid growth of the gonads, development of the secondary sexual characteristics and production of spermatozoa in the male and sexual cycles in the female. Puberty refers to the time at which these changes have progressed to the point that reproduction is possible (Ganong, 1991). The quiescent period has survival value in that it delays the onset of reproduction until the parents are better able to care for the young. Animals that conceive early in their first breeding season have a greater probability of weaning more, and heavier offspring during their life-time (Lesmeister et al. 1973).

Most breeds of goats reach puberty between 8 and 10 months of age (Sands and Dowell, 1978; Shelton, 1978). There are however considerable differences between breeds. It has been reported that Pygmy goats may reach puberty as early as 3 months (Rogers *et al.,* 1969). The presence and teasing activity of the buck have also been reported to modify age at puberty (Shelton, 1977).

Work in sheep provided evidence that the mechanism holding puberty in check resides in the central nervous system (Foster, *et al.,* 1986). It appears most likely to be by a neural mechanism that keeps the gonadotrophin releasing hormone (GnRH) pulse generator quiescent resulting in little or no stimulation of the release of gonadotrophins from the anterior pituitary until just before puberty (Imakawa, *et al.,* 1986; Ganong, 1991). The mechanism of this inhibition remains to be fully determined. However, it has been shown in pre-pubertal and anoestrous females that oestradiol, from growing follicles exerts a negative feedback on the hypothalamus and pituitary, which in turn inhibits the release of pituitary gonadotrophins. Puberty is therefore believed to be modulated through changes in the hypothalamus inhibition (Imakawa, *et al.,* 1986; Kinder, *et al.,* 1987) in a gradual and interactive process involving maturation of the hypothalamo-pituitary-gonadal axis (Pineda, 1983). During the peri-pubertal period, responsiveness to negative feedback effects of oestrogen decreases, and secretion of gonadotrophins increases so that ovarian follicular growth is stimulated. Oestradiol secretion by the follicles is then enhanced and in turn the pre-ovulatory surge of gonadotrophins leads to the final growth, maturation and rupture of the follicles.

Oestrogen is the hormonal stimulus for oestrus in both sheep and goats (Lindsay, 1991). In the sheep, it has been shown that a period of 6-8 days of progesterone priming is essential for the ovary to be fully sensitized to oestrogen (Moran *et al.,* 1989). Having had no corpus luteum and hence progesterone from a previous ovulation, the first ovulation at puberty and at the beginning of the breeding

season or after a short cycle in ewes is generally not accompanied by behavioural oestrus (Robinson, 1954). Progesterone is therefore needed for the initiation of regular cyclic activity.

In goats, the blocking effect of oestradiol on the maturing hypothalamus has not been conclusively determined. It has been reported that, in contrast to ewes, does do not require a long period of progesterone priming prior to oestradiol in order to exhibit oestrus (Chemineau, 1983). It has been observed however, that both pubertal and adult goats at the beginning of the breeding season display a short cycle of about 8 days. This is due to a short luteal phase lasting only 5-6 days, followed by a second cycle which is usually of normal length (Pineda, 1983). This suggests that the hypothalamic-pituitary-gonadal axis in the goat also requires progesterone for the initiation of regular cyclic activity.

1.1.2 The oestrous cycle

Seasonality of oestrous behaviour

The goat shows a seasonal cycle of reproductive activity depending on photoperiodicity, nutrition, social interactions or a combination of these factors. Thus, in temperate zones most breeds of goats behave as short daylight breeders with a clear anoestrous period (Gill and Dev, 1972; Corteel, 1975 & 1977; Shelton, 1978). By contrast, in the tropics and the subtropics there is an extended period of breeding including continuous year-round sexual activity (Sahni and Roy, 1967). In between these two extremes are breeds that show elements of photoresponsiveness that may be overridden or intensified by other cues such as buck effect and nutrition or by a combination of any of these factors (Lindsay, 1991). The occurrence of seasonal anoestrus, in breeds that are subject to high photoperiodicity such as the Angora, may

delay exhibition of sexual maturity by doe kids with the result that goats do not show sexual activity until their second year.

Seasonality of breeding activity probably evolved in regions where climate and nutrition change throughout the year, in order to ensure that offspring are born at the time most favourable for survival. High environmental temperatures have been reported to influence reproductive performance particularly in temperate-breeds of goat (Jainudeen and Hafez 1987). Suckling may also delay the resumption of ovarian activity in goats (Jainudeen and Hafez 1987).

The breeding season in animals is characterised by the capacity of the females to regulate the pulsatile release of LH at frequencies and amplitudes appropriate to bring about the luteal and follicular phases of the oestrous cycle (Lindsay, 1991). By contrast, anoestrus is characterised by regular but infrequent pulses of LH (Martensz *et al.*, 1979). Sensitivity of the pulse-generating mechanism in the brain to the action of oestradiol is the key to the mechanism of this switch in does as in ewes. It has been observed in mature Saanen goats that, as the first ovulation of the breeding season approaches, LH pulse frequency progressively increases by 67% and mean levels by 47% (Chemineau *et al.*, 1988). LH pulse frequency was found to be 54% lower during the anoestrous season than during the breeding season in ovariectomized oestradiol-treated goats. Mean LH concentration was affected in a similar manner (Chemineau *et al.*, 1988). For the pulse-generating mechanism to increase and sustain the frequency of tonic release of LH, even in the face of increased production of oestradiol from the ovary, it has to overcome its sensitivity to oestradiol sufficient to evoke the first ovulation of the breeding season. It is thought that the effect of short days (Karsh *et al.*, 1984), the male effect, and possibly improved nutrition (Scaramuzzi and Radford, 1983) renders the mechanism less sensitive to oestradiol thereby allowing it to increase the frequency of tonic releases of LH. Conversely, the effect of lengthening days, poor

nutrition, or other external factors increases the sensitivity to oestradiol and the animal becomes anoestrus.

Length of oestrous cycle

Reports on the length of the oestrous cycle in the goat are numerous. The majority of cycles are 19-21 days in length (Camp *et al.*, 1983; Pathiraja, *et al.*, 1991). The oestrous cycle in goats has been reported to be significantly shorter during periods of the year with moderate climatic conditions compared to extremely cool dry and hot wet periods (Prasad and Bhattacharyya, 1979). Multiparous females have also been reported to exhibit shorter oestrous cycles than those which are primiparous or biparous (Riera, 1982). The incidence of short cycles (less than 9 days) is relatively common in goats and usually occurs at the beginning of the breeding season and during the post-partum period (Riera, 1982; Camp, *et al.*, 1983; Chemineau, 1983). Short cycles of less than 17 days may however occur during the breeding season particularly when does are continuously exposed to bucks (Pineda, 1983; Camp *et al.*, 1983). There is little evidence of prooestrus or metoestrus in goats and sheep. From external observation the oestrous cycle can therefore be divided into the period of oestrus and the interoestrous, or dioestrous period. At the ovarian level the cycle can be divided into the follicular phase covering the 2-3 days in which ovulatory follicles grow and release their ova, and the luteal phase characterised by the presence of one or more corpora lutea on the ovaries.

Duration of oestrus

The duration of oestrus in goats is about 24-36 hours, but considerable variation associated with breeds occurs (Devendra and Burns, 1970). The duration of oestrous behaviour may be shorter in young maiden than in older multiparous ewes and

does. Environmental stress factors may shorten the duration (Doney and Gunn, 1981) and may also extend the interval between the ovulatory LH surge and ovulation (Cumming, 1976). Oestrus may occur at any time during the day (Sahni and Roy 1967). Behavioural and physical signs of receptivity and oestrus are much more pronounced in female goats than in sheep. The hormonal stimulus for oestrus in both goats and sheep is oestradiol. Progesterone priming is however essential for the hypothalamo-pituitary gonadal axis to be fully sensitive to oestradiol. This period of priming is probably shorter in goats than in sheep (Chemineau, 1983).

Postpartum Anoestrus

In goats, the time taken for uterine involution and the interval to the first postpartum ovulation have not been fully established. In sheep, uterine involution takes about 27 days and the time to first ovulation is 20 days or less (Jainudeen and Hafez, 1987). The postpartum interval to the first oestrus in ewes has been reported to be influenced by season, breed, ram contact and possibly lactation (Hunter, 1968). Some reports have shown that inadequate nutrition and poor body condition before the beginning of the postpartum period result in delayed oestrus and ovulation without behavioural oestrus. These effects are accentuated in heavily lactating animals and in primiparous, relative to multiparous, animals (Rattray, 1977). There are some studies however, that demonstrate little or no effect of nutritional restriction on the onset of breeding (Ducker and Boyd, 1974; Dufour and Wolynetz, 1977). The mechanisms involved in the onset of breeding activity after kidding are believed to be similar to those of puberty, and following seasonal anoestrus (Ferrel, 1991).

1.1.3 Folliculogenesis

Reports on the pattern of follicular growth and atresia occurring in the ovaries of sheep during the oestrous cycle have been published (Turnbull *et al.*, 1977, Dufour *et al.*, 1979, Cahill and Mauléon 1980). There is a continuous process of new follicles commencing growth from about Day 20 of foetal life. Only very few of these remain healthy and eventually produce ova. The majority die or undergo atresia at some stage of their development before they reach the point of ovulation (Peters *et al.*, 1975). Folliculogenesis in sheep takes about 6 months from the recruitment of an oocyte from the primordial population to final ovulation. Most of this time is spent in the pre-antral stage during which time there is little atresia. When the follicles enter the antral phase, development is more rapid but atresia is more frequent. It was estimated that approximately 3 follicles per day enter the antral phase and at any stage in the ovary of a mature female there are 5-24 antral follicles though the majority will become atretic (Turnbull *et al.*, 1977). The physiological need for this is unclear.

In attempts to understand the control of folliculogenesis in animals early studies, using histological examination of ovaries obtained from cows slaughtered on predetermined dates of the oestrous cycle, concluded that follicles grow throughout the oestrous cycle resulting in the development of a dominant preovulatory follicle (Hammond, 1927). Later studies in sheep and cows proposed two waves of follicles development following observations of second large dominant follicles towards oestrus (Rajakoski, 1960; Hutchison and Robertson, 1966; Smeaton and Robertson, 1971; Brand and De Jong, 1973; Ireland *et al.*, 1979). Three waves of follicular development rather than two were reported in a few studies in sheep and cows (Smeaton and Robertson, 1971; Ireland and Roche, 1987). Other studies however, detected no such wave pattern of follicular development in sheep and cows and concluded that the rate of follicular growth is independent of the stage of the oestrous cycle and the patterns

observed earlier were due to follicles growing and regressing asynchronously during the luteal phase (Donaldson and Hansel, 1968; Turnbull *et al.*, 1977). Detailed information on the cyclic changes occurring in the ovaries of the goat is very sketchy. In a study on Angora goats, Pretorius (1971) reported that ovarian follicular volume increased sharply during the first part of the luteal period (day one to day 10). This was followed by another short but intense follicle growth wave towards oestrus, which terminated in ovulation, thus supporting the two-wave pattern of ovarian follicular development. The largest number of follicles was recorded just prior to and following the onset of oestrus. Mean follicular diameter was also higher around the time of oestrus compared to the rest of the dioestrous period. The incidence of large and medium sized follicles varied with the stage of the cycle but never exceeded 15% of the total number of the ovarian follicles. The development of real-time ultrasound has made it possible to successfully monitor the growth patterns of individual follicles on a daily basis. Studies using this technique in cows have confirmed that follicular growth and development occur in waves during the oestrous cycle rather than being continuous and independent of the stage of the cycle (Savio *et al.*, 1988; Knopf *et al.*, 1989).

What initiates follicular growth in animals is not yet fully understood. Poor vascularization of the pre-antral and early antral follicles probably makes them dependent on locally produced growth factors like EGF and FGF rather than on endocrine factors like FSH. Mariana *et al.* (1991) suggested the differentiation of follicular cells to be a multi-step and multi-regulated process as follows: Granulosa cells replicate actively in these follicles and steroidogenesis is low. However, as the follicles become larger and well vascularized dependency on gonadotrophins becomes greater. Small and medium sized follicles become highly sensitive to FSH which acts by increasing steroidogenesis and various synthetic processes in the follicular cells. FSH is essential in aromatization of oestradiol from androgen precursors. In the absence of

FSH, androgens produced by theca cells under the influence of LH will hasten atresia (Mariana *et al.*, 1991). The oocyte in this phase is maintained in a meiotically-arrested stage. Increased levels of oestradiol and probably IGF-1 enhance the stimulatory effects of FSH. Granulosa cells replicate less actively, lose their sensitivity to growth factors and become differentiated. In several domestic animals, one or a few follicles develop further and dominate the others which become atretic. In the ewe, the dominant follicle or follicles are believed to secrete large quantities of oestradiol and inhibin which cause a negative feed-back on the pituitary leading to a modest fall in the plasma concentration of FSH about 48 hours prior to the preovulatory LH surge (Baird, *et al.*, 1981). The dominant follicle then deprives the others of this limited FSH probably by taking up most of the hormone, or by the production of local inhibiting factors that restricts the growth of adjoining follicles (Cahill, 1984). This leads to the granulosa cells of the other follicles becoming incapable of converting androgens to oestradiol, and their demise is accelerated (Baird, *et al.*, 1975; Baird and McNeilly, 1981). Follicles destined to ovulate are probably protected from the deleterious effects of low FSH levels by their high intra-follicular concentration of the hormone. In sheep however, the similarities in the ovulatory responses of ovaries to PMSG administration before and after the emergence of the dominant follicle in the follicular phase, and in the presence or absence of a large healthy or atretic follicle during the luteal phase (Driancourt *et al.*, 1991) suggest that follicular dominance is probably not operative or that it is essentially passive and can easily be overcome by raising gonadotrophin concentration artificially in this species.

The process of follicular dominance in goats has not been investigated. It has however been observed in does that both the largest and the second largest follicles grow rapidly in size during the early luteal phase of the oestrous cycle. However, from day 12 of the cycle until early oestrus of the next cycle a slight but continuous decrease in size of the second largest follicle occurred (Pretorius, 1971), suggesting some form

of dominance. In sheep, the size of the second largest healthy follicle after the ovulatory follicle has emerged never exceeds 2.5-2.7 mm. (Brand and de Jong, 1973; Driancourt *et al.*, 1989).

The final development and maturation of the follicles is probably dependent on the action of LH on the highly vascularized follicles. It had been suggested that FSH and oestradiol together are needed for the production of LH receptors in the granulosa cells of the follicle. Without a good population of LH receptors, the follicle can not respond to the preovulatory surge of LH by ovulating (Richard, 1979). It has been shown in cows and ewes, that large antral follicles, healthy or atretic, have functional LH receptors on their theca cells. This has been demonstrated by their ability to ovulate following human chorionic gonadotrophin administration (Driancourt *et al.*, 1989). In contrast, oestradiol content, or the ability of the follicles to produce oestradiol is blunted during anoestrus (McNatty *et al.*, 1984) or before puberty (Sonjaya and Driancourt, 1989). This indicates that active oestradiol production during terminal follicular growth is a prerequisite for growth or acquisition of LH receptors. Increased secretion of oestradiol by the antral follicle is believed to finally result in the initiation of the preovulatory LH surge.

1.1.4 Ovulation and ovulation rate

The doe, like the ewe is a spontaneous ovulator and capable of multiple ovulations at oestrus. The time of ovulation in relation to the onset of oestrus in goats varies particularly with breed. Most breeds ovulate between 24 and 48 hours after the onset of oestrus (Harrison, 1948; Salama, 1972; Gonzalez, 1977; Rao and Bhattacharyya, 1980). The Nubian goat however ovulates later probably due to the longer oestrous cycle characteristic of this breed (Jainudeen and Hafez, 1987). A number of studies in goats have reported the right ovary to be generally more active

than the left (Taneja, 1959; Lyngset, 1971;). It has also been reported that in cases of multiple ovulations there appears to be a greater tendency for the first ovulation to occur in the right ovary (Lyngset, 1971).

Ovulation rate in goats is generally high with an average of 2 to 3 follicles ovulating at each oestrus (Pineda, 1989). The ovulation rate may however vary, particularly with breed and management conditions, from 1-4 follicles (Lyngset, 1968a; Bhattacharyya and Prasad, 1974; Rao and Bhattacharyya, 1980; Pineda, 1983). Ovulation rate increases with age and reaches a maximum at 3-6 years, then declines gradually. Higher ovulation rates have been reported earlier rather than later in the breeding season.

Both oocyte maturation and follicle rupture are believed to be triggered by the preovulatory surge of LH. Immediately following this surge, the follicles become hyperaemic and subsequently oedematous. It has been postulated (Lipner, 1988) that protein synthesis initiated by LH may be responsible for cellular differentiation of the membrana granulosa to lutein cells and secretion of steroids and plasminogen activator. Activation of adenylate cyclase may initiate other responses including prostaglandin secretion. The theca interna may also be stimulated by LH to increase the secretion of progesterone and androgens as well as prostaglandins and plasminogen activator. The adrenergic neurons in the follicle wall may be activated either by LH or neurologically and secrete norepinephrine. Histamine released from mast cells and the alpha-adrenergic agonist may act to enhance hyperaemia by affecting the contractility of the endothelial cells, the pericytes, and the post-capillary venules. Plasminogen activator converts plasminogen in the follicular fluid and extracellular oedema fluid to plasmin that acts on collagen. Induced collagenolysis and serine proteases then complete the proteolysis of the collagen. The tensile strength of the follicular wall is then decreased to the point at which rupture occurs because of the existing intrafollicular pressure.

The physiological basis of variation within and between breeds in the number of ova shed is not yet understood. Studies in sheep have revealed an inverse relationship between the population of primordial follicles and the subsequent ovulation rate and a positive correlation between the number of non-atretic antral follicles and ovulation rate (Cahill *et al.*, 1979). Although it is apparent that late development of follicles is under the control of the hypophyseal hormones, few significant relationships between plasma hormone concentrations and ovulation rate have been established. Studies in sheep (Findlay and Cumming, 1976; Bindon *et al.*, 1979) have failed to reveal any difference in the level of FSH that could explain the observed difference in ovulation rate between and within breeds. Pituitary and plasma FSH concentrations were found to be higher in Booroola and Romanov ewes which had a high ovulation rate (3-5) than in controls (ovulation rate 1-2) (Cahill *et al.*, 1981; McNatty, *et al.*, 1987). However no such difference was observed between Finn (ovulation rate 2-4) and Suffolk ewes (ovulation rate 1-2) (Wheaton *et al.*, 1988). In goats, no positive relationship was found between the magnitude of LH surge and the corresponding number of ovulations (Pelletier et al, 1982).

1.1.5 The corpus luteum and luteolysis

Luteinization and the formation of the corpus luteum (CL) are a natural extension of follicular growth and ovulation initiated by dramatic increases in serum levels of LH associated with the preovulatory surge of the hormone (Niswender *et al.*, 1988). Luteinization is a series of morphological and biochemical changes in the cells of the theca interna and membrana granulosa of the preovulatory follicle. Two distinct types of steroidal cells are readily distinguished in the CL of most species. These are called the large luteal cells, also referred to as granulosa-lutein, comprising about 4.2% of the total cells of the CL and about 25% to 35% of the volume in sheep and the small luteal cells, or theca-lutein comprising about 18.5% of total cells and 12% to 18% of

the volume (Rodgers *et al.,* 1984). The 2 cell types appear to be derived from at least 2 different types of follicular steroid-secreting cells. It has been suggested that large luteal cells in sheep are of follicular granulosa cell origin, while small luteal cells derive from follicular thecal cells (O'Shea *et al.,* 1979b; O'Shea *et al.,* 1984). It has also been proposed that small luteal cells can transform into large luteal cells following observations that the ratio of small to large dissociated luteal cells declined during the life span of ovine CL (Fitz *et al.,* 1981), and following reports of cells intermediate between large and small lutea cells on ultrastructural studies (Priedkalns and Weber, 1968). Fibrocytes, endothelial cells and pericytes, vascular laminae comprise the remaining cells of the CL (Rodgers *et al.,* 1984).

In domestic animals, LH is the important luteotropin. This has been shown in goats by the demonstration that hypophysectomy of goats at any time during pregnancy results in the demise of the corpus luteum and abortion (Hansel, 1988; Heap *et al.,* 1988). Both in pregnant and non-pregnant animals the CL is maintained by a relatively slow pulsatile pattern of LH release (one pulse in 2-3 hours) (Heap *et al.,* 1988). The role of prolactin in the maintenance of the CL in domestic animals is not very clear. Although there is evidence of its importance in the maintenance of the CL in hypophysectomized does (Buttle, 1978) and ewes (Niswender *et al.,* 1988), confirming data in intact animals are not available.

The major primary endocrine secretion of the CL is progesterone which is required for the maintenance of pregnancy. On a per cell basis, the large luteal cell secretes 4 times more progesterone than the small luteal cell (Fitz *et al.,* 1982; Rodgers *et al.,* 1983). The regulation of progesterone secretion by the CL is complex. Progesterone secretion by the small luteal cells is regulated by LH, the action of which is mediated through specific receptors residing in the plasma membrane of the cell (Fitz *et al.,* 1982; Hoyer and Niswender, 1985). In contrast, LH receptors are barely

detectable in the large luteal cells and progesterone secretion by the large cells is apparently independent of LH regulation (Fitz *et al.,* 1982; Hoyer and Niswender, 1985).

The CL in goats increases rapidly in size and weight during the first 12 days of the luteal period (Pretorius, 1971). Regression of the CL begins at Day 12 such that by the time the next oestrus begins the CL has decreased about 35% in size and 76% in weight (Pretorius, 1971). Central cavities ranging from 1.9 to 8.9 mm. (Pretorius, 1971) have been observed in the CL of normally cycling does without any associated reproductive disorders.

Luteolysis

Prostaglandin (PG) $F_{2\alpha}$ produced by the uterus is the luteolytic agent in ruminants. In goats, $PGF_{2\alpha}$ concentrations have been observed to show a pulsatile pattern with peaks increasing markedly as progesterone levels fell and oestrus approached (Homeida and Cooke, 1982; Homeida and Khalafalla, 1990). Active immunization against oxytocin during Days 10 to 21 of the oestrous cycle (Cooke and Homeida, 1985) or the administration of an oxytocin antagonist between Days 12 and 18 of the cycle were shown to block the release of $PGF_{2\alpha}$ and prolong the oestrous cycle in goats confirming that endogenous oxytocin has a facilitatory role in luteolysis via prostaglandin production. It was suggested that oxytocin-induced oestrus may also occur via testosterone secretion (Cooke and Homeida, 1985). What initiates $PGF_{2\alpha}$ synthesis that leads to luteolysis is not well understood. One possible explanation is that oestrogen from antral follicles causes the initial synthesis and release of $PGF_{2\alpha}$ (Beard and Lamming, 1992; Cooke and Ahmad, 1992). It is thought that in sheep this initial synthesis and release of $PGF_{2\alpha}$ affects the corpus luteum to cause a reduction in progesterone production and the release of luteal oxytocin. Oxytocin then interacts

with receptors in the uterus to initiate another round of $PGF_{2\alpha}$ synthesis (Fairclough *et al.,* 1984). $PGF_{2\alpha}$ synthesis has been shown to cease within 6-12 hours after progesterone concentrations have become basal (i.e. with the completion of luteolysis).

Premature regression of the CL and hence early return to oestrus is relatively common in goats and usually occurs at the beginning or end of the breeding season (Armstrong *et al.,* 1983a; Pendleton *et al.,* 1992) particularly following the teasing activity of a buck and in the period postpartum (Corteel, 1977). Premature luteal regression or failure of luteal development has been reported more frequently following superovulation of does with pregnant mare serum gonadotrophin (PMSG) than with FSH (Armstrong *et al.,* 1983a; Pendleton *et al.,* 1992). The cause of this phenomenon is not fully understood but is believed to be caused by the premature release of $PGF_{2\alpha}$ (Battye *et al.,* 1988) or due to failure of ovulation of a large follicle in response to the preovulatory surge of LH (Pendleton *et al.,* 1992). Oestrogen from the large unovulated follicle could probably have risen sufficiently to induce a second oestrus in the presence of low levels of progesterone. It has been suggested that premature regression of the CL may be prevented by the use of specific prostaglandin inhibitors to suppress the release of $PGF_{2\alpha}$ and thereby prolong luteal activity (Battye *et al.,* 1988). The reinsertion of progesterone implants into the vagina of superovulated donors at the time at which the natural increase in progesterone concentration would normally occur on Days 4-5 of the cycle has been suggested as another approach of providing an exogenous source of progesterone for the uterus. This may aid the retention of embryos within the uterine lumen until collection for embryo transfer (McKelvey, 1990).

1.1.6 Fertilization, embryo survival and development

Transport and survival of gametes

In domestic animals, freshly ovulated oocytes are picked up by the infundibulum and directed rapidly down the oviduct to the site of fertilization in the ampulla. The primary mechanism for this transport is believed to be by the action of ciliated cells lining the fimbriae which helps to direct the oocytes in their associated cumulus mass of cells into the oviduct. The passage of the oocyte into the fimbriae is facilitated by the distension of the fimbriated infundibulum which fills with blood and becomes contractile, surrounding the ovary. Peristaltic and segmental muscular contractions may also be involved in the transport of the ovum down the oviduct. Hormonal mechanisms involving the oestrogen-progesterone ratio are believed to partly coordinate the contractile activity of both the fimbriae and the smooth muscles in the wall of the oviduct which facilitates the transport of the ovum. It is not known for how long the fertilized ovum in goats remains above the isthmoampullary junction. However, entry into the uterus takes place in 3-4 days after ovulation.

Fertilization and early embryonic development

Fertilization is usually highly successful in domestic animals and is seldom a significant source of reproductive loss once the basic conditions of normality of the spermatozoa and ova and the presence of both in the ampulla at exactly the same time have been met (Kelly, 1984). Fertilization of one ovum seems to be invariably accompanied by fertilization of the others following multiple ovulations. There is no information on the length of time that goat ova remain fertile in the female tract after ovulation. Reports of the fertilizable life of the ova of the ewe range from 10 to 48 hours after ovulation (Jainudeen and Hafez, 1987; Anderson, 1991). Penetration of the

ovum by the sperm cell results in the erection of a barrier to prevent fertilization by more than one sperm cell. It also stimulates resumption and completion of meiosis and activates the ovum to initiate cleavage.

The rate of cleavage of the fertilized ovum varies among species. In goats, the fertilized ovum develops into 2 cells in 0-1 day after ovulation, 4 cells in 1-2 days, 8 cells in 2-3 days, early morula in 2-4 days, compact morula in 4-5 days, early blastocyst in 5-6 days, blastocyst in 6-7 days, expanded blastocyst in 7-8 days and hatching blastocyst in 7-9 days. (Betteridge, 1977; Harper, 1988). Cell divisions during cleavage are nearly synchronous. The dividing cells, also referred to as blastomeres become progressively smaller and equal in size, but with no net increase in the size of the embryo.

Uterine environment

Prior to blastulation the embryo appears to develop independent of its uterine environment (Heap *et al.,* 1979). However, for further embryonic development to proceed normally the uterine environment has to be adequately prepared to receive a blastocyst of a corresponding age. It has been shown in ewes that the uterine environment is under the influence of progesterone secreted in the luteal phase of the oestrous cycle preceding mating, and of oestrogen secreted immediately before, and during oestrus (Miller *et al.,* 1977). It has been suggested that oestradiol secreted at oestrus regulates endometrial sensitivity to subsequent luteal progesterone through its influence on the progesterone receptor population in the uterus of sheep (Miller *et al.,* 1977; Murphy *et al.,* 1977).

Maternal recognition of pregnancy

Recognition of pregnancy in the goat is believed to occur between Days 15 and 17 of pregnancy following the observation that surgical removal of the conceptus on Day 17 and at times thereafter resulted in prolongation of interoestrous intervals, whereas removal on Days 13 and 15 had no effect (Gnatek, *et al.*, 1989). For pregnancy to be established in domestic animals, the presence of the embryo in the uterus must be recognized by the mother and translated into prolongation of the active life of the CL and its continued secretion of progesterone. Oestrogen synthesis by the embryo is one way the endometrium may be informed of the presence of an embryo. Theoretically the life of the CL can be prolonged either by blocking the synthesis and release of endometrial $PGF_{2\alpha}$, the major luteolytic factor, or through the synthesis of compounds that protect the CL from its luteolytic activity. Agents believed to suppress the secretion of endometrial $PGF_{2\alpha}$ have been described in fluids or extracts from the conceptus in the ewe soon after the blastocyst enters the uterus (Findlay, *et al.*, 1982). Antiluteolytic proteins of embryonic origin in sheep and cattle referred to as trophoblastin or ovine and bovine trophoblast protein-1 (oTP-1 or bTP-1) respectively, were isolated prior to Day 14 of pregnancy and are believed to be important for the establishment of pregnancy (Stewart, 1992). Similar proteins believed to comprise a caprine trophoblast protein-1 (cTP-1) complex have recently been isolated in does between Days 16 and 21 but not afterwards (Gnatek, *et al.*, 1989). They comprise an acidic protein consisting of two isotopes with molecular weights of about 17,000 as well as two other low molecular weight acidic proteins shown by immunoprecipitation to react with anti-ovine trophoblast protein-1 (oTP-1) serum. Even though these proteins disappear by Day 21 of pregnancy, it has been suggested that their activity may last several months (Martal, *et al.*, 1979). Polypeptide growth factors are increasingly implicated in the response of the uterus to these trophoblast protein-1 signals. Growth factors are important constituents of uterine tissues and their

secretions and include the insulin-like growth factors (IGFs) -I and -II, epidermal growth factors (EGF), acidic and basic fibroblast growth factors aFGF, bFGF), transforming growth factors (TGF)-alpha and beta, and colony stimulating factor (Simmen *et al.,* 1993). The insulin-like family of growth factors appear to function as key mediators of the coordinated development of the uterus and conceptus during early pregnancy by virtue of their ability to influence directly or indirectly, the synthesis and secretion of uterine and conceptus secretory proteins. The modes of action of the IGFs are modulated within the uterine microenvironment by Type I IGF receptors and IGF binding proteins (IGFBP) which are themselves subject to local control within the uterus and conceptus (Simmen *et al.,* 1993).

Transuterine migration of ova

The incidence of transuterine migration of ova is where an ovum is shed from the ovary opposite to the uterine horn in which the foetus develops. This phenomenon occurs in all domestic animals but relatively infrequently in the cow (Boyd *et al.,* 1944). The reported frequency of transuterine migration in goats ranges from 7 to 41% in single pregnancies (Boyd *et al.,* 1944; Taneja, 1959; Basu *et al.,* 1961; Lyngset, 1968b). Transuterine migration does not appear to be a cause of embryonic mortality in sheep (Sittmann, 1972), However, the efficiency of the spacing of embryos could influence subsequent placental size and thereby foetal growth.

The mechanism by which the migration occurs is not clear in goats. The migration has been reported to occur around Day 14 of pregnancy in sheep (Abnes and Woody, 1971). This coincides with the phase of rapid trophoblastic enlargement with a possible exertion of some form of repulsion between embryos. Intrauterine migration appear to be modulated through peristaltic contractions of the myometrium stimulated

by the developing embryo. Oestrogens, histamines and prostaglandins are embryonic products that could stimulate myometrial activity.

Embryo attachment

The factors that initiate the process of attachment of the embryo to the endometrium is uncertain. The time of attachment of the goat embryo remains to be ascertained but is probably around Day 15-16 of ovulation. In sheep, the attachment of the trophoblast to the caruncular epithelium is initiated on the 13th day of gestation (Wooding, and Staples, 1981). The development of adhesive properties by the coats of the uterine lumen and the trophoblastic cells (Guillomot *et al.,* 1981) and the protrusion of trophectoderm papillae into intercaruncular openings facilitate attachment. Prior to this attachment, a transient (Guillomot *et al.*, 1981) increase in uterine blood flow has been reported (Greiss and Anderson, 1970) between Days 13 and 15, a marked suppression in spontaneous myometrial activity (Fleet and Heap, 1981) probably associated with the continuation of luteal function (Lye and Porter, 1978) on Day 15 and increased content of the luminal fluids between Days 13 and 17 (Ellinwood, *et al.,* 1979) probably the result of local action by the embryo on the endometrial protein synthesis (Findlay *et al.,* 1982). How the presence of the embryo in the uterus brings about these changes is not known. The type of placentation in goats, as in the ewe, is syndesmochorial. The total number of caruncles reported in does range from 120 to 200 (Amoroso, 1952; Lyngset, 1968a), with similar average numbers in the two horns (Lyngset, 1968a). The total volume of foetal fluids seems to increase throughout pregnancy (Lyngset, 1971).

In goats, the time placental development begins has not been determined. In sheep however, placental development begins about 30 days after conception with the fusion of the chorion to the caruncles to form placentomes which together constitute

the placenta (Mellor, 1969). The number of placentomes associated with each foetus is fixed at this stage, but the total weight of the placentomes increases until about 90 days of gestation (Robinson *et al.*, 1977). The number of placentomes per embryo generally decreases as litter size increases. There is however a compensatory hypertrophy of individual placentomes as the number per conceptus decreases as a result of increase in the number of foetuses. This compensatory hypertrophy occurs before about 90 days of gestation (Mellor, *et al.*, 1977).

Placental size and function are regulated by complex interrelationships between the foetal and maternal systems that are not well understood. A large amount of natural variation occurs both within and between breeds in domestic animals (Ferrel, 1991). Placental size and function are also influenced by environmental factors such as heat or cold stress, parity of dam, number of foetuses and maternal nutrition (Ferrel, 1991). There is evidence in sheep, that placental weight limits foetal growth before the end of the pregnancy (Mellor and Murray, 1981 and 1982a).

Early embryo loss

Embryo loss in all farm animals is usually much higher than can be attributed to chromosomal abnormalities (Robinson, 1986). Loss of embryos, particularly those in the preimplantation and early implantation phase, between ovulation and 30 days of gestation can be considerable. Loss from the time of complete attachment of the placenta at about 30 days until parturition is relatively low (Lindsay, 1991). In sheep, 20 to 40% or more of mated ewes were reported not to be represented by live births (Wilmut, *et al.*, 1986). Chromosomal abnormalities and cracked zona pellucida have been reported to cause the loss of 6-8% of all embryos (Long and Williams,1980) or a quarter to a third of total embryo loss in sheep (Robinson, 1986). In goats, Casida *et al.*, (1966) and Lyngset, (1968b) recorded embryo losses of 19.6 and 2.4%

respectively, using data derived from genitalia obtained from the abattoir. Factors which have been reported to affect embryo mortality in animals include chromosomal abnormalities, environmental and/or disease factors, age of the animal, superovulation, level of feeding and other dietary deficiencies. It has also been suggested that some embryos die because of a spontaneously-arising asynchrony with their uterine environment (Wilmut and Sales, 1982).

The consequence of embryo loss is return to oestrus at a normal interval when embryos die early before implantation signalling, and delayed return to oestrus if after implantation signalling (Robinson, 1986). Other consequences are reduction of the number of offspring born and the birth of smaller offspring or increases in the within-litter variability in foetal growth when embryos die after implantation (Rhind *et al.*, 1980; Macdonald *et al.*, 1981; McKelvey and Robinson, 1986).

Gestation, foetal growth and development

It has been suggested (Winters, *et al.*, 1942) that prenatal life in domestic animals be subdivided into 3 periods:

1). The period of the ovum- is the period during which the developing zygote sheds the zona pellucida and becomes a blastocyst and lasts until it makes its first loose attachment to the endometrium.

2). The period of the embryo- time from blastocyst development until there is differentiation of the organ system in the embryo and more complete placenta formation.

3). The period of the foetus- time during which most of the growth of the placenta and foetus occurs and lasts until parturition.

Gestation in goats lasts an average of 150 days but may range from 146 to 155 days depending upon breed, environmental conditions and number of kids born (Pineda, 1983). Singleton pregnancies tend to have longer gestation than those of twins or triplets. Foetal growth in sheep and goats has been known to be influenced by numerous factors such as fecundity, sex, parity, breed, heat or cold stress, diseases and maternal nutrition. Most of the mathematical equations used to describe the growth of the sheep foetus have been purely empirical and provide a reasonable description of growth only in late gestation. The Gompertz equation has however been demonstrated (Robinson and McDonald, 1979) to describe foetal growth for all ages of foetuses. The following relationship was obtained when the equation was fitted to data for twins, triplets and quadruplets sheep foetuses from ewes with an average body weight at mating of 70 kg.

$$\ln W = 9.649 - 17.574e\text{-}0.0176t - 0.00079\ ft$$

where W = foetal weight in g, t = time in days from conception, and f = number of foetuses. Robinson and McDonald (1979) also proposed a model for the description of growth-retarded foetuses. It was an extension of the Gompertz equation to include the allometric relationship with the following general form.

$$\ln y = a + be\text{-}ct + d\ (\ln W - \ln W_t)$$

where y is a measurement of the size or weight of some foetal component, t is foetal age, W is foetal weight and W_t is the expected foetal weight at age t and is obtained from a Gompertz equation relating W to t. Exponential patterns of foetal growth have been described for sheep and other domestic species (Lyngset, 1971; Koong *et al.*, 1975, Ferrel *et al.*, 1976; Robinson *et al.*, 1977;). It has been demonstrated in goats too, that foetal growth did not follow a first order relationship (Kadu and Kaikini,

1987) and that the patterns of embryonic and foetal development in the species were on the whole similar to those in sheep (Lyngset, 1971; Kadu and Kaikini, 1987).

1.2 Factors that affect reproduction in goats

Introduction

Several factors are known to affect the underlying physiological mechanisms of reproduction in livestock. Considerable variation in reproductive performance exists between different genotypes of goats. A wide range of environmental and management factors such as nutrition, body condition, photoperiod, exposure to the male, temperature and other stresses have been reported to influence reproductive performance in goats. Several techniques have been employed to manipulate these factors with a view to increasing productivity. The most important of these factors are discussed below with greater emphasis on nutrition.

Genotype

The reproductive performance of goats varies considerably throughout the world. Variations within and between breeds exist in components of reproductive performance such as age at puberty, or the time of onset and duration of the breeding season in the adult, age at first kidding, kidding interval, ovarian activity or ovulation rate and multiple birth rate. Knowledge of the heritability of these reproductive traits is proving to be important in crossbreeding programs for rapid expansion of limited gene pools.

Photoperiodicity

Photoperiodicity (or photoresponsiveness) appears to be an innate adaptation of breeds from high latitudes which is retained when these breeds are moved to tropical latitudes or subjected to constant photoperiods (Davendra and Burns, 1970; Thimonies and Chemineau, 1988). The animal's perception of daylight does not force it into breeding activity however, but entrains or "fine-tunes" an innate circannual rhythm that determines, at least approximately, when it will start or stop breeding regardless of the light pattern or number of hours of daylight (Karsh and Wayne, 1988). The mechanism of photoperiodism is not fully understood in animals. There are indications that the photoperiodism effect involves a direct action on the hypothalamic-pituitary-axis and a change in the sensitivity of the central nervous system to negative feedback from steroids. Injection of oestradiol into castrated sheep has reduced gonadotrophins to undetectable levels during the non-breeding season but caused only a slight depression during the normal breeding season or under short days (Legan *et al.*, 1977). However, in spayed mares in which negative feedback from sex steroids does not occur, gonadotrophin levels have been observed to reach a maximum during the normal breeding season and decrease during the non-breeding season (Garcia and Ginther, 1976).

How photoperiodic signals are converted to neuroendocrine messages is not well understood. It has however been documented in sheep (Arendt, 1986) that the pineal gland and melatonin mediate changes in neuroendocrine-gonadal activity in response to a changing photoperiod. In mares, it has been observed that both superior cervical ganglionectomy and pinealectomy altered the ability to respond to a stimulatory photoperiod (Hart, *et al.*, 1984). Melatonin levels have been found to be high during dark periods and low during light periods (Maeda *et al.*, 1988). The main production of melatonin by the pineal gland occurs during the dark phase of the natural

24 hour light-dark cycle or photoperiod while synthesis is suppressed by light (Deveson *et al.*, 1990). It appears that neural impulses generated as a result of light incident upon photoreceptors in the eye are transmitted to the pineal gland where they regulate the synthesis and secretion of melatonin. The differences in the pattern of melatonin secretion probably act as a signal indicating daylength to the neuroendocrine axis (Maeda *et al.*, 1988).

There are indications that the goat foetus is capable of detecting photoperiod before birth probably via the maternal melatonin signal. This suggestion followed the observation that onset of puberty in females and sexual development in males have been delayed in goat kids exposed to 20 hour light and 4 hour darkness *in utero* (Deveson, *et al.*, 1992).

Photoperiodicity has been overcome in both sheep (Arendt, 1986; Staples, 1989) and goats (Deveson *et al.*, 1990) by the administration of exogenous melatonin. This is of great potential in advancing the breeding season and thereby reducing the fluctuations in milk, meat and fibre production.

The male effect

Introduction of does to the male after a period of separation has been known to induce rapid LH pulses that lead to a preovulatory surge and ovulation. Exposure of does to the billy goat has been used to advance the breeding season by up to 3 weeks (Pineda, 1983). Most does begin to cycle within 3 to 9 days after the exposure. The induction of breeding activity by the male was effective whether anoestrus was due to photoperiod (Ott, *et al.*, 1980) or to nutrition (Chemineau, 1983; Mgongo, 1988). This effect of the male seems to be inversely related to the degree of latitude of the region of the genotype. Sheep and goats from high latitudes respond to the male during the

few weeks immediately preceding the breeding season in seasonal breeders. Short cycles have however been commonly reported following the male-induced ovulation (Chemineau, 1983). The teasing activity of the male apparently induces luteolysis in the cycling doe, thereby shortening the luteal phase of the cycle. The short cycles appear to be associated with lack of sufficient progesterone priming from the anoestrous ovary at the beginning of the breeding season, and can be prevented by the injection of synthetic progestogen Fluorogestone acetate (Chemineau, 1985). The mechanism involved in the male-induced luteolysis remains to be fully determined.

Environmental temperature and other stresses

The effects of high ambient temperature on reproduction have been well documented in domestic livestock. The occurrence of ovulation may not be adversely affected by exposure of animals to continuously high ambient temperatures (Robertson, 1977), although some delay or suppression was recorded when ewes were exposed to cooling environments. Extremely high ova mortality rates, up to 100% have been described in experimentally-induced conditions of heat stress in sheep (Edey, 1976), while significant increase in ova loss has also been associated with exposure to cold in the period just prior to or immediately after mating (Doney and Gunn, 1981). The effects of continuous daily exposure of pregnant sheep to high ambient temperatures on birth weight were reported to be apparent as early as the end of the third month of pregnancy and to be accompanied by a reduction in the weight of the foetal cotyledons (Alexander, 1964). Other environmental stressors such as wind and rain may reduce ovulation rate by as much as 20% (Doney and Gunn, 1981). Several aspects of normal husbandry practice such as excessive handling, transportation or the mixing of strange animals have also been suggested as possible causes of increased ova wastage (Doney *et al.*, 1981).

The degree of the effect of environmental stressors on reproduction may depend on their nature, individual differences in tolerance, the degree to which the animals are accustomed to the specific stressor and the timing of the exposure (Doney *et al.*, 1981). The physiological pathways through which these effects are mediated are not clearly understood.

1.3 Nutritional influences on reproduction

Introduction

Nutritional influences on reproduction have been recognized in domestic animals for many years. The influences reported include effects on attainment of puberty, duration of post-partum anoestrus, gametogenesis, ovulation rate, conception rate, embryonic mortality, prenatal development and sexual behaviour (Wentzel, 1987; Ferrell, 1991). Both body condition and food intake are aspects of nutrition that can affect reproduction. Body condition is a consequence of food intake over several weeks or months and may be described as having a medium-term nutritional effect (Gunn and Rhind, 1983). The level of intake may be described as having a short-term effect. Effects of body condition and level of food intake are frequently compounded.

1.3.1 Effect on the onset of puberty

Onset of puberty in goats has been related to body weight which in turn depends on the level of nutrition, age, number of kids at birth and the season of year kids are born (Epstein and Hertz, 1964; Singh and Singh, 1974; Shelton, 1978; Smith, 1988). An increased plane of nutrition favours an earlier puberty. This is particularly true for non-seasonal breeding animals (Pineda, 1989). Poor nutrition in general, and energy deficiency in particular, has been reported to delay the onset of puberty in Jamunapari

and Barbari breeds of goats in India (Singh and Sengar, 1970; Sachdeva *et al.,* 1973). Such an effect was however not observed in Boer goats in South Africa (Greyling and Van Niekerk, 1990). Although there can be delays to the extent that the age of puberty is doubled, the eventual onset of puberty cannot be prevented by poor nutrition (Pineda, 1989). Diseases which can influence growth rate, either directly or because of interference with feeding and utilization of nutrients, may also delay the onset of puberty.

Age and weight at first behavioural oestrus has been shown to be markedly influenced by level of nutrition or postweaning rate of weight gain in domestic animals (Allen and Lamming, 1961 Sacco *et al.,* 1987). The concept that a threshold value for age, weight and body condition or fatness must be attained before puberty can occur in animals has received considerable support (Kirkwood & Aherne, 1985). However, the observation that a positive energy balance was necessary for onset of puberty in rats supported the suggestion that net flow of energy rather than fatness *per se* modulates the attainment of puberty (Frisch an Vercoe, 1977). The effects of dietary protein on puberty have been attributed primarily to its relationship to somatic growth and that within ranges in the diet compatible with an adequate growth rate it does not appear substantially to affect the time of onset of puberty (Ferrel, 1991).

Level of nutrition affects the age at the onset of puberty in lambs through modulation of gonadotrophin secretion (Ferrel, 1991). When growth was retarded in ewe lambs the frequency of LH pulses was reduced and oestradiol continued to exert a negative feedback action on secretion of LH (Foster, *et al.,* 1986). Sensitivity to oestradiol negative feedback was decreased and LH secretion increased when ewe lambs that had been fed restricted amounts of feed were fed *ad-libitum* (Foster and Olster, 1985). Similar responses were observed in ovariectomized ewe lambs, indicating that effects of nutrition can occur independent of feedback effects of ovarian

steroids (Foster *et al.,* 1989). These effects seem to be specific for pituitary gonadotrophin secretion since long term restricted feeding of ovariectomized ewe lambs caused decreased frequency of FSH and LH release, had no effect on serum prolactin (PRL) concentrations, and increased serum growth hormone concentrations (Foster *et al.,* 1989; Thomas *et al.,* 1990). In addition *ad-libitum* feeding of previously feed restricted ovariectomized ewe lambs was associated with increased pituitary concentrations of messenger (m)RNA for LH and FSH subunits, decreased mRNA for growth hormone (GH), and no change in mRNA for PRL. These changes were consistent with changes in LH, FSH, GH and PRL concentrations in serum (Landefield *et al.,* 1989). Restricted feed intake also resulted in a suppression of the preovulatory surge of LH secretion that occurred following oestradiol administration. This suggests that pituitary reserves of LH were less, or pituitary sensitivity to LH releasing hormone was reduced, in lambs fed restricted levels of energy. These and the reports by Foster, et al. (1989) suggested that in ewe lambs, influence of nutritional level on puberty attainment appears to be mediated, at least partially, by sensitivity of the hypothalamus and pituitary to oestradiol feedback, by pituitary responsiveness to LH-RH, and by pituitary expression of gonadotrophin genes.

1.3.2 Effect on the breeding season

In the absence of photoperiodic cues, nutritional control of the breeding season is more important in the tropics than in the temperate latitudes (Lindsay, 1991). In the Niger republic, Red Sokoto goats kidded all year round, but 51% of the parturitions occurred in February to April, presumably as a result of better nutrition in the preceding rainy season (Haumesser, 1975). There is also evidence that poor nutrition can shorten the length of the breeding season in ewes (Oldham, 1980).

The mechanism of the effect of poor nutrition on the length of the breeding season is not very clear. Poor nutrition is believed to prolong the anoestrous season by prolonging the sensitivity of the GnRH pulse generating mechanism to oestradiol.

1.3.3 Effect on oestrous cycle and oestrous behaviour

There are no specific reports of the effects of nutrition on the duration of the oestrous period and on oestrous behaviour in goats. It has however been reported (Wentzel, 1987) that inadequate nutrition can lead to absence of overt oestrus or low intensity oestrus in goats. In sheep, it has been demonstrated (Rattray, 1977; Doney and Gunn, 1981; Ferrel, 1991) that exceptionally low body condition and liveweight or severe undernutrition immediately prior to mating are associated with delayed onset or suppression of seasonal or post-partum oestrus, ovulation without oestrus or "silent oestrus," and lengthening of the oestrous cycle. The mechanism of these effects is unclear but are probably mediated through changes in the hypothalamic-pituitary-gonadal axis.

1.3.4 Effect on ovulation and ovulation rate

The effects of undernutrition on ovulation and ovulation rate in goats have not been well documented. Nutritional status, body weight and size or condition have all been reported as capable of influencing ovulation rate in goats (Wentzel, 1987). Overall reduced fertility has been reported in does on poor energy rations (Singh and Sengar, 1970; Sachdeva *et al.,* 1973) or with poor body condition (Wentzel 1987). Low body mass, which may result from inadequate nutrition has been reported to influence reproductive efficiency. This is especially pronounced in the case of young does in their first breeding season (Shelton, 1961; Van der Westhuysen, 1981; Wentzel, 1987). "Flushing," or increasing the level of feeding in the period prior to

mating to improve twinning has been practised successfully in goats (Economides and Louca, 1981). In sheep, positive relationships have been demonstrated between ovulation rate and body weight or body condition (Gunn *et al.*, 1979a; Gunn, 1983) and level of premating feed intake (Gunn *et al.*, 1979a; Rhind *et al.*, 1985). In general, ovulatory responses due to changes in nutrition are small and usually no more than 0.1-0.5 ovulations per ewe, which is less than could be expected using hormonal techniques (Lindsay, 1991). Twinning rates achieved by nutritional flushing (30-40%) however, approximate that resulting from hormonal treatments without the risk of producing some quadruplets or quintuplets which is quite often a consequence of hormonal treatment (Wentzel, 1987).

Despite the observed relationships between nutrition and ovulation rate, the physiological link between the two remains tenuous. Changes in gonadotrophic hormones in response to level of nutrition or body condition, where discernible, have been small and at times inconsistent. A positive relationship between body condition and FSH concentration in sheep was reported by Rhind and McNeilly (1986) however, in another experiment in the same species, none was observed (Rhind *et al.*, 1989a). It is feasible however, that subtle changes in the hormones during the reproductive cycle are sufficient to produce the ovulatory responses observed due to changes in nutrition (Lindsay, 1991).

1.3.5 Effect on embryo loss

The influence of nutrition on embryo loss in goats has not been investigated. Studies in sheep however have demonstrated detrimental effects to embryo survival by extremes of nutrition (Robinson, 1986). Fasting of ewes for up to 3 days in the first fortnight of pregnancy was reported to produce little if any effect on embryo survival (Blockey *et al.*, 1974). Various low levels of food intake after mating have however

been found to lead to reduced embryo survival (Cumming, 1972; Gunn *et al.*, 1972; McKelvey and Robinson, 1986), extended interval to repeat oestrus suggesting embryo mortality after Day 12 (Edey, 1970; MacKenzie and Edey, 1975b), retarded embryo development (Parr *et al.*, 1982) and reduced embryo growth or size (Parr and Williams, 1982). A high plane of feeding immediately after mating, particularly in ewes poorly fed before mating, has been reported to improve embryo survival (Gunn *et al.*, 1979b). A high plane of feeding in fat ewes or those already in good body condition can however also reduce embryo survival (El-Sheikh *et al.*, 1955; Gunn *et al.*, 1972).

The mode of action of nutrition on embryo survival and growth is only speculative. The modifying effect of nutrition on asynchrony has yet to be established (Robinson, 1986). Survival of the embryo can be affected only by extreme nutritional restriction considering the extremely small nutrient requirement of the embryo. The action of nutrition on embryo survival may presumably operate through alterations in the endocrine status of the mother (Robinson, 1986). An inverse relationship between the plane of nutrition and peripheral plasma concentrations of progesterone has been demonstrated by Cumming *et al.*, (1971), Parr *et al.*, (1982) and Williams and Cumming (1982). The growth of sheep embryos in the first two weeks of pregnancy has been shown to be influenced by progesterone concentrations in maternal plasma (Lawson 1977). Enhancing the circulating concentrations of plasma progesterone either directly by supplementation or by administration of hCG during the luteal phase of the oestrous cycle, can improve fertility of ewes (Kittok *et al.*, 1983). It has been suggested (Wilmut, *et al.*, 1986) that pregnancy depends on a specific sequence of concentrations of progesterone and oestrogen, and embryo loss may be caused by excesses or inadequate amounts. Limitation in the supply of maternal glucose in response to nutritional restriction has also been implicated in reduction of embryo growth and probably survival (Parr and Williams, 1982). It is difficult to explain the mechanism of the detrimental effect of a high level of nutrition on the survival of the

embryo in ewes that are in good condition at mating. The observation that embryos are particularly vulnerable to small increases in maternal temperature (Edey, 1976) has prompted the implication of heat stress in the mechanism (Robinson, 1986).

1.3.6 Effects on foetal growth

There are no reports of the effect of nutrition on the growth of the goat foetus. Most studies on nutritional influences on the growth of sheep foetus concentrate on the later stages of pregnancy in which about 80% of growth normally takes place (Wallace, 1948; Alexander, 1964; Mellor, 1983). Because of the small size of the foetus and its very small nutritional requirements in early gestation, growth in this period was thought to have little relevance. It has been shown however (McKelvey and Robinson, 1986), that growth in this period is important in establishing foetal growth trajectories in late pregnancy. Previous reports have indicated that severe postmating deficiencies may retard growth in the early stages of embryo development (Parr *et al.*, 1982; Parr *et al.*, 1986). Evidence has also been provided of an adverse effect of poor body condition on embryo growth (McKelvey and Robinson, 1986).

The mechanism by which nutrition affects the growth of the foetus is not fully determined. However, despite the large amount of natural variation in placental size that exist within breeds, placental weight and foetal growth rate were found to be correlated positively during periods of maternal undernutrition which started between 90 and 112 days of gestation in ewes (Mellor and Murray, 1981; Mellor and Murray, 1982a). The cotyledonary component of the foetus was found to be more sensitive to undernutrition than even the foetus itself (Clark and Speedy, 1980; Rattray *et al.*, 1980). This suggests that the effect of undernutrition on the foetus is mediated through effects on the placenta.

The effects of undernutrition during the mid- and late pregnancy has been well investigated in Angora goats. Energy deficiency in particular was identified as the primary cause of abortion at this stage of pregnancy in the breed (Wentzel, 1987).

1.4 Possible mechanisms for the effects of nutrition on reproduction.

Several mechanisms that potentially contribute to altered reproductive function caused by nutrition have been suggested. Increased plane of nutrition could result in increased liver size and increased concentration of NADPH-dependent mixed function oxidase enzymes that metabolise naturally-occurring steroids in the liver microsomes (Thomas *et al.*, 1987; Thomford and Dziuk, 1988). This could potentially result in increased liver capacity for oxidative metabolism of sex steroids, which may in turn, result in decreased feedback on the hypothalamic and pituitary systems previously discussed. It has also been suggested that changes in circulating concentrations of certain amino acids and precursors to brain neurotransmitters may result in altered gonadotrophin release (Ferrel, 1991). Energy and protein intake have been demonstrated to influence the levels of insulin, thyroxine, and insulin-like growth factor-1 (IGF-1). A role for these hormones in hypothalamic, pituitary and ovarian function have been proposed. Other hormones suggested to play roles which are however, not well defined include epidermal growth factor (EGF), transforming growth factor-beta (TGF-β); fibroblast growth factor (FGF) and inhibin.

1.4.1 Mode of action of feed intake and body condition on reproduction

Studies with ovariectomized ewes, without steroid replacement therapy (Rhind *et al.*, 1989b), showed that the release of LH and FSH in response to a GnRH challenge was not higher in ewes with a high food intake than in ewes with a moderate intake. Further studies with ovariectomized ewes with steroid replacement therapy (Rhind *et al.*, 1991) demonstrated a higher mean LH concentration, pulse frequency and pulse amplitude in ewes on high feed intake compared with ewes on moderate intake. Gonadotrophin release following injection of a high dose of GnRH in ewes was not greater in ewes on high feed intake than in those on moderate intake. These observations indicate that level of food intake did not have a positive direct effect on GnRH pulse frequency and hypothalamic activity or on pituitary responsiveness to GnRH, and the differences in LH profiles reflect a differential sensitivity to oestradiol feedback at the hypothalamic level. Similar experiments with ewes in high and low body conditions showed that higher mean LH concentration and pulse amplitude in ewes with high body condition were due partly to differences in pituitary responsiveness to GnRH. This suggests that the pituitary in ewes with low body condition is more sensitive to the inhibitory effects of oestradiol. In addition to the indirect effect of body condition on the hypothalamic activity, Rhind *et al.*, (1989b) demonstrated that there is also a small effect on hypothalamic activity which is not steroid mediated.

1.5 Artificial regulation of reproductive performance in goats

In addition to exploitation of those factors which naturally affect the reproductive cycle in order to improve productivity in goats, increased understanding

of the physiological mechanisms of reproduction has also made it possible to use artificial techniques which involve either physical intervention or administration of exogenous substances.

1.5.1 Oestrus synchronization

Synchronization of oestrus during the normal breeding season aids management control by permitting the design of an economic and efficient feeding regime for late pregnancy, concentrates the labour intensive parturition period and allows for the efficient operation of large scale artificial insemination and embryo transfer programmes (Doney *et al.*, 1982; Henderson, 1987).

There are 2 types of pharmaceutical products which may be used to control the timing of breeding: those based on $PGF_{2\alpha}$ and those based on progesterone or synthetic equivalents of the two natural hormones. $PGF_{2\alpha}$-based products cause CLs present on the ovaries to undergo premature lysis, bringing the animals back into oestrus (Allison, 1991). The CL of does was reported to be sensitive to the luteolytic effects of $PGF_{2\alpha}$ as early as Day 4 following oestrus, and does with active CLs will normally come into oestrus within 24-48 hours of treatment. This is sooner than either the cow or sheep responds (BonDurant, 1981; Mews, 1981). A disadvantage of this method however, is that animals without a responsive CL, those just coming to oestrus anyway and those which have just been in oestrus, and which are still forming a CL will not respond. Reasonable fertility has however, been reported when $PGF_{2\alpha}$ was administered in two doses 9 to 11 days apart (Henderson, 1987; Ishwar and Pandey, 1992). Doses of prostaglandin routinely used for luteolysis in goats are 8 to 15 mg $PGF_{2\alpha}$ (Ott *et al.*, 1980). However, doses as low as 1.25 mg $PGF_{2\alpha}$ have been found to be effective (Bretzlaff *et al.*, 1981).

The progesterone-based products are administered as an external source of progesterone for long enough to outlast any ovarian CL source. At the time the external source is removed, blood progesterone concentrations fall and the animal will come into oestrus. The progesterone method of synchronization is effective in all cycling animals even though the whole treatment process is longer than with the prostaglandins. The usual method of administering progesterone to ruminants is by some sort of progesterone releasing device. Progestogen impregnated intravaginal pessaries or `sponges' are the most widely used products for the induction of oestrus and ovulation in goats (Henderson, 1987). They can be used both in the breeding season and during anoestrum. When used in conjunction with small doses of gonadotrophins (200-300 iu) at the time of sponge withdrawal, the time of oestrus can be predicted with great accuracy. The sponges may be inserted for between 12 to 21 days (Corteel, 1977; Henderson, 1987). Alternatively, progesterone preparations may be given by injection, subcutaneous implants, or by mouth (Allison, 1991).

1.5.2 Extension of the breeding season

The seasonal nature of goats' reproduction leads to seasonal fluctuation in the products from these animals, such as milk, meat and fibre. One method of helping to overcome the seasonal variation in goat products especially with regards to milk, is for the females to kid as early in the year as possible. In general too, the earlier the female kids the more milk it will produce in that season (Mews, 1981). The breeding season has been successfully advanced by artificially mimicking both the action of the CL by the administration of progestogens, and that of the anterior pituitary by administration of pregnant mare serum gonadotrophin (PMSG) about 48 hours before the removal of the progestogen. The deeper into anoestrus the animal is, the higher the dose of PMSG required (up to a maximum of around 750-1000iu), and the poorer the results (Henderson,1987).

The breeding season in the goat has been successfully advanced by mimicking long-day light followed by melatonin treatments. Successful treatments have been reported (Deveson *et al.*, 1990) in dairy goats by simulating 2 months of long-day light, during the winter, followed immediately by 3 months of spring melatonin treatment by feeding, drenching or implantation. Better results were reported using this method than with conventional methods such as progesterone sponges, male effect and prostaglandin treatments (Deveson *et al.*, 1990). In addition, melatonin increases fecundity, by increasing the number of fertile oestrous cycles, ovulation rate and subsequent kidding rate (Kennaway *et al.*, 1987; Chemineau *et al.*, 1988).

One problem posed by the treatment of goats with melatonin in spring is the growth of a thick winter coat during the summer which may lead to heat stress and an inadequate summer coat during the winter which may lead to goats suffering from cold exposure unless they are housed (Deveson *et al.*, 1990). Use of melatonin to manipulate seasonal coat growth cycles may however, be of great potential in the cashmere and mohair industries. Further studies are however needed to define the minimum length of light and melatonin treatment for successful advancement of oestrus in goats.

1.5.3 Superovulation, artificial insemination and embryo transfer in goats

Superovulation (or multiple ovulation), artificial insemination (AI) and embryo transfer (ET) techniques have not had wide application in goats in the past. Recently however, increased demand for milk and high quality fibre from goats has led to heightened interest in genetically-improved breeds of the species and therefore increased attention to these techniques.

The use of these techniques makes it possible to obtain more offspring from a few top performing animals in a herd or group of herds, and allows them to leave more progeny by the time they reach a given age. This will lead to the shortening of generation intervals facilitating a dramatic increase in the rate of genetic progress that can be made in breeding programmes. These techniques are however costly, and might lead to genetic defects being spread more widely throughout a breed. The widespread use of bucks through AI will also increase the rate of inbreeding.

For successful AI and ET programmes, accurate synchronization of the onset of oestrus is essential. The methods of achieving this have been discussed earlier, and are the same for both the donor and recipient does. The processes of collection, handling, processing, chilling and freezing of semen for AI in goats have been reviewed elsewhere (McKelvey, 1990).

Superovulation has been achieved in goats using PMSG. However, the results obtained using this drug were poor due to its long half life and high LH content (Tervit *et al.,* 1985) leading to the production of multiple unovulated follicles in the ovaries and poor synchronization of ovulation time. FSH is currently the drug of choice for achieving superovulation in goats. FSH of porcine and ovine sources are available. Because of its short half life FSH is administered twice daily over a period of 4 days, usually 3 days before and one day after progestogen pessary removal. The high incidence of premature regression of the CL in superovulated does may be suppressed by the use of specific prostaglandin inhibitors or the reinsertion of progesterone pessaries at the time the natural increase of progesterone will normally occur (Battye *et al.,* 1988; McKelvey, 1990).

The techniques of embryo recovery, evaluation, storage and transfer in goats have been reviewed (Armstrong *et al.,* 1983a; Kiessling *et al.,* 1986; Mckelvey, 1990;

Amoah and Gelaye, 1991). Embryos for transfer are normally recovered from superovulated does 4-7 days after oestrus. Surgical methods of embryo recovery involve laparotomy and exposure of the uterine horns through a ventral mid-line incision cranial to the udder attachments. Using a needle inserted at the tip of the uterine horn, each uterine horn is back-flushed towards the cervix with culture medium. The ovum culture fluid is collected through a 10-French Gauge Foley catheter placed in each of the uterine horns slightly cranial to the bifurcation (Mckelvey, 1990; Amoah and Gelaye, 1991).

Non-surgical or laparoscopic recovery of embryos involve the injection of culture medium into the tip of each of the uterine horns under laparoscopic visualization. The medium is collected via a cervical catheter. Embryos are transferred to recipients surgically at laparotomy or non-surgically by laparoscopy. Embryos are normally placed at the tip of the uterine horn (Amoah and Gelaye, 1991). Both collection and transfer procedures are performed under general anaesthesia.

CHAPTER 2

Materials and Methods

2.1 Experimental Animals

British Saanen, Toggenburg, crosses of the two and Angora does, approximately 2-3 years of age were used in the experiments in this study. All animals were moved into individual pens at least 2 months before the commencement of each experiment to allow for adjustment to feed and experimental environment and to attain a body condition score of approximately 2 on a scale from 0 to 5, based on the amount of fat and muscle cover over the back and lumbar bones, behind the last rib (MAFF, 1984). Animals in all the experiments were fed on goat nuts [Dry matter (DM)= 87%; Metabolizable energy (ME) = 11.5 Megajoules (MJ) per Kg of DM; Digestible crude protein (DCP) = 13.1g] and pelleted barley straw [DM = 90%; ME = 9.1MJ/KgDM; DCP = 1g/KgDM, Nutrition International Ltd., Basingstoke, U.K.]. Fresh water was provided *ad libitum*. All animals were weighed weekly throughout experimental periods.

2.2 Surgical procedures

2.2.1 Blood sampling

Frequent blood samples (every 15 minutes for 3, 8, 10 or 24 hours) were collected through an indwelling jugular catheter (Vygon U.K. Ltd. Cirencester) inserted one day earlier. The catheters were frequently flushed with sterile heparinised saline during the sampling period. The catheters did not cause any obvious discomfort or distress and were removed immediately after sampling was finished.

Infrequent blood samples (once daily) were collected by venepuncture from the jugular vein. All blood samples were collected into heparinised tubes and within 30 minutes of collection centrifuged at 4°C, 2000*g* for 30 minutes. Plasma was separated and stored at -20°C pending hormone assay.

2.2.2 Laparoscopy

Laparoscopy was carried out to determine ovulation rate in does. Animals were fasted and water withdrawn for 24 hours before laparoscopy. Animals were intubated under general anaesthesia induced with 3mg/kg saffan. Anaesthesia was maintained with halothane. The ventral abdominal region was shaved and disinfected. Animals were restrained in a cradle which was tilted at an angle of approximately 45% so that the hind quarters of the does was higher than the forequarters. Trocars and cannulae were inserted into the pelvic cavity at a position slightly cranial to the udder. A laparoscope inserted through one of the holes was used to view the ovary while a Semm's forceps inserted through the other hole was used to manipulate the ovaries. Number of CL was counted. Cannulae were removed after laparoscopy and topical antibiotic (Terramycin spray, Pfizer Ltd., Sandwich, Kent) was applied to the puncture wounds. Long acting Penicillin was injected intramuscularly (i.m.). Two does were lost following anaesthetic complications. In the rest of the does there was no obvious complications following the procedure.

2.2.3 Embryo collection

Embryos were recovered from donor does on Day 6 after the onset of oestrus, by a technique described previously (Armstrong *et al.*, 1983a). Briefly, laparotomy was performed under general anaesthesia. The uterine horns were exposed through a ventral mid-line incision cranial to the udder attachments. Ovum culture medium which

had been maintained in an incubator at 37°C was used to flush each uterine horn back towards the cervix, using a needle inserted at the tip of the uterine horn. The ovum culture fluid was collected through a 10-French Gauge Foley catheter placed in each uterine horn slightly cranial to the bifurcation. Number of CLs on the ovaries was counted during embryo collection. The peritoneum and abdominal muscles were sutured with catgut and the skin incision wound was closed using metal clips. Topical antibiotic (Terramycin spray, Pfizer Ltd., Sandwich, Kent) was applied to the incision site and long acting penicillin and 10mg $PGF_{2\alpha}$ were given to animals by intramuscular injection.

All transferable embryos at the late morula and early blastocyst stages were pooled together for subsequent transfer to recipients on the same day. The donors were each used twice for the collection of embryos. A period of two months elapsed between collections.

2.2.4 Transfer of embryos

Reproductive tracts of recipients does were exposed at laparotomy and the number of CLs counted. Two embryos, randomly picked, in about 0.02 ml medium were transferred to one uterine horn using a tom cat catheter (Sherwood Medical, St. Louis, USA) inserted deep into the uterine horn ipsilateral to the corpus luteum. The incision wounds were handled in the same way as in the donors.

2.3 Radioimmunoassays

Assays for LH and FSH were performed in 12 x 75mm plastic tubes (Elkay Products Inc., Boston, U.S.A.). All tubes were run in duplicate.

2.3.1 Assay validation

Validation of the assays were carried out as described by Abraham *et al.*, (1972). Assay sensitivity was derived from the variation of the counts in the zero standard (containing no unlabelled hormone). Sensitivity of the assays was calculated as twice the standard deviation from the zero standard. Accuracy of assays was determined by adding known quantities of hormone to samples of plasma which were then assayed. Quantitative recovery of the added hormones was indicated by the slope of the curve. Precision of the assays was assessed by measuring the within-assay (intra-assay) and between-assay (inter-assay) variations. The intra-assay variation was obtained by calculating the coefficient of variation of 10 replicate measurements performed in a single assay. The inter-assay variation was obtained by calculating the coefficient of variation of a plasma pool placed in each assay. Parallelism of the assays was obtained by serially diluting a sample of known quantity of concentration of hormone and comparing the slope of the curve with that of the standard curve using correlation analysis.

2.3.2 Assay buffers

Assay buffers were freshly prepared for each assay using fresh distilled water as diluent.

(1) 0.5M phosphate buffer, pH 7.5 (phosphate buffer)

This was prepared by adjusting the pH of 0.5M di-sodium hydrogen orthophosphate (anhydrous) (BDH Chemicals Ltd., Poole, England) to 7.5 using 0.5M potassium di-hydrogen orthophosphate (BDH Chemicals Ltd., Poole, England).

(2) 1.0% Bovine serum albumin/Phosphate buffer saline (1.0% BSA/PBS)

This buffer was made up of 9g of sodium chloride, 10g BSA (Boehringer Mannheim GmBH, Germany), 10ml phosphate buffer and 1 litre distilled water.

(3) 0.1% Bovine serum albumin/phosphate buffer saline (0.1% BSA/PBS)

This buffer contained 9g sodium chloride, 10ml phosphate buffer 1g BSA and 1 litre distilled water.

(4) 0.9% Phosphate buffer saline (0.9% PBS)

This was made up of 9g sodium chloride, 10ml phosphate buffer and 1 litre distilled water.

2.3.3 Luteinizing hormone (LH)

LH concentrations were determined in plasma samples by the double-antibody RIA using the techniques described by McNeilly *et al.*, (1986). Purified ovine LH (NIADDK-oLH-1-3) was used for iodination and NIADDK-LH-23 for preparation of reference standards. The antiserum was NIADDK-anti-oLH-1 diluted in assay buffer (0.1% BSA) to an initial dilution of 1:1,000,000. Specificity of the antiserum in terms of its reactivity (relative potency) in RIA with pituitary hormones was as follows: oLH NIADDK-23 = 1, oLH NIDDK-FSH-RP-1 = 0.054, oPRL NIADDK-1-2 = 0.00005, oGH NIADDK-1-4 = 0.0057, 8-Arg Vasopressin = 0.000001, bTSH NIADDK-1-1 = 0.0015.

Iodination of the LH was carried out by the addition of 10µl ^{125}I (sodium iodide: Amersham International plc., Buckinghamshire England), 10µl lactoperoxidase and 10µl freshly prepared hydrogen peroxide to 50µl of LH (50µg/µl) in a reaction vial. The vial was reacted on a vortex for 20 seconds. Cysteine hydrochloride (10µl) and 1% BSA/PBS (1ml) were added and the vial reacted on a vortex for 10 seconds. The mixture was pipetted onto a sephadex G 100 column. From the bottom of the column, approximately 1ml of the mixture was collected in 12 x 75mm glass tubes (Elkay Products Inc., Boston, U.S.A.). The head of the column was maintained with 0.1% BSA/PBS once it had reached the bed-line. All tubes were counted and those that form the first peak counts were retained.

Standard solutions (100µl) ranging from 0.5 to 100ng/ml and samples (100µl) were added to 200µl assay buffer. Antiserum (100µl) was added and the tubes were incubated for 24 h. ^{125}Iodine-labelled LH (100µl - approximtely 12,000cpm) was then added and the tubes were incubated for a further 48 h at 4°C. Normal rabbit serum (NRS: diluted 1:400)(100µl) and donkey anti-rabbit serum (DARS: diluted 1:64)(100µl) were added. The tubes were incubated overnight at 4°C. One millilitre 0.9% saline was added and the tubes were centrifuged at 2000g for 30 minutes at 4°C. The supernatants were decanted, the tubes were dried and the precipitates were counted on a gamma counter (CliniGamma, Pharmacia, Turku, Finland).

The sensitivity of the assay was 0.25ng/ml and intra- and inter-assay coefficients of variation were 12.3 and 13.6% respectively. Concentrations of LH recovered following spiking of plasma samples were highly correlated with the known quantities added ($r = 0.990$, $P = 0.000$). Graph plots of gonadotrophin recovered versus gonadotrophin added were nearly straight lines (Figure 2.1).

Figure 2.1 Comparison of a standard curve for LH and dilution-response curve of a goat plasma sample of known concentration

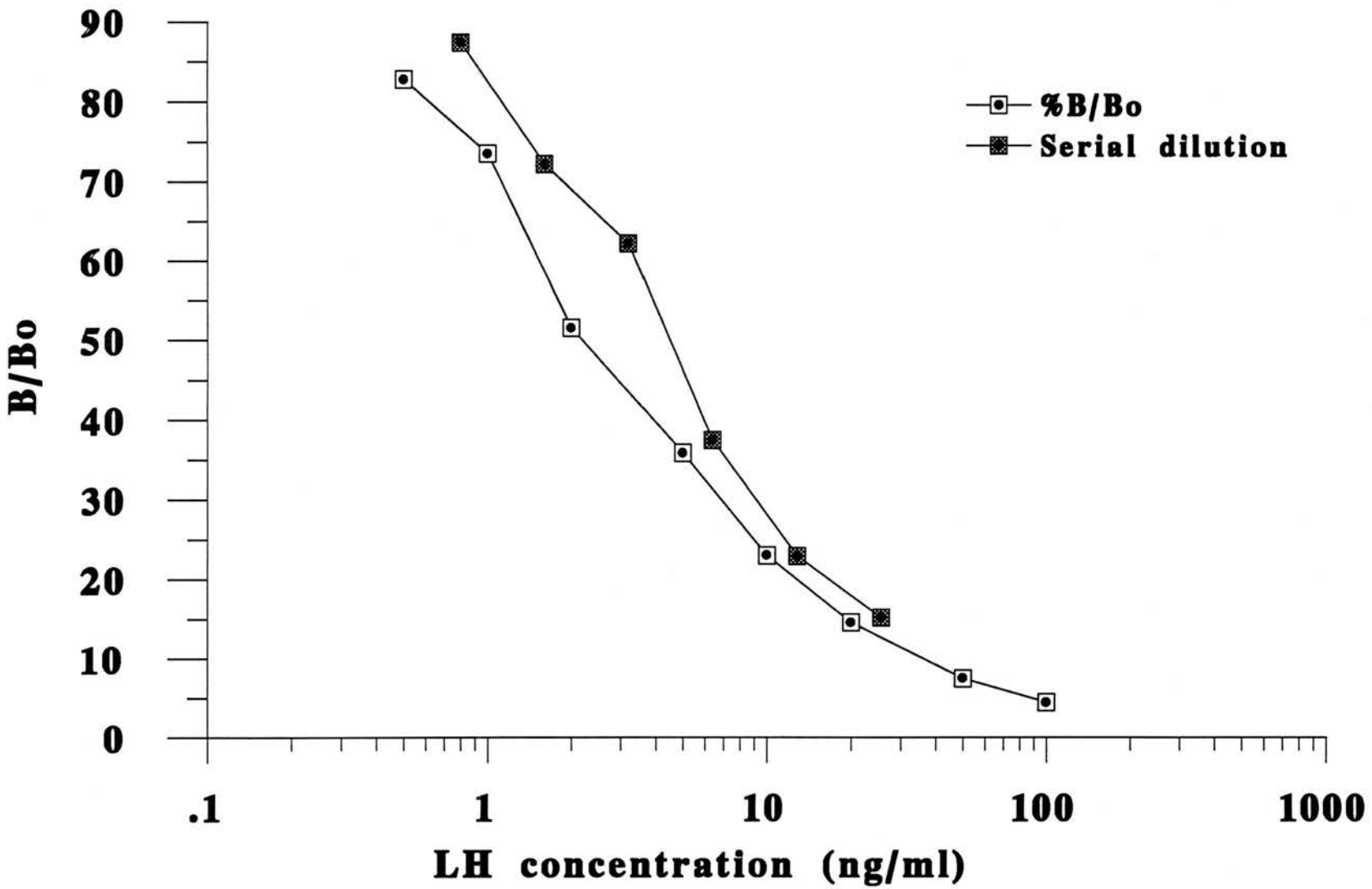
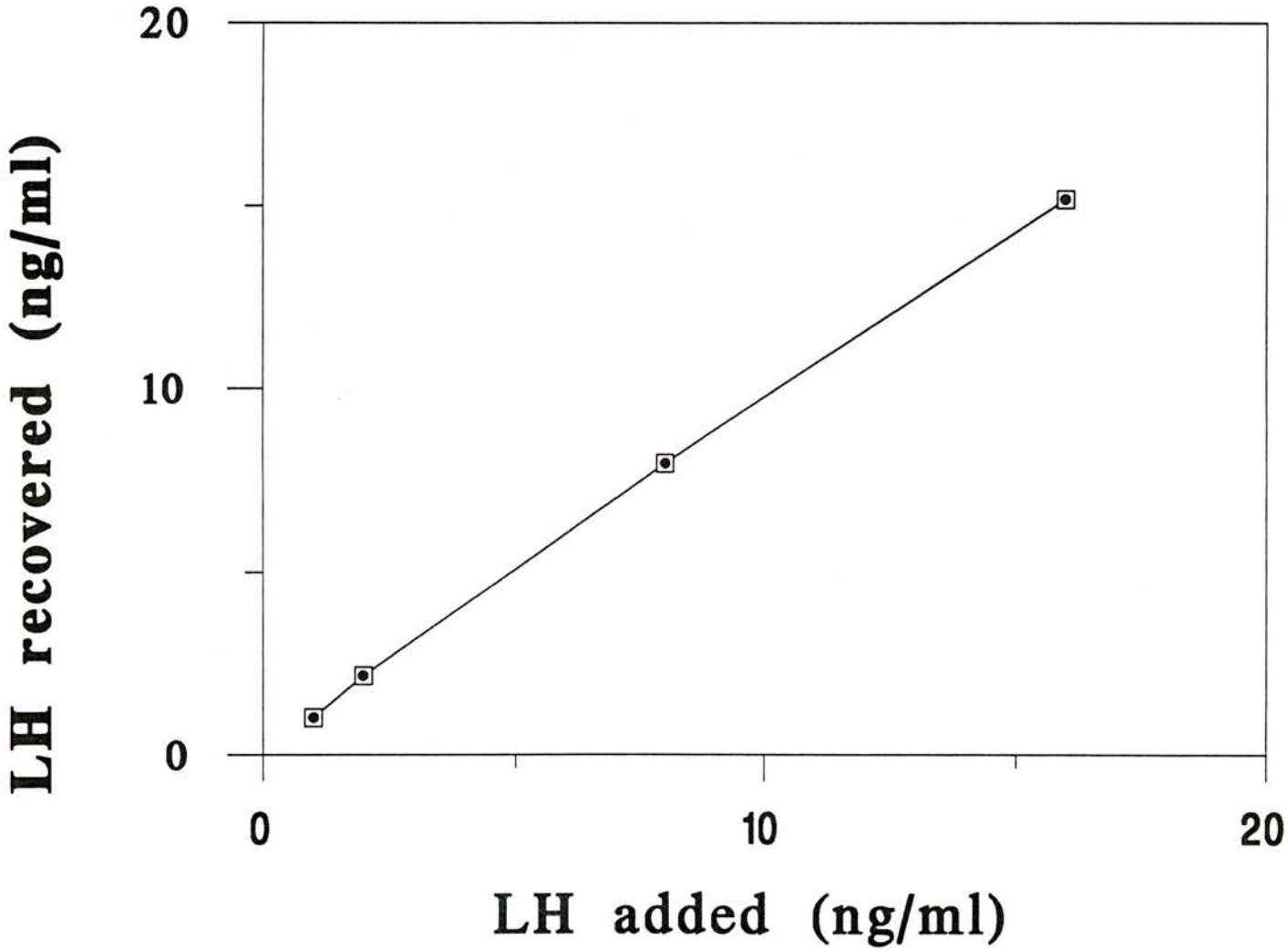

90
80
70
60
50
40
30
20
10
0
B/Bo
%B/Bo
Serial dilution
.1
1
10
100
1000
LH concentration (ng/ml)

Figure 2.2 Quantitative recovery of known amounts of LH added to anoestrous goat plasma


20
10
0
0
10
20
LH recovered (ng/ml)
LH added (ng/ml)

2.3.4 Follicle stimulating hormone (FSH)

FSH concentrations were determined in plasma samples by the double-antibody RIA using the techniques described by McNeilly *et al.*, (1976). Purified ovine FSH (NIDDK-oFSH-I-1) was used for iodination, and (NIDDK-FSH-RP-1) for preparation of reference standards. The antiserum was NIDDK-anti-oFSH-1 diluted in assay buffer (0.1% BSA) to an initial dilution of 1:40,000. Specificity of the antiserum in terms of its reactivity (relative potency) in RIA with pituitary hormones was as follows: oFSH NIDDK-FSH-RP-1 = 1, oLH NIAMDD-23 = 0.00167, oPRL AFP-4328C = 0.00001, oGH AFP-5285C = 0.00027, 8-Arg Vasopressin = 0.00003, hACTH = 0.00001.

Iodination of the FSH was carried out by the addition of 5µl sodium iodide (sodium iodide: Amersham International Plc., Buckinghamshire England), 10µl lactoperoxidase and 10µl freshly prepared hydrogen peroxide to 50µl of FSH (50µg/µl) in a reaction vial. The vial was reacted on a vortex for 20 seconds. Cysteine hydrochloride (10µl) and 1% BSA/PBS (1ml) were added and the vial reacted on a vortex for 10 seconds. The mixture was pipetted onto a sephadex G 100 column. Approximately 1ml of the mixture is collected in tubes from the bottom of the column. The head of the column was maintained with 0.1% BSA/PBS once the head had reached the bed-line. All tubes were counted and those with peak counts were retained.

Standard solutions (150µl) ranging from 0.5 to 100ng/ml and samples (150µl) were added to 300µl assay buffer. Antiserum (100µl) was added and the tubes were incubated for 24 h. ^{125}Iodine-labelled FSH (100µl - approximately 12,000cpm) was then added and the tubes were incubated for a further 48 h at 4^{o}C. Normal rabbit serum (NRS: diluted 1:400); (100µl) and donkey anti-rabbit serum (DARS: diluted 1:64); (100µl) were added. The tubes were incubated overnight at 4^{o}C. One millilitre

0.9% saline was added and the tubes were centrifuged at 2000g for 30 minutes at 4°C. The supernatants were decanted, the tubes were dried and the precipitates were counted on a gamma counter.

The sensitivity of the assay was 0.5ng/ml and intra- and inter-assay coefficients of variation were 9.6 and 12.8% respectively. Concentrations of FSH recovered following spiking of plasma samples were highly correlated with the known quantities added ($r = 0.997$, $P = 0.000$). Graph plots of gonadotrophin recovered versus gonadotrophin added were nearly straight lines (Figures 2.3).

2.3.5 Progesterone

Radioimmunoassy to determine progesterone concentration was performed using a kit (RSL ^{125}I progesterone, ICN Biomedicals, Inc. Thames, UK) according to the manufacturers instructions. The progesterone kit standards (ranging from 0 - 50 ng/ml) have been prepared from a human serum-based matrix. All reagents were brought to room temperature prior to use. Diluent buffer (phosphate buffer saline, pH 7.6 containing rabbit gamma globulin and 0.1% sodium azide - 500µl) was added to tubes numbered 1 and 2. Progesterone kit standard (0ng/ml - 100µl) was added to tubes numbered 1, 2, 3 and 4. Progesterone kit standards (ranging from 0.2 to 50ng/ml - 100µl), goats progesterone standards prepared in anoestrous goat plasma (ranging from 0 to 50ng/ml) for comparison with kit standards, controls (100µl) and samples (100µl) were added to their respective tubes in duplicate. ^{125}I-Progesterone (200µl) was added to all tubes and progesterone antiserum (500µl) was added to all tubes except 1 and 2. All tubes were reacted on a vortex and incubated at 37°C for 60 minutes. Precipitant solution (goat anti-rabbit gamma globulin and polyethylene glycol - 500µl) was added to all tubes and the contents mixed on a vortex. The tubes were

Figure 2.3 Comparison of a standard curve for FSH and dilution-response curve of a goat plasma sample of known concentration

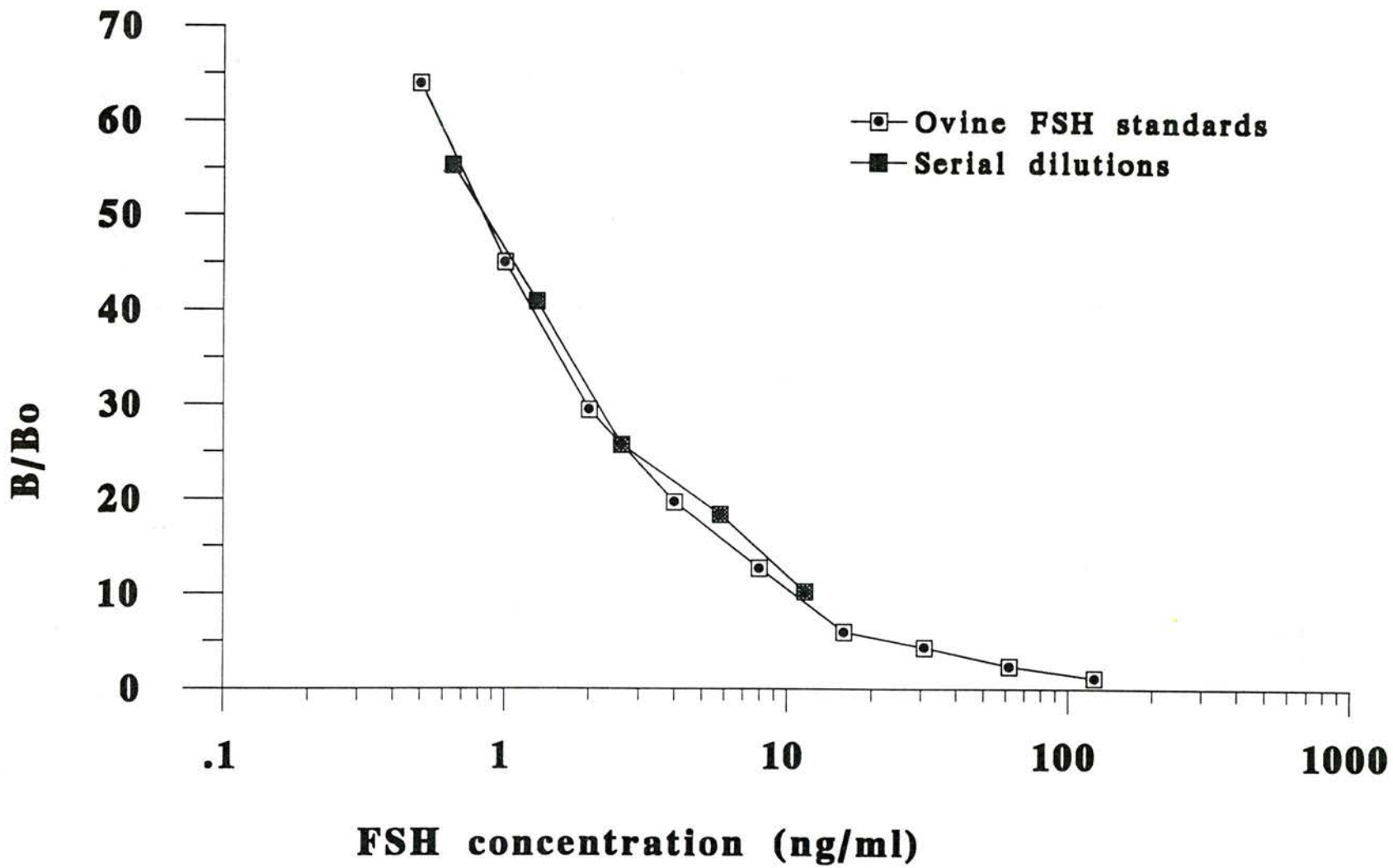
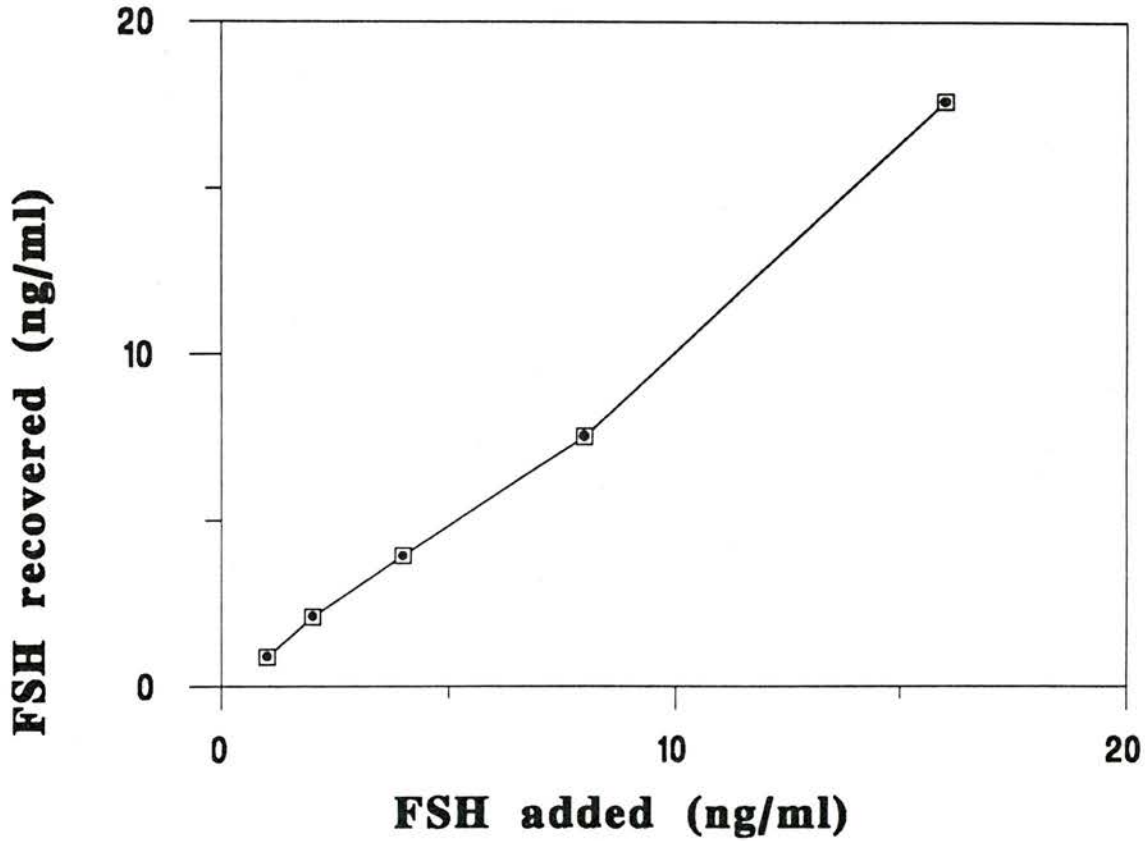

70
60
50
40
30
20
10
0
B/Bo
.1
1
10
100
1000
FSH concentration (ng/ml)
Ovine FSH standards
Serial dilutions

Figure 2.4 Quantitative recovery of known amounts of FSH added to anoestrous goat plasma


20
10
0
FSH recovered (ng/ml)
0
10
20
FSH added (ng/ml)

centrifuged at 1000*g* for 20 minutes at 4°C. The supernatants were decanted, the tube were blotted and the precipitates remaining in the tubes were counted on a gamma counter (CliniGamma: Pharmacia, Turku, Finland).

The sensitivity of the assay was 0.3ng/ml and intra- and inter-assay coefficients of variation were 14.6 and 20.8%, respectively. The regression of the curve obtained from the kit's progesterone standards (y = 21.5 - 0.214x) and that of prepared goat standards (y = 21.5 - 0.232x) were significantly correlated (P = 0.99); (Figure 2.5).

2.3.6 Assay calculations

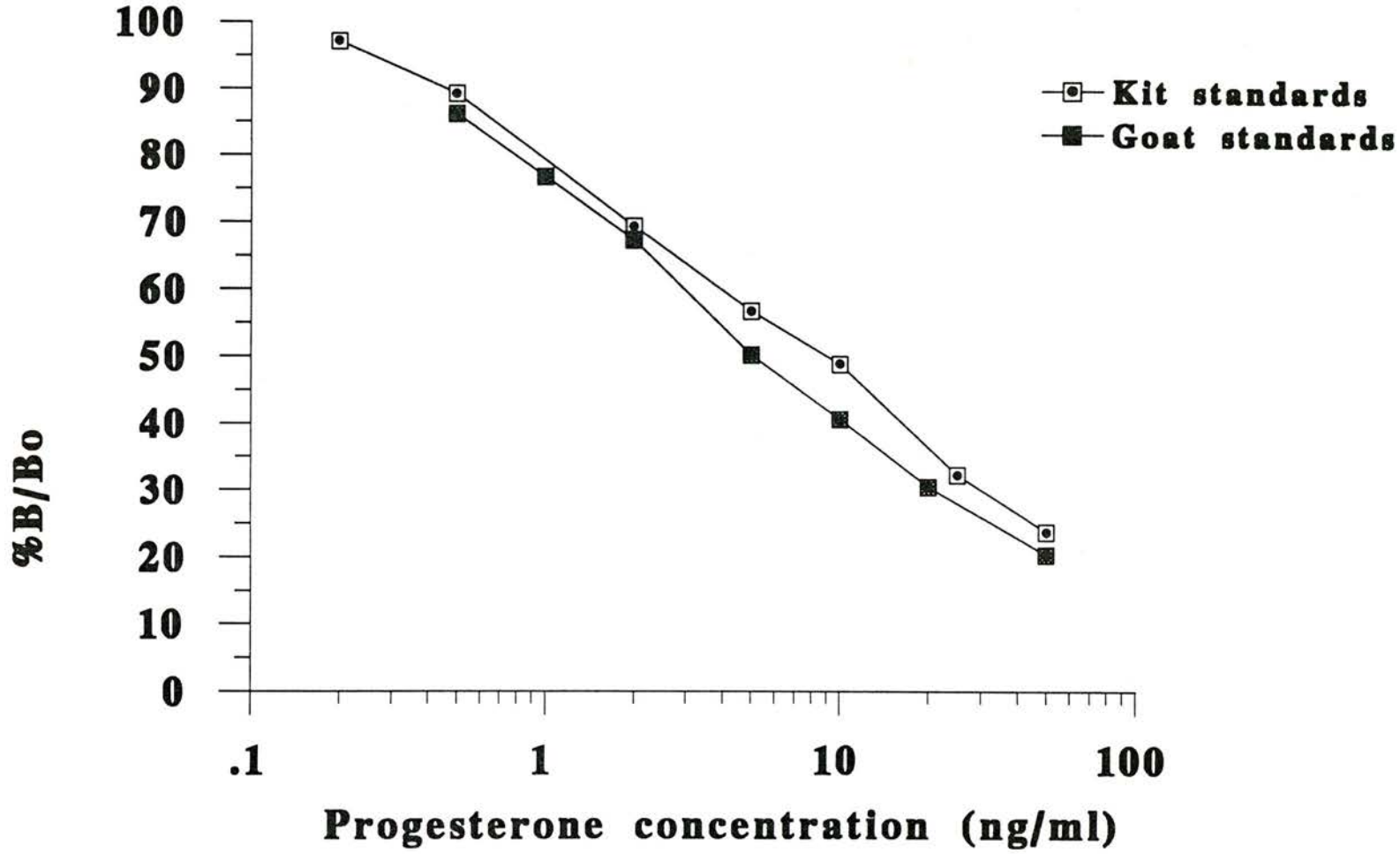
All radioimmunoassays were analysed using the Super Serial Card programme of Apple II computer (Apple Computer Inc., Cupertino, California). Assay sensitivities, within- and between-assay coefficients of variation for each hormone were calculated from all individual assays for that hormone.

2.4 Enzymeimmunoassay (EIA)

2.4.1 Progesterone

Plasma concentration of progesterone was determined by enzyme immunoassay using progesterone EIA kits for plasma (Ridgeway Science Ltd., Gloucestershire, U.K.) and the procedure described by Boland *et al.*, (1985). Standards ranging from 0 to 20 ng progesterone/ml were prepared by addition of progesterone to anoestrous goat plasma. The wells of the microtitre plates were already lined with antiserum, the cross-reactivity of which has been described previously (Sauer, *et al.*, 1982). Standards (10µl) or plasma (10µl) were added to individual wells in duplicate. Progesterone-enzyme label in PAS-gelatine buffer (Phosphate/azide/saline/gelatin, 200µl) was then

Figure 2.5 Comparison of progesterone RIA kit standard curve and goat plasma progesterone standard curve


100
90
80
70
60
50
40
30
20
10
0
%B/Bo
.1
1
10
100
Progesterone concentration (ng/ml)
Kit standards
Goat standards

added and the plates were left for 2 hours at room temperature. The plates were washed 2 times in PAS-gelatine buffer and once in deionised water. Alkaline phosphatase substrate in DEM (diethanolamine/magnesium chloride/azide-200µl) was added and the plates were left for 30 minutes at room temperature. Optical density of the wells was measured and concentrations were computed from standard curves. The results were expressed as ng progesterone/ml of plasma. The limit of detection of the assay was 0.1ng/ml. The intra-assay and inter-assay coefficients of variation were 3.72% and 22.64%, respectively. Figure 2.6 shows representative progesterone standard curve using progesterone EIA kit.

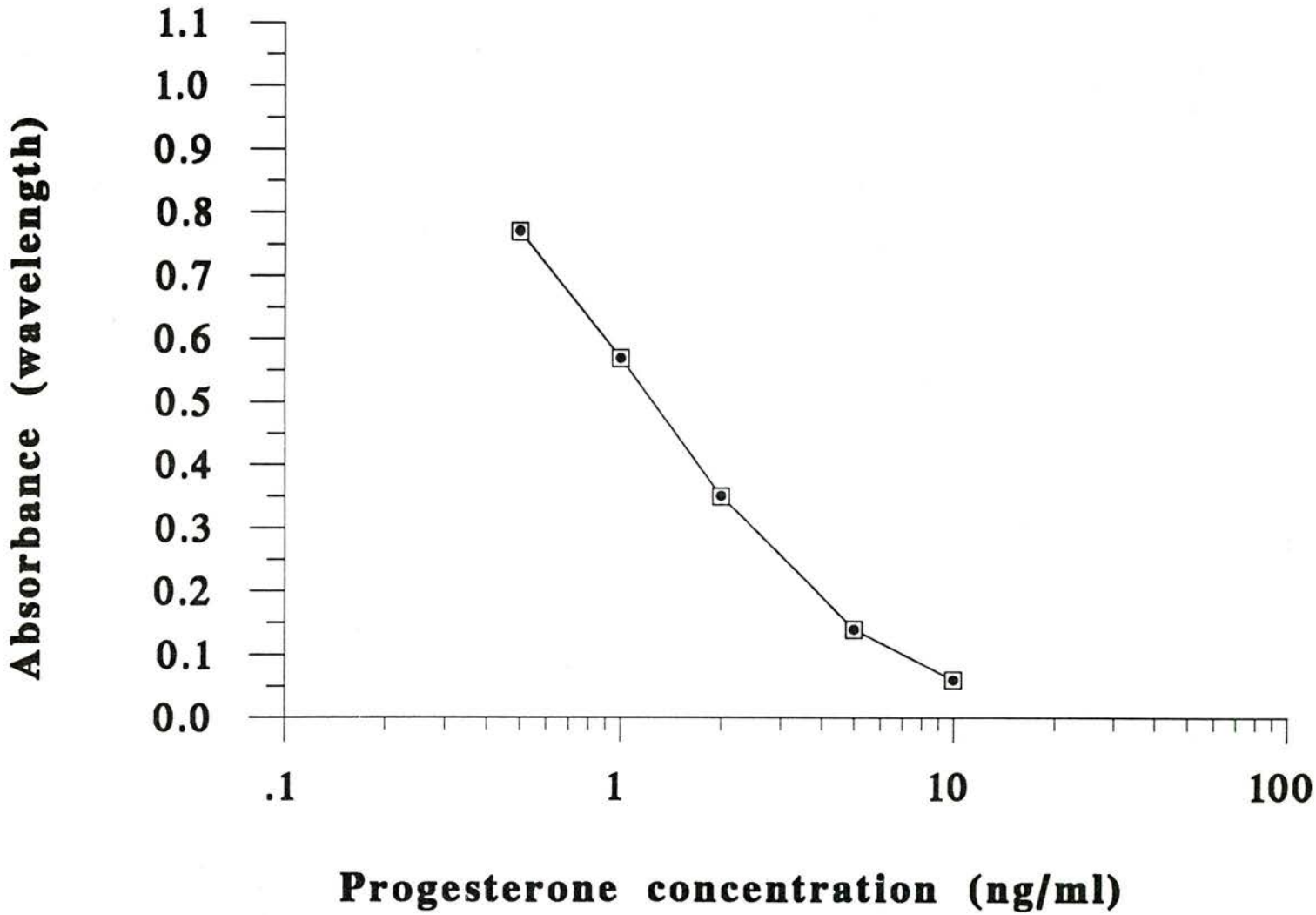
2.5 Light microscopy

Corpora lutea samples for light microscopy were collected at slaughter. The sections were fixed in Bouins solution and submitted for haematoxylin and eosin staining. Cellular counts and measurements were carried out under the light microscope.

2.6 Electron microscopy

Corpora lutea for transmission electron microscopy were collected at slaughter, cut into several pieces of approximately 1 mm^3 and placed in bijou bottles containing 3% gluteraldehyde in cacodylate buffer at 4^oC. The samples were sent for processing on the same day. The processed sections were viewed under the electron microscope.

Figure 2.6 Representative progesterone standard curve using progesterone EIA kit


Absorbance (wavelength)
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
.1
1
10
100
Progesterone concentration (ng/ml)

2.7 Gonadotrophin binding assay

Corpora lutea samples for gonadotrophin binding assays were collected at slaughter, frozen in liquid nitrogen and stored at -20°C until required. LH binding to the goat CL was determined as described by Bramley *et al.,* (1987). In brief, the CL tissue was weighed and minced with scissors. One millilitre homogenizing buffer [0.3M sucrose, 1mM EDTA, 10mM Tris-hydrochloride (Tris-HCl), pH 7.4] was added per 100g tissue. A Polytron (Kinematica, Lucerne, Switzerland) homogeniser was used to mince the tissue. The homogenate was filtered through 4 layers of cheesecloth and 2ml aliquots were stored at -20°C. For the assay, 3 total binding (Bo), 2 non-specific binding (NSB) and 6 total counts tubes were included. BSA 5% (Sigma A-2425 made up in 40mM Tris-HCl, pH 7.4 - 100µl) and assay buffer (40mM Tris-HCl, pH 6.5 - 650µl) were added together into assay tubes. Human LH tracer (Chelsea Reagent, specific activity = 100µCi/µg, diluted in assay buffer so that 100µl gave 100,000 cpm - 100µl) and 10µl goat CL homogenate were added and the total volume in the tubes adjusted to 1ml with assay buffer. Human chorionic gonadotrophin (Chorulon: Intervet, Cambridge, UK. - 50iu - 100µl) was added to NSB tubes. The tubes were reacted on a vortex and incubated at room temperature (20°C) overnight (16h). Separation was performed on ice by adding 0.5ml 0.5% IgG (Globulin, Sigma G-5009 made up in 40mM Tris-HCl, pH 7.4) followed immediately by 1ml 25% PEG. The tubes were mixed vigorously on a vortex and centrifuged at 3000*g* for 30 minutes. The supernatants were aspirated and the pellets were counted in a gamma counter. Specific binding was calculated by subtracting average NSB counts from average Bo counts. DNA concentration of the luteal tissue homogenate was measured by a modified method of Setaro and Morley (1976). The results of the hormone binding assays were expressed as picograms (pg) of [I^{125}]-labelled hormone bound specifically per milligram of DNA.

2.8 Statistical analyses

All arithmetic means were presented as ± S.E.M. Depending on the type of data, Student's *t* test, Chi square test and variations of ANOVA and non-parametric analyses were used to test statistical differences between treatment groups. Correlations between parameters were determined by regression analyses. Differences were regarded as significant when $P<0.05$. LH pulses and their characteristics were analysed by the Hormone Pulse-Profile Analysis programme (Elsevier Biosoft, Cambridge, UK) using the regional coefficient of variation algorithm as described previously by Veldhuis *et al.*, (1986). A pulse was preceded by a rise which exceeded the 'rise threshold' (expressed as a multiple of the regional coefficient of variation) and followed by a fall which exceeded the 'fall threshold' (again expressed as a multiple of the regional coefficient of variation).

All statistical analyses were performed using either the Minitab statistical software (University of Lancaster, U.K.) or Genstat 5 (copyright 1990, Agricultural Trust, Rothamsted Experimental Station).

CHAPTER 3

The effects of undernutrition on the onset of oestrus, ovulation rate and embryo survival and development following synchronization of oestrus and superovulation in goats

3.1 Introduction

The concept of "flushing" or increasing the level of feeding in the period prior to mating as a means of improving kidding rates has long been known and practised in goats (Economides and Louca, 1981). In ewes, ovulation and lambing rates have been shown to be affected by the premating food intake (Gunn *et al.*, 1979a; Rhind *et al.*, 1985) as well as on body condition at mating (Gunn *et al.*, 1979a; Gunn, 1983). By contrast, poor body condition has been associated with delay or suppression of oestrus and with a high return-to-service rate in the same species (Gunn and Doney, 1975 & Gunn *et al.*, 1979a).

Reports of the effects of low level of nutrition on oestrus onset, ovulation rate and embryo loss in goats are not readily available. Workers in India have shown that a higher percentage of multiple births occurred in does in winter than in summer and this has been attributed to better nutrition during the monsoon (Prasad *et al.*, 1972). Significantly poorer kidding rates have been reported in does on low energy intake (Singh and Sengar, 1970) and provision of a high energy/medium protein diet has been shown to increase kidding rates (Sachdeva *et al.*, 1973). Wentzel, (1987) has suggested that inadequate nutrition could decrease pregnancy rate in does probably as a result of conditions such as anovulation or retarded ovulation.

Experiment 1 of this study was carried out to provide data on the effects of a low level of feeding on the onset of oestrus following synchronisation with $PGF_{2\alpha}$, on ovulation rate and on embryo loss in early pregnancy in goats. Experiment 2 was conducted to provide information on the effect of undernutrition on ovulation rate and ovum development following multiple ovulation.

3.2 Materials and methods

3.2.1 Experiment 1- The effects of undernutrition on the onset of oestrus, ovulation rate and embryo survival in goats

3.2.1.1 Animals and experimental procedure

Forty multiparous, nonlactating, British Saanen and Toggenburg does aged between 2 and 3 years, were chosen from a stock of 59 for the purpose of the experiment. The does chosen had all attained a body score of approximately 2. The goats were individually penned in a large shed throughout the study. Oestrus was synchronized in the early breeding season (September to October) of 1990 by the insertion of intravaginal sponges containing 45 mg fluorogestone acetate (Chronogest, Intervet U.K. Ltd., U.K.) for 17 days. Onset of oestrus was detected by exposure of the does to vasectomized bucks every 4 hours after removal of sponges. The does were then randomly allocated to 2 groups. From Day 0 (first day of oestrus), the does in one group (Group R, n = 24) were fed on rations calculated to provide 25% of their metabolic energy and digestible crude protein requirements for maintenance (National Research Council, 1981) until 25% of their initial body weight was lost. Thereafter these does were fed to maintain their new weight. The other group (Group M, n = 16) was fed on rations providing 100% of their daily requirements. All does were weighed weekly throughout the duration of the study. Sixteen days after onset of oestrus, 10

mg $PGF_{2\alpha}$ (Lutalyse, Upjohn Ltd., Crawley, U.K.) was administered as a single intramuscular injection to all does. Onset of oestrus was determined as described earlier. The does were mated every 4 hours with 2 different Saanen bucks throughout the duration of oestrus. Ovulation rate was determined by counting CL at laparoscopic examination performed 6 to 10 days after the onset of oestrus. All does were slaughtered by stunning and exsanguination between 57 and 65 days after mating, and the foetuses were counted and measured.

3.2.2 Experiment 2- The effects of undernutrition on ovulation rate and ova development following multiple ovulation with porcine FSH

3.2.2.1 Animals and experimental procedure

Nine British Saanen goats from the same stock as those in experiment 1, and sharing similar body conditions were divided into 2 groups. Oestrus was synchronized with progestagen sponges as in experiment 1. From the day of sponge insertion does in the 2 groups were fed on the same rations respectively as the 2 groups in experiment 1. Commencing from Day 16, a day before removal of sponges, the does were injected intramuscularly twice daily with 22mg porcine follicle stimulating hormone (pFSH; Sigma Chemical Company, St. Louis, U.S.A.) divided into 4 descending doses. The does were hand mated to different Angora bucks at 6 hourly intervals throughout the duration of the ensuing oestrus. The does were slaughtered by stunning and exsanguination at Day 6 after oestrus. The uterine horns were immediately exteriorised and ova were flushed out by a technique described previously (Armstrong *et al.*, 1983a). The ova were studied under the microscope and the unfertilized ova, morulae, and blastocyst stages were counted.

3.2.3 Statistical analyses

Data for the time from administration of $PGF_{2\alpha}$ or removal of progestagen pessaries to onset of oestrus, ovulation rates and potential kidding rates were analyzed using the Student's t-test while the rest of the data were analyzed using the Chi-square test. All means are given as ± SEM. The small number of does involved in Experiment 2 precluded statistical comparisons between the two groups.

3.4 Results

3.4.1 Experiment 1

3.4.1.1 Liveweight and body condition changes

Does fed the restricted diet lost a significant amount of body weight during the experiment (Figure 3.1). Mean body condition score also went down from 1.87 ± 0.044 at the beginning, to 1.08 ± 0.059 at the end of the experiment in this group (Figure 3.2).

3.4.1.2 Oestrus onset

Thirty-nine of 40 does (97.5%) came into oestrus within 96 hours following the withdrawal of intravaginal progestagen sponges. The average time of onset of oestrus was 54.2 ± 2.06 hours. One doe in Group M did not exhibit oestrus until 9 days later and was not included in the calculation of the mean. The proportion of does in oestrus within 96 hours of $PGF_{2\alpha}$ administration (Figure. 3.3) was lower in Group R than in Group M (71.0% and 87.5%, respectively), however the difference was not significant

Figure 3.1 Mean weekly liveweights of does on maintenance and restricted feeding throughout the period of controlled feeding

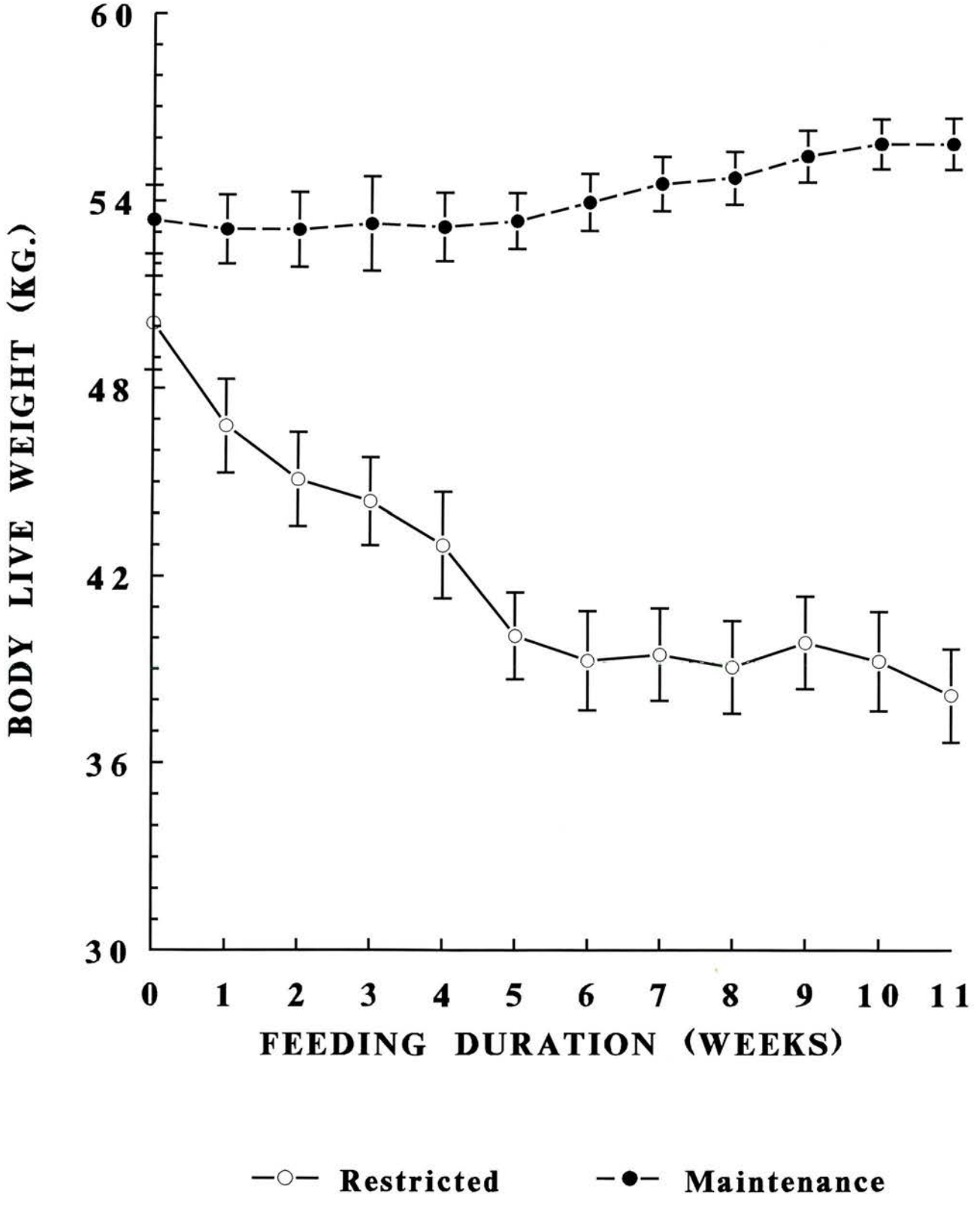
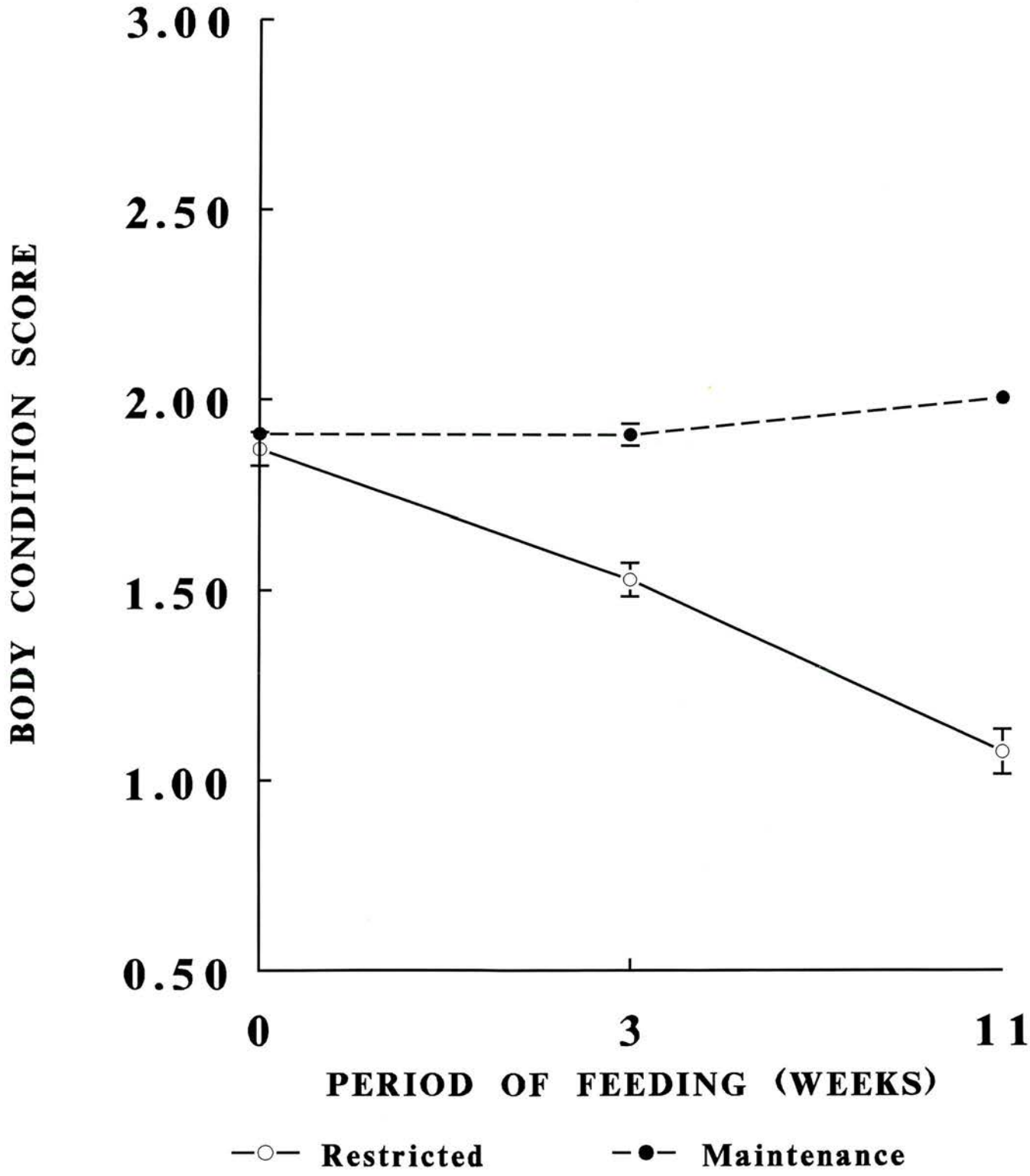

60
54
48
42
36
30
BODY LIVE WEIGHT (KG.)
0 1 2 3 4 5 6 7 8 9 10 11
FEEDING DURATION (WEEKS)
Restricted
Maintenance

Figure 3.2 Mean body conditions of goats on maintenance and restricted feeding from the beginning to the end of the experiment


3.00
2.50
2.00
1.50
1.00
0.50
BODY CONDITION SCORE
0
3
11
PERIOD OF FEEDING (WEEKS)
Restricted
Maintenance

(P=0.5). The length of time from $PGF_{2\alpha}$ administration to the onset of oestrus tended to be longer (P=0.12) in Group R (62.4 ± 4.13 hours) than in Group M (54.6 ± 2.7 hours). Two does in Group R and the remaining two in Group M came into oestrus later than 96 hours and were mated, while 5 does in Group R showed no overt signs of oestrus and were not mated.

3.4.1.3 Ovulation rate

The number of ovulations per doe, the ovulation rate and the side of the ovary involved are shown in Table 3.1. Ovulation occurred in all the does in Group M and in 83.5% of those in Group R. Seventy-one percent and 50% of twin ovulations in Groups R and M, respectively, occurred one on each ovary, while 28.6% and 50.0% occurred on the same ovary. Four does (16.7%) in Group R that did not show overt signs of oestrus after $PGF_{2\alpha}$ were found to have ovulated. Two does in Group R that showed signs of oestrus did not ovulate, while 2 others in the same group neither showed signs of oestrus nor ovulated. No significant difference was found in the overall ovulatory activity between the left and right ovary within each group.

3.4.1.4 Pregnancy rate and embryo loss

The proportion of does pregnant at slaughter, potential kidding rate and the proportion of embryos lost are shown in Table 3.2. One of the does in Group M showed evidence of a foetus lost after implantation, which was represented by a haemorrhagic remnant surrounded by a zone devoid of cotyledons with 2 other viable foetuses. Embryos from single ovulations were lost in Group R but not in Group M. Fewer embryos were lost in does with multiple ovulations in Group R than in Group M, although the difference was not significant (P<0.5). More does with single ovulations failed to get pregnant than those with multiple ovulations in Group R

Figure 3.3 Period of time from administration of $PGF_{2\alpha}$ to first standing oestrus in does on maintenance or restricted rations

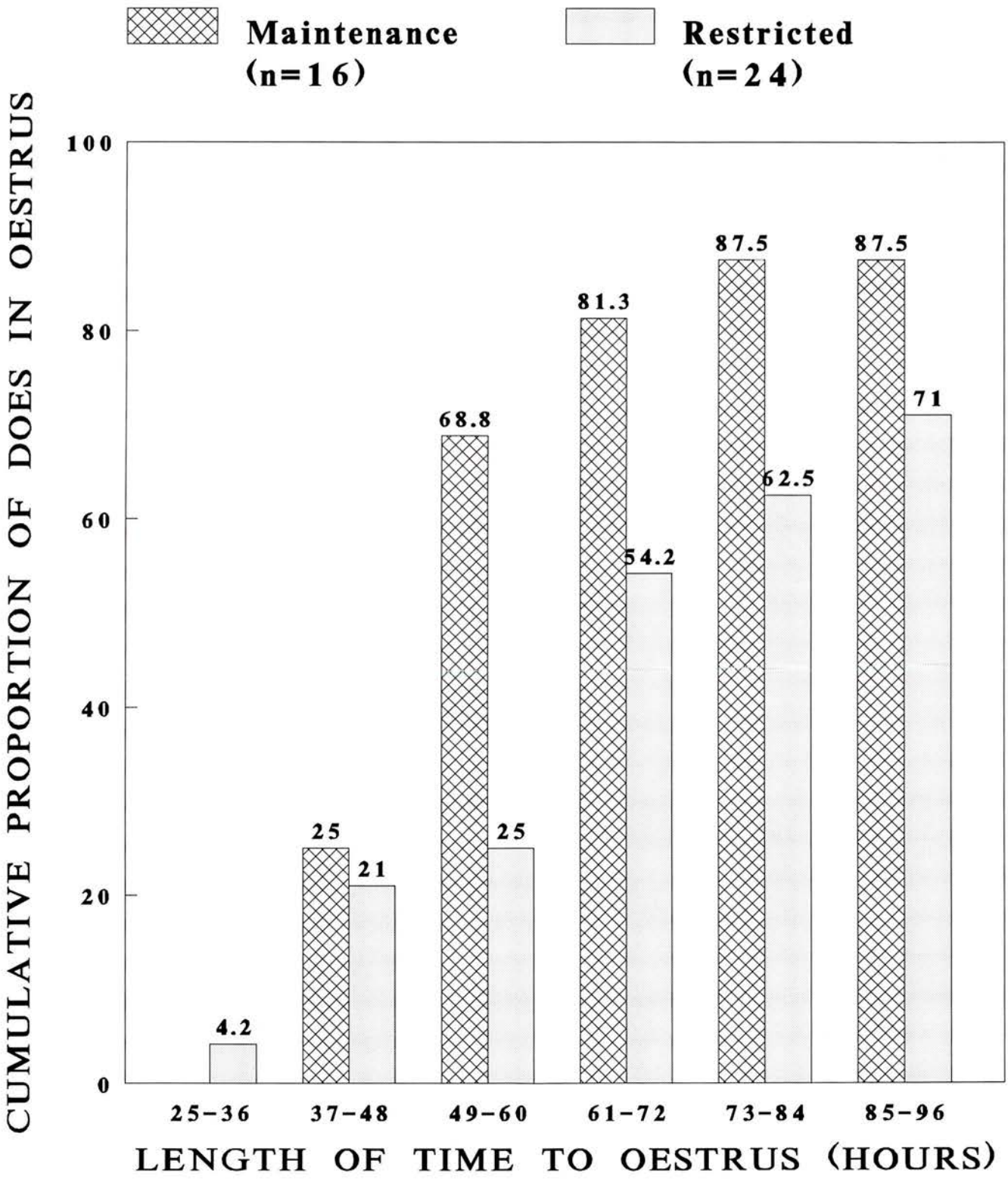

Maintenance
(n=16)
Restricted
(n=24)
CUMULATIVE PROPORTION OF DOES IN OESTRUS
100
80
60
40
20
0
4.2
25
21
68.8
25
81.3
54.2
87.5
62.5
87.5
71
25-36
37-48
49-60
61-72
73-84
85-96
LENGTH OF TIME TO OESTRUS (HOURS)

Table 3.1 Ovulation rate[a] and side of ovary involved in goats on maintenance and restricted rations

Feeding level	Maintenance	Restricted	P
Total no. of does	16	24	
No. of ovulations			
0	0 (0.0%)	4 (16.7%)	<0.1
1	2 (12.5%) (R=2)	12 (50.0%) (R=6) (L=6)	<0.025
2	12 (75.0%) (RR=3) (LL=3) (RL=6)	7 (29.2%) (RR=1) (LL=1) (RL=5)	<0.005*
3	2 (12.5%) (RRL=2)	1 (4.2%) (RLL=1)	
Total no. of ovulations	32	29	
Ovulation rate (O.R.)	2.00±0.13	1.21±0.16	<0.0005
O.R. Right ovary	1.13±0.18	0.58±0.12	=0.018
O.R. Left ovary	0.88±0.18	0.63±0.13	=0.27

R= CL on the right ovary.
L= CL on the left ovary.
[a]Ovulation rate was calculated from the total number of corpora lutea divided by the number of does in each group.
* = 2 and 3 ovulations combined.

($P<0.05$). Similarly, the proportion of embryo loss was higher ($P<0.01$) in single than in multiple ovulations in the restricted group. Embryo loss from twin ovulations were not different in the two groups whether the ovulations occurred on the same ovary or on different ovaries.

3.4.1.5 Embryo migration

Embryo migration (i.e. when a corpus luteum was found in one ovary and the foetus in the opposite uterine horn) occurred in 33.3% of does in Group R and in 41.7% of those in Group M. This migration occurred in both groups in all does with twin ovulations on one ovary. No migration was observed in either group in does with single ovulations.

3.4.2 Experiment 2

Only 5 out of 9 goats responded to superovulation with pFSH. There were no differences in the mean ovulation rate, ova recovery rate and the proportions of ova that were unfertilized, in morula or in blastocyst stages of development between Group M and Group R does (table 3.3).

Table 3.2 Proportion of does pregnant at slaughter, potential kidding rates and embryo losses in goats on maintenance and restricted rations

Feeding level	Maintenance	Restricted	P
No. of does mated	13	18	
Total no. of ova shed (ov. rate)	25 (1.92)	24 (1.33)	=0.015
No. of does which failed to ovulate	0	4	
No. and proportion of does pregnant[a]	12 (92.5%)	9 (50.0%)	<0.025
Percent pregnant of those which ovulated	92.5	64.3	
Total no. of foetuses (as % of ovulations)	19 (76.0%)	16 (66.7%)	
Potential kidding rate per doe mated[b]	1.46±0.18	0.87±0.24	=0.069
Potential kidding rate per doe pregnant at slaughter[c]	1.58±0.15	1.78±0.22	=0.48
Ova not represented by foetuses at slaughter (embryo loss)	6 (24.0%)	8 (33.3%)	<0.5
Embryo loss (left ovary)	2 (16.7%)	5 (38.5%)	<0.5
Embryo loss (right ovary)	4 (30.8%)	3 (27.3%)	<0.9

[a]Proportion of does mated which were pregnant at slaughter.
[b]Total number of foetuses divided by number of does mated.
[c]Total number of foetuses divided by the number of does pregnant at slaughter.
Data from 6 does in Group R and 3 does in Group M were not included due to failure to exhibit oestrus or to unexpected deaths.

Table 3.3 **Effect of undernutrition on superovulation with pFSH in goats (M = Maintenance ration, R = Restricted ration)**

Feeding level	Group M	Group R
Number of does that responded	3/5	2/4
Total number of ovulations	39	30
Mean ovulation rate in responders	13.0 ± 1.0	15.0 ± 4.0
Number of ova recovered (%)	30 (77)	27 (90)
Ova recovery per doe	10.0 ± 0.0	13.5 ± 0.5
Morulae (% of total)	24 (80)	22 (81.5)
Blastocysts (% of total)	5 (16.7)	4 (14.8)
Unfertilized (% of total)	1 (3.3)	1(3.7)

3.5 Discussion

The changes in mean live-weight and body condition score recorded in this study indicated that nutritional status of the does on the restricted ration was substantially different from that of does on the maintenance ration. The oestrus response of the does in this study to treatment with intravaginal progestagen-impregnated sponges (97.5% of does) and to $PGF_{2\alpha}$ administration (87.5% in Group M and 71.0% in Group R, respectively) supports the previous reports of the efficiency of these drugs as oestrus synchronizing agents (Bretzlaff *et al.*, 1983; Ritar *et al.*, 1984). The length of time from sponge withdrawal to onset of oestrus (54.2 + 2.06 hours) was slightly longer than in previous observations of 16 to 48 hours (Corteel, 1977; Bongso *et al.*, 1982) but it was shorter than the 67.6 hours reported in Boer goats (Greyling *et al.*, 1985). Although not statistically significant, a higher proportion of does on restricted rations failed to exhibit any sign of oestrus following $PGF_{2\alpha}$ administration, and those that did tended to do so later than those on maintenance ration. The observation that four of the does that did not show signs of oestrus actually ovulated indicated that silent ovulation is a feature of undernutrition in goats. Similar findings have been reported in sheep, in which delay or suppression of oestrus is associated with poor body condition (Gunn and Doney, 1975 and Gunn *et al.*, 1979a).

These results indicate that a low level of nutrition with a drop in body weight and condition in goats was accompanied by a significant reduction in mean ovulation rate, in the occurrence of multiple ovulations and in the proportion of does pregnant. These findings are consistent with the observed poorer kidding rate in does on low energy intake (Singh and Sengar, 1970) and a higher kidding rate and larger percentage of multiple births in does on good nutrition than those on low nutrition (Prasad *et al.*, 1972; Sachdeva *et al.*, 1973). The results are also similar to those on the effects seen in sheep (Gunn and Doney, 1975; Gunn *et al.*, 1979a). The ovulation rates

following oestrus synchronization with $PGF_{2\alpha}$ in the does on maintenance ration in this study were similar to those reported in dairy goats (Ott *et al.*, 1980; Mori and Kano, 1984).

Even though one doe was observed with a haemorrhagic remnant in the uterine horn, suggesting a loss of a foetus after the process of implantation had started, the absence of foetal and/or placental remnants in the rest of the does suggests that the losses in these does occurred before implantation. This observation supports the report in sheep (Robinson, 1986) that because of the very low nutrient requirements of embryos at this early stage, only extreme nutritional regimens affect their survival.

There are very few reports of the effects of feed restriction on embryo loss in goats. In sheep, significant embryo loss was reported when feed was restricted from 2 weeks before mating, while no evidence of an effect was found when feed was restricted from a day after mating (Parr and Cumming, 1982). It has been suggested that because of their lower mean ovulation rate, sheep on restricted feeding should normally have a lower overall incidence of ova wastage than those on normal feeding (Edey, 1969). In this study, however, embryo loss tended to be high in feed-restricted does in addition to the low ovulation rate. The figures of embryo loss recorded in this study were higher than those reported by others (Taneja, 1959; Lyngset 1968a). However, in these latter studies the figures were probably low because they were derived from goats slaughtered at the abattoir and comprised only those where the goats were pregnant with at least one foetus, but where the number of CL surpassed the number of foetuses. The cases where a single ovulation had occurred and the embryo was lost were therefore not recorded.

The findings in the feed-restricted group of this study contrasted with most reports in goats and sheep, where a greater embryo loss from multiple than single-shed

ova was reported (Lyngset 1968a; Gunn *et al.*, 1979b). Previous reports in goats of a tendency for 1 embryo to die when 2 follicles have ovulated from the same ovary (Lyngset 1968a), and of a higher loss in twin ovulations in the right than in the left ovaries (Casida *et al.*, 1966; Lyngset 1968a) were also not confirmed by this study.

A marked tendency was observed in the present study for one or more embryos to migrate to the opposite uterine horn when more than one ovum was shed from an ovary. This phenomenon, also referred to as transuterine migration, has been reported to occur in all domestic animals but rarely in the cow (Boyd *et al.*, 1944). The migration did not appear to be affected by level of nutrition. A frequency of transuterine migration ranging from 7 to 41% in single pregnancies has been reported (Taneja, 1959; Basu *et al.*, 1961; Lyngset, 1968b) however none was recorded in this study. Studies in sheep have indicated that the migration does not appear to be a cause of embryonic mortality (Abnes and Woody, 1971); however, the efficiency of the spacing of embryos could influence subsequent placental size and thereby foetal growth. Migration has been reported to occur around Day 14 of pregnancy in sheep (Gadsby *et al.*, 1980), which coincides with the phase of trophoblastic enlargement with a possible exertion of some form of repulsion between embryos.

The consequence of poor ovulation rate and higher embryo loss is reduced kidding rate. Other detrimental effects of embryo loss include the birth of smaller than average kids. This may occur when embryos die during implantation and the surviving embryo(s) fail to utilize the maternal cotyledons vacated by those that died (Rhind *et al.*, 1980).

The low response of the does to superovulation with pFSH in Experiment 2 could probably be due to stress associated with change of housing and inclement weather, or the lateness in the breeding season (February/March) in which the

experiment was conducted. Of the does which responded, ovulation rate and ova recovery rate figures in the 2 groups of does were similar to those reported for healthy goats in studies elsewhere (Drost, 1986; Pendleton *et al.*, 1992; Rosnina *et al.*, 1992). The preliminary data suggested that undernutrition at the level applied had no effect on the response of does to superovulation with pFSH, fertilization and early development of the fertilized ova. This indicates that administration of FSH to the does had offset the detrimental effects of low level of feeding seen in Experiment one and suggests a role for FSH in the mediation of nutritional effects on ovulation rate in goats.

The exact mechanism by which nutrition affects reproduction in domestic animals is not fully understood. However, there is evidence in sheep that body condition may have a direct effect on hypothalamic and pituitary activity (Rhind *et al.*, 1989), while both body condition and level of food intake may affect gonadotrophin profiles and reproductive activity through indirect effects on hypothalamic/pituitary sensitivity to steroid feedback and/or inhibin (Rhind *et al.*, 1991). It is likely that changes in gonadotrophin profiles act together with other nutritionally controlled factors within the ovary to determine the pattern of follicular development and ovulation rate. The complex relationship between nutrition and embryo growth and survival may also depend on interactions between genotype, fecundity, degree of restriction of intake and, perhaps, the period of restriction (Rhind *et al.*, 1991).

In conclusion, low level of feeding in goats resulted in reduced reproductive performance through delay and suppression of oestrus; it also reduced the ovulation rate, incidence of twin and triple ovulations and the proportion of does that became pregnant. Preliminary data from this study suggest that low level of feeding for 17 days prior to mating had no effect on the superovulatory response to pFSH and on early development of the ova in goats.

CHAPTER 4

The effects of undernutrition on the components of the gravid uterus in goats.

4.1 Introduction

Low kid birth weights have been shown to lead to higher neonatal mortality rates than in heavier kids of the same breed (Bajhau and Kennedy, 1990). Absolute growth rates are also lower in those kids that survive than in heavier kids. Even though knowledge of the precise regulation of foetal growth rate has remained limited, the plane of nutrition and the size of the placenta are well recognized as major determinants, acting either singly or simultaneously (Mellor, 1983). The previous chapter demonstrated the detrimental effects of undernutrition on oestrus, ovulation rate, pregnancy rate and embryo survival in goats. Death of embryos during implantation has been shown to have a detrimental effect on birth weight as a result of the inability of the surviving foetus(es) to utilize the maternal cotyledons vacated by the embryo(s) that died (Rhind *et al.,* 1980), and as a result of a disturbance of the natural balance in the distribution of embryos between the two horns of the uterus.

In sheep, the general pattern of foetal growth under different nutritional conditions has been extensively studied (Robinson *et al.,* 1977; Mellor and Murray, 1981, 1982a and b; Parr *et al.,* 1982; Mckelvey and Robinson, 1986). Changes in foetal growth rate in ewes given a variety of nutritional treatments indicate a far greater foetal sensitivity to maternal underfeeding and to differences in placental size than had been suspected previously (Mellor, 1983). There is however a dearth of information in this field with respect to goats.

The aim of this experiment was to determine the effects of restricted feed intake in does from 19 days before mating to 60 days after mating on the size and weight of the foetus and other components of the gravid uterus. Because 80% of foetal growth takes place in the last 2 months of pregnancy, growth in early pregnancy has been regarded as having little relevance. Evidence has however been provided that events in this period are important in establishing foetal growth trajectories in late pregnancy (Mckelvey and Robinson, 1986). This study is therefore important because it will provide information on the impact feed restriction has during a stage of gestation that has not previously been examined in goats.

4.2 Materials and methods

4.2.1 Experiment 3

4.2.1.1 Animals and experimental procedure

Thirty-one of the does in Experiment 1, Chapter 2 that were mated following synchronization with $PGF_{2\alpha}$ were used for this experiment. Management and feeding of the does were described in Experiment 1. All mated does were slaughtered between 57 and 65 days after mating (mean = 61.8 $\pm$ 0.50 days). Following slaughter, the gravid uterus was removed and weighed. The ovaries with the corpora lutea (CL) were removed, weighed and dissected, and the CL were counted. Each horn of the uterus was opened through an incision along the dorsal length. The foetal cotyledons were carefully separated from the maternal caruncles by gentle traction and the placentomes were counted. The foetal membranes were punctured and the fluids drained and weighed. The umbilical cord was ligated and cut. The foetus was removed and blotted. The weight, crown-rump length, curved crown-rump length and the head length of the foetus were measured and the sex was recorded. The foetal membranes plus

cotyledons were weighed, the foetal cotyledons were clipped from the membranes and each was weighed separately. The weight of the empty uterus was obtained by calculating the difference in weight between the gravid uterus and the foetus, foetal fluids, and the foetal membranes plus cotyledons combined.

4.2.1.2 Statistical analyses

Using a model-based approach it was possible to perform one analysis with all the foetal data taking into account the foetal number and gestational length and the fact that foetuses from the same doe are more alike than foetuses from different does. This is a "mixed model" analysis with feeding treatment having a fixed effect and doe a random effect. Covariates also having fixed effects were foetal number and gestational length. The parameters of this model were estimated using the Restricted Maximum Likelihood (REML) directory of Genstat 5 (copyright, 1990 Agricultural Trust, Rothamsted Experimental Station). All means are quoted ± SEM.

4.3 Results

4.3.1 Liveweight and body condition changes

Does on the restricted ration lost 24.1% of initial body liveweight and 39.4% of initial body condition, while those on maintenance ration gained 4.8% body weight and 5.6% body condition during the course of the experiment.

4.3.2 Pregnancy rate

The proportion of does found to be pregnant at slaughter and the foetal numbers are shown in Table 4.1. Significantly more does in Group M were pregnant than in

Group R (P<0.02). The proportion of pregnant does with twin foetuses was not different in the two groups. None of the foetuses recovered showed any sign of abnormality.

4.3.3 Foetal and placental measurements

Analysis of the measurements of the foetuses and the other gravid uterus components revealed that the difference in gestational length at which the does were slaughtered had a significant effect on the weight of foetuses, crown-rump length, curved crown-rump length, head length, weight of cotyledons, and the mass of total foetal fluids but had no effect on the number of placentomes and weight of placental membranes and empty uterus. Foetal number also had a significant effect on the number of placentomes, weight of empty uterus, mass of total foetal fluids and weight of the ovary with corpora lutea but had no effect on the weight of the foetuses, crown-rump length, curved crown-rump length, head length, weight of placentomes and weight of foetal placental membranes. Table 4.2 shows the coefficient of regression of both foetal number and gestational length on the measurements while the means adjusted for foetal number and gestational length on the measurements are shown in Table 4.3.

Foetuses were found to constitute 4.04 and 4.78% of the weight of the gravid uterus in Group M and Group R, respectively. Foetuses of does in Group R were significantly lighter (P<0.05), had a shorter crown-rump length (P<0.05) and tended to have a shorter curved crown-rump length and head length (P<0.1, respectively) than those from does in Group M. The ratio between curved crown-rump length and crown-rump length was 1.08 and 1.1 for Group M and Group R, respectively. No significant difference was found in the weight of empty uterus and the number of foetal cotyledons in the placenta of the two groups (P<0.2 and P<0.4, respectively), however

the total weight of the cotyledons and the weight of empty foetal placental membranes tended to be lower ($p<0.1$ and $p<0.1$ respectively) in the placenta of Group R does. The weight of foetuses was correlated with weight of cotyledonary component of the placenta ($r = 0.86$). The mass of foetal fluids was significantly less ($P<0.02$) in Group R does. The foetal fluids were found to be unevenly distributed between foetuses in some twin pregnancies. The weights of the ovaries with CL were poorly correlated with the number of CL ($r=0.36$; $P<0.1$) and tended to be lighter ($P=0.1$) in Group R does.

Table 4.1 Pregnancy rate and foetal numbers in does on maintenance and restricted rations

Feeding treatment	Maintenance	Restricted
No. of does mated	13	18
No. of pregnant does	12(92.3)	9(50.0)
No. of pregnant does with multiple foetuses	7(58.3)	6(66.7)
No. of pregnant does with single foetuses	5(41.7)	3(33.3)
Total no. of foetuses	19	16

Figures in parentheses represent percentages.

Table 4.2 **Regression coefficient of foetal number and gestational length on the means of measurements of the foetus and other components of the gravid uterus in goats**

	Foetal number		Gestational length	
Measurement	Coefficient	SED	Coefficient	SED
Foetal weight (g)	0.82	2.26	4.56	0.55**
Crown-rump length (cm)	-0.15	0.20	0.33	0.05**
Curved crown-rump length (cm)	0.04	0.17	0.35	0.04**
Head length (cm)	-0.04	0.07	0.12	0.02**
No. of placentomes per pregnancy	-18.34	4.36**	1.97	1.06
Total weight of cotyledons (g) per pregnancy	-10.19	7.48	8.90	1.79**
Weight of placental membrane without cotyledons (g)	-4.20	6.09	2.28	1.46
Weight of empty uterus (g)	122.00	23.30**	8.20	6.01
Mass of foetal fluids (g)	537.00	84.60**	36.00	21.80
Weight of ovary with CL (g)	1.15	0.48*	-0.10	0.12

** $P<0.001$.
* $P<0.05$.

Table 4.3 Effects of undernutrition on the foetus and components of the gravid uterus in goats on maintenance and restricted feeding (Means adjusted for foetal number and gestational length)

Feeding treatment	Maintenance	Restricted	SED.
No. of pregnant does	12	9	
No. of foetuses	19	16	
Foetal weight (g)	49.01	42.99	2.51*
Crown-rump length (cm)	10.51	9.95	0.23*
Curved crown-rump length (cm)	11.34	10.97	0.193
Head length (cm)	3.83	3.71	0.078
No. of placentomes per foetus	58.37	62.9	34.83
Weight of cotyledons (g) per foetus	88.49	74.36	8.26
Weight of empty placental membrane (g)	51.82	38.84	6.72
Weight of empty uterus (g)	279.00	240.00	26.20
Mass of foetal fluids (g)	746.00	504.00	95.0**
Weight of ovary with CL (g)	4.39	3.30	0.54

* $P<0.05$.

4.4 Discussion

The average weight of foetuses in this study was less than figures reported for slightly older caprine foetuses (Kadu and Kaikini, 1987). It has been reported in a previous study (Kadu and Kaikini, 1987) that the relative percentage of growth rate of both single and twin foetuses increased from 59.72% in the first stage of gestation (up to 15 days) to 81.12% in the fifth stage (61 to 75 days) and decreased from 67.62% in the sixth stage (76 to 90 days) to 53.18% in the ninth stage (beyond 121 days). It is apparent therefore that the pregnant does in the present study were slaughtered in a period of rapid foetal growth. This may explain the highly significant effect of gestational length on foetal weight observed in this study even though all the does were slaughtered within a gestational range of 1 week. The detrimental effects of undernutrition on foetal weight observed in our study supports observations in sheep where body condition at the time of embryo transfer and plane of nutrition in the first 2 months of gestation were significantly correlated with foetal weight (Parr *et al.*, 1982; Mckelvey and Robinson, 1986).

In agreement with previous work (Kadu and Kaikini, 1987) the present study found that foetal numbers had no significant effect on foetal weight in early pregnancy. This is probably because of the small size and hence low nutritional requirements of the foetus at this stage. However, as term approaches foetal growth rate tends to decrease because of susceptibility to hypoglycaemia during underfeeding (Mellor, 1983).

The crown-rump length figures recorded in the 2 groups in this study closely resemble figures reported elsewhere (Lyngset, 1971; Kadu and Kaikini, 1987). The difference in foetal weight and crown-rump length in this study is significant while that in curved crown-rump length and head length are almost significant. Changes in curved crown-rump length, crown-rump length and head length reflect skeletal growth,

whereas weight changes indicate growth of soft tissues. The degree of retardation of skeletal and soft tissue growth may be different during the period of underfeeding. Observations in sheep (Mellor, 1983) indicated that overall, restricted nutrition affected the earlier maturing tissues less than the later maturing tissues, suggesting that any reduction in foetal growth measured with reference to changes in crown-rump length will underestimate the effects on the weight of foetuses.

The ratio of curved crown-rump length to crown-rump length observed in this study is low compared with another observation elsewhere (Mellor, 1983), in which the ratio increased to 1.6 in the third stage, and decreased later on to about 1.1 in the last stage of gestation. The fluctuation in ratio and poor correlation between the two have led to the suggestion that curved crown-rump length may not be a satisfactory measure for studying growth curve of goat foetuses (Mellor, 1983).

It has been reported (Kadu and Kaikini, 1987) that the percentage weight of caprine foetuses to the gravid uterus increased progressively from 0.82% in the first 15 days to 49.16% over 121 days of pregnancy, while foetal membranes decreased from 13.26 times the foetal weight at the end of 1 month to as low as 0.21 times at about 135 days. The ratio of foetal weight to gravid uterus observed in the present study was higher than those reported earlier for similar gestational age (Kadu and Kaikini, 1987). The previously reported figures, however, were derived from samples collected in the abattoir and the exact days of pregnancy at slaughter could not be obtained. Both daily average and relative growth rates are known to be high at about the time the does in this study were slaughtered.

The number of placentomes utilized by a foetus is fixed at about 30 days after conception, but the total weight of the placentomes increases until about 90 days of gestation (Robinson *et al.*, 1977; Mellor, 1983). The number of placentomes per foetus

generally decreases as foetal number increases because the number of potential implantation sites available to each individual in the uterus is reduced with each additional chorionic envelop in the uterus (Rhind *et al.*, 1980). The results of our study reflect this general trend. Undernourished does, however, tended to have reduced foetal cotyledon and placental membrane weight and a reduced mass of foetal fluids. Everitt (1964) and Alexander and Williams (1971) have demonstrated a similar effect on placental growth in ewes moderately underfed during the first 90 days of pregnancy. There is also some indication that underfeeding during late pregnancy reduces placental weight in twin-bearing ewes but not in ewes with single foetuses (Mellor and Murray, 1981).

It has been reported that the cotyledonary component of the placenta in sheep is more sensitive to undernutrition than the foetus in mid and late pregnancy (Clarke and Speedy, 1980; Rattray *et al.*, 1980). The response of the placenta to undernutrition in early pregnancy is not clear. A compensatory hypertrophy of the placental cotyledons between 35 and 60 days of gestation has been reported in ewes on low feeding regimens (50% maintenance), indicating that the initial reaction of the foeto-placental unit to hypoglycaemia is to attempt to increase its uptake of nutrients by increasing its placental absorptive area (Mckelvey and Robinson, 1986). In the present study, however, no hypertrophy of any of the placental components was observed.

The mean weight of the ovaries in the 2 groups in this study was higher than figures reported elsewhere (Lyngset, 1971), and it tended to be affected by foetal number and to be lighter in the undernourished does. Earlier studies in goats (Lyngset, 1971) have demonstrated a significant increase in the size (diameter) of the CL with increasing foetal size, and a significant difference in the size of CL depending on the number in the ovary. This study demonstrated a poor correlation between the number of CL and weight of the ovaries; however, no further comparison could be made as the

size and weight of the CL were not measured. It has been observed, however, that the size of the CL is by no means always connected with its secretory activity (Lyngset, 1968a).

Whether the growth lost by the foetus in early pregnancy as a result of low level of feeding can be fully recovered before birth if does are refed has not been investigated. Studies in sheep (Rowson and Moor, 1966; McKelvey and Robinson, 1986) have demonstrated that small and growth-retarded embryos from thin ewes can survive and develop as well as large embryos from well fed ewes if both were transferred to an optimal uterine environment. In mid and late pregnancy, however, it has been shown (Mellor and Murray, 1981; 1982a & b) that the foetal response to refeeding of ewes after periods of underfeeding was largely a function of the degree and duration of the underfeeding. Thus, refeeding ewes after 16 days or less of severe underfeeding resulted in an immediate increase in the growth rate of the foetuses. This was however, followed by a progressive decrease in growth rate until birth, and, despite the earlier increase in growth rate the growth lost during the period of underfeeding was not made good before birth. No increase in growth rate was observed when ewes were refed after 21 days of severe underfeeding. Moderate underfeeding from 35 to 120 days of gestation reduced foetal growth rate apparently, irreversibly as did a period of 21 days of severe underfeeding (Mellor and Murray, 1982b). Refeeding such ewes at 120 days of gestation did not result in increased foetal growth rate but prevented a further decrease in growth rate. It is unlikely that foetuses of does on severe and long term underfeeding regimen such as in this study, can when refed, fully recover the growth lost during the period of underfeeding.

The consequences of foetal growth retardation and therefore low birth weight, on kid survival and post-natal growth have been described (Bajhau and Kennedy, 1990). Both kid survival and growth rate were dependent on birth weight. Birth weight had

more than twice the effect of milk intake and more than 10 times the effect of breed on kid growth.

The mechanism by which the effects of undernutrition on the foetus and the other components of the gravid uterus are mediated is not fully understood. It is suggested that the major cause of this growth rate response is a reduction in the maternal supply of glucose to the uterus (Liggins, 1989). The amount of glucose available to the foetus depends on its concentration in the maternal blood stream, which is maintained within a narrow limit by a complex control system involving several endocrine organs.

In conclusion, substandard nutrition in goats resulted in retardation of the growth of the foetuses, reduction in foetal fluid mass, and a tendency to reduce the weight of the cotyledons and placental membranes.

CHAPTER 5

The effects of undernutrition before or after embryo transfer on pregnancy rate in does.

5.1 Introduction

The previous experiments have demonstrated detrimental effects of combined pre-mating and post-mating undernutrition on ovulation rate, pregnancy rate and on the products of conception at 60 days post-mating. Evidence has been provided in ewes (Mckelvey and Robinson, 1986) that nutritional status in the pre-mating period and/or during the first few days of embryo development can have long-term effects on the subsequent development of the conceptus, and that these effects are independent of any carry-over effect of pre-mating nutrition on the post-mating period. In goats, there is little information on the effects of undernutrition either before and/or after mating on pregnancy rate and embryo survival. From the results of the previous experiments, it was not possible to discern the separate effects of undernutrition in these periods.

The aim of the present study was to investigate the separate effects of undernutrition before and after mating on pregnancy rate and embryo survival in the goat.

Embryo transfer was used for the purpose of this experiment. Embryos from does on good nutrition were transferred to recipients on different feeding regimens. This technique provided the means of separating the effects of fertilization and early embryo development from later embryo development.

5.2 Materials and methods

5.2.1 Experiment 4

5.2.1.1 Animals and experimental procedure

Fifty seven multiparous non-lactating Angora cross goats which were 2 or 3 years old were available for the experiment. The goats' rations were adjusted so that each animal achieved a body condition score of approximately 2. For the purpose of embryo transfer the goats were randomly allocated to a group of donors of embryos or to one of 3 groups of recipients. All does were weighed weekly throughout the duration of the study. The ration of the donors (n=12) was adjusted to provide their calculated maintenance requirements of energy and protein (National Research Council, 1981) throughout the experiment. Recipient does in Group A were fed a restricted diet calculated to provide about 25% of their maintenance requirement (National Research Council, 1983) from 35 days before, followed by full maintenance diet for about 60 days after embryo transfer (n=16). Group B does were fed a full maintenance diet before, followed by the restricted diet for 60 days after embryo transfer (n=15). Does in Group C were fed a full maintenance diet both before and after embryo transfer (n=14).

5.2.1.2 Oestrus synchronization and superovulation

Oestrus was synchronized in the donor does by insertion of intravaginal pessaries containing 45 mg fluorogestone acetate (Chronogest, Intervet International, B.V. Boxmeer, Holland). The sponges were left in place for 17 days. Superovulation was achieved by the intramuscular injection of 22 mg porcine follicle stimulating hormone (pFSH); (Follicle stimulating hormone, Sigma Chemical Company, St. Louis,

U.S.A.) divided into 4 descending doses twice daily, commencing 2 days before the withdrawal of sponges. The donors were hand-mated to different Angora bucks at 6 hourly intervals throughout the duration of the ensuing oestrus. The recipient does were synchronized in oestrus using the same method as in the donors. The sponges were however removed 12 hours earlier in the recipients than in the donors. Three hundred international units of PMSG (Folligon, Intervet U.K. Ltd., Cambridge, U.K.) was injected intramuscularly into each recipient at the time of sponge withdrawal to ensure synchrony of ovulation. Vasectomized Saanen bucks were used for the detection of oestrus in all the does. Embryo collection from the donor does, and transfer to the recipient groups were performed as described earlier (Chapter 2).

5.2.1.3 Slaughter procedure

All does were slaughtered at 60 days after embryo transfer as described in experiment 1 and the foetuses were counted. Ovaries with CL were immediately dissected and the CL peeled out, weighed, frozen in liquid nitrogen and stored at -20°C for use in a later experiment (see Chapter 7).

5.2.1.4 Statistical analyses

The proportion of does that ovulated following oestrus synchronization in the 3 groups were compared using the Chi-square test, ovulation rates were compared using the two-tailed Analysis of variance (ANOVA) while pregnancy rate and embryo survival data were analyzed using Fisher's exact test for 2 x 2 tables. All means are $\pm$ SEM.

5.3 Results

5.3.1 Ovulation rate

The proportion of does that ovulated, the mean ovulation rate and the number of does with regressed CL in the donor and recipient groups are shown in Table 5.1. Regressed CL were included in the calculation of the ovulation rate. The proportion of does that ovulated and ovulation rates were lower in group A does that received the restricted ration before the embryo transfer than in Groups B and C does that received full maintenance ration. The differences were however not significant (P = 0.225; P = 0.483, respectively).

5.3.2 Ova recovery

Two hundred and twenty nine ova out of 340 ovulations in the donor group were recovered (67.35% or 9.96 $\pm$ 6.29 ova per doe). One hundred and sixty (69.87% or 6.96 $\pm$ 6.31 per doe) ova recovered were fertilized while 69 (30.13%) were unfertilized. Fifty (31.25%) of the fertilized ova were at the blastocyst stage, 106 (66.25%) were morulae and 8 (1.75%) were at the eight-cell stage. Three does yielded only unfertilized ova, while 9 does had all their recovered ova fertilized. The number of embryos recovered from the does was correlated with the number of CL counted (ovulation rate) (r=0.678; P=0.001).

5.3.3 Pregnancy rate

The number of does that received embryos, the proportion pregnant, and the proportion of live and dead foetuses at 60 days after embryo transfer are shown in

Table 5.2. Does with regressed CL and those with very large unovulated follicles were not used for embryo transfer. Three of the does in Group A that received embryos had to be removed from the experiment as a result of their inability to recover their appetite following the period of restricted feeding. The proportion of does pregnant at 60 days after embryo transfer in Groups B tended to be lower than that in Group C (P=0.070). Pregnancy rate was also lower in Group A than in Group C, the difference however was not significant (P=0.314). Significantly fewer live foetuses (P<0.032) were found in both Group A (P=0.032) and Group B (P=0.0004) does, however Group B does had a higher proportion of dead foetuses than Group C does (P=0.009). There was no difference in the number of live foetuses between Group A and Group B does (P=0.540).

Table 5.1 Ovulation rate in donor and recipient does

	Donors	Group A	Group B	Group C
No. of does	24	16	15	14
No. of does that ovulated	23(95.8)	10(62.5)	12(80.0)	12(85.7)
No. of does with regressed CL	0	1	2	3
Total no. of CL	340	19	23	21
Total no. of regressed CL	0	1	3	4
Mean ovulation rate	14.2±8.11	1.2±0.29	1.5±0.34	1.5±0.23

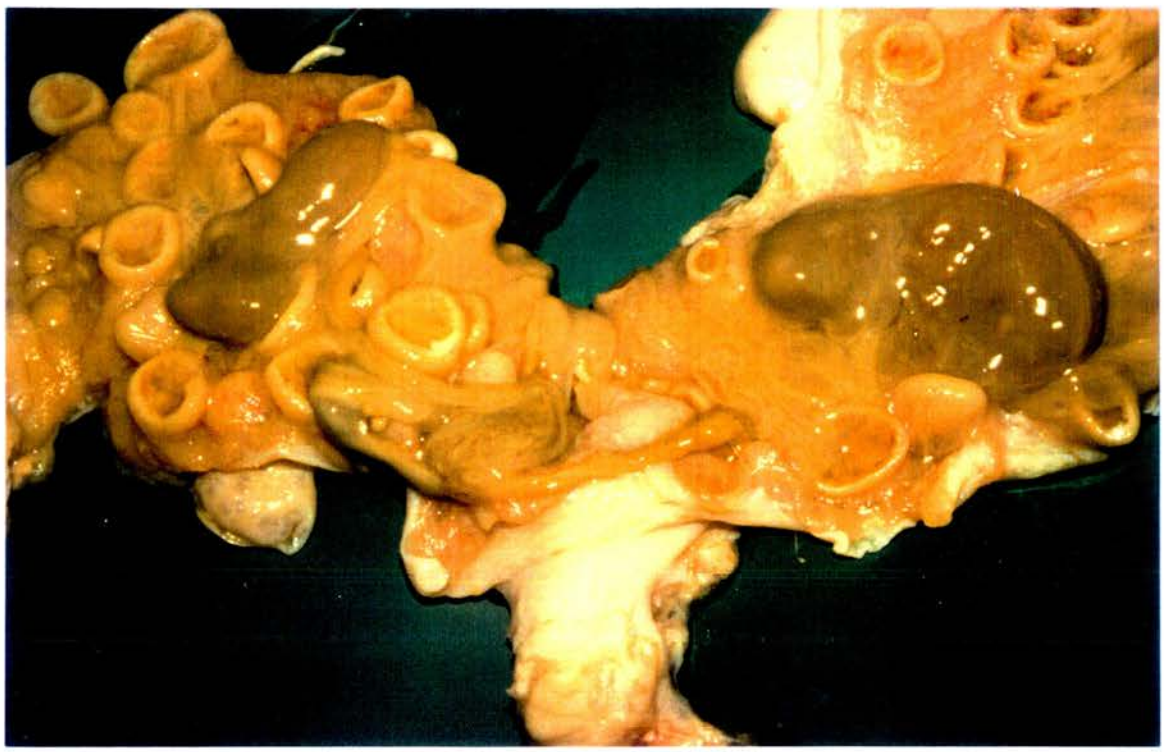
percentage in parentheses

Table 5.2 **Pregnancy rate and embryo survival in recipient does on restricted ration before and maintenance ration after embryo transfer (Group A), maintenance ration before and restricted rations after embryo transfer (Group B) and maintenance rations before and after embryo transfer (Group C)**

	Group A	Group B	Group C
No. of does that received embryos	6	10	9
No. of embryos transferred	12	20	19
No. of does pregnant	2(33.3)	2(20.0)	6(66.7)
No. of live foetuses	3(25.0)	1(0.05)	11(57.9)
No. of dead foetuses	1	3	0
Total no. of foetuses	4	4	11

percentage in parentheses.

Plate 5.1 Mummified twin foetuses from a doe on maintenance feeding before embryo transfer and restricted feeding after embryo transfer



5.4 Discussion

Ovulation rate and embryo recovery rate following treatment with intravaginal sponges and superovulation with pFSH observed in this study were similar to those in previous studies employing the same techniques (Armstrong *et al.*, 1983a and b; Drost, 1986; Amoah and Gelaye, 1991; Pendleton *et al.*, 1992; Rosnina *et al.*, 1992). The proportion of does that ovulated and the ovulation rate following injection of 300 IU PMSG at sponge withdrawal in the recipient does were slightly lower in the does on restricted rations before embryo transfer but were not significantly different between the 3 groups. The results were also similar to those reported for does treated with the same dose (Rosnina *et al.*, 1992), but considerably lower than in does treated with higher doses of 750-1250 IU PMSG (Armstrong *et al.*, 1983a; Ritar *et al.*, 1989; Pendleton *et al.*, 1992). Six does in the recipient groups had prematurely regressed CL at 6 days after the onset of oestrus compared with none in the pFSH superovulated donors. Premature luteal regression/or failure of normal luteal development has been reported to occur more frequently in does superovulated with PMSG than with FSH (Armstrong *et al.*, 1983b; Pendleton *et al.*, 1992). The cause of the premature regression/or failure of normal luteal development is not fully understood. Premature release of $PGF_{2\alpha}$ has recently been implicated as the cause of the early luteal regression in superovulated goats (Battye *et al.*, 1988). A high rate of early returns to oestrus has also been reported following superovulation of does with PMSG (up to 44%), in the postpartum period (Corteel, 1977) and early or late in the breeding season in naturally cycling goats (Armstrong *et al.*, 1983a; Pendleton *et al.*, 1992). The early return to oestrus is believed to be as a result of altered endocrine patterns associated with superovulation (Armstrong *et al.*, 1983b). It has also been suggested that PMSG-treated does prematurely return to oestrus due to failure of ovulation of large follicles in response to the preovulatory surge of luteinizing hormone (LH). Oestrogen from the

large, unovulated follicles could possibly have risen sufficiently to induce a second oestrus in the presence of low levels of progesterone (Pendleton *et al.*, 1992).

Current reports indicate that pregnancy rates following transfer of goat embryos range from 45 to 80%. Several factors including the quality of embryos, nutritional status of does and transfer expertise (Godke *et al.*, 1985) are believed to influence the success of embryo transfer, however, there is little information on the specific effect of each of these factors in goats. The pregnancy rate recorded for does on maintenance feeding all throughout the experiment in this study is comparable with those reported elsewhere (Moore, 1979; Godke *et al.*, 1985). Very low pregnancy rates were however recorded in both the does poorly fed before, and those poorly fed after embryo transfer. The low pregnancy rate in the does poorly fed before embryo transfer did not differ significantly from that of does on adequate rations probably because of the lower number of does in the group that recovered and survived to 60 days after embryo transfer. Our findings were similar to those reports in sheep (Edey, 1970; Cumming, 1972; MacKenzie and Edey, 1975a; Parr *et al.*, 1982; Parr and William, 1982) in which levels of underfeeding, ranging from 0.15M to 0.7M for durations of 0 to 37 days postmating, were found to extend the interval to repeat oestrus, suggesting embryo mortality and retarded embryo development. A contrasting study (McKelvey and Robinson, 1986) however, reported no significant effect on pregnancy rates in ewes despite a nutritionally-induced change of 20% in the weight of the ewes in the first 60 days of pregnancy. It is likely that a threshold of feed restriction and/or duration has to be exceeded before the survival of the embryo is endangered.

Because of the small number of animals that received embryos and were pregnant, and the very few foetuses these animals yielded in the two feed-restricted groups, it was not possible to assess the separate effects of feed restriction before and after embryo transfer. It is however obvious from the low pregnancy figures and the

high proportion of dead foetuses in the two group of does that feed restriction in either of the two periods was detrimental to the survival of the conceptus.

The mechanism by which nutrition affects embryo survival and growth are not fully understood. The effect may presumably be through the alteration in the intrauterine environment, which may affect embryo survival and the growth capacity of the embryo through the development of the placentae and the transfer of nutrients between the maternal and the foetal circulations (El-Sheikh *et al.,* 1955). It has been suggested that these effects may operate through an alteration in the endocrine balance of the mother (Robinson, 1986). Evidence has been provided that progesterone plays a key role in the initiation and maintenance of pregnancy (Wilmut *et al.,* 1986), with an inverse relationship demonstrated between the plane of nutrition and peripheral plasma progesterone concentrations in sheep (Parr *et al.,* 1982; Williams and Cumming, 1982; McKelvey and Robinson, 1986). Feed intake is believed to modify the plasma concentration of progesterone through a change in the rate of steroid metabolism brought about by an alteration in hepatic blood flow (Parr *et al.,* 1982). Reduced blood glucose supply to the foetus may also influence the growth and survival of the foetuses (Parr *et al.,* 1982). The effects of undernutrition on the endocrine organs and their secretions which are involved in the complex system of controlling maternal blood glucose concentrations have not been fully investigated in goats.

In conclusion, this study demonstrated detrimental effects of restricting feeding both before and after embryo tranfer in the recipient does, on the survival of the transferred embryo. Because embryo transfer was not successful under poor nutrition it was not possible to assess the separate effects of undernutrition before or after the transfer. It is recommended that embryo transfer should not be used unless adequate feeding of animals can be guaranteed.

CHAPTER 6

The effects of undernutrition on gonadotrophin profiles, time and duration of preovulatory gonadotrophin surge and total releasable gonadotrophin following gonadotrophin-releasing hormone administration in goats

6.1 Introduction

Experiments 1 and 2 have demonstrated that ovulation rates and embryo survival in does are influenced by level of nutrition before mating and before and after mating, respectively. The physiological link between nutrition and reproduction has been investigated in sheep (Gunn, 1983; Rhind and McNeilly, 1986; Rhind *et al.*, 1989a&b; Rhind *et al.*, 1991) but not in goats. The effects of level of feed intake in the premating period on ovulation rate could be mediated through changes in hypothalamic activity or secretion of gonadotrophins from the pituitary in response to GnRH, either of which could affect circulating gonadotrophin profiles. Differences in FSH profiles in sheep with different body condition scores (Rhind and McNeilly, 1986) and differences in LH pulse frequency have been reported and implicated in the control of ovulation rate. Ovulation rate could also be influenced by changes in the ovarian response to gonadotrophin (Rhind *et al.*, 1989a).

In goats, LH pulse frequency and mean concentrations have been studied during the anoestrous and breeding seasons in ovariectomized, oestradiol-treated goats (Chemineau *et al.*, 1988). However, reports on normal FSH profiles and the effect of nutrition on gonadotrophin profiles in the different phases of the oestrous cycle are not available. The aim of this experiment therefore was to determine the effects of

undernutrition in goats on (i) LH and FSH profiles in the luteal, follicular and preovulatory phase before the LH surge of the oestrous cycle; (ii) time of onset and duration of preovulatory gonadotrophin surge in relation to sponge removal and (iii) the total releasable gonadotrophin content of the pituitary following administration of GnRH.

6.2. Materials and Methods

6.2.1 Experiment 5: The effects of undernutrition on gonadotrophin profiles in the luteal, follicular and preovulatory phases.

6.2.1.1 Animals and experimental procedure

The same does were used as in Experiment 1. Animal management, feeding and oestrus synchronization were described earlier. On Day 10 after the first synchronized oestrus (luteal phase) and the commencement of feed treatment, blood samples were collected into heparinized tubes via jugular catheters at 15 minute intervals for 10 hours from 10 animals in each group. On Day 16 after the first oestrus, 10 mg $PGF_{2\alpha}$ was administered intramuscularly to all the does. From 20 hours (follicular phase) and 40 hours (preovulatory phase) following sponge removal blood samples were again collected at 15 minute intervals for 8 and 10 hours, respectively. All blood samples were centrifuged at 2000*g* for 30 minutes and the plasma was separated and stored at -20°C pending radioimmunoassay.

6.2.2 Experiment 6: The effects of undernutrition on the time of onset and duration of gonadotrophin surge in relation to sponge removal and the total releasable gonadotrophins following GnRH administration.

6.2.2.1 Animals and experimental procedure

Sixteen mature, non-lactating British Saanen does aged between 2 and 3 years were used for the experiment. Between August and September, 1992, the does were fed on the same rations as those in Experiment One until a body condition score of approximately 2 was achieved. The does were randomly allocated to 2 groups and were penned individually. In October, the rations of the does were adjusted so as to provide 100% and 25% energy and protein requirements for maintenance (National Researc Council, 1981) for Group M and Group R, respectively. Seventeen days after the commencement of differential feeding, all the does were synchronized in oestrus using progestagen sponges containing 45 mg fluorogestone acetate intravaginally and left in place for 17 days. On the day of the sponge removal 10 mg $PGF_{2\alpha}$ was administered intramuscularly to all the does. The does were checked for oestrus at six-hourly intervals using a vasectomised buck. Blood samples were collected from the does through a jugular catheter at 15 minutes intervals for 24 hours from the time standing oestrus was first detected. Ten days after the ensuing oestrus 13 µg GnRH (Receptal, Hoechst, U.K. Ltd., Milton Keynes) was administered intravenously and blood samples collected at 15 minute intervals for 3 hours. All blood samples were handled as described in Experiment 5. All plasma samples except those collected between 2 and 10 days after oestrus in experiment 2 were assayed for LH and FSH concentrations.

6.2.3 Statistical analyses

LH pulses were identified by the Munro pulse analysis programme (Elsevier-Biosoft, Cambridge, UK) using the regional coefficient of variation algorithm as described by Veldhuis *et al* (1986). A "pulse" was preceded by a rise which exceeded 5 times the regional coefficient of variation (CV). A smoothing window of 30 minutes was used to determine the CV and a minimum pulse interval of 30 minutes. Overall mean LH and FSH concentrations (mean of plasma concentration), mean LH pulse amplitude and mean LH pulse frequency of the two groups of goats in each of the 3 phases were compared using the one-way analysis of variance with treatment having a fixed effect and doe a random effect. Number of does on heat following feed treatment and synchronization of oestrus with sponges and $PGF_{2\alpha}$ were compared using Fisher's exact test for 2 x 2 tables.

Preovulatory surge was assumed to have occurred when concentrations rose above and returned below 5 and 20 ng/ml for LH and FSH respectively. Peak concentrations during the gonadotrophin surge were defined as the highest concentrations which were not preceded by a decrease in concentration. Indices of the area under the gonadotrophin peaks during the preovulatory gonadotrophin surge and following GnRH administration were obtained by summation of the means of all the elevated values.

The lengths of time from sponge removal and GnRH administration to the onset of gonadotrophin surge and to peak concentrations, the duration of the surge, total area under the surge and peak concentrations were compared between the 2 groups using the two sample unpaired t-test. All means are presented $\pm$ SEM. Differences are regarded significant when $P<0.05$.

6.3 Results

6.3.1 Experiment 5

6.3.1.1 Body liveweight changes

By the end of the last blood collection period, the mean body weight and condition score of goats in Group R had dropped from 50.1 ± 1.5 Kg and 1.87 ± 0.04 to 44.4 ± 1.4 Kg and 1.53 + 0.04, respectively while those of goats in Group M had changed from 53.4 ± 1.1 Kg and 1.91 ± 0.02 to 53.3 ± 1.5 Kg and 1.91 ± 0.03 respectively.

6.3.1.2 Luteinizing hormone

Figures 6.1 and 6.2 show representative LH profiles during the luteal, follicular and preovulatory phase (before the preovulatory surge of LH) of a doe on maintenance feeding and a doe on restricted feeding. In both groups, mean LH concentrations were significantly higher in the follicular phase and preovulatory phase than in the luteal phase (Figure 6.3). Undernutrition had no significant effect on mean LH concentration in the three phases of the cycle. Pulse frequency of LH in both groups of goats was higher in the follicular and preovulatory phases than in the luteal phase (Figure 6.4), but was not affected by undernutrition. Amplitude of LH pulses was not affected by cycle stage or nutritional status (Figure 6.5).

6.3.1.3 Follicle stimulating hormone

The secretion of FSH in the 3 periods in this study was irregular with no well-defined pulses. Mean FSH concentrations were similar in the three phases of the

oestrous cycle in the two groups of goats (Figure 6.6). There was no significant drop in concentration in the preovulatory phase compared with the follicular phase. There was no significant effect of undernutrition on FSH concentrations in the luteal and preovulatory phases, however they tended to be lower in the follicular phase in goats on restricted feeding than in adequately fed goats.

6.3.2 Experiment 6

6.3.2.1 Body liveweight changes

The mean body liveweight and condition score of does in Group R dropped from 41.37 ± 2.47 Kg and 1.99 ± 0.066 at the beginning, to 35.44 ± 2.28 Kg and 1.76 ± 0.080 at the end of the experiment, respectively. Does in Group M improved in weight from 41.56 ± 1.44 to 45.56 ± 1.28 Kg and body condition score from 1.97 ± 0.049 to 2.04 ± 0.046 in the same period.

6.3.2.2 Oestrus onset and commencement of blood sample collection

Blood sample collection was begun at 32h after sponge removal and $PGF_{2\alpha}$ administration when 5 does were seen to show signs of oestrus. Blood samples were not collected from one doe in Group M whose sponge could not be completely removed from the vagina, and another in Group R that had problems with its intravenous catheter.

In Group M, 5 (71.4%) out of the 7 does sampled exhibited oestrous signs before the end of the 24 hour sampling period (between 32 and 56 h after sponge withdrawal and the administration of $PGF_{2\alpha}$). In 4 of these does, the entire duration of the preovulatory LH surge occurred within the sampling period, while in one, the

Figure 6.1 LH profile during the luteal phase (10h on Day 10 after oestrus), follicular phase (20 to 28h after $PGF_{2\alpha}$ administration) and preovulatory phase before gonadotrophin surge (40 to 50h after $PGF_{2\alpha}$ administration) in a goat on maintenance feeding (Goat number C1)

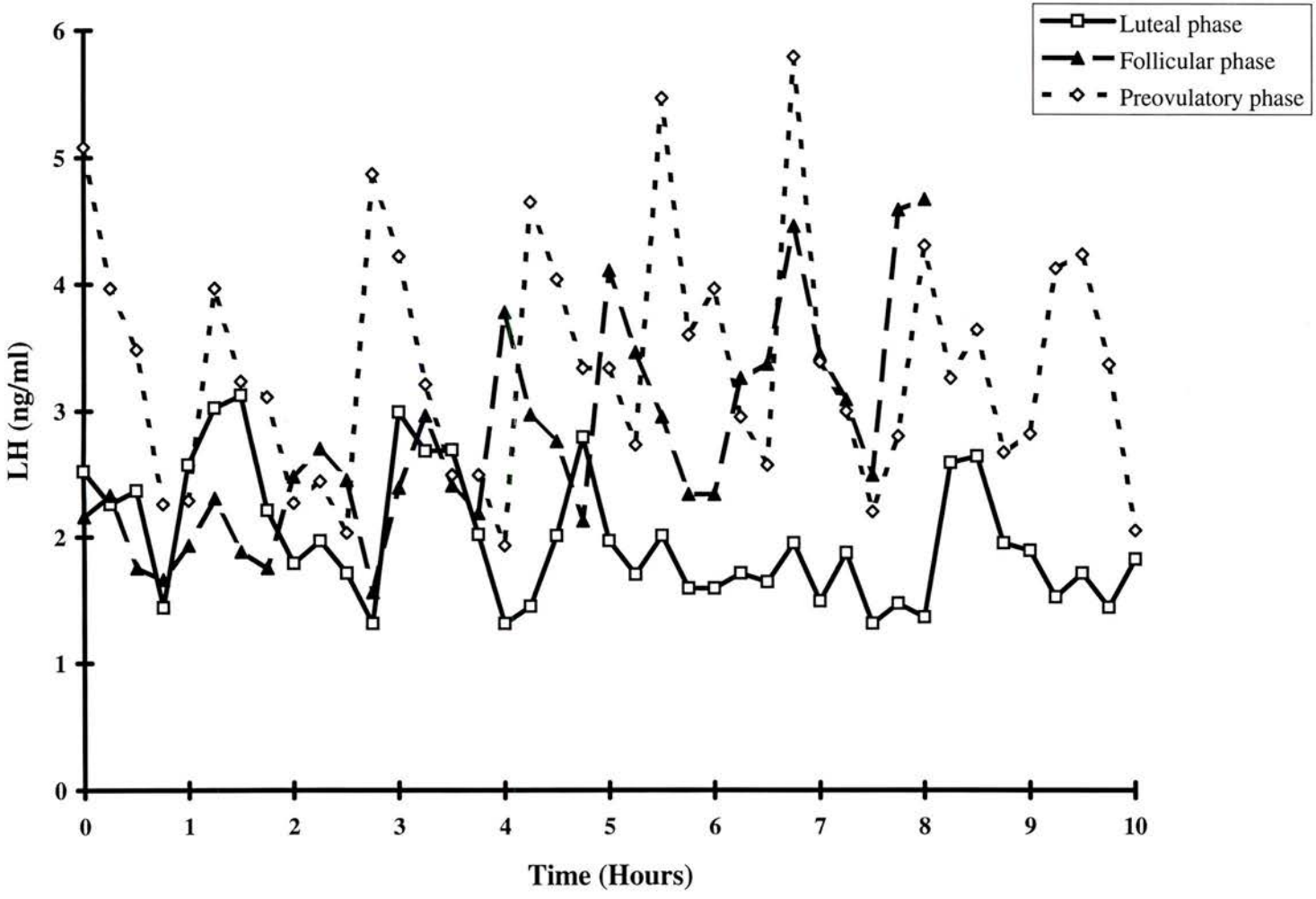

Luteal phase
Follicular phase
Preovulatory phase
LH (ng/ml)
Time (Hours)
0
1
2
3
4
5
6
0
1
2
3
4
5
6
7
8
9
10

Figure 6.2 LH profile during the luteal phase (10h on Day 10 after oestrus), follicular phase (20 to 28h after $PGF_{2\alpha}$ administration) and preovulatory phase before gonadotrophin surge (40 to 50h after $PGF_{2\alpha}$ administration) in a goat on restricted feeding (Goat number B8)

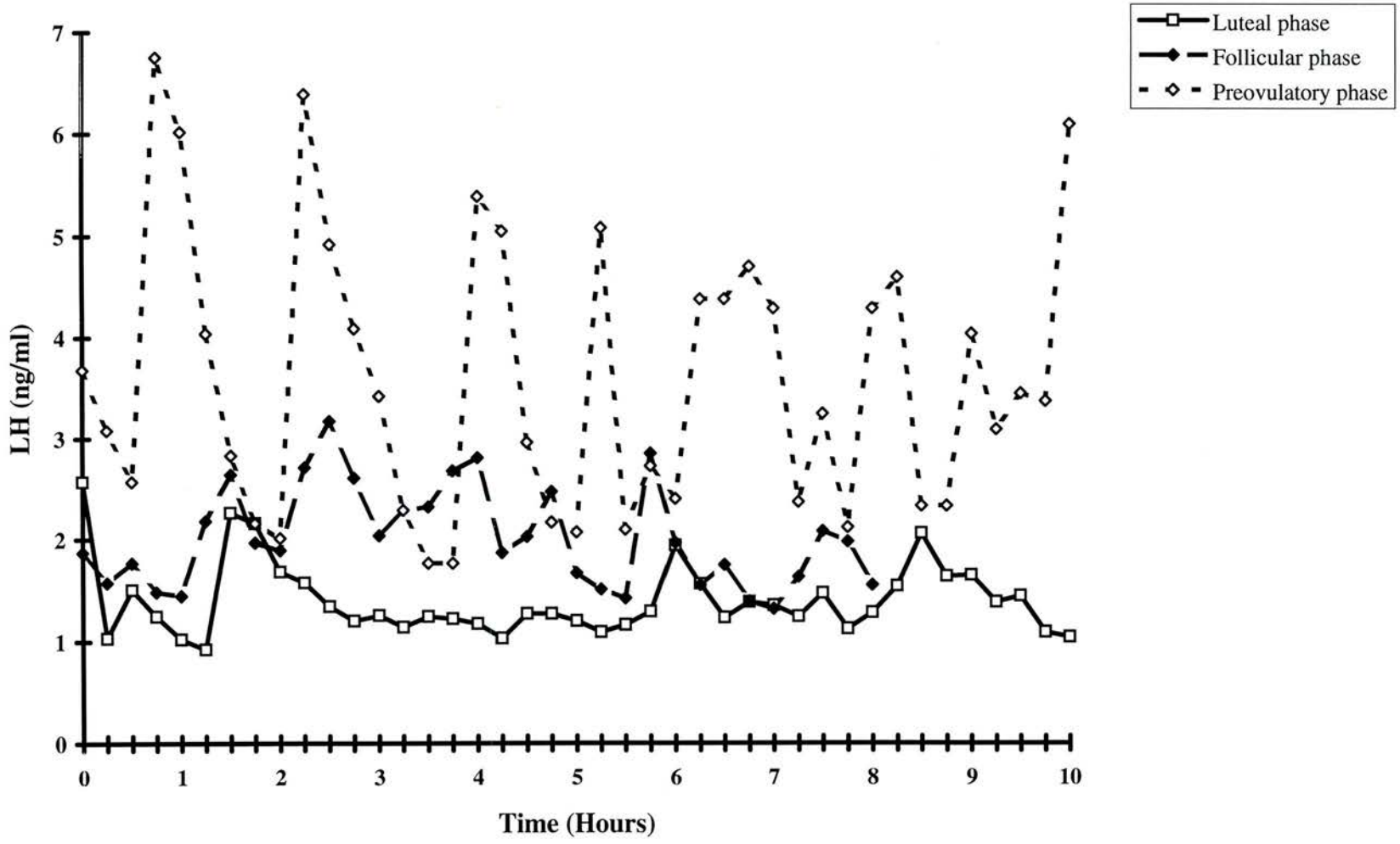
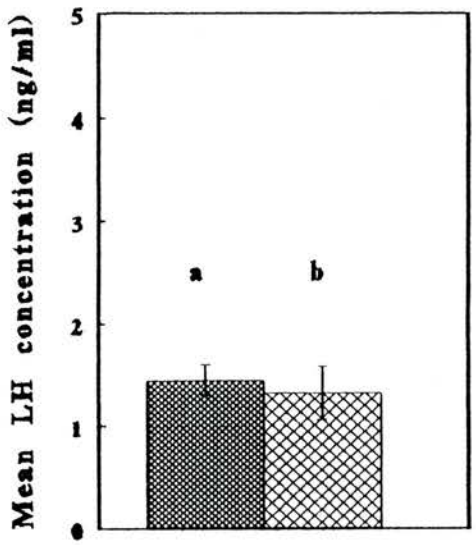
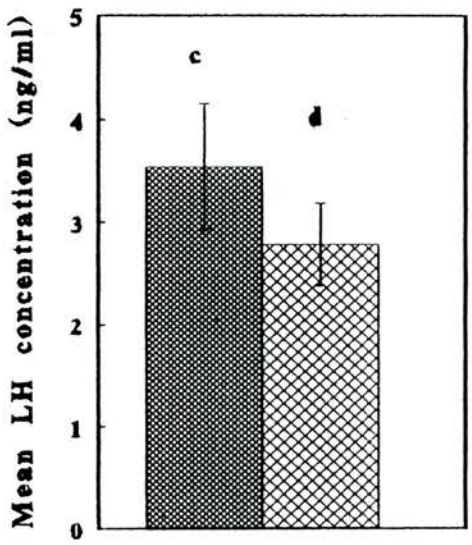

Luteal phase
Follicular phase
Preovulatory phase
LH (ng/ml)
0
1
2
3
4
5
6
7
0
1
2
3
4
5
6
7
8
9
10
Time (Hours)

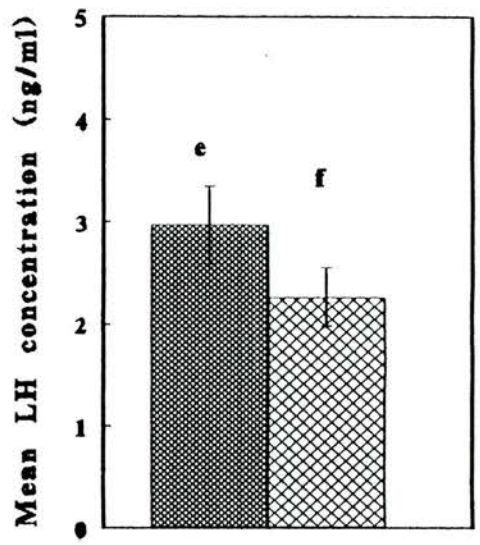
Figure 6.3 Mean LH concentration during the luteal phase (10h on Day 10 after oestrus), follicular phase (20 to 28h after $PGF_{2\alpha}$ administration) and preovulatory phase before the gonadotrophin surge (40 to 50h after $PGF_{2\alpha}$ administration) in goats on maintenance (▓▓) and restricted feeding (▒▒).



Luteal phase



Follicular phase



Preovulatory phase

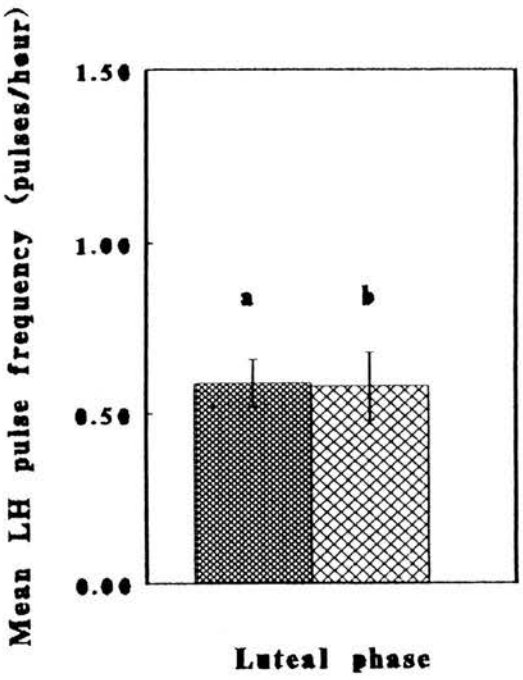
a,c P=0.008

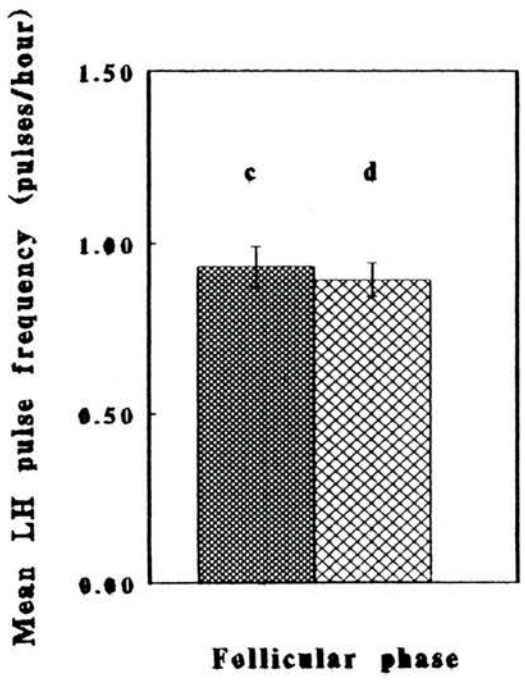
a,e P=0.008

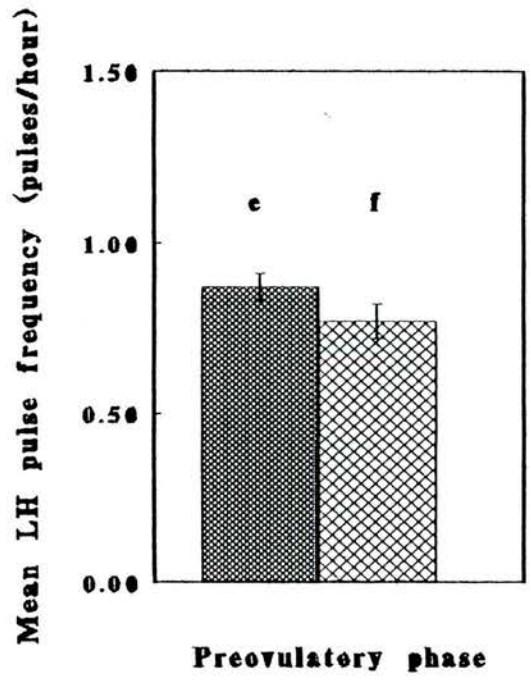
b,d P=0.020

b,f P=0.044

Figure 6.4 Mean LH pulse frequency during the luteal phase (10h on Day 10 after oestrus), follicular phase (20 to 28h after $PGF_{2\alpha}$ administration) and preovulatory phase before gonadotrophin surge (40 to 50h after $PGF_{2\alpha}$ administration) in goats on maintenance (▩) and restricted feeding (▨).







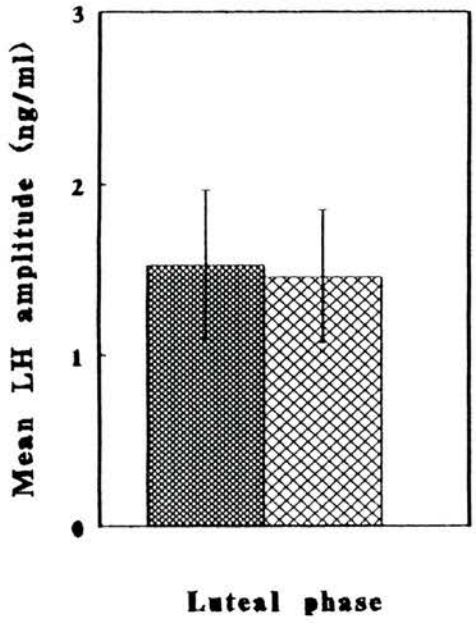
a,c P=0.031

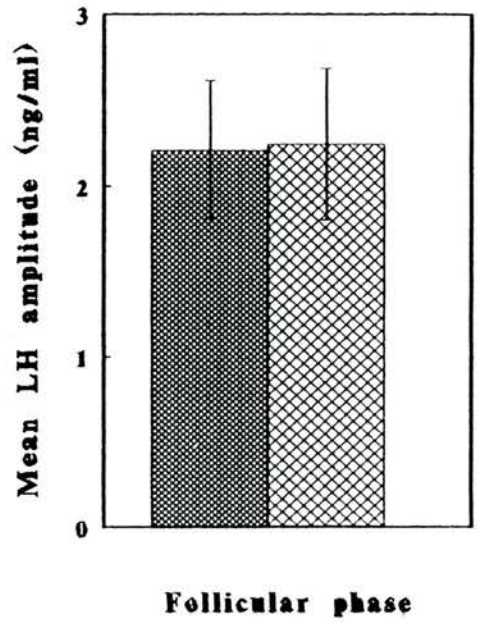
a,e P=0.036

b,d P=0.030

b,f P=0.056

Figure 6.5 Mean LH pulse amplitude during the luteal phase (10h on Day 10 after oestrus), follicular phase (20 to 28h after $PGF_{2\alpha}$ administration) and preovulatory phase before gonadotrophin surge (40 to 50h after $PGF_{2\alpha}$ administration) in goats on maintenance (▩) and restricted feeding (▨).


Mean LH amplitude (ng/ml)
3
2
1
0
Luteal phase


Mean LH amplitude (ng/ml)
3
2
1
0
Follicular phase

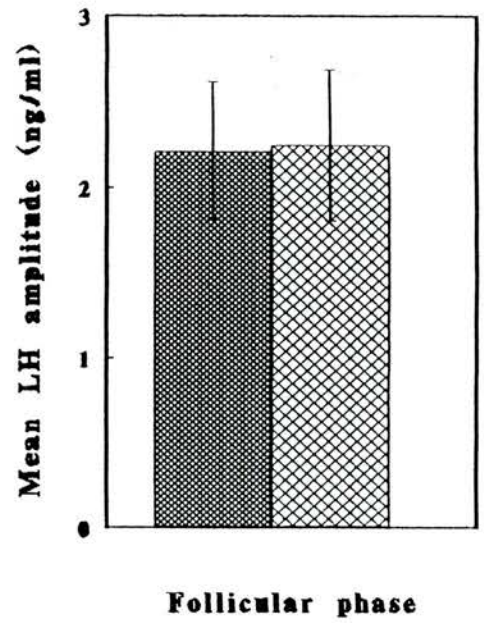
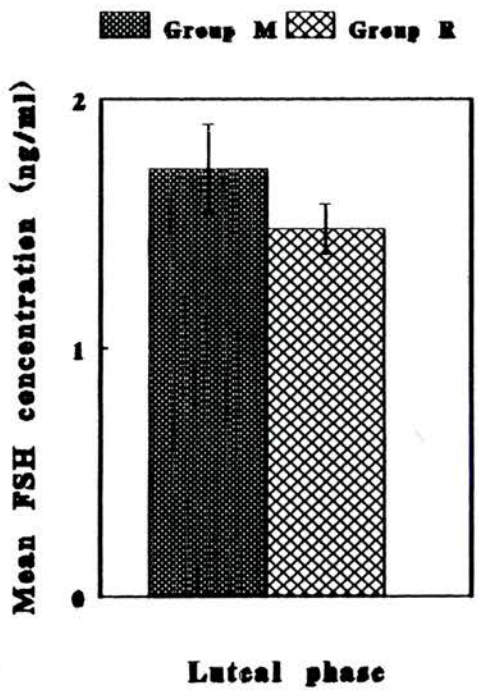
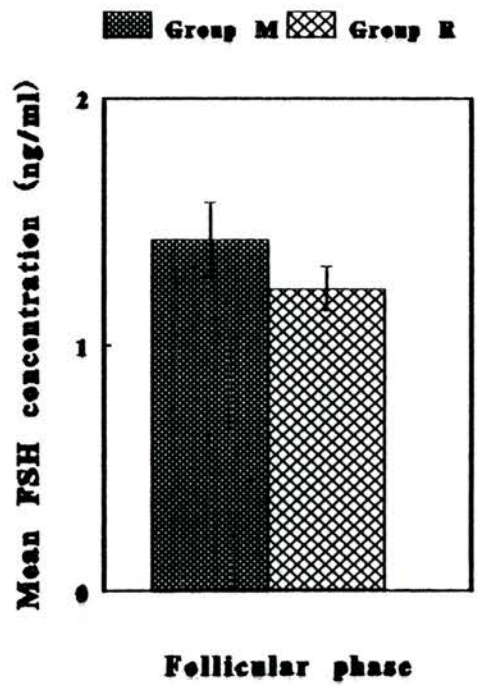
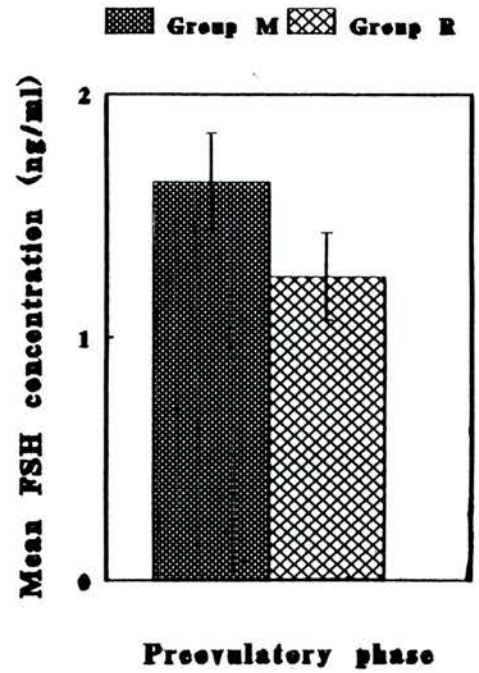

Mean LH amplitude (ng/ml)
3
2
1
0
Follicular phase

Figure 6.6 Mean FSH concentration during the luteal phase (10h on Day 10 after oestrus), follicular phase (20 to 28h after $PGF_{2\alpha}$ administration) and preovulatory phase before gonadotrophin surge (40 to 50h after $PGF_{2\alpha}$ administration) in goats on maintenance (▓▓) and restricted feeding (▒▒).


Group M
Group R
Mean FSH concentration (ng/ml)
2
1
0
Luteal phase


Group M
Group R
Mean FSH concentration (ng/ml)
2
1
0
Follicular phase


Group M
Group R
Mean FSH concentration (ng/ml)
2
1
0
Preovulatory phase

greater part of the surge occurred before this period. The remaining 2 does in Group M exhibited oestrus at 64 hours after sponge withdrawal and $PGF_{2\alpha}$ administration (8 hours after the end of the 24 hour blood collection period). Of the 8 does in Group R, only 2 (25%) exhibited oestrus. These two does, out of the 7 sampled, exhibited an LH surge before the end of the sampling period (P=0.100). One other doe in the group came into oestrus 68 hours after sponge withdrawal. The remaining 5 does in this group did not show signs of oestrus. The difference in exhibition of oestrus in the two groups of does just failed to reach significance (P=0.070).

Only does in which the entire preovulatory LH surge occurred within the sampling period were included in preovulatory gonadotrophin surge analyses in this study.

6.3.2.3 Preovulatory luteinizing hormone surge following oestrus synchronization

Representative LH profiles following luteolysis induced by sponge removal and $PGF_{2\alpha}$ administration in does on maintenance and restricted rations are shown in Figure 6.7. Table 6.1 shows the relationship between undernutrition and the mean values of the periods of time from luteolysis induced by sponge removal to the onset of oestrus, preovulatory LH surge and peak LH concentration, the duration of the surge, the area under the LH surge and the value of the peak concentration. The mean periods from sponge removal to the onset of oestrus, preovulatory LH surge and the occurrence of the LH peak were shorter in the feed-restricted group but the differences were not significant. The mean duration of the LH surges were similar in the two groups. In Group M, the mean periods from the onset of oestrus to the LH surge and to the occurrence of peak concentration were more than two-fold greater than in Group R, however because of wide variation within the two groups the differences

were not significant. The area under the LH surge and the peak LH concentrations were greater in Group M than in Group R (P=0.006, P=0.001, respectively). The mean periods from sponge removal to the onset of the LH surge and to the peak concentration were highly correlated with the mean periods from oestrus onset to LH surge (r=0.955, P=0.0002 and r=0.992, P=0.0001, respectively) and from oestrus to the LH peak (r=0.882, P=0.0004 and r=0.954, P=0.0002, respectively).

6.3.2.4 Preovulatory follicle stimulating hormone concentrations following oestrus synchronization

Figure 6.8 shows representative FSH profiles of a does on maintenance feeding and 2 on restricted feeding following luteolysis induced by sponge removal and administrations of $PGF_{2\alpha}$. The mean periods from sponge removal to the beginning of the preovulatory increase in FSH concentrations and to the mean peak FSH concentrations were shorter in the feed-restricted group than in the adequately fed group, the differences were however not significant (Table 6.2). The mean peak FSH concentration was also lower in the feed-restricted group than in the adequately fed group but the difference was not significant. The mean duration of the increased FSH concentrations was similar in the 2 groups, however, the total FSH released in this period was more than two-fold greater in the adequately fed does than in the feed-restricted does.

6.3.2.5 Luteinizing hormone surge following gonadotrophin-releasing hormone administration

Administration of 13µg GnRH induced a surge in the concentration of LH in all the does. Figure 6.9 shows representative profiles of two does on maintenance and restricted feeding. After reaching a peak, LH did not fully return to basal

concentrations by the end of the 3 hour sampling period. LH concentrations in Group M rose faster (P=0.031) from pre-injection levels to greater than 10 ng/ml than did those in Group R (Table 6.3). The mean total LH released following GnRH administration was significantly higher in Group M than in Group R. The mean peak LH concentration attained during the surge was higher in Group M than in Group R does, however the difference was not significant (P>0.100). There was no difference in the mean period of time from GnRH administration to LH peak concentration between the two groups.

6.3.2.6 Follicle stimulating hormone concentrations following gonadotrophin-releasing hormone administration

Figure 6.10 shows representative FSH profiles of two does on maintenance and restricted feeding following GnRH administration. The time taken for FSH to rise from pre-injection level to greater than 20 ng/ml was not different in the 2 groups (Table 6.4). The mean amount of FSH released and the peak concentrations attained were higher in does in Group M than those in Group R. The mean time from GnRH administration to FSH peak concentration was significantly shorter in the does in Group R.

Figure 6.7 LH profile of goat K (maintenance feeding) and goat J (restricted feeding) showing preovulatory LH surge following sponge withdrawal and administration of 10mg $PGF_{2\alpha}$, and goat R (restricted feeding) showing no LH surge during the 24-hour sampling period

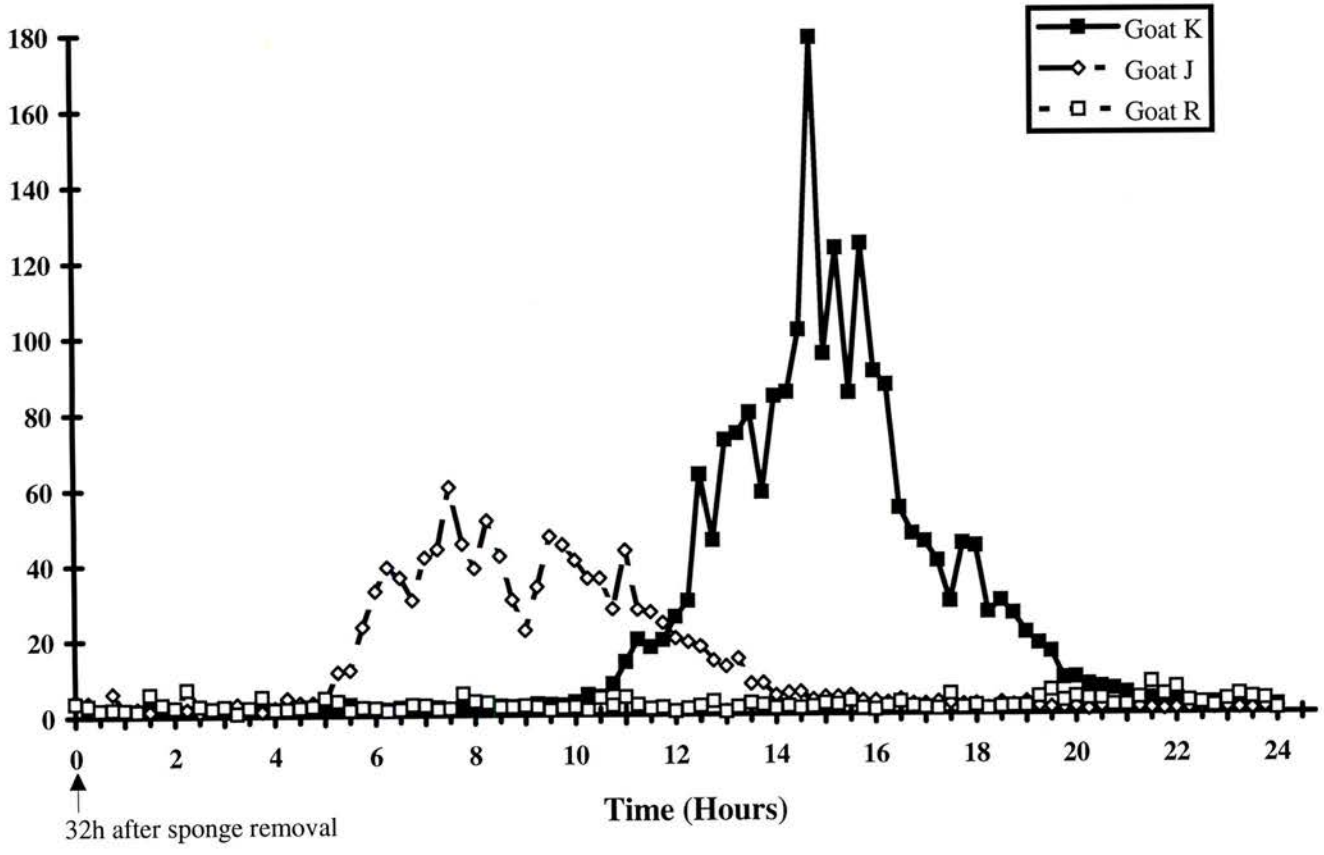

Goat K
Goat J
Goat R
180
160
140
120
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
18
20
22
24
Time (Hours)
32h after sponge removal

Figure 6.8 FSH profile of goat K (maintenance feeding) and goat J (restricted feeding) showing preovulatory FSH surge following sponge withdrawal and administration of 10mg $PGF_{2\alpha}$, and goat H (restricted feeding) showing no FSH surge during the 24-hour sampling period.

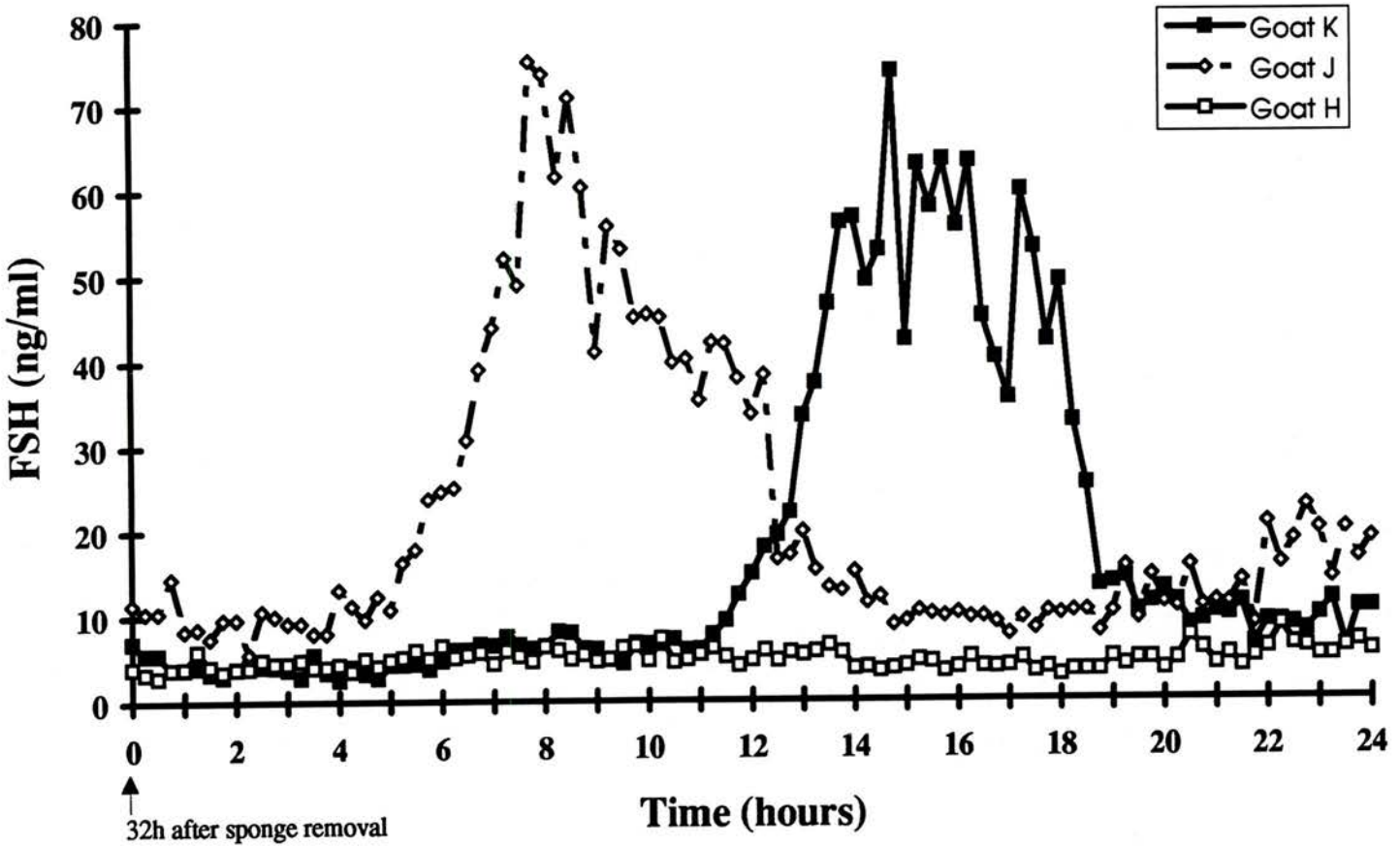

Goat K
Goat J
Goat H
FSH (ng/ml)
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
20
22
24
32h after sponge removal
Time (hours)

Figure 6.9 Luteinizing hormone profile in the three hours following gonadotrophin releasing hormone administration in a goat on maintenance feeding (Goat A) and another on restricted feeding (Goat F)

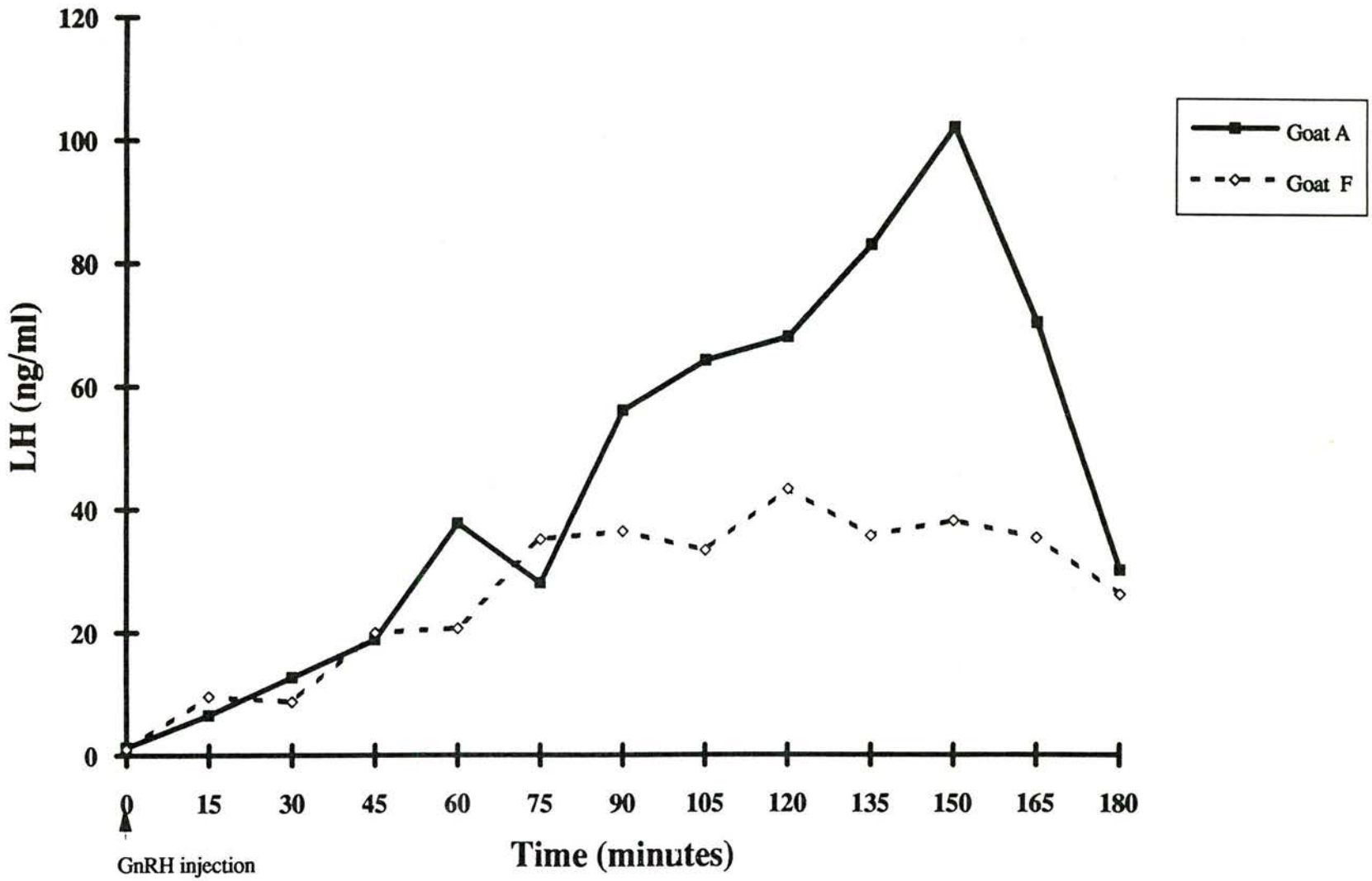

120
100
80
60
40
20
0
LH (ng/ml)
Goat A
Goat F
0
15
30
45
60
75
90
105
120
135
150
165
180
GnRH injection
Time (minutes)

Figure 6.10 Follicle stimulating hormone profile in the three hours following gonadotrophin releasing hormone administration in a goat on maintenance feeding (Goat B) and another on restricted feeding (Goat Q)

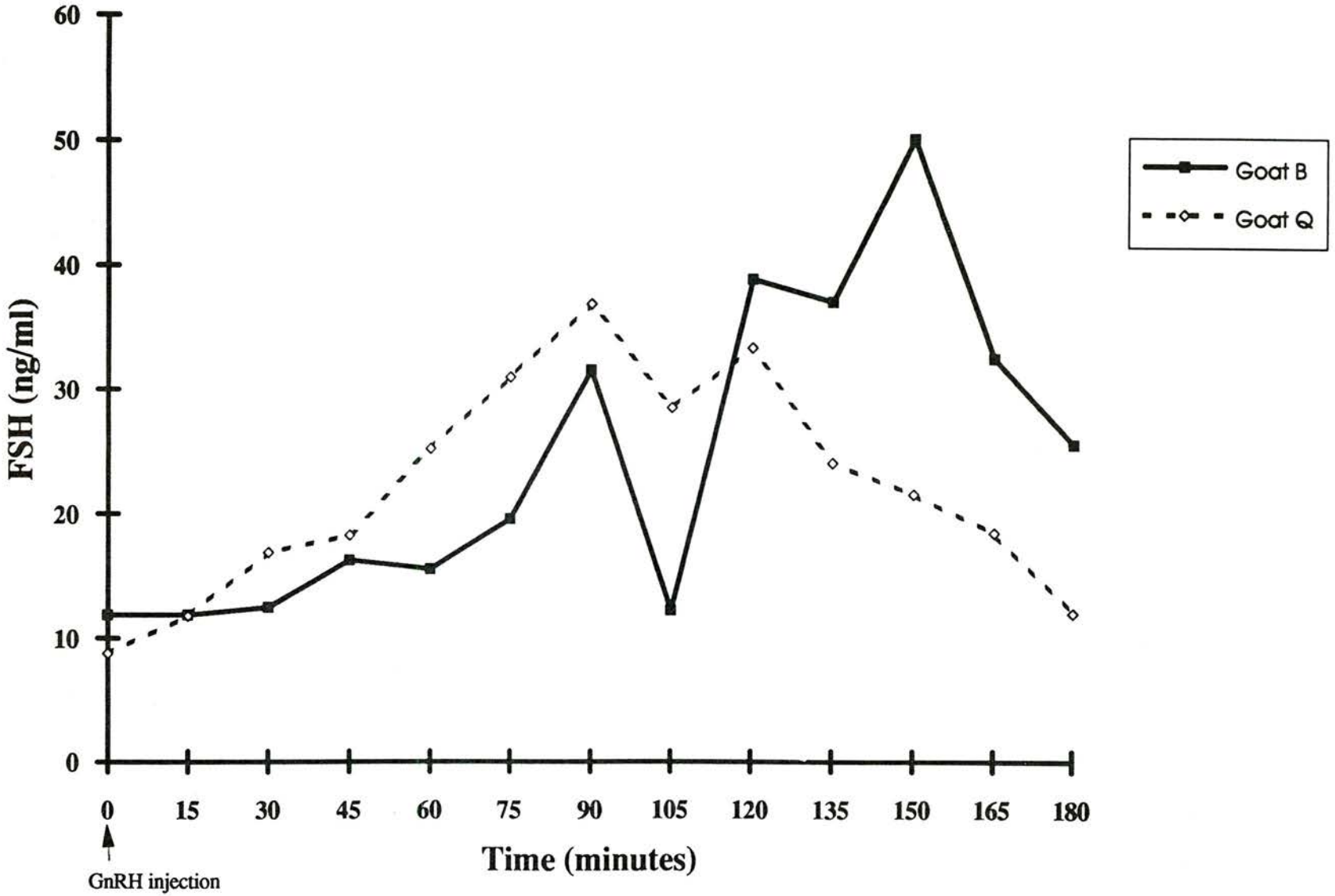

60
50
40
30
20
10
0
FSH (ng/ml)
0
15
30
45
60
75
90
105
120
135
150
165
180
Time (minutes)
GnRH injection
Goat B
Goat Q

Table 6.1 **Effects of undernutrition on the time of onset of oestrus, preovulatory surge of LH, its peak concentration and duration following luteolysis induced by sponge removal and $PGF_{2\alpha}$ administration in does**

	Group M (n=4)	Group R (n=2)	P
Sponge removal to oestrus onset (h)	31.25 ± 0.75	32.37 ± 2.38	0.576
Sponge removal to the beginning of LH surge (h)	33.83 ± 1.12	35.12 ± 3.12	0.670
Sponge removal to LH peak (h)	37.00 ± 0.52	36.12 ± 3.37	0.759
Onset of oestrus to the beginning of LH surge (h)	4.81 ± 1.99	2.25 ± 0.25	0.440
Onset of oestrus to LH peak concentration (h)	8.19 ± 2.22	3.75 ± 1.00	0.260
Duration of LH surge (h)	9.75 ±0.72	7.62 ± 1.12	0.171
Area under the LH surge (ng)	1974 ± 116	913 ± 151	0.006
Mean peak LH value (ng/ml)	178.4 ± 8.20	67.75 ± 7.25	0.001

Table 6.2 Effects of undernutrition on the relationship between induced luteolysis, oestrus onset and preovulatory increase in FSH concentrations in goats

	Group M (n=4)	Group R (n=2)	P
Sponge removal to oestrus onset	31.25 ± 0.75	33.37 ± 2.38	0.576
Sponge removal to the beginning of increased FSH concentrations (h)	37.88 ± 2.48	35.00 ± 2.75	0.520
Sponge removal to FSH peak concentration (h)	39.87 ± 2.45	36.50 ± 3.25	0.47
Onset of oestrus to the beginning of increased FSH concentrations (h)	6.31 ±2.06	2.62 ± 0.37	0.299
Onset of oestrus to FSH peak concentration (h)	8.62 ± 2.05	4.25 ± 1.00	0.236
Duration of increased FSH concentrations (h)	5.81 ± 0.06	5.87 ± 0.62	0.885
Area under increased FSH concentrations (ng)	1082 ± 58.2	986 ± 179	0.531
Mean peak FSH value (ng/ml)	97.3 ±26.1	62 ± 12.5	0.426

Table 6.3 Effects of undernutrition on the total LH released following intravenous administration of 13μg GnRH and the time and value of the peak concentration

	Group M (n=8)	Group R (n=8)	P
Mean LH level before GnRH administration (ng/ml)	1.53 ± 0.332	1.59 ± 0.280	>0.80
Mean time from GnRH administration to >10 ng/ml (min)	28.12 ± 3.40	43.13 ± 5.26	=0.03
Total LH released within 3 h. of GnRH administration (ng)	508.9 ± 38.7	391.6 ± 38.2	>0.05
Time from GnRH administration to LH peak concentration (min)	131.87 ± 7.56	133.1 ± 10.4	>0.80
Mean peak LH value (ng/ml)	82.21 ± 5.29	66.82 ± 6.94	>0.10

Table 6.4 **The effects of undernutrition on the total FSH released following intravenous administration of 13μg GnRH and the time and value of peak concentration**

	Group M (n=8)	Group R (n=8)	P
Mean FSH level before GnRH administration (ng/ml)	11.4 ± 1.54	7.98 ± 0.87	= 0.07
Time from GnRH administration to >20 ng/ml (min)	80.6 ± 10.6	97.5 ± 11.0	= 0.29
Total FSH released within 3 hours of GnRH administration (ng)	297.2 ± 27.5	226.4 ± 16.5	>0.05
Time from GnRH administration to peak FSH concentration (min)	135.0 ± 7.50	123 ± 6.8	=0.025
Mean peak FSH value (ng/ml)	42.42 ± 3.61	30.25 ± 3.05	= 0.02

6.4 Discussion

There are very few reports on gonadotrophin profiles in the different phases of the oestrous cycle in goats. The detection of LH pulses in the luteal phase of the two groups of goats in Experiment 6 of this study is in contrast to a study in Saanen goats by Knight *et al.*, (1988) in which LH pulses were undetectable during the luteal phase. In that study progestagen sponges were still present in the animals during the luteal phase when blood samples were collected. Artificially high circulating concentrations of progestagens may have interfered with hypothalamic-hypophyseal function. In the present study, mean LH pulse frequency was higher in both follicular and luteal phases than that reported in British White goat (Llewelyn *et al.*, 1993), but similar to that reported in the ewe (Rhind *et al.*, 1991). In the present study, mean concentrations and pulse frequency of LH were greater after luteolysis than during the luteal phase, in agreement with previous reports in does (Mori and Kano, 1984; Llewelyn, *et al.*, 1993) and suggests that, as in ewes (Karsch *et al.*, 1983) progesterone regulates LH pulse frequency.

Mean FSH concentrations in the present study were not significantly different in the period between 20 and 28h compared with 40 and 50h after sponge withdrawal, or compared with the luteal phase. By contrast, in ewes FSH concentrations decreased over the 3 days prior to LH surge (Rhind *et al.*, 1985). However in ewes, endogenous FSH concentrations do not appear to influence ovulation rates (Driancourt *et al.*, 1988; Adams *et al,* 1988), probably due to the large variability in FSH concentrations which are measured even within ewes of the same breed with the same ovulation rate (Picton and McNeilly, 1991). It seems likely that ovulation rate depends more on follicular sensitivity to gonadotrophins than on plasma gonadotrophin concentrations (Driancourt and Fry, 1989).

While there do not appear to be any other reports on the effect of undernutrition on gonadotrophins in goats, reports in sheep are contradictory. The results of this study broadly agree with those of Rhind *et al.* (1985 and 1989b) and Rhind and McNeilly (1986) in which different levels of feeding and body condition did not significantly affect basal concentrations of LH in the oestrous cycle. The secretion of gonadotrophic hormones, particularly LH, is known to be closely related to GnRH secretion (Clarke and Cummings, 1982) and it is known that LH is important in controlling terminal follicular growth and differentiation (Rhind *et al.,* 1985). The absence of a significant nutritional effect on LH profiles during the luteal phase suggests that undernutrition, at the level and duration applied in the present study did not significantly alter hypothalamic activity nor did it affect pituitary responsiveness in this period. In spite of these hormonal profiles, however, laparoscopic examination of the ovaries of these does demonstrated a significant effect of undernutrition on ovulation rate (Experiment 1, Chapter 3). This suggests that the effects of undernutrition on reproductive performance in goats were not mediated through changes in LH secretion in the luteal, follicular or the preovulatory period before the gonadotrophin surge. The failure to detect changes in LH concentration in does on restricted feeding may have been due to the does being in relatively good condition at the start of the experiment. In heifers, restricting dietary energy intake in animals with low body condition resulted in alterations to circulating LH patterns. However, heifers in good body condition did not show changes in LH concentrations in blood (Roberson *et al.,* 1992).

In the present study diet did not affect FSH concentrations. This observation is in agreement with an observation in sheep in which undernutrition did not produce changes in FSH concentrations (Rhind *et al.,* 1989b). In contrast however other studies in sheep have reported body condition (Rhind and McNeilly) and level of food

intake (Rhind *et al.*, 1985; Rhind and McNeilly, 1986) to influence FSH concentrations.

In the present study the tendency for more does on maintenance feeding to exhibit oestrus than those on restricted feeding is in agreement with the previous results (Experiment 1, Chapter 2). In the underfed does, one of the animals which failed to show oestrus actually ovulated, therefore underfeeding was associated with silent oestrus as well as anovulation. The number of does that exhibited a preovulatory gonadotrophin surge followed by ovulation was also reduced in does receiving restricted feeding. In does on restricted diet, failure of ovulation was clearly associated with the absence of a preovulatory surge of gonadotrophins during the sampling period. The decreased magnitude of the preovulatory gonadotrophin surge in does on restricted feeding which did ovulate suggested that restricted feeding may have depleted pituitary gonadotrophin reserves and/or reduced the sensitivity of the pituitary to GnRH in this period.

Even though only two does on restricted feeding exhibited oestrus and a gonadotrophin surge within the sampling period, there was no significant difference in the time from luteolysis to onset of oestrus, to gonadotrophin surge and to peak concentrations between these does and those on maintenance feeding. The mean duration of the LH surge observed in does on maintenance ration in the present study was similar to that observed in the British White goats (Llewelyn *et al.*, 1993), while that in restricted does was shorter. The duration of the surge was however slightly longer in the does on maintenance ration in the present study than in Shiba goats (Mori and Kano, 1984).

A wide range in preovulatory peak LH concentrations has been reported in both sheep (Rhind *et al.*, 1980) and goats (Ritar, *et al.*, 1984; Llewelyn, *et al.*, 1993).

While a number of studies in sheep demonstrated significantly higher preovulatory mean LH peak values in ewes with multiple ovulations than those with single ovulations (Rhind *et al.,* 1985), others have been unable to demonstrate any relationship between the magnitude of preovulatory LH peak and ovulation rate (Haresign, 1981; Rhind *et al.,* 1985). The biological significance of the large difference in the area under the LH surge and the preovulatory mean peak LH concentrations recorded between does on restricted feeding and those on maintenance feeding in the present study is therefore difficult to assess. However, it has been shown that LH increases blood flow to the ovary (Niswender *et al.,* 1976; Murdoch *et al.,* 1983). Increase in the blood flow to the developing follicles could act to increase local ovarian gonadotrophin concentration and presumably influence ovulation rate.

Preovulatory LH surges of reduced magnitude have been observed in association with abnormal corpora lutea and short oestrous cycles in goats following induced ovulation (Bretzlaff *et al.,* 1988; Camp *et al.,* 1983). The observation in this study of a tendency for does on restricted feeding to have an LH surge of lower magnitude and to have inadequate subsequent luteal function as indicated by a fall in progesterone levels soon after the initial rise appears to support this view. However, abnormal corpus luteum formation has also been observed in anoestrous ewes in which the induced preovulatory LH surge was comparable with that in cycling ewes (McNeilly *et al.,* 1981). The importance of the magnitude of the preovulatory LH surge to subsequent luteal function therefore remains to be clearly defined.

Response to administration of a pharmacological dose of GnRH is highly correlated with pituitary reserves of LH, therefore, we administered a large dose to evaluate total releasable pituitary gonadotrophin (Crowder *et al.,* 1982). The results of the present study in which restricted feed intake significantly reduced the release of LH after GnRH stimulation are in agreement with reports by Beal *et al.* (1978) in which

the pituitary LH content in intact cows was decreased in response to administration of GnRH. Similarly, in heifers, pituitary content of LH declined with increased weight loss in animals which were initially in good body condition (Roberson *et al., 1992*). The role of steroid feedback on the pituitary response to GnRH could not be determined in this study since both groups had ovaries. The observation in the present study suggested that undernutrition acted directly on the pituitary by depleting the gonadotrophin reserves.

The results from this study have shown that undernutrition tended to suppress oestrus and to reduce or eliminate the preovulatory gonadotrophin surge following luteolysis induced following sponge removal and $PGF_{2\alpha}$ administration. Total releasable concentration of gonadotrophins following administration of GnRH were also reduced in feed-restricted goats.

CHAPTER 7

The effects of undernutrition on progesterone secretion during early luteal phase and mid gestation in goats.

7.1 Introduction

Progesterone has long been known to be essential for the establishment of pregnancy in animals. There is evidence that enhancing the circulating concentrations of progesterone during the luteal phase of the oestrous cycle can improve fertility in ewes (Kittok *et al.*, 1983). The growth of sheep embryos in the first 2 weeks of pregnancy has also been shown to be influenced by the progesterone concentrations in the maternal plasma (Lawson, 1977). In the goat, the ovaries have been shown to be the main source of progesterone during pregnancy (Meites *et al.*, 1951).

It has been shown that the timing of the increase in progesterone concentrations following oestrus, controls the time of changes in uterine protein synthesis (Miller and Moore, 1976; Lawson and Cahill, 1983). The failure to establish pregnancy if embryos are transferred between ewes that were not on heat at the same time shows that embryos must develop in a uterus that is at an appropriate stage (Wilmut and Sales, 1981; Lawson and Cahill, 1983). Studies in ewes have demonstrated that very few animals ovariectomised after mating can remain pregnant if they are given 4 mg progesterone per day or less, whereas pregnancy will be established in a majority of ewes given 10 mg/day or more (Moore and Rowson, 1959; Trouson and Moore, 1974; Parr, *et al.*, 1982). This observation indicates that pregnancy will be maintained only if a certain minimal level of progesterone is present during the luteal phase. Two discrete phases were identified after mating when

progesterone influences uterine function: A period from oestrus until Day 4 or 5 when the level should be low, followed by an increase to levels typical of the luteal phase (Wilmut *et al.*, 1985). Later in pregnancy a certain minimum level of progesterone is essential for pregnancy maintenance in ewes (Trounson and Moore 1974; Parr *et al.*, 1982).

The previous experiments (1, 3 and 4) have demonstrated a tendency for undernutrition to reduce pregnancy rates and embryo survival and growth in goats. Since an association has been demonstrated between progesterone profiles and embryo survival in healthy, well managed ewes (Wilmut *et al.*, 1985), it is possible that the effects of undernutrition observed earlier were mediated through effects on progesterone concentrations after mating. The aim of the experiments in this chapter therefore was to determine the effects of undernutrition on progesterone concentration during the early luteal phase and mid-gestation in the goat. Experiment 7 provides information on progesterone levels during the early luteal phase in unmated does, while Experiment 8 provides similar information in mated does in the early luteal and mid-gestation periods.

7.2 Materials and methods

7.2.1 Experiment 7 The effects of undernutrition on progesterone concentrations in early luteal phase in unmated goats.

7.2.1.1 Animals and experimental procedure

Goats used in Experiment 6 (Chapter 6) were used for this experiment. The management and feeding of the goats have been described earlier (Chapter 6). Plasma

samples were collected once daily from Day 2 after synchronized oestrus until Day 10. Plasma progesterone concentrations were determined in all samples using the progesterone radioimmunoassay kit (RSL ^{125}I progesterone, ICN Biomedicals, Inc. Thames, UK) according to the manufacturer's directions.

7.2.2 Experiment 8. The effects of undernutrition on progesterone concentrations in mated goats.

7.2.2.1 Animals and experimental procedure

The does in Experiment 1 (Chapter 3) were used in this experiment. The management and feeding of the does were described earlier (Chapter 3). Plasma samples were collected daily from Day 1 after the second oestrus to Day 10. Samples from 4 does pregnant at 60 days after mating in Group M, 3 pregnant in Group R and 4 non pregnant in Group R were analysed for progesterone concentration by enzyme immunoassay using progesterone EIA kits for plasma and the procedure described by Bretzlaff *et al.*, (1989). Plasma samples were also collected from Day 51 to Day 60 after mating in 6 pregnant does in Group M and 4 in Group R and analysed.

7.2.3 Statistical analyses

Daily plasma progesterone concentrations after ovulation were compared between the two groups by the Mann-Whitney-Wilcoxon two sample test for ranked observations while those in mid-gestation were compared using the Kruskal-Wallis test. All means are presented as $\pm$ SEM.

7.3 Results

7.3.1 Experiment 7

Weight loss and body condition score changes of the goats have been described in Experiment 6 (Chapter 6).

Only 1 goat out of the 8 in the feed-restricted group exhibited elevated plasma progesterone concentrations (over 1ng/ml) which were sustained throughout the sampling period. Two other does in this group exhibited an initial rise in progesterone levels which, however, fell to undetectable levels soon afterwards. Plasma samples from two does in Group M were not analysed for progesterone because their intravaginally inserted sponge could not be completely removed. Plasma progesterone levels from Day 2 to Day 10 after oestrus in does on maintenance feeding and the one doe on restricted feeding are shown in Table 7.1. Progesterone concentrations in the doe on restricted feeding were lower than those on maintenance feeding however statistical comparison could not be made because of the small numbers of goats involved.

7.3.2 Experiment 8

Weight loss and body condition score changes were reported in Experiment 1 (Chapter 3).

Plasma progesterone levels from Days 1 to 10 in does on maintenance feeding that were pregnant at 60 days and those pregnant and non-pregnant on restricted feeding are shown in Table 7.2. There was a wide variation in progesterone concentrations especially in feed-restricted does. There was no difference in

progesterone concentrations between pregnant does on maintenance feeding and pregnant and non pregnant does on restricted feeding in the (P=0.235). Plasma progesterone concentrations between Days 51 and 60 of gestation was slightly, but significantly, higher (P=0.0315) in the feed restricted goats compared with those on adequate feeding (Tables 7.3).

Table 7.1 **The effect of undernutrition on plasma progesterone concentration ± SEM (ng/ml) from 2 to 10 days after oestrus in does (Group M = maintenance rations, Group R = restricted rations)**

Days post-oestrus	Group M (n=6)	Group R (n=1)
2	0.06 ± 0.04	0.00
3	0.92 ± 0.34	0.13
4	3.26 ± 1.09	0.95
5	5.01 ± 1.53	1.85
6	8.59 ± 1.81	4.07
7	11.34 ± 3.80	3.86
8	16.88 ± 4.54	3.63
9	11.68 ± 4.72	5.06
10	17.68 ± 5.26	4.81

Table 7.2 **Plasma progesterone concentration ± SEM (ng/ml) from 1 to 10 days after mating in does on maintenance (Group M) and restricted rations (Group R) that became pregnant and those on restricted rations that did not**

	Group M	Group R	
	Pregnant (n=4)	Pregnant (n=3)	Non-pregnant (n=4)
Mean Ov. rate	1.75 ± 0.25	1.75 ± 0.25	1.50 ± 0.29
Days post-mating			
1	1.5 ± 0.82	0.5 ± 0.25	0.1 ± 0.10
2	1.6 ± 0.46	0.4 ± 0.22	1.4 ± 1.17
3	2.6 ± 1.77	1.6 ± 0.285	1.4 ± 1.28
4	2.9 ± 1.42	3.8 ± 0.62	2.3 ± 2.06
5	7.8 ± 3.06	7.37 ± 3.00	4.8 ± 2.58
6	10.2 ± 2.42	10.4 ± 2.60	5.5 ± 2.00
7	9.6 ± 3.03	7.8 ± 3.10	5.9 ± 1.99
8	8.9 ± 1.55	10.5 ± 0.50	8.4 ± 0.80
9	10.1 ± 2.46	11.5 ± 0.50	10.9 ± 2.75
10	7.9 ± 4.47	13.5 ± 0.50	8.1 ± 3.25

Table 7.3 **Progesterone concentration ± SEM (ng/ml) between Days 51 and 60 after mating in does on maintenance (Group M) and restricted rations (Group R)**

	Group M (n=6)	Group R (n=4)
Mean ov. rate	1.83 ± 0.17	1.75 ±0.25
Days post mating		
51	13.3 ± 1.59	14.1 ± 2.09
52	13.3 ± 1.58	13.2 ± 1.68
53	13.8 ± 1.90	13.4 ± 1.83
54	11.5 ± 1.37	12.3 ± 1.43
55	12.3 ± 1.50	14.7 ± 1.45
56	14.8 ± 1.91	16.4 ± 1.37
57	12.1 ± 2.20	15.0 ± 1.11
58	12.8 ± 1.86	17.4 ± 1.97
59	12.8 ± 2.05	15.8 ± 0.21
60	13.7 ± 1.52	16.4 ± 1.87

7.4 Discussion

The plasma progesterone patterns, both in the luteal and the mid-gestation period observed in does in this study are in agreement with reports elsewhere for different breeds of goats (Blom and Lyngset, 1971; Irvine *et al.,* 1972; Jones and Knifton, 1972; Wentzel *et al.,* 1979; Llewelyn *et al.,* 1987; Pathiraja *et al.,* 1991). In Experiment 7, the failure of all but one doe in the feed-restricted group to demonstrate an elevation of progesterone level for 10 days after oestrus highlights the importance of adequate nutrition during the period before and immediately after oestrus in goats. The low progesterone concentrations in the doe on restricted feeding indicate minimal luteal function, however statistical comparisons could not be made with does on maintenance feeding. In Experiment 8, there was no difference in progesterone concentrations in the ten days after oestrus between pregnant does on adequate feeding and either pregnant or non-pregnant does on restricted feeding, probably because of the small number of animals in the groups and the wide variation in progesterone concentrations. The drop in progesterone concentrations after an initial rise observed in 2 out of 3 does on restricted feeding that exhibited an elevation in concentration, indicates that undernutrition may be associated with premature loss of luteal function. The mechanism through which the effect of undernutrition on luteal function is mediated is not clear. In Chapter 6, it was observed that the preovulatory LH surge was of lower magnitude in does on restricted nutrition. Previous work in goats has suggested that an insufficient LH surge may lead to abnormal luteal development (Armstrong *et al.,* 1983b). It has also been suggested that restricting energy intake reduces the CL's responsiveness to stimulation by LH resulting in the CL synthesizing and releasing less progesterone (Gombe and Hansel, 1973; Apgar et al., 1975). The action of LH has been shown to be mediated via a specific receptor residing in the plasma membrane of the luteal cells. Modifying the availability of these receptors can modify the effects of LH on the ovary (Hoyer and Niswender, 1985).

The higher progesterone concentrations recorded in the period between Day 51 and 60 of gestation in the feed-restricted goats compared with those on adequate feeding in the present study is in agreement with a number of studies in pregnant sheep (Cumming *et al.,* 1971; Wilmut *et al.,* 1985), which have reported an inverse relationship between plane of nutrition and peripheral plasma progesterone. Reduced metabolic clearance rate or mobilization of stores of progesterone have been suspected to be the cause (Cumming, et al. 1971) but, the mechanism and significance of the increase have yet to be explained.

In conclusion, although undernutrition did not appear to affect circulating concentrations of progesterone early in gestation, by mid-gestation there was an inverse relationship between level of nutrition and plasma progesterone concentrations.

CHAPTER 8

The effects of undernutrition on ovarian follicle populations, gonadotrophin and prolactin binding to the cells of the corpus luteum, and the cellular morphology of the goat CL.

8.1 Introduction

Poor ovulation rates have been recorded for goats on low, compared with those on adequate levels of feeding (Experiment 1). From the dynamics of folliculogenesis, it is evident that variations in ovulation rate must arise from differences in the number of follicles entering the antral phase of growth and/or differences in the prevailing rate of atresia. Either and/or both of these differences would alter the potential number of follicles, sufficiently developed, to ovulate in response to the required gonadotrophin stimulus. It has been suggested (Cahill, 1981) that long-term treatments which influence ovulation rate (e.g. breed, season and age) are likely to act primarily by increasing the number of non-atretic antral follicles, while short-term treatments, that change the ovulation rate per ovary (e.g. PMSG stimulation, unilateral ovariectomy and short-term nutritional effects), probably act primarily by changing the number of follicles that undergo atresia in the final stages of growth. In goats, numbers of large and medium sized follicles have been reported to vary with the stage of the cycle but never exceeded 15% of the total number of ovarian follicles (Pretorius, 1971). There are however, no reports in this species on the effect of undernutrition on follicular populations at any stage of the oestrous cycle.

The CL plays a crucial role in the reproductive process of mammals. Progesterone, which is required for the maintenance of pregnancy is the major primary endocrine secretory product of the CL. Abnormal luteal function has been implicated in failure of implantation and embryo wastage (Niswender *et al.*, 1985). This view was supported by evidence from Experiment 8 (Chapter 7) of this study in which a higher proportion of feed-restricted goats than those on maintenance feeding either failed to exhibit elevation of progesterone levels following oestrus synchronization or demonstrated a drop to undetectable levels soon afterwards.

Studies on the relative and absolute numbers and volumes of the luteal cells have been conducted in sheep (Rodgers *et al.*, 1984; Niswender *et al.*, 1985) but not in goats. Despite several similarities in the reproductive process between sheep and goats, there are important differences that makes specific research in the goat important. For instance, in contrast to the sheep where the CL can be dispensed with after Day 50 of pregnancy without causing abortion, the goat's CL is essential throughout pregnancy (Cooke and Knifton, 1980). The CL of domestic animal species consists of 2 distinct types of endocrine cells -small and large luteal cells. Basal secretion of progesterone by large luteal cells is at least 4 times that of small luteal cells on a per cell basis (Fitz *et al.*, 1982; Rodgers *et al.*, 1983). The regulation of progesterone secretion by the CL in mammalian species is complex. In goats, as in most domestic animal species, LH is believed to be the main luteotrophin. An absolute requirement for LH in the maintenance of a functional CL has been demonstrated in both pregnant and non-pregnant goats (Heap *et al.*, 1988). The action of LH, like that of other protein hormones is mediated via a specific receptor residing in the plasma membrane of the luteal cell. The availability of these receptors can therefore modify the effects of the hormone (Hoyer and Niswender, 1985). Even though the large luteal cells' contribution to luteal progesterone secretion is significant, this

contribution is apparently independent of LH regulation. Receptor concentrations for LH were reported to be 10 times greater in small than in large luteal cells, in which receptors are barely detectable (Fitz *et al.,* 1982; Hoyer and Niswender, 1985). It has been suggested that LH may play a role in regulating the differentiation of small luteal cells into large luteal cells (Donaldson and Hansel, 1965; Niswender *et al.,* 1985).

Prolactin has been reported to act synergistically with LH in maintaining pregnancy in hypophysectomised goats (Buttle, 1983), suggesting its role in the maintenance of the CL. Confirming data in intact animals are however not available. In rats prolactin has been reported to be necessary for maintenance of normal numbers of receptors for LH in the developing CL. Specific receptors for prolactin have been reported in human (Saito and Saxena, 1975; Poindexter *et al.,* 1979; McNeilly et al., 1980; Ben-David and Schenker, 1982) and porcine CL (Rolland *et al.,* 1976). In porcine luteal cells, prolactin appears to influence the number of receptors for low-density lipoprotein (Murphy and Rajkuman, 1985) and may therefore play a role in regulating substrate availability for steroidogenesis.

The results of previous experiments in this study demonstrated a reduced preovulatory surge of LH and FSH in feed-restricted goats. The experiments in this chapter were therefore carried out to look at the effects on the CL. The objectives were to study the effects of undernutrition (i) on gonadotrophin and prolactin receptor populations in the CL, (ii) on morphological ultrastructure of the luteal cells and (iii) on ovarian follicle populations during the early luteal phase in goats.

8.2 Materials and methods

8.2.1 Experiment 9 Effect on ovarian follicle populations, LH and prolactin binding to CL and cellular morphology of CL

8.2.1.1 Animals and experimental procedure

Samples for determination of ovarian follicle populations, LH and prolactin receptor assay, light and transmission electron microscopy were obtained at the end of Experiment 6 (Chapter 6). The animals, their feeding, and management have been described earlier (Chapter 6, Experiment 6). Six days after oestrus does were slaughtered by stunning and exsanguination. The ovaries from 7 goats in Group M and 8 from Group R were immediately recovered and weighed. The ovaries were dissected longitudinally and follicles with diameters of 1 to 5mm, 5 to 10mm and greater than 10mm were counted. Six whole CL from 5 does in Group M and 9 from 7 goats in Group R were dissected out, weighed, snap frozen in liquid nitrogen as soon as they were obtained and stored at $-20^{o}C$ pending assays for gonadotrophin and prolactin binding to the cells of the corpus luteum. Slices of CL from 4 does in each of the two groups were collected, placed in Bouins solution and submitted for haematoxylin and eosin staining. Slices of CL from 2 goats each in Group M and R were cut into several pieces of approximately 1 mm^3 and placed in bijou bottles containing 3% gluteraldehyde in cacodylate buffer at $4^{o}C$ and immediately sent for processing for electron microscopy.

Binding of LH, FSH and prolactin to CL were determined using the technique described by Bramley *et al.,* (1987). DNA concentrations of luteal tissues were measured using the method of Setaro and Morley (1976).

Sections of CL were viewed under the oil immersion lens of a light microscope. Populations of large and small luteal cells were counted in 30 randomly selected fields in each CL section. The diameter of 12 large luteal cells randomly selected in each section were measured. Sections were also viewed under the electron microscope for the study and subjective comparisons of the ultrastructure of the luteal cells.

8.2.1.2 Statistical analyses

Follicle populations, numbers and weights of CL, luteal cell numbers and sizes and LH and prolactin receptor concentrations were analysed using the one-way ANOVA. The proportion of the two luteal cell types were compared using the Chi-square test.

8.3 Results

The changes in body weight and condition score during the course of the experiment have been described in Chapter 6, Experiment 6.

8.3.1 Ovulation rate and weight of CL

Mean ovary weight, number of CL per ovary, weight of CL and ovulation rate in does on maintenance feeding and those on restricted feeding are shown in Table 8.1. Ovulation rate, as determined by counting the number of CL at slaughter, was significantly

higher (P=0.018) in Group M than in Group R does. The mean weight of ovaries and the number of CL per ovary were also higher in Group M than in Group R does (P=0.050 and 0.017, respectively). There was however no difference in the weight of individual CL in the two groups.

8.3.2 Ovarian follicle populations

Table 8.2 shows the effect of undernutrition on follicle populations in goats. Does in Group M tended to have a larger number of follicles between 1 and 5 mm (P=0.094), between 5 and 10 mm in diameter (P=0.089) and total overall follicles greater than 1 mm in diameter (P=0.063). There were no follicles greater than 10 mm in does on maintenance feeding and only 2 in one doe on restricted feeding. Follicles between 1 and 5 mm in diameter constituted 87.3 and 87.0 % of all follicles greater than 1 mm in Groups M and Group R, while those between 5 and 10 mm constituted 12.8 and 10.6%, respectively.

8.3.3 Luteinizing hormone and prolactin binding to luteal tissue

Determination of FSH receptor concentrations was not successful because the non-specific bindings were too high. The levels of specific LH binding were in general higher than those of prolactin.

The effects of undernutrition on LH and prolactin binding to CL are shown in Table 8.3. The mean DNA concentration per CL was similar in the 2 groups. Mean LH binding per CL and per unit DNA were higher in Group M than in Group R but with the huge variation in both groups, the differences were not significant (P=0.52; P=0.49,

Table 8.1 **Weight of the ovary and CL and ovulation rate in does on maintenance (Group M) and restricted feeding (Group R)**

	Group M (n=7)	Group R (n=8)	P
Mean ovary weight (g)	2.82 ± 0.32	1.88 ± 0.15	0.017
Mean ovulation rate	2.43 ± 0.30	1.25 ± 0.31	0.018
Mean weight of individual CL (g)	0.26 ± 0.03	0.28 ± 0.180	0.770

Table 8.2 Ovarian follicle populations in does on maintenance (Group M) and restricted feeding (Group R)

	Group M (n=7)	Group R (n=8)	P
Mean number of follicles 1 to 5 mm in diameter per ovary	12.6 ± 1.53	8.7 ± 1.56	0.094
Mean number of follicles 5 to 10 mm in diameter per ovary	1.86 ± 0.39	1.06 ± 0.25	0.089
Mean number of follicles greater than 10 mm in diameter per ovary	0	0.12 ± 0.85	0.369
Total population of follicles over 1 mm per ovary	14.43 ± 1.59	10.00 ± 1.62	0.063

respectively). Mean prolactin binding per CL and per unit DNA was higher in Group R than in Group M but the differences were not significant.

8.3.4 Morphology of luteal cells

Under the light microscope, sections of CL from does on maintenance feeding and those on restricted feeding revealed small spindle-shaped cells interspersed between the large luteal cells in most regions of the CL sections (Plate 8.1). The small spindle-shaped cells also occurred almost exclusively in certain regions of the CL and in areas around the trabeculae (Plate 8.2).

The large luteal cells were mostly spherical or polyhedral with lightly staining cytoplasm and a round or slightly elongated nucleus that was centrally located in most of the cells (Plates 8.1 and 8.2). There was no difference (P=0.88) in the diameter of the large luteal cells between does on maintenance feeding (27.9 $\pm$ 0.30) and those on restricted feeding (27.8 $\pm$ 0.27). The small spindle-shaped cells varied greatly in length and width and had a dark staining cytoplasm and a round or elongated nucleus The small luteal cells were not readily distinguishable from amongst these spindled-shaped cells therefore there sizes were not measured.

The ultrastructural morphology of the small and large luteal cell types of the goats on maintenance and restricted feeding was not different under the electron microscope. The large luteal cell (Plate 8.3.) contained a large, slightly irregular nucleus with usually one distinct nucleolus. Mitochondria were observed all over the cytoplasm but were particularly numerous close to the nucleus. The mitochondria were varied in shape and size (Plate 8.4) but the majority were spherical or slightly elongated. Smooth endoplasmic

reticulum was abundant (Plate 8.6) and mostly found near the periphery of the cell. Rough endoplasmic reticulum was however relatively scarce. Neither the smooth nor the rough endoplasmic reticula were arranged in sheets or whorls. Vacuolated Golgi complexes, in active synthesis, were also seen in close proximity to the nucleus. There were abundant membrane-bound, densely staining secretory granules of varied sizes and staining intensity (Plate 8.5). Lipid droplets of various sizes were also seen in the large luteal cells. Old large luteal cells appear side by side with younger cells. The old cells had lighter staining cytoplasm, fewer lipid droplets and a rounder nucleus containing less prominent chromatin granules than the younger cells (Plates 8.6). The smooth endoplasmic reticulum was more abundant in the younger cells than in the older ones (Plate 8.6).

Under the electron microscope the small luteal cells were recognizable and contained a more irregularly shaped nucleus and a less distinct nucleolus than the large luteal cell (Plates 8.7). The small luteal cells showed a much lower proportion of cytoplasm than the large luteal cells. The nucleus occupied a far higher percentage of the cell than in the large luteal cells. Spherical or elongated mitochondria were observed but were fewer in number than in the large luteal cells. The smooth endoplasmic reticulum was more abundant than the rough endoplasmic reticulum. The Golgi complexes were less elaborate than in the large luteal cells. Secretory granules were virtually absent in the small luteal cells however lipid droplets were more abundant and larger than in the large luteal cells (Plate 8.6). Connective tissue cells, endothelial cells of blood vessels and numerous red blood cells, were observed in the CL sections. These cells were elongated and did not possess the steroidogenic structures of the luteal cells (Plate 8.3).

Table 8.3 **Luteinizing hormone and prolactin binding to CL in does on maintenance (Group M) and restricted feeding (Group R)**

	Group M (n=5)	Group R (n=7)	P
Number of CL	6	9	
Mean DNA binding per CL (μg)	5.8 ± 1.30	6.5 ± 0.49	0.56
Mean LH binding per CL (pg)	209± 68	157 ± 45	0.52
Mean LH binding per unit DNA (pg/μg)	33.7 ± 5.20	26.3 ± 7.80	0.49
Mean prolactin binding per CL (pg)	14.1 ± 4.80	19.4 ± 2.80	0.37
Mean prolactin receptor concentration per unit DNA (pg/μg)	2.3 ± 0.33	3.01 ± 0.42	0.21

Plate 8.1 Light micropscopic section of goat CL showing small spindle-shaped cells (SP) interspersed between large luteal cells (L).-X900

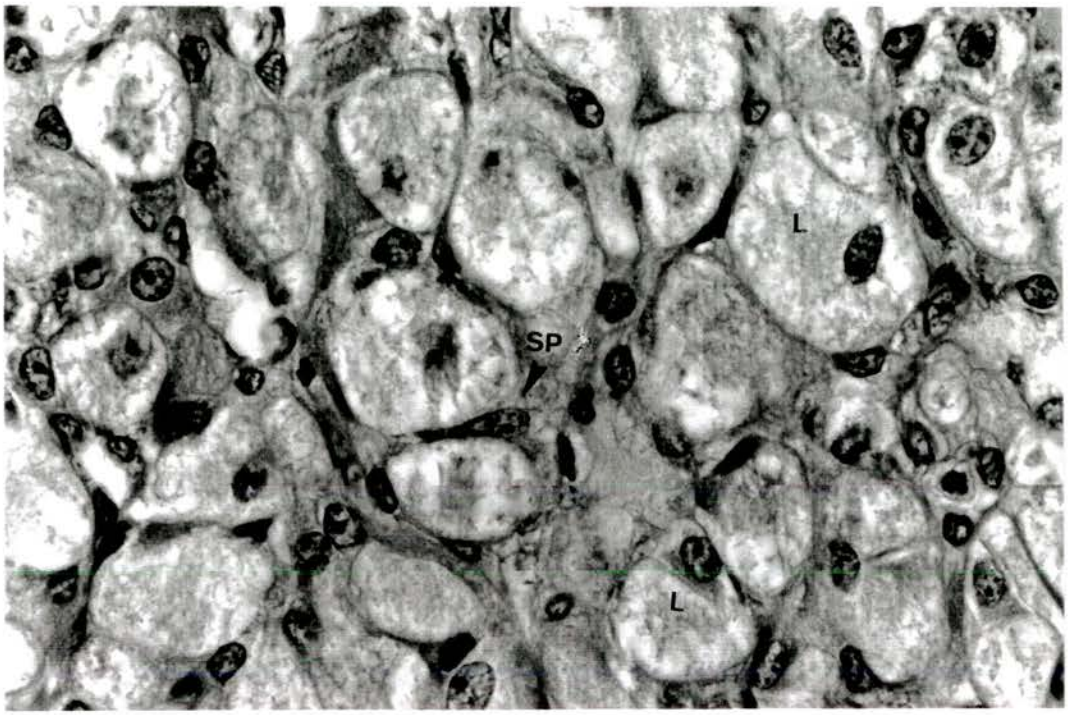

L
SP
L

Plate 8.2 Light microscope section of a goat's CL showing regions of small spindle-shaped cells interspersed between large luteal cells (L) and regions of predominantly small spindle-shaped cells (SP).-X144

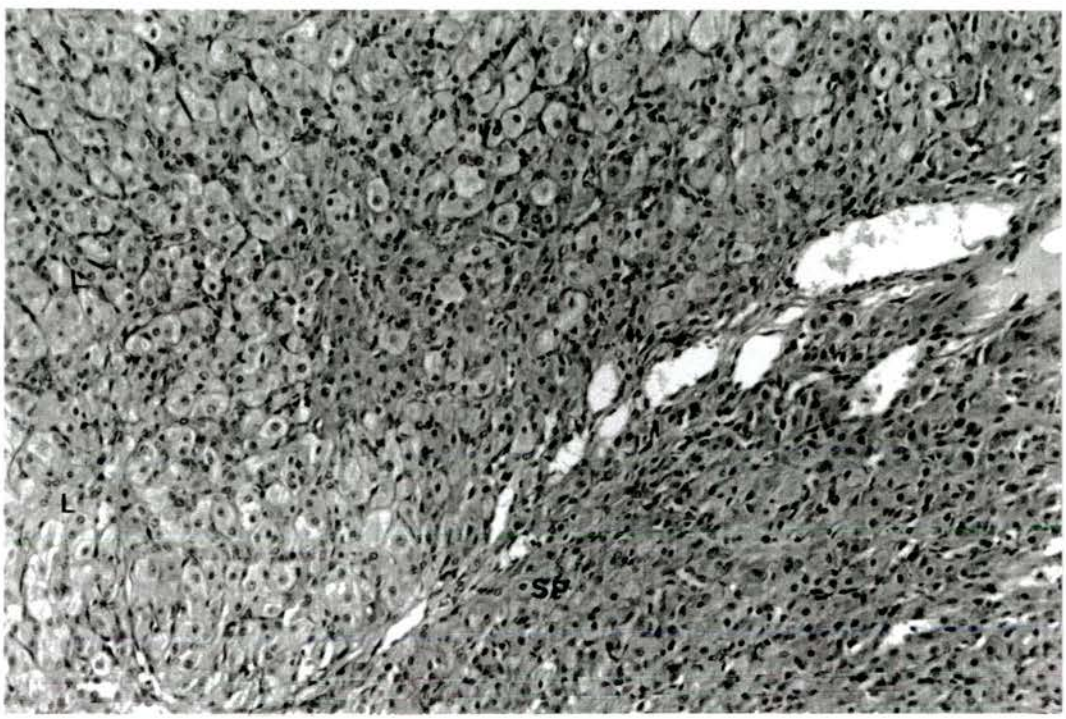

L
SB

Plate 8.3 Electron micrograph of a goat's CL showing a portion of a large luteal cell containing a nucleus (N), several mitochondria (M), lipid droplets (LD) and secretory granules (SG). Endothelial cells (ED) and red blood cells (RBC) were observed near the large luteal cell.-X6846

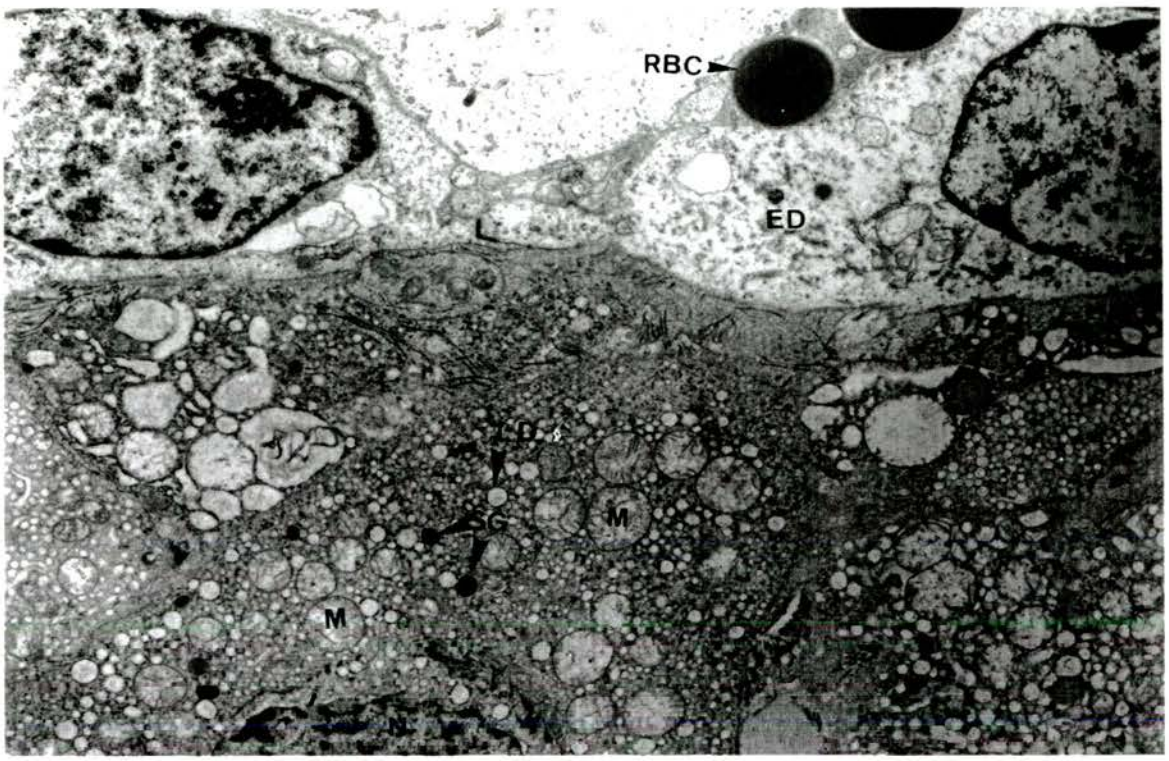

RBC
ED
L
G
M
M
N

Plate 8.4 Electron micrograph showing an enlarged portion of a large luteal cell with mitochondria (M) and lipid droplets (LD) of different shapes and sizes.- X33000

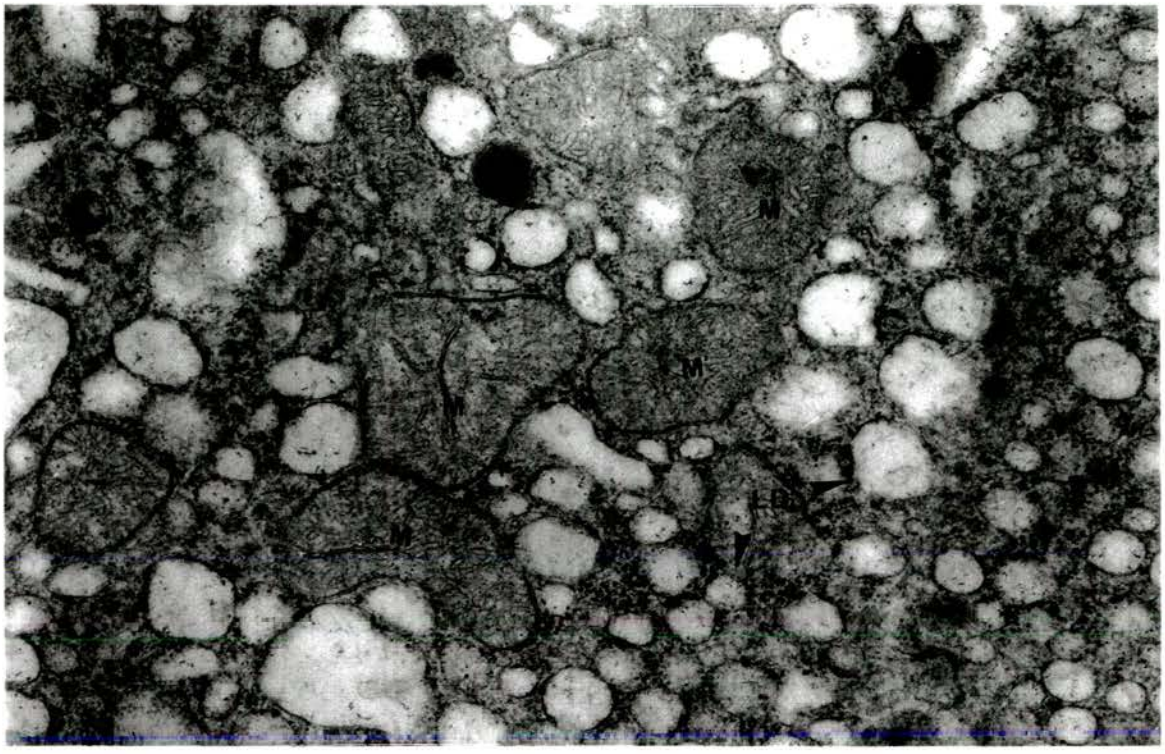


Plate 8.5 Electron micrograph showing an enlarged portion of a large luteal cell with active Golgi complexes (G) and secretory granules (SG) of different sizes and staining intensity.-X30800

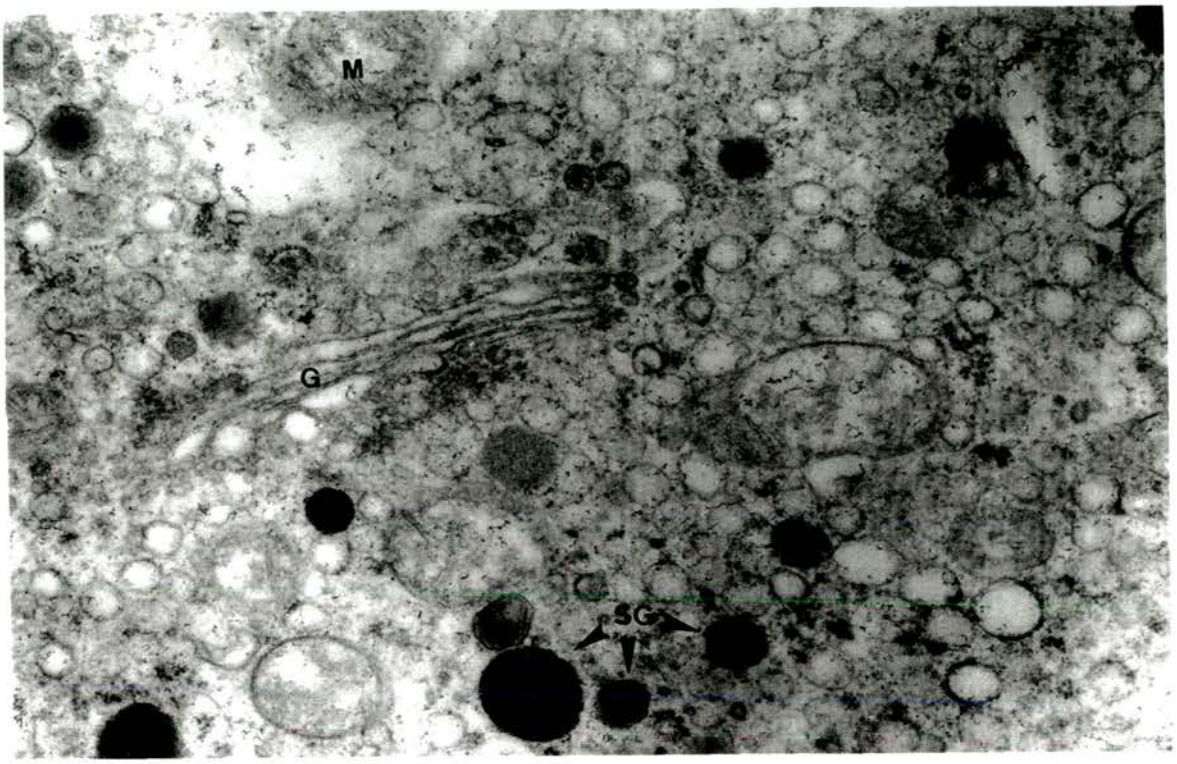

M
G
SG

Plate 8.6 Electronmicrograph showing a young and old large luteal cells. In the older large luteal cell the nucleus (N) was rounder, the cytoplasm (CT) was paler with less smooth endoplasmic reticulum (SER) and there were fewer lipid droplets (LD).-X6410

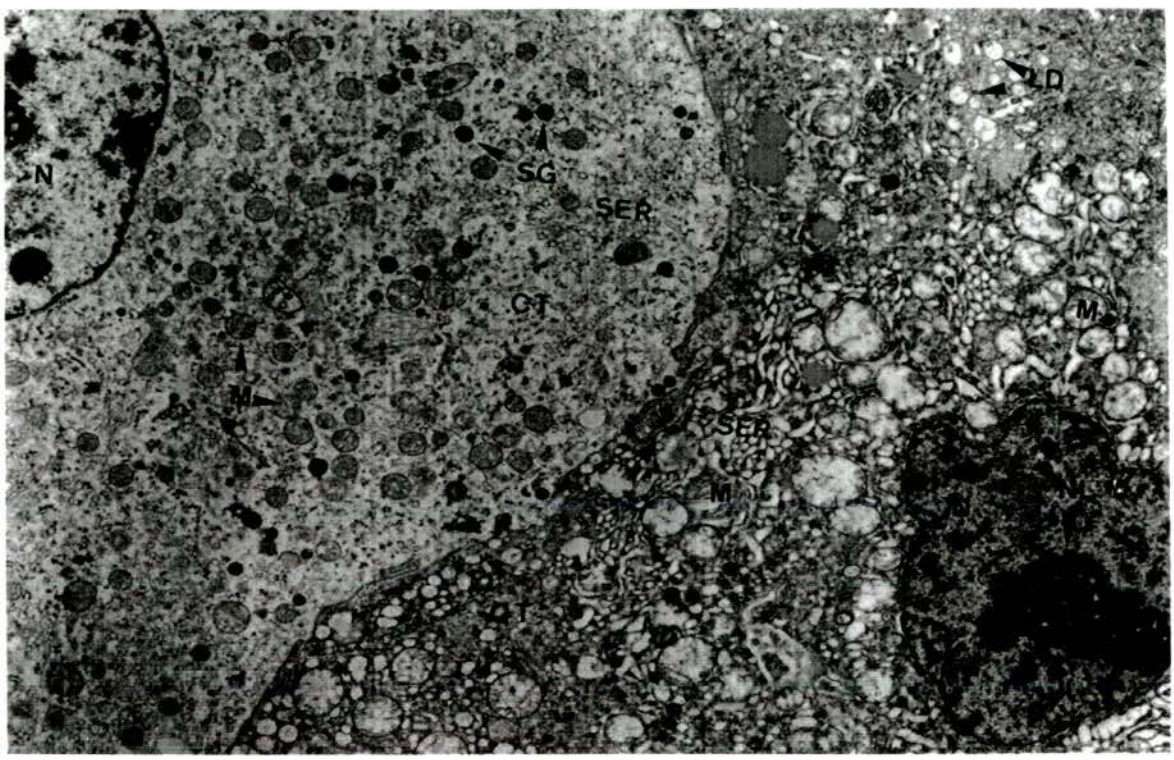
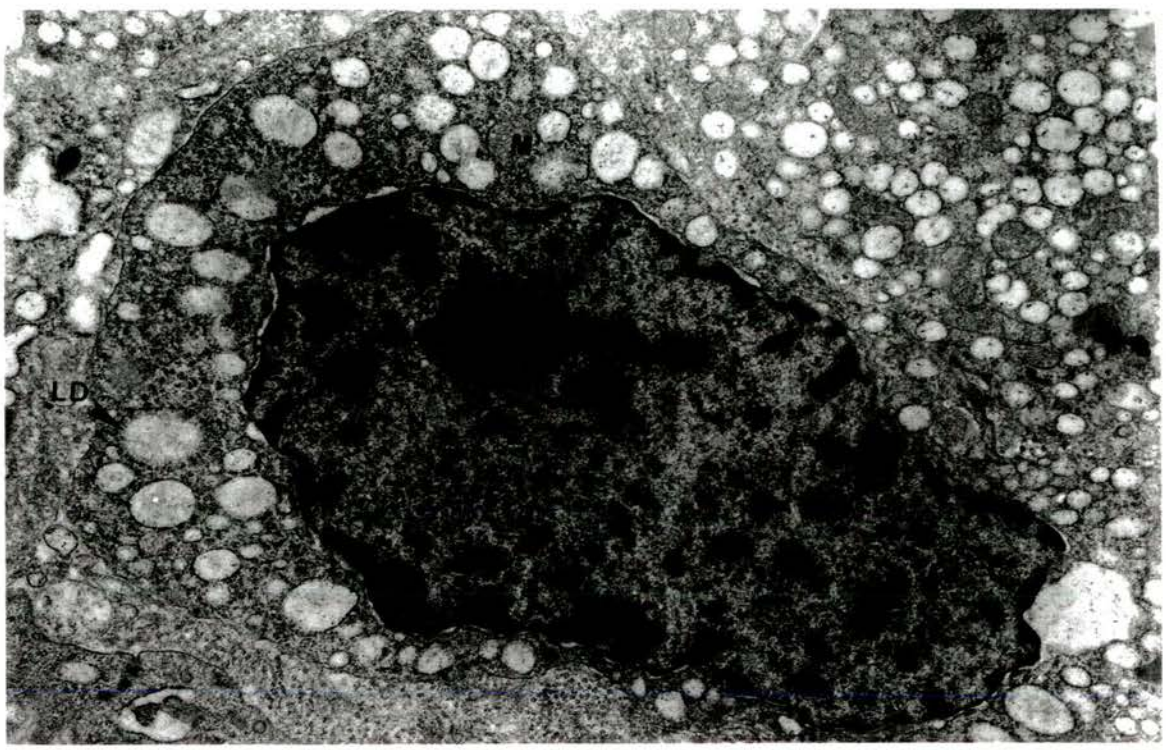

N
SG
SER
CT
M
LD
M
SER
M

Plate 8.7 Electron micrograph showing a small luteal cell. The nucleus occupied a much greater proportion of the cell than in the large luteal cells. There was relatively less cytoplasm in the small luteal cells compared with the large luteal cells. The nucleolus was less distinct, the mitochondria (M) were fewer and the lipid droplets (LD) were larger than in the large luteal cells. Secretory granules were virtually absent.-X14010


N
LD

8.4 Discussion

The CL of the doe was reported to attain a maximum size and weight at around Day 10 after oestrus, which is somewhat later than in ewes (Pretorius, 1971). Growth and the changes that normally occur in the CL were reported to be similar among goat breeds (Harrison, 1948) and to resemble those reported in sheep (Hutchison and Robertson, 1966). The average weight of the CL in this study was slightly lower than that reported for Angora goats at a similar stage of the reproductive cycle (Pretorius, 1971). In a study in Angora goats, Pretorius (1971) recorded the largest number of follicles and the highest mean follicle diameter around the time of oestrus. The incidence of large and medium sized follicles in that study, varied with the stage of the cycle but never exceeded 15% of the total number of the ovarian follicles. The number of follicles recorded in the does on maintenance feeding in the present study is greater than that reported for Angora does at a similar stage of the cycle (Pretorius, 1971) but the proportions are similar. The difference in number of follicles compared with Angora does probably reflects the higher ovulation rates in dairy breeds of goats.

The present study demonstrated a significant effect of undernutrition on ovulation rate and a tendency for it to affect the number of small (1 to 5 mm) and medium (5 to 10 mm) sized follicles in the ovary of the goat. The effects on ovulation rate is consistent with an earlier observation (Experiment 1, Chapter 2), and with reports of overall reduced fertility in does on inadequate nutrition (Singh and Sengar, 1970; Sachdeva *et al.*, 1983), with low body mass or in poor body condition (Shelton, 1961; Van der Westhuysen, 1981, Wentzel, 1987). The apparent decrease in numbers of both small and medium sized follicles in the feed-restricted group in the present study indicates that the effect of undernutrition on ovulation rate begins to manifest itself early in the development of the follicles. This observation differs from those reported following nutritional flushing of sheep in which the numbers of small follicles

from 18h after oestrus were not affected but those of follicles >3mm in diameter were greater in ewes on higher plane of nutrition than those on maintenance diet (Haresign 1981; Nottle *et al.,* 1986; Rhind and McNeilly, 1986; Xu, et al.; 1989). Haresign (1981) suggested that nutritional flushing of ewes did not affect the number of small follicles, but increased ovulation rate by preventing atresia of 2 to 3mm follicles. The apparent decrease in the number of follicles 1 to 5mm and 5 to 10mm in diameter in does on restricted feeding in the present study therefore indicates that undernutrition might have resulted in increased atresia of follicles. It was also possible that undernutrition reduced the pool of preantral and early antral follicles from which those for subsequent development were recruited. The underlying mechanism(s) by which undernutrition affects follicle populations in the ovary remains unclear (Smith, 1988). In the previous experiments (Experiment 5, Chapter 6), FSH profiles during the luteal and follicular phases were not different in adequately fed goats that subsequently had a higher ovulation rate compared with those in the feed-restricted goats. However, earlier studies in ewes suggested that the effect of undernutrition is mediated either through alteration in the ovarian sensitivity to gonadotrophins rather than by alteration of gonadotrophin profiles (Findlay and Cumming, 1976; Scaramuzzi and Radford, 1983; Ritar and Adams, 1988), or that subtle changes in gonadotrophin profiles during the reproductive cycle could be sufficient to produce the observed effects (Lindsay, 1991). It is also possible that the effects of undernutrition on follicle populations and ovulation rate may be mediated through changes in other factors which act either directly on the ovary or indirectly by modulating the actions of gonadotrophins. Several of such factors have been implicated, among which are the ovarian steroids and proteins, locally produced substances, growth factors such as EGF and FGF, which appear to control the development of preantral and early antral follicles, and IGF-1, which probably enhances the stimulatory effects of FSH autocatalytically (Mariana *et al.,* 1991).

The number of LH receptors was reported to increase from Days 2 to 10 of the oestrous cycle in ewes, remained high through Day 14 and then decline dramatically by Day 16 (Niswender *et al.*, 1985). The total LH binding in this study was correlated with the weight of the CL, an observation similar to that in ewes (Niswender *et al.*, 1985). There was large variability however in LH binding in both feed-restricted does and those on normal feeding. Despite significantly reducing ovulation rates at the previous oestrus, and tending to lower total ovarian follicular populations, undernutrition in this study did not clearly affect mean LH binding in the CL in goats. It has been shown in ewes, that the total LH binding to the CL is highly correlated with secretion of progesterone (Niswender *et al.*, 1985). Progesterone levels were not recorded in the period prior to CL collection in this study, and therefore, the relationship between the LH receptor concentration and progesterone secretion by the same set of CL could not be determined. In cattle it was shown that ovarian hypofunction under conditions of restricted energy intake was not due to reduced circulating levels of LH (Gombe and Hansel, 1973). It was suggested that the first effect of restricted feed intake is a reduced ability of the ovarian tissue to respond to LH (Gombe and Hansel, 1973). The possibility that other local factors in the ovary could play a role in the mediation of nutritional effects on luteal function cannot be discounted.

This study demonstrated prolactin binding to the goat's CL. There was however no significant difference in binding with level of nutrition. Previous reports on prolactin receptor concentrations in CL of sheep and goats are not available. Prolactin receptor protein has been reported to be similar in sequence to growth-hormone receptor which may indicate that they have a similar, yet-to-be-determined mechanism of action (Edery *et al.*, 1989). The frequency of release of prolactin and pituitary secretion of mRNA for prolactin have been reported not to be affected by long-term undernutrition in ovariectomized ewe lambs in contrast to LH and FSH and their

mRNAs (Foster *et al.*, 1989; Thomas *et al.*, 1990). Even though prolactin has been reported to be necessary for the maintenance of normal numbers of receptors for LH in the developing CL in rats (Richards and Williams, 1976), its role in regulating luteal function in other domestic ruminants is not very clear. It does not appear to influence luteal function in cattle (Hansel *et al.*, 1973; Hoffman *et al.*, 1974) or sheep (Karsh *et al.*, 1971; Niswender, 1974). Both long term restricted feeding and subsequent *ad-libitum* refeeding of ovariectomized ewe lambs had no effect on serum prolactin and pituitary expression of its mRNA (Foster *et al.*, 1989; Thomas *et al.*, 1990). The exact role of prolactin and its receptors in regulating luteal receptors for LH in species other than rodents and the significance of the observation in this study will require to be further investigated.

The failure to demonstrate FSH binding to the tissues of the CL in the present study indicates that receptors for this hormone were not present in the goat CL. Future investigations are needed to demontrate the presence of these receptors in the follicles and there relationship with ovulation rate and level of nutrition.

The gross weight of individual CL in this study were unaffected by feed-restriction. Both light and electron microscopic studies also did not reveal any differences. The gross, microscopic and ultrastructural morphology of goat luteal cells are in general, similar to those described in other domestic ruminants (Donaldson and Hansel, 1965; Niswender *et al.*, 1976; O'Shea *et al.*, 1979a and b; Fitz *et al.*, 1982; Rodgers *et al.*, 1984 Niswender and Nett, 1988). The spindle shape of the small cells observed under the light microscope indicates that most are probably not steroidogenic but endothilial cells and fibroblasts. Minor difference in the relative populations of small and large luteal cells had been noted between the outer and the inner regions of the sheep CL (Rodgers *et al.*, 1984). The differences observed in the distribution of the large luteal cells and the small spindle-shaped cells in the present study however, were

not restricted to between these two regions of the CL. The observations in this study support a previous report that the smooth endoplasmic reticulum in the large luteal cell of goats, even though tubular, did not appear in the form of sheets or fenestrated cisternae as in other domestic species (Gemmel and Stacy, 1979). The contents of the secretory granules in the large luteal cell of the goat have not been fully identified. It has been suggested that they may contain more than one secretory product in sheep (Niswender and Nett, 1988). The observation of areas with different staining densities in the secretory granules of the CL in this study indicates that this is probably the case in goats too. Components of the secretory granules identified include oxytocin in sheep (Wathes *et al.*, 1983) and cows (Rodgers *et al.*, 1983) and relaxin in cows (Fields *et al.*, 1980).

In conclusion, the results of this experiment suggest that the effect of undernutrition on ovulation rate in goats manifests itself as a reduced population of small follicles. The study could however not demonstrate any changes in the gross, microscopic or ultrastructural morphology of the CL or its LH and prolactin binding to account for the observed effects of undernutrition on reproduction.

GENERAL DISCUSSIONS AND CONCLUSION

The results of this work have demonstrated the detrimental effects of undernutrition on reproduction in goats.

Studies on the onset of oestrus, ovulation rate, pregnancy rate and embryo loss following oestrus synchronization with progestagen sponges and $PGF_{2\alpha}$ (Experiment 1, Chapter 3) confirmed previous observations in other species (Gunn and Doney, 1975; Gunn *et al.*, 1979a) that low levels of feeding delayed or suppressed the onset of oestrus, reduced ovulation rate and the incidence of multiple ovulations and reduced the proportion of goats that became pregnant. The absence of evidence of embryo loss after implantation suggests that losses occurred before implantation. The absence of a significant effect of undernutrition on embryo loss supports the view (Robinson, 1986) that because of the very low nutrient requirements of embryos at this early stage, only extreme nutritional regimens affect their survival. The seeming lack of effect of undernutrition on the superovulatory response of goats to porcine FSH (Experiment 2) indicates that administration of gonadotrophins may offset effects of undernutrition on ovulation rate.

Foetal and placental measurements in pregnant goats at 60 days after mating (Chapter 4) confirmed the general trend that nutrition had a significant effect on foetal size and weight and on placental weight at this stage of gestation. Foetal numbers however, had no significant effect on either foetal size and weight or placental weight (Kadu and Kaikini, 1987), probably because of the small size and hence, low nutritional requirements of the foetuses. Furthermore, the study confirmed the positive correlation between foetal measurements and cotyledon weight. The study revealed a significant reduction in foetal measurements and a tendency for undernutrition to

reduce the weight of the cotyledons. This suggests that the detrimental effects of undernutrition on foetal measurements were probably mediated through effects on the cotyledons. Because foetal growth rate in early gestation provides the trajectory for growth in late pregnancy (McKelvey and Robinson, 1986), effects in early gestation will invariably affect survival and later growth and presumably, birthweight of foetuses.

Transfer of embryos from does on adequate rations to 3 groups of does receiving adequate rations before and after embryo transfer, adequate ration before and restricted rations after embryo transfer and restricted rations before and adequate rations after embryo transfer (Chapter 5), revealed the detrimental effects of undernutrition both before or after embryo transfer on the establishment of pregnancy and survival of the embryo. Because of the poor pregnancy and embryo survival records following the procedure, the separate effects of restricting feed in the two periods could not be assessed.

Study of basal gonadotrophin profiles during the luteal, follicular and preovulatory phases before the surge of gonadotrophins demonstrated no significant effect of undernutrition in any of the 3 phases. This suggests that the effects of undernutrition on reproductive performance were not mediated through changes in gonadotrophin profiles in these periods but probably through altered sensitivity of the hypothalamic-pituitary-gonadal axis. The tendency of undernutrition to suppress oestrus and to reduce or eliminate the preovulatory gonadotrophin surge in goats suggests that undernutrition either acted directly on the pituitary by depleting the gonadotrophin reserves or by decreasing the sensitivity of the pituitary to GnRH or indirectly by increasing the negative feedback of ovarian steroids on the pituitary that even the GnRH dose used in the present study was not sufficient to override. The incidence of loss of luteal function soon after ovulation in feed-restricted does which had a preovulatory LH surge of reduced magnitude supports previous reports

elsewhere (Bretzlaff *et al.*, 1988; Camp *et al.*, 1983) that suggested a relationship between undernutrition and subsequent luteal development and function. This relationship needs to be investigated fully.

The failure of 5 out of the 8 does in the feed-restricted group to exhibit oestrus, and the failure by all but one, to maintain luteal function for the 10 days after oestrus strongly highlighted the importance of nutrition in the periods just before and after oestrus for normal development and function of the CL.

The tendency for undernutrition to reduce the populations of follicles of 1 to 5 and 6 to 10mm in diameter observed in the ovaries of goats during early luteal phase in Experiment 8, probably resulted from increased atresia of the developing follicles or from a reduction in the pool of preantral and early antral follicles from which those destined for subsequent development were recruited. The mechanisms through which these effects were mediated are difficult to explain since no changes in the basal profiles of gonadotrophins during the luteal phase were observed in feed restricted goats in Chapter 6. It is likely that other factors such as ovarian steroids and growth factors are involved. These factors have been implicated in the development of preantral and early antral follicles through direct influence at the ovarian level and/or by modulating gonadotrophin actions on ovaries (Pell and Bates, 1990; Spicer *et al.*, 1990; Mariana *et al.*, 1991).

The absence of any significant effect of undernutrition on LH and prolactin binding to receptors in the CL, the absence of changes in luteal cells, and the slight increase in progesterone concentration observed in the period between 51 and 60 days of gestation in pregnant feed-restricted goats highlight the complexity of the mediation of nutritional effects on reproductive performance. The failure to demonstrate FSH

binding to luteal tissues in the present study indicates the absence of receptors for the hormone.

Collectively, the results in this study have demonstrated that the detrimental effects of undernutrition on reproduction in goats were manifested during the oestrous cycle through delay and suppression of oestrus, reduced ovulation rates, pregnancy rates and survival of embryos. Furthermore, the numbers of small and medium sized ovarian follicles appear to be reduced by undernutrition while the sizes, relative populations and ultrastructural morphology of luteal cells appeared not to be affected. During pregnancy, undernutrition reduced foetal and placental growth in early gestation. Undernutrition appeared to have affected the occurrence and magnitude of the preovulatory surge of gonadotrophin which in turn affected the incidence of ovulation and probably the formation and maintenance of the corpus luteum. However, changes in the basal levels of gonadotrophins during the luteal and follicular periods, and in the morphology of the CL and in LH receptor content of the CL during the luteal phase were not sufficient to account for all the observed effects of undernutrition. This indicates that the mechanism through which undernutrition affects reproduction is complex and is unlikely to be wholly through changes in gonadotrophin levels, but also through changes in the sensitivity of the target organs to gonadotrophins. The presence of FSH receptors on the goat's follicles and their relationship to nutrition and ovulation rate needs to be investigated in future. If the quest to unravel the mechanism of action of the well known interrelationship between nutrition and reproduction is to succeed therefore, the role of growth factors which act either directly on the ovary or indirectly by influencing the actions of gonadotrophins will also have to be fully investigated.

BIBLIOGRAPHY

Abnes, F.B. and Woody, C.O. (1971). Embryo migration in ewes. *J. Anim. Sci. abstr.* **33**, 314 .

Abraham, G.E., Odell, W.D., Swerdloff, R.S. and Hopper, K. (1972). Simultaneous radioimmunoassay of plasma FSH, LH, progesterone 17-hydroxyprogesterone, and estradiol 17β during the menstrual cycle. *J. Clin. Endocr.* **34,** 312.

Alexander, G. (1964). Studies on the placenta of the sheep (*Ovis aries*): placental size. *J. Reprod. Fert.* **7,** 289-305.

Alexander, G. and Williams, A.H. (1971). Heat stress and the development of the conceptus in domestic sheep. *J. Agric. Sci. Cambridge.* **76**, 53-72.

Allen, D.M. and Lamming, G.E. (1961). Nutrition and reproduction in ewes. *J. Agric. Sci. Camb.* **56**, 69.

Allison, J.R.D. (1991). CIDRs and advances in breeding control in goats. *Goat Vet. Soc. J.* **12**, 17-21.

Amoah, E.A. and Gelaye, S. (1991). Embryo recovery, evaluation, storage and transfer in goats. *Small Rumin. Res.* **6**, 119-129.

Amoroso, E.C. (1952). Placentation In: *Marshall's Physiol. of Reprod. Vol. II, 3rd Ed.* Longmans, New York, pp. 127-311.

Anderson, G.B. (1991). Fertilization, early development, and embryo transfer. In: P.T. Cupps (ed.), *Reproduction in Domestic Animals.* Academic Press, San Diego. pp. 280-313.

Apgar, J., Aspros, D., Hixon, J.E., Saatman, R.R. and Hansel, W. (1975). Effect of restricted feed intake on the sensitivity of the bovine corpus luteum to LH *in vitro. J. Anim. Sci.* **41**, 1120.

Arendt, J. (1986). Role of the pineal gland in seasonal reproductive function in mammals. Oxford Rev. *Reprod. Biol.* **8**, 266-320.

Armstrong, D.T., Pfitzner, A.P. Warnes, G.M. and Seamark, R.F. (1983a). Superovulation treatment and embryo transfer in Angora goats. *J. Reprod. Fert.* **67**, 403-410.

Armstrong, D.T., Pfitzner A.P., Warnes, G.M., Ralph, M.M. and Seamark, R.F. (1983b). Endocrine responses of goats to superovulation with PMSG and FSH. *J. Reprod. Fert.* **67**, 395-401.

Arthur, G.H., Noakes, D.E. and Pearson, H. (1989). Oestrus cycle and its control. In: *Veterinary Reproduction and Obstetrics*. Bailliere Tindall. London. pp. 3-45.

Baird, D.T., Baker, T.G., McNatty, K.P. and Neal, P. (1975). Relationship between the secretion of the corpus luteum and the length of the follicular phase of the ovarian cycle. *J. Reprod. Fert.* **45**, 611-619

Baird, D.T. and McNeilly, A.S. (1981). Gonadotrophic control of follicular development and function during the oestrus cycle of the ewe. *J. Reprod. Fert. suppl.* **30**, 119-133.

Baird, D.T. and Scaramuzzi, R.J. (1976). Changes in the secretion of ovarian steroids and pituitary luteinizing hormone in the peri-ovulatory period in the ewe; the effect of progesterone. *J. Endocr.* **70**, 237-245.

Baird, D.T., Swanston, I.A. and McNeilly, A.S. (1981). Relationship between LH, FSH and prolactin concentration and the secretion of androgens and estrogens by the preovulatory follicle in the ewe. *Biol. Reprod.* **24**, 1013-1025.

Bajhau, H.S. and Kennedy, J.P. (1990). Influence of pre- and postpartum nutrition on growth of goat kids. *Small Rumin. Res.* **3**, 227-236.

Baril, G, and Vallet, J.C. (1990). Time of ovulations in dairy goats induced to superovulate with porcine follicle stimulating hormone during and out of the breeding season. *Theriogenology* **34**, 303-311.

Basu, S., Goswami, S.K. and De, S.K. (1961). Studies on the genitalia of the she goat. *Indian Vet. J.* **38**, 302-304.

Bhattacharyya, N.K. and Prasad, S.P. (1974). Ovulation characteristics in nullipara Barbari nannies. *Proc. 26th Congr. physiol. Sci.* New Delhi, **11**, 392.

Battye, K.M., Fairclough, R.J., Cameron, A.W.N. and Trounson, A.O. (1988). Evidence of prostaglandin involvement in early luteal regression of the superovulated nanny goat (Capra hircus). *J. Reprod. Fert.* **84**, 425-430.

Beal, W.E., Short, R.E., Staigmillar, R.B., Bellows, R.A., Kaltenbach, C.C. and Dunn, T.G. (1978). Influence of dietary energy intake on bovine pituitary and luteal function. *J. Anim. Sci.* **46**, 181-188.

Beard, A.P. and Lamming, G.E. (1992). The influence of oestradiol level on the development of the oxytocin receptor and the oxytocin induced $PGF_{2\alpha}$ release in the ewe. *J. Reprod. Fert. Abstr.* **9**, 111.

Ben-David, M. and Schenker, J.G. (1982). Human ovarian receptors to human prolactin: implications in infertility. *Fertility and Sterility* **38**, 182-186.

Betteridge, K.J. (1977). Superovulation. In: K.J. Betteridge (ed.), *Embryo Transfer in Farm Animals, Monograph 16,* Canada Dept. Agric. Ottawa, pp. 1-9.

Bindon, B.M. (1971). The role of progesterone in implantation in the sheep. *Aust. J. Biol. Sci.* **24**, 149-158.

Bindon, B.M., Blanc, M.R., Pelletier, J., Terqui, M and Thimonier, J. (1979). Periovulatory gonadotrophin and ovarian steroid patterns in sheep of breeds with different fecundity. *J. Reprod. Fert.* **55**, 15-25.

Blockey, M.A., Cumming, I.A. and Blaxter, R.W. (1974). The effect of short term fasting in ewes on early embryonic survival. *Proc. Aust. Soc. Anim. Prod.* **10**, 265-269.

Blom, A.K. and Lyngset, O. (1971). Plasma progesterone levels in goats during pregnancy measured by Competitive Protein Binding. *Acta Endocr.* **66**, 471-477.

Boland, M.P., Foulkes, J.A., MacDonnell, H.F. and Sauer, M.J. (1985). Plasma progesterone concentrations in superovulated heifers determined by enzymeimmunoassay and radioimmunoassay. *Br. Vet. J.* **141**, 409-415.

BonDurant, R.H. (1981). Reproductive physiology in the goat. *Modern Vet. Prac.* **6**, 525-529.

Bongso, T.A., Fatimah, I. and Dass, S. (1982). Synchronization of estrus of goats treated with progestogen impregnated intravaginal sponges and P.M.S.G., and reproductive performance following natural mating or artificial insemination with frozen semen. *Anim. Reprod. Sci. abstr.* **5**, 111.

Borque, C., Pintado, B., Perez, B., Gutierrez, A., Munoz, I. and Mateos E. (1993). Progesterone levels in superovulated Murciana goats with or without successful embryo collection. *Theriogenology.* **39**, 192.

Boyd, J.D., Hamilton, W.J. and Hammond, J.R. (1944). Transuterine (internal) migration of the ovum in sheep and other mammals. *J. Anat.* **78**, 5-14.

Boyd, J.S. and Ducker, M.J. (1983). A method of examining the cyclic changes occurring in the sheep ovary using endoscopy. *Vet. Rec.* **93**, 40-43.

Bramley, T.A., Stirling, D., Swanston, I.A., Menzies, G.S. and Baird, D.T. (1987). Specific binding sites for LH/chorionic gonadotrophin, low density lipoprotein, prolactin and FSH in homogenates of human corpus luteum. I: Validation of methods. *J. Endocr.* **113**, 305-315.

Brand, A. and De Jong, W.H.R. (1973). Qualitative and quantitative micromorphological investigations of the tertiary follicle population during the oestrous cycle in sheep. *J. Reprod. Fertil.* **3**, 431-439.

Bretzlaff, K.N., Elmore, R.G. and Nuti, L.C. (1989). Use of an enzyme immunoassay to determine concentrations of progesterone in caprine plasma and milk. *J. Am. Vet. Med. Assoc.* **5**, 664-668.

Bretzlaff, K.N., Hill, A. and Ott, R.S. (1983). Induction of luteolysis in goats with prostaglandin $F_{2\alpha}$. *Am. J. Vet. Res.* **44**, 1162-1164.

Bretzlaff, K.N., Ott, R.S., Weston, P.G., and Hixon, J.E. (1981). Doses of prostaglandin $F_{2\alpha}$ effective for induction of estrus in goats. *Theriogenology.* **16**, 587-591.

Bretzlaff, K.N., Weston, P.G., Hixon J.E. and Ott, R.S. (1988). Plasma luteinizing hormone and progesterone concentrations in goats with estrus cycle of normal or short duration after prostaglandin $F_{2\alpha}$ administration during dioestrus or pregnancy. *Am. J. Vet. Res.* **49**, 939-943.

Buttle, H.L. (1978). The maintenance of pregnancy in hypophysectomised goats. *J. Reprod. Fert.*, **52**, 255-260.

Buttle, H.L. (1983). The luteotrophic complex in hysterectomized and pregnant goats. *J. Physiol.* **342**, 399-407.

Cahill, L.P. (1981). Folliculogenesis in the sheep as influenced by breed, season and oestrous cycle. *J. Reprod. Fert. suppl.* **30**, 135-142.

Cahill, L.P. (1984). Folliculogenesis and ovulation rate in sheep. In: D.R.Lindsay and D.T. Pearce (eds.), *Reproduction in sheep.* Camb. Univ. Press, Sydney, pp. 92-98.

Cahill, L.P., Mariana, J.C. and Mauléon, P. (1979). Total follicular populations in ewes of high and low ovulation rates. *J. Reprod. Fert.* **55**, 27-36.

Cahill, L.P. and Mauléon, P. (1980). Influence of season, cycle and breed on follicular growth rate in sheep. *J. Reprod. Fert.* **58**, 321-328.

Cahill, L.P., Saumande, J., Ravault, J.P., Blanc, M., Thimonier, J., Mariana, J.C. and Mauléon, P. (1981). Hormonal and follicular relationships in ewes of high and low ovulation rates. *J. Reprod. Fert.* **62,** 141-150.

Camp, J.C., Wildt, D.E. and Howard, P.K. (1983). Ovarian activity during normal and abnormal length estrus cycles in the goat. *Biol. Reprod.* **28**, 673-681.

Casida, L.E., Woody, C.O. and Pope, A.L. (1966). Inequality in function of the right and left ovaries and uterine horns of the ewe. *J. Anim. Sci.* **25**, 1169-1171.

Chemineau, P. (1983). Effect on estrus and ovulation of exposing Creole goats to the male at three times of the year. *J. Reprod. Fert.* **67**, 65-72.

Chemineau, P. (1985). Effects of a progestagen on buck-induced short ovarian cycle in the Creole meat goat. *Anim. Reprod.* Sci. **9**, 87-94.

Chemineau, P., Pelletier, J., Guerin, Y., Colas, G., Ravault, J.P., Toure, G., Almeida, G., Thimonier, J. and Ortavant, R. (1988). Photoperiodic and melatonin treatment for control of seasonal reproduction in sheep and goats. *Reprod. Nutr. Dev.* **28**, 409-422.

Clarke, I.J. and Cumming, J.T. (1982). The temporal relationship between gonadotrophin releasing hormone (GnRH) and Luteinizing hormone (LH) secretion in ovariectomized ewes. *Endocrinology*, **111**, 1737-1739.

Clarke, C. and Speedy, A.W. (1980). The effects of pre-mating condition and protein/energy nutrition on foetal growth and body reserves in Scottish half-bred ewes. *Anim. Prod. abstr.* **30**, 485.

Cooke, R.G. and Ahmad, N. (1992). Effects of oetradiol-17β and progesterone on uterine sensitivity to oxytocin in ovariectomized goats. *J. Reprod. Fert. Abstr.* **9**, 121.

Cooke, R.G. and Homeida, A.M. (1985). suppression of prostaglandin $F_{2\alpha}$ release and delay of luteolysis after active immunization against oxytocin in the goat. *J. Reprod. Fert.* **75**, 63-78.

Cooke, R.G. and Knifton, A. (1980). The effect of intra-aortic prostaglandin $F_{2\alpha}$ on uterine motility in pregnant goats. *J. Reprod. Fert.*, **59**, 347-350.

Corteel, J.M. (1975). The use of progestagens to control the oestrous cycle of the dairy goat, *Ann. Biol. Anim. Bioch. Biophys.* **15**, 353-363.

Corteel, J.M. (1977). Management of artificial insemination of dairy seasonal goats through oestrus synchronization and early pregnany diagnosis. In: Management of Reproduction in Sheep and Goats. University of Wisconsin, Madison, pp. 24-25.

Crowder, M.E., Gilles, P.A., Tamanini, C. Moss, G.E. & Nett, T.M. (1982). Pituitary content of gonadotrophins and GnRH-receptors in pregnant, post-partum and steroid-treated OVX ewes. *J. Anim. Sci.* **54,** 1235-1242.

Cumming, I.A. (1972). The effect of nutritional restriction on embryo survival during the first three weeks of pregnancy in Perendale ewes. *Proc. Aust. Soc. Anim. Prod.* **9**, 199-203.

Cumming, I.A. (1976). Synchronization of ovulation. In: G.J. Tomes, D.E. Robertson and R.J. Lightfoot (eds.), *Sheep Breeding, Proceedings of the 1976 International Congress,* Muresk, Western Australia Institute of Technology. pp. 430-448.

Cumming, I.A., Mole, B.J., Obst, J., Blockey, M.A.Deb., Winfield, C.G. and Goding, J.R. (1971).Increase in plasma progesterone caused by undernutrition during early pregnancy in the ewe. *J. Reprod. Fert.* **25**, 146-147.

Devendra, C. and Burns, M. (1970). Goat Production in the Tropics. *Technical Communication No. 19 of the Commonwealth Bureau of Animal Breeding and Genetics*. Edinburgh.

Deveson, S., Arendz, J. and Forsyth, I.A. (1990). The effects of melatonin on seasonal breeding in goats. *Goat Vet.* J. **2**, 45-51.

Deveson, S., Forsyth, I.A. and Arendz, J. (1992). Retardation of pubertal development by prenatal long days in goat kids born in autumn. J. *Reprod. Fert.* **95**, 629-637.

Donaldson, L. and Hansel, W. (1965). Histological study of bovine corpora lutea. *J. Dairy Sci.* **48**, 905-909.

Donaldson, L. and Hansel, W. (1968). Cystic corpora lutea and normal and cystic Graafian follicles in the cow. *Austr. Vet. J.* **44**, 304-308.

Doney, J.M., and Gunn, R.G. (1981). Nutritional and other factors in breeding performance of ewes. In: D.P. Gilmore and B. Cooke (eds.), *Environmental Factors in Mammalian Reproduction.* Macmillan, London, pp. 169-177.

Doney, J.M., Gunn, R.G. and Horak, F. (1982). Reproduction. In: I.E. Coop (Ed.), *World Animal Science, Production-system Approach 1, Sheep and Goat Production.* Elsevier Scientific Publication Company, Amsterdam. pp. 57-80.

Driancourt, M.A., Bodin, L., Boomarov, O., Thimonier, J. and Elson, J.M. (1990). Number of mature follicles ovulating after a challenge of human chorionic gonadotropin in different breeds of sheep at different physiological states. *J. Anim. Sci.* **68**, 719-724.

Driancourt, M.A. and Fry, R.C. (1989). Mechanisms involved in the control of the differentiation of the ovulatory follicle in sheep. *J. Reprod. Fert., Abstr. Ser.* **3**, 54.

Driancourt, M.A. Webb, R. and Fry, R.C. (1991). Does follicular dominance occur in ewes? *J. Reprod. Fert.* **93**, 63-70

Drost, M. (1986). Embryo transfer. In: Robertson S.J. (ed.), *Veterinary Obstetrics and Genital Diseases (Theriogenology).* Woodstock, Vermont, pp. 927-941.

Ducker, M.J. and Boyd, J.S. (1974). The effects of day length and nutrition on the oestrus cycle and ovulatory activity of Greyface ewes. *Anim. Prod.* **18**, 159-167.

Dufour, J.J., Cahill, L.P. and Mauléon, P. (1979). Short and long term effects of hypophysectomy and unilateral ovariectomy of ovarian follicular populations in sheep. *J. Reprod. Fert.* **57**, 302-309.

Dufour, J.J. and Wolynetz, M. (1977). The effects of energy levels imposed before or during the oestrous season on rates and locations of ovulation in the sheep. *Canadian J. Anim. Sci.* **57**, 169-176.

Economides, S. and Louca, A. (1981). The effects of the quantity and quality of feed on the performance of pregnant and lactating goats. In: P. Morand-Fehr, A. Bourbouze and M. de-Simiane (ed.), *Nutrition and Systems of Goat Feeding*. ITOVIC-INRA Symposium International, Tours, France, pp. 12-15.

Edery, M., Mills, K.T. and Bern, H.A. (1989). Effects of testosterone on morphology and on progestin and estrogen receptor levels in the mouse uterus and mammary gland. *Biol. of the Neonates*. **56**, 324-331.

Edey, T.N. (1969). Prenatal mortality in sheep: a review. *Anim. Breed. Abstr.* **37**, 173.

Edey, T.N. (1970). Nutritional stress and preimplantation mortality in Merino sheep. *J. Agric. Sci.* **74**, 193-198.

Edey, T.N. (1976). Embryo mortality. In: G.J. Tomes, D.E. Robertson and R.J. Lightfoot (eds.), *Sheep Breeding. Proceedings of the 1976 International Congress,* Muresk, Western Australian Institute of Technology, New England University Press, Armadale, pp. 400-410.

Ellinwood, W.E., Nett, T.M. and Niswender, G.D. (1979). Maintenance of the corpus luteum of early pregnancy in the ewe II. Prostaglandin secretion by the endometrium *in vitro* and *in vivo*. *Biol. Reprod.* **21**, 845-856.

El-Sheikh, A.S., Hulet, C.V., Pope, A.L. and Casida, L.E. (1955). The effect of level of feeding on reproductive capacity of the ewe. *J. Agric. Sci.* **14**, 919-929.

Epstein, B.H. and Hertz, A. (1964). Fertility and birthweight of goats in a subtropical environment. *J. Agric. Sci.* Camb. **62**, 237-244.

Everitt, G.C. (1964). Maternal undernutrition and retarded foetal development in Merino sheep. *Nature*. **201**, 1341-1342.

Fairclough, R.J., Moore, L.G., Peterson, A.J. and Watkins, W.B. (1984). Effect of oxytocin on plasma concentration of 13, 14-dihydro 15-keto-prostaglandin F and the oxytocin-associated neurophysin during the oestrus cycle and early pregnancy in the ewe. *Biol. Reprod.* **31**, 36-43.

Ferrel, C.C. (1991). Nutritional influences on reproduction. In: P.T. Cupps (ed.), *Reproduction in Domestic Animals*. Academic Press, San Diego, pp. 577-603.

Ferrel, C.L., Garret, W.N., Hinman, N. and Grichting, G. (1976). Energy utilization in pregnant and non-pregnant heifers. *J. Anim. Sci.* **42**, 937-950.

Fields, M.J., Fields, P.A., Castro-Hernandez, A. and Larkin, L.H. (1980). Evidence of relaxin in corpora lutea of late pregnant cows. *Endocrinology*, **107**, 869-876.

Findlay, J.K., Clarke, I.J., Swaney, J., Colvin, N. and Doughton, B. (1982). Oestrogen receptors and protein synthesis in caruncular and intercaruncular endometrium of sheep before implantation. *J. Reprod. Fert.* **64**, 329-339.

Findlay, J.K. and Cumming, I.A. (1976). FSH in the ewe: effect of season, liveweight and plane of nutrition on plasma FSH and ovulation rate. *Biol. Reprod.* **15**, 335-342.

Fitz, T.A., Mayan, M.H., Sawyer, H.R. and Niswender, G.D. (1982). Characterization of two steroidogenic cell types in the ovine corpus luteum. *Biol. Reprod.* **27**, 703-711.

Fitz, T.A., Sawyer, H.R. and Niswender, G.D. (1981). Characterisation of two steroidogenic cells types in the ovine corpus luteum. *Biol. Reprod. Suppl. 1,*. **24**, 54A.

Fleet, I.R. and Heap, R.B. (1982). Uterine blood flow, myometrial activity and their response to adenosine during the peri-implantation period in sheep. *J. Reprod Fert.* **65**, 195-205.

Foster, D.L., Ebling, F.J.P., Micka, A.F., Vannerson, L.A., Bucholtz, D.C., Wood, R.I., Suttie, J.M. and Fenner, D. (1989). Metabolic interfaces between growth and reproduction. 1. Nutritional modulation of gonadotrophin, prolactin and growth hormone secretion in the growth-limited female lamb. *Endocrinology*, **125**, 342-350.

Foster, D.L., Karsch, F.J., Olster, D.H., Ryan, K.D. and Yellon, S.M. (1986). Determinants of puberty in a seasonal breeder. *Recent progr. in horm. res.* **42**, 331-378.

Foster, D.L. and Olster, D.H. (1985). Effect of restricted nutrition on puberty in the lamb: patterns of tonic luteinizing hormone (LH) secretion and competency of the LH surge system. *Endocrinology,* **116**, 375-381.

Frisch, J.E. and Vercoe, J.E. (1977). Food intake, eating rate, weight gain, metabolic rate and efficiency of feed utilisation in *Bos taurus* and *Bos indicus* crossbred cattle. *Anim. Prod.* **25**, 343-358.

Fry, R.C., Cahill, L.P., Cummins, J.T., Bindon, B.M., Piper, L.R. and Clarke, I.J. (1987). The half-life of follicle stimulating hormone in ovary-intact and ovariectomized Booroola and control merino ewes. *J. Reprod. Fertil.* **81**,611-615.

Gadsby, J.E., Heap, R.B. and Burton, R.D. (1980). Estrogen production by blastocyst and early embryonic tissue of various species. *J. Reprod. Fert.* **60**, 409-417.

Ganong, W.F. (1991). Role of the nervous system in reproduction. In: P.T. Cupps (ed.), *Reproduction in Domestic Animals*. Academic Press, San Diego, pp. 1-24.

Garcia, M.C. and Ginther, O. J. (1976). Effects of ovariectomy and season on plasma luteinizing hormone in mares. *Endocrinology* **98**, 958-962.

Gemmel, R. and Stacy, B.D. (1979). Ultrastructural study of granules in the corpora lutea of several mammalian species. *Am. J. Anat.* **155**, 1-14.

Gill, G.S. and Dev, D.S. (1972). Performance of two exotic breeds of goats under Indian conditions. *Ind. J. Anim. Prod.* **3**, 173.

Gnatek, G.G., Smith, L.D., Duby, R.T. and Godkin, J.D. (1989). Maternal recognition of pregnancy in the goat: Effects of conceptus removal on interestrus interval and characterization of conceptus protein production during early pregnancy. *Biol. Reprod.* **41**, 655-663.

Godke, R.A., Overskei, T.L. Voelkel, S.A. (1985). The potential of micromanipulation and embryo transfer in breeding goats. *Dairy Goat J.* **63**, 154-157.

Gombe, S. and Hansel, W. (1973). Plasma luteinizing hormone (LH) and progesterone levels in heifers on restricted energy intake. *J. Anim. Sci.* **37**, 728-733.

Gonzalez S. C. (1977). Reproductive performance in arid districts of Venezuela. In: *Symposium Sobre la Cabra en los Países Mediterraneos*, Malaga, 3-7 Octubre. pp. 317-321.

Greyling, J.P.C. and Van Niekerk, C.H. (1990). Ovulation in the Boer goat doe. *Small Rumin. Res.* **3**, 457-464.

Greyling, J.P.C., Van Niekerk, C.H. and Grobbelaar, J.A.N. (1985). Synchronization of oestrus in Boer goat doe: The response to the use of intravaginal progestagen and PMSG. *South African J. Anim. Sci.* **15**, 52-55.

Greiss, F.C. and Anderson, S.G. (1970). Uterine blood flow during early ovine pregnancy. *Am. J. Obstet. Gynec.* **106**, 30-38.

Guillomot, M., Flétchon, J.E. and Wintenberger-Torres, S. (1981). Conceptus attachment in the ewe: an ultrastructural study. *Placenta.* **2**, 169-182.

Gunn, R.G. (1983). The influence of nutrition on the reproductive performance of ewes. In: Haresign, W. (ed.), *Sheep Production.* Butterworth, London, pp. 99-110.

Gunn, R.G. and Doney, J.M. (1975). The interaction of nutrition and body condition at mating on ovulation rate and early embryo mortality in Scottish Blackface ewes. *J. Agric. Sci.* **85**, 465-470.

Gunn, R.G., Doney, J.M. and Russel, A.J.F. (1972). Embryo mortality in Scottish Blackface ewes as influenced by nutrition and body condition at mating. *J. Agric. Sci. Camb.* **79**, 19-25.

Gunn, R.G., Doney, J.M. and Smith, W.F. (1979a). Fertility in Cheviot ewes: the effects of body condition at mating on ovulation rate and early embryo mortality in North and South Country Cheviot ewes. *Anim. Prod.*.**29**, 11-16.

Gunn, R.G., Doney, J.M. and Smith, W.F. (1979b). Fertility in Cheviot ewes. 2. The effect of level of pre-mating nutrition on ovulation rate and early embryo mortality in North Country Cheviot ewes in moderately-good condition at mating. *Anim. Prod.* **29**, 17-23.

Gunn, R.G., Doney, J.M. and Smith, W.F. (1979c). Fertility in Cheviot ewes. 3. The effect of level of nutrition before and after mating on ovulation rate and early embryo mortality in South Country Cheviot ewes in moderate condition at mating. *Anim. Prod.* **29**, 25-31.

Gunn, R.G. and Rhind, S.M. (1983). Manipulation of ovulation rate and lambing rate in the ewe. In: *HFRO Biennial Report 1982-83,* pp. 131-139.

Hammond, J. (1927). The physiology of reproduction in the cow. Cambridge University Press, Cambridge.

Hansel, W. (1988). Establishment of pregnancy: Regulation of the corpus luteum. *11th Int. Congr Anim. Reprod. Artl. Insem.* Dublin, Ireland, 1988. Univ. Coll. Dublin. Vol. 5 pp. 61-70.

Hansel, W., Concannon, P.W. and Lukaszewska, J.H. (1973). Corpora lutea of the large domestic animals. *Biol. Reprod.* **8**, 222-245.

Haresign, W. (1981). The influence of nutrition on reproduction in the ewe. I. Effects on ovulation rate, follicular development and luteinizing hormone release. *Anim. Prod.* **32**, 197-202.

Harper, M.J.K. (1988). Gamete and zygote transport. In: E. Knobil and J. Neill (eds.), *The Physiology of Reproduction.* Raven Press, New York, pp. 103.

Harrison, R.J. (1948). The changes occurring in the ovary of the goat during the oestrous cycle and early pregnancy. *J. Anat.* **83**, 21-48.

Hart, P.J., Squires, E.L., Emel, K.H. and Nett, T.M. (1984). Seasonal variation in hypothalamic content of gonadotrophin-releasing hormone (GnRH), Pituitary receptors for GnRH, and pituitary content of luteinizing hormone and follicle stimulating hormone in the mare. *Biol. Reprod.* **30**, 1055-1062.

Haumesser, J.B. (1975). Queques aspect de la reproduction chez la chevre rousse de Maradi. Comparison avec d'autres races tropicals ou subtropicais. *Rev. Elev. Med. Vet. Pays Trop.* **28**, 225-234.

Heap, R.B., Davis, A.J., Fleet, I.R., Goode, J.A., Hamon, M., Nowak, R.A., Stewart, H.J., Whyte, A. and Flint, A.P.F. (1988). Maternal recognition of pregnancy. *Proc. 11th int. congr. Anim. Reprod. A.I.* Dublin **5**. 55-60.

Heap, R.B. and Flint, A.P.F. (1984). Hormone production by the ovary. In: C.R. Austin and R.V. Short (eds.), *Reproduction in Mammals: 3. Hormonal Control of Reproduction.* Camb. Univ. Press, Cambridge, pp. 165-166.

Heap, R.B., Flint, A.P.F., Gadsby, J.E. and Rice, C. (1979). Hormones, the early embryo and the uterine environment. *J. Reprod. Fert.* **55**, 267-275.

Henderson, D.C. (1987). Manipulation of the breeding season in goats-a review. *Goat Vet. Soc. J.* **8**, 7-16.

Hoffman, B., Schams, D., Bopp, R., Ender, M.L., Gimenez, T. and Karg. H. (1974). Luteotrophic factors in the cow: evidence for LH rather than prolactin. *J. Reprod. Fert.* **40**, 77-85.

Homeida, A.M. and Cooke, R.G. (1982). Pheripheral plasma concentration of 13, 14-dihydro-15-keto prostaglandin $F_{2\alpha}$ and progesterone around luteolysis and during early pregnancy in the goat. *Prostaglandins,* **24**, 313-321.

Homeida, A.M. and Khalafalla, A.E. (1990). Effects of oxytocin and oxytocin antagonist on testosterone secretion during the oestrus cycle of the goat (*Capra hircus*). *J.Reprod. Fert.* **89**, 347-350.

Hoyer, P.B. and Niswender, G.D. (1985). The regulation of steroidogenesis is different in the two types of ovine luteal cells. *Can. J. Physiol. Pharmacol.* **63,** 240-248.

Hunter, G.L. (1968). Increasing frequency of pregnancy in sheep. *Anim. Breed. Abstr.* **36**, 347-378.

Hutchison, J.S.M. and Robertson, H.A. (1966). The growth of the follicle and corpus luteum in the ovary of the sheep. *Res. Vet. Sci.* **7,** 17-24.

Imakawa, K., Day, M.L., Garcia-Winder, M., Zelesky, D.D., Kittock, R.J., Schanbacher, B.D. and Kinder, J.E. (1986). Endocrine changes during restoration of estrous cycles following induction of anestrus by restricted nutrient intake in beef heifers. *J. Anim. Sci.* **63**, 565-571.

Ireland, J.J., Coulson, P.B. and Murhree, R.L. (1979). Follicular development during four stages of the estrus cycle of beef cattle. *J. Anim. Sci.* **49**, 1261-1269.

Ireland, J.J. and Roche, J.F. (1987). Hypothesis regarding development of dominant follicles during bovine estrus cycle. In: J.F. Roche and D. Callaghan (Eds.), *Follicular Growth and Ovulation Rate in Farm Animals,* Martinus Nijhoff Publishers, Dordrecht. pp, 1-8.

Irvine, G. Jones. D.E. and Knifton, A. (1972). Progesterone concentration in the peripheral plasma of pregnant goats. *J. Endocr.* **53**, 447-452.

Ishwar, A.K. and Pandey, J.N. (1992). Oestrus synchronisation and fertility in Black bengal goats following administration of progesterone/prostaglandin and gonadotrophins. *Res. Vet. Sci.* **52**, 141-146.

Jainudeen, M.R. and Hafez, E.S.E. (1987). Reproductive cycles: sheep and goats. In: E.S.E Hafez (ed.), *Reproduction in Farm Animals,* Lea and Febiger, Philadelphia, pp. 315-323.

Jones, D.E. and Knifton, A. (1972). Progesterone concentration in the peripheral plasma of goats during the oestrous cycle. *Res. Vet. Sci.* **13**, 193-195.

Kadu, M.S. and Kaikini, A.S. (1987). Prenatal development of caprine fetus. *Ind. J. Anim. Sci.* **57**, 962-969.

Karsch, F.J., Bittman. E.L., Foster, D.L., Goodman, R.L., Legan, S.J. and Robinson J.E. (1984). Neuroendocrine basis of seasonal reproduction. *Rec. Prog. Horm. Res.* **40**, 185-232.

Karsch, F.J., Cook, B., Ellicott, A.R., Foster, D.L., Jackson, G.L. and Nalbandov, A.V. (1971). Failure of infused prolactin to prolong the lifespan of the corpus luteum of the ewe. *Endocrinology*, **89**, 272-275.

Karsch, F.J., Foster, D.L., Bittman, E.L., Goodman, R.L., (1983). A role for oestradiol in enhancing luteinizing hormone pulse frequency during the follicular phase of the estrus cycle of sheep. *Endocrinology.* **113**, 1333-1339.

Karsch, F.J. and Wayne, N.L. (1988). Interplay of endogenous rhythms and environmental cues in oprganizing the seasonal reproductive cycle of the ewe. In: *11th Intern. Congr. Anim. Reprod., Art. Insem.* University College Dublin Ireland, June 26-30, **5**, 220-227.

Kelly, R.W. (1984). Fertilization failure and embryonic wastage In: D.R. Lindsay and D.T. Pearce (eds.), *Reproduction in Sheep.* Cambridge University Press, Cambridge, pp. 127-133.

Kennaway, D.J., Dunstan, E.A. and Staples, L.D. (1987). Photoperiodic control of the onset of breeding activity and fecundity in ewes. *J. Reprod. Fert.* **34**, 187-199.

Kiessling, A.A., Hughes, W.H. and Blankevoort, M.R. (1986). Superovulation and embryo transfer in the dairy goat. *J. Am. Vet. Med. Assoc.* **188**, 829-832.

Kinder, J.E., Day, M.L. and Kittock, R.J. (1987). Endocrine regulation of puberty in cows and ewes. *J. Reprod. Fertil.* Suppl. **34**, 167-186.

Kirkwood, R.N. and Aherne, F.X. (1985). Energy intake, body composition and reproductive performance of the gilt. *J. Anim. Sci.* **60**, 1518-1529.

Kittock, R.J., Stellflug, J.N. and Lowry, S.R. (1983). Enhanced progesterone and pregnancy rate after gonadotrophin administration in lactating ewes. *J. Anim. Sci.* **56**, 652-655.

Knight, C.H., Wilde, C.J., McLeod, B.J., Haresign, W. (1988). Exogenous GnRH induces ovulation in seasonally anoestrus lactating goats (*Capra hircus*). *J. Reprod. Fert.* 83, 679-686.

Knopf, L., Kastelic, J.P., Schallenberger, E. and Ginther, O.J. (1989).Ovarian follicular dynamics in heifers: test of two-wave hypothesis by ultrasonically monitoring individual follicles. *Domestic Anim. Endocr.* **6**, 111-119.

Koong, L.J. Garrett, W.N. and Rattray, P.V. (1975). A description of the dynamics of foetal growth in sheep. *J. Anim. Sci.* **41**, 1065-1068.

Landefield, T.D., Ebling, F.J.P., Suttie, J.M., Vannerson, L.A., Padmanabhan, V., Beitins, I.Z. and Foster, D.L. (1989). Metabolic interfaces between growth and reproduction. II. characterization of changes in messenger ribonucleic acid concentrations of gonadotrophin subunit, growth hormone and prolactin in nutritionally growth-limited lambs and the differential effects of increased nutrition. *Endocrinology,* **125**, 351-356.

Lawson, R.A.S. (1977). Research application of embryo transfer in sheep and goats. In: K.J. Betteridge (ed.), *Embryo Transfer in Farm Animals. Monograph 16.* Canada Dept. Agric.

Lawson, R.A.S. and Cahill, L.P. (1983). Modification of the embryo maternal relationship in ewes by progesterone treatment early in the oestrus cycle. *J. Reprod. Fert.* **67,** 473-475.

Lawson, R.A.S. Parr, R.A. and Cahill, L.P. (1983). Evidence of maternal control of blastocyst growth after asynchronous transfer of embryos to the uterus of the ewe. *J. Reprod. Fert.* **67**, 477-483.

Legan, S.J., Karsch, F.J. and Foster, D.L. (1977). The endocrine control of seasonal reproductive function in the ewe: A marked change in response to the negative feedback action of oestradiol on luteinizing hormone secretion. *Endocrinology,* **101**, 818-824.

Legan, S.J. and Robinson, J.E. (1984). Neuroendocrine basis of seasonal reproduction. *Rec. Prog. Horm. Res.* **40**, 185-232.

Lesmeister, J.L., Burfening, P.L. and Blackwell, R.L. (1973). Date of first calving in beef cows and subsequent calf production. *J. Anim. Sci.* **36**, 1-6.

Liggins, G.C. (1989). The Fetus. In: Austin, C.R. and R.V. Short (eds.), *Reproduction in Mammals 2: Embryonic and Fetal Development.* Camb. Univ. Press, Cambridge, pp. 114-117.

Lindsay, D.R. (1991). Reproduction in the sheep and goat. In: P.T. Cupps (ed.), *Reproduction in Domestic Animals.* Academic Press, San Diego, pp. 491-515.

Lipner, H. (1988). Mechanisms of mammalian ovulation. In: E. Knobil, J.D. Neill et al. (eds.), *The Physiology of Reproduction.* Ravens Press, New York, pp. 447-488.

Llewelyn, C.A., Luckins, A.G., Munro, C.D. and Perrie, J. (1987). Effect of *T. congolense* on the oestrus cycle of the goat. *Br. Vet. J.* **143**, 423-431.

Llewelyn, C.A., Perrie, J., Luckins, A.G. and Munro, C.D. (1993). Oestrus in the British white goat: timing of plasma luteinizing hormone surge and changes in behavioural and vaginal traits in relation to onset of oestrus. (1993). *Br. Vet. J.* **149**, 171-182.

Long, S.E. and Williams, C.V. (1980). Frequency of chromosomal abnormalities in early embryos of the domestic sheep (*Ovis aries*). *J. Reprod. Fert.* **58**, 197-201.

Lye, S.J. and Porter, D.G. (1978). Demonstration that progesterone `blocks' uterine activity in the ewe *in vivo* by a direct action on the myometrium. *J. Reprod. Fert.* **52**, 87-94.

Lyngset, O. (1968a). Studies on reproduction in the goat III; the functional activity of the ovaries of the goat. *Acta Vet. Scand.* **9**, 268-276.

Lyngset, O. (1968b). Embryonic mortality in the goat. *VIth Cong. Int. Reprod. Anim. Insem. Artif.* **1**, 39-441.

Lyngset, O. (1971). Studies on reproduction in the goat. V11. Pregnancy and the development of the fetal accessories of the goat. *Acta Vet. Scand.* **12**, 185-201.

Macdonald, I., Robinson, J.J. and Fraser, C. (1981). Studies in reproduction of prolific ewes. 7. Variability in the growth of individual foetuses in relation to intrauterine factors. *J. Agric. Sci. Camb.* **96**, 187-194.

Mackenzie, A.J. and Edey, T.N. (1975a). Short-term Undernutrition and prenatal mortality in young and mature Merino ewes. *J. Agric Sci.* **84**, 113-117.

Mackenzie, A.J. and Edey, T.N. (1975b). Effects of pre-mating undernutrition on oestrus, ovulation and pre-natal mortality in Merino ewes. *J. Agric. Sci.* **84**, 119-122.

Maeda, K., Mori, Y. and Kano, Y. (1988). Involvement of melatonin in the seasonal changes of the gonadal function and prolactin secretion in female goats. *Reprod. Nutr. Dev.* **28**, 487-497.

Mariana, J.C., Monniaux, D., Driancourt, M.A. and Mauléon, P. (1991). Folliculogenesis. In: P.T. Cupps (ed.), *Reproduction in Domestic Animals.* Academic Press, San Diego, pp. 119-171.

Martal, J., Lacroix, M.C., Loudes, C., Saunier, M. and Wintenberger-Torres, S. (1979). Trophoblastin, an antiluteolytic protein present in early pregnant sheep. *J. Reprod. Fert.* **56**.63-73.

Martensz, N.D., Scarramuzzi, R.J. and Van Look, P.F.A. (1979). Plasma concentrations of luteinizing hormone, follicle-stimulating hormone and progesterone during anaestrus in ewes actively immunized against oestrdiol-17β, oestrone or testosterone. *J. Endocri.* **81**, 261-269.

McKelvey, W.A.C. (1990). Reproductive techniques in the goat. *Goat Vet. Soc. J.* **11**, 33-40.

McKelvey, W.A.C. and Robinson, J.J. (1986). Embryo survival and growth in the ewe: recent studies on the effects of nutrition and on novel techniques for the recovery and transfer of embryos. *The Rowett Res. Instit. Annual Report,* pp. 9-25.

McLeod, B.J., Haresign, W. and Lamming, G.E. (1982).The induction of ovulation and luteal function in seasonally anaestrus ewes treated with small dose multiple injections of GnRH. *J. Reprod. Fert.* **65**, 215-221.

McNatty, K.P., Heath, D.A., Henderson, K.M., Lun, S., Hurst, P.R., Ellis, L.M., Montgomery, G.W., Morrison, L. and Thurley, D.C. (1984). Some aspects of thecal and granulosa cell function during follicular development in the bovine ovary. *J. Reprod. Fert.* **72**, 39-53.

McNatty, K.P., Hudson, N., Henderson, K.M., Gibb, M., Morrison, L., Ball, K. and Smith, P. (1987) Differences in gonadotrophin concentrations and pituitary responsiveness to GnRH between Booroola ewes which were homozygous (FF), heterozygous (F+) and non-carriers (++) of a major gene influencing their ovulation rate. *J. Reprod. Fert.* **80**, 577-588.

McNeilly, A.S., Hunter, M., Land, R.B. and Frazer, H.M. (1981). Inadequate corpus luteum function after the induction of ovulation in anoestrous ewes by LH-RH or an LH-RH agonist. *J. Reprod. Fert.* **63,** 137-144.

McNeilly, A.S., Jonassen, J.A. and Frazer, H.M. (1986). Suppression of follicular development after chronic LHRH immunoneutralization in the ewe. *J. Reprod. Fert.* **76**, 481-490.

McNeilly, A.S., Kerin, J., Swanston, I.A., Bramley, T.A. and Baird, D.T. (1980). Changes in the binding of human chorionic gonadotrophin/luteinizing hormone, follicle stimulating hormone and prolactin to human corpora lutea during the menstrual cycle and pregnancy. *J. Endocr.* **87**, 315-325.

McNeilly, J.R., McNeilly, A.S. and Cunningham, F.J. (1976). Development and application of heterologous radioimmunoassay for ovine follicle stimulating hormone. *J. Endocr.* **70**, 69-79.

Meites, J., Webster, H.D., Young, F.W., Thorp, F. and Hatch, R.N. (1951). Effects of corpora lutea removal and replacement with progesterone on pregnancy in goats. *J. Anim. Sci.* **10**, 411-416.

Mellor, D.J. (1969). Vascular anastomosis and fusion of foetal membranes in multiple pregnancies in the sheep. *Res. Vet Sci.* **10**, 361.

Mellor, D.J. (1983). Nutrition and placental determinants of foetal growth rate in sheep and consequences for the newborn lamb. *Br. Vet. J.* **139**, 307-324. *abstr.*

Mellor, D.J., Mitchell, B. and Matheson, I.C. (1977). Reduction in lamb weight caused by pre-mating carunclectomy and mid-pregnancy placental ablation. *J. Comp. Path.* **87**, 629-633.

Mellor, D.J. and Murray, L. (1981). Effects of placental weight and maternal nutrition on the growth rates of individual foetuses in single and twin bearing ewes during late pregnancy. *Res. Vet. Sci.* **30**, 198-204.

Mellor, D.J. and Murray, L. (1982a). Effects of long term undernutrition on the ewe on the growth rates of individual foetuses during late pregnancy. *Res. Vet. Sci.* **32**, 177-180.

Mellor, D.J. and Murray, L. (1982b). Effects on the rate of increase in foetal girth of refeeding ewes after short periods of severe undernutrition during late pregnancy. *Res. Vet. Sci.* **32**, 377-382.

Mews, A.R. (1981). Breeding and fertility in goats. *Goat. Vet. Soc. J.* 2, 2-11.

Mgongo, F.O.K. (1988). Stress of nutritional origin and its effects on peripheral concentration of plasma hormones in goats. *Ind. Vet. J.* **65**, 486-490.

Miller, B.G. and Moore, N.W. (1976). Effects of progesterone and oestradiol on RNA and protein metabolism in the genital tract and on survival of embryos in the ovariectomised ewe. *Aust. J. Biol. sci.* **29**, 565-573.

Miller, B.G., Murphy, L. and Stone, G.M. (1977). Hormone receptor levels and hormone RNA and protein metabolism in the genital tract during the oestrous cycle in the ewe. *J. Endocrinology,* **73**, 123-130.

Ministry of Agriculture Fisheries and Food. (1984). Condition scoring of ewes. MAFF (publications). Alnwick, Northumberland, U.K. Leaflet 787.

Moore, N.W. (1974). Multiple ovulation and ovum transfer in the goat. *Proc. Aust. Soc. Anim. Prod.* **10**, 246-249.

Moore, N.W. (1979). Embryo transfer in the Angora goat. *Aust. J. Agric. Sci.* **30**, 973-981.

Moore, N.W. and Rowson, L.E.A. (1959). Maintenance of pregnancy in ovariectomised ewes by means of progesterone. *Nature,* **184**, 1410.

Moran, C., Quirke, J.E. and Roche, J.F. (1989). Puberty in heifers: a review. *Anim. Reprod. Sci.* **18**, 167-182.

Mori, Y. and Kano, Y. (1984). Changes in plasma concentration of L.H., Progesterone and estradiol in relation to the occurrence of luteolysis, estrus and time of ovulation in the Shiba goat (Capra hircus). *J. Reprod. Fert.* **72**, 223-230.

Murdoch, W.J., Nix, K.J. and Dunn, T.G. (1983). Dynamics of ovarian blood supply to preovulatory follicles of the ewe. *Biol. Reprod.* **28**, 1001-1006.

Murphy, B.D. and Rajkuman, K. (1985). Prolactin as a luteotrophin. *Can. J. Physiol. Pharmacol.* **63**, 257-264.

Murphy, L., Stone, G.M., Miller, B.G. and Moore, N.W. (1977). Oestradiol and progesterone: soluble receptor levels and metabolism in the uterus. of the ovariectomized ewe. *Aust. J. Biol. Sci.* **30**, 225-228.

National Research Council (1981) Nutrient Requirements of Domestic Animals. Nutrient Requirements of Goats: Angora, Dairy and Meat Goats in Temperate and Tropical Countries. National Academy Press, Washington, D.C.

Niswender, G.D. (1974). Influence of 2-Br-α-ergocryptine on the serum levels of prolactin and the oestrous cycle in sheep. *Endocrinology,* **94**, 612-615.

Niswender, G.D. and Nett, T. (1988). The corpus luteum. In: E. Knobil and J. Neill (eds), *The physiology of Reproduction.* Raven Press Ltd. New York, pp. 489-525

Niswender, G.D., Reimers, T.J., Diekman, M.A. and Nett, T.M. (1976). Blood flow: a mediator of ovarian function. *Biol. Reprod.* **14**, 64-81.

Niswender, G.D., Schwall, R.H., Fitz, T.A., Farin, C.E. and Sawyer, H.R. (1985). Regulation of luteal function in domestic ruminants: new concepts. *Rec. Prog. Horm. Res.* **41**, 101-142.

Nottle, M.B., Setchell, B.P. and Seamark, R.F. (1986). Supplementation with lupin grain for six days can increase induced ovulation rate in the ewe. *Proc. Nutri. Sci. Aust.* **11**, 239.

Oldham, C.M. (1980). A study of sexual and ovarian activity in Merino sheep. Ph.d. thesis, University of Western Australia.

O'Shea, J.D., Al-Obaidi, S.A.R., Hillard, M.A., Bindon, B.M., Cummins, L.J. and Findlay, J.K. (1984). Increased ovulation rate in Merino ewes and advancement of puberty in Merino lambs immunized with a preparation of enriched in inhibin. In: D.R. Lindsay and D.T. Pearce (eds.), *Reproduction in sheep.* Cambridge University Press. pp, 335-337.

O'Shea, J.D., Cran, D.G. and Hay, M.F. (1979a). The small luteal cell of the sheep. *J. Anat.* **128**, 239-251.

O'Shea, J.D., Cran, D.G. and Hay, M.F. (1979b). Fate of the theca interna following ovulation in the ewe. *Cell and Tissue Res.* **210**, 305-319.

Ott, R.S., Nelson, D.R. and Hixon, J.E. (1980). Peripheral serum progesterone and luteinizing hormone concentrations of goats during synchronization of estrus and ovulation with $PGF_{2\alpha}$ *Am. J. Vet. Res.* **41**, 1432-1434.

Parr, R.A. and Cumming, I.A. (1982). Effects of maternal nutrition and plasma progsterone concentrations on survival and growth of the sheep embryo in early gestation. *J. Agric. Sci.* **98**, 39-46.

Parr, R.A., Cumming, I.A., and Clarke, I.J. (1982). Effects of maternal nutrition and plasma progesterone concentration on survival and growth of sheep embryo in early gestation. *J. Agric.* **98**, 39-46.

Parr, R.A. and Williams, A.H. (1982). Nutrition of the ewe and embryo growth during early pregnancy. *Aust. J. Biol. Sci.* **35,** 271-276.

Parr, R.A., Williams, A.H., Campbell, I.P., Witcombe, G.F. and Roberts, A.M. (1986). Low nutrition of ewes in early pregnancy and the residual effect on the offspring. *J. Agric. Sci. Cambridge.* **106**, 81-87.

Pathiraja, N., Oyepide, E.O., Gyang, E.O. and Obasi, A. (1991). Plasma progesterone level during oestrus cycle and their relationship with the ovulation rate in Red sokoto (Maradi) goats. *Br. Vet. J.* **147,** 57-62.

Pell, J.M. and Bates, P.C. (1990). The nutritional regulation of growth hormone action. *Nutr. Res. Rev.* **3**, 163-192.

Pelletier, J., Gonzalez-Stagnaro, C.Baril, G. and Corteel, J.M. (1982). [Induced preovulatory discharge of LH during the seasonal anaestrus of the goat]. *Seance Acad. Sci.* 294, 867-870 *(Abstr.)*.

Pendleton, R.J., Youngs, C.R., Rorie, R.W., Pool, S.H., Memon, M.A. and Godke, R.A. (1992). Follicular stimulating hormone versus pregnant mare serum gonadotropin for superovulation of dairy goats. *Small Rumin. Res.* **8,** 217-224.

Peters, H., Byskov, A.G., Himelstein-Braw, R. and Faber, M. (1975). Follicular growth: the basic event in the mouse and human ovary. *J. Reprod. Fert.* **45**, 559-566.

Picton, H.M. & McNeilly, A.S. (1991). Administration of FSH to ewes during chronic treatment with GnRH agonist: evidence to support an FSH-threshold theory for follicle selection. *J. Reprod. Fert.* **93,** 43-51.

Pineda, M.H. (1983). Reproductive patterns of sheep and goats. In: L.E. McDonald (ed.), *Vet. Endocr. Reprod.* Lea and Febiger, pp. 428-443.

Pineda, M.H. (1989). Reproductive patterns of sheep and goats. In: MacDonald, L.E. and Pineda, M.H. (ed), *Veterinary Endocrinology and Reproduction.* Lea and Ferbiger, Philadelphia, U.S.A. pp. 428-447

Poindexter, A.N., Buttram, V.C. Jr., Besch, P.K. and Smith, R.G. (1979). Prolactin receptors in the ovary. *Fertility and Sterility* **31**, 273-277.

Prasad, S.P. and Bhattacharyya, N.K. (1979). Oestrus cycle and behaviour in different seasons in Barbari nannies. *Ind. J. Anim. Sci.* **49**, 1058-1062.

Prasad S., Roy, A. and Pandey, M. (1972). Influence of age, body weight, parity and season on the reproductive performance of Barbari goats. *Agri Univ. J. Res.* **20**, 31-39.

Pretorius, P.S. (1971). Gross ovarian changes in the cycling and anoestrus Angora goat doe. *S. Afr. J. Anim. Sci.* **1**, 63-66.

Priedkalns, J. and Weber, A.F. (1968). Ultrastructural studies of the bovine Graafian follicle and corpus luteum. *Zeitschrift für Zellforchung und mikroskopische Anatomie,* **91**, 554-573.

Rajakoski, E. (1960). The ovarian follicular system in sexually mature heifers with special reference to seasonal, cyclical and left-right variations. *Acta Endocr.* (suppl.) **52**, 1-68.

Rao, V.H. and Bhattacharyya, N.K. (1980). Ovulation in Black Bengal nanny goats. *J. Reprod. Fert.* **58**, 67-69.

Rattray, P.V. (1977). Nutrition and reproductive efficiency. In: H.H. Cole and P.T. Cupps (ed.), *Reproduction in Domestic animals*. Academic Press, New York, San Francisco, London. pp. 553-575.

Rattray, P.V., Trigg, T.E. and Urlich, C.F. (1980). Energy changes in twin pregnant ewes. In: Mount, L.E. (ed.), *Energy Metabolism*. Butterworth, London, pp. 325-328.

Rhind, S.M., Leslie, I.D., Gunn, R.G. and Doney, J.M. (1985). Plasma FSH, LH, prolactin and progesterone profiles of Cheviot ewes with different levels of intake before and after mating, and associated effects on reproductive performance. **Anim. Reprod. Sci. 8**, 301-313.

Rhind, S.M., Martin, G.B., McMillen, S., Tsonis, C.G. and McNeilly, A.S. (1989a). Effect of level of food intake of ewes on the secretion of LH and FSH and on the pituitary response to gonadotrophin-releasing hormone in ovariectimized ewes. *J. Endocr.* **121**, 325-330.

Rhind, S.M., McMillen, S. and McKelvey, W.A.C. (1991). Effects of levels of food intake and body condition on the sensitivity of the hypothalamus and pituitary to ovarian steroid feedback in ovariectomized ewes. *Anim. Prod.* **52**, 115-125.

Rhind, S.M., McMillen, S., McKelvey, W.A.C., Rodriguez-Herrejon, F.F. and McNeilly, A.S. (1989b). Effect of the body condition of ewes on the secretion of LH and FSH and the pituitary response to gonadotrophin-releasing hormone. *J. Endocr.* **120**, 497-502.

Rhind, S.M., and McNeilly, A.S. (1986). Follicle populations, ovulation rates and plasma profiles of LH, FSH and prolactin in Scottish Blackface ewes in high and low levels of body condition. *Anim. Reprod. Sci.* **10**, 105-115.

Rhind, S.M., Robinson, J.J. and MacDonald, I. (1980). Relationships among uterine and placental factors in prolific ewes and their relevance to variation in fetal weight. *Anim. Prod,.* **30**, 115-124.

Richards, J.S. and Williams, J.L. (1976). Luteal cell receptor content for prolactin (PRL) and luteinizing hormone (LH). Regulation by LH and PRL. *Endocrinology*, **99**, 1571-1581.

Riera, G.S. (1982). Reproductive efficiency and management in goats. *Proc. 10th. Int. Conf. Goat. Prod. Dis.* Tucson 162-174.

Ritar, A.J. and Adams, N.R. (1988). Increased ovulation rate, but not FSH or LH concentrations, in ewes supplimented with lupin grain. *Anim. Prod. Aust.* **17**, 310-313.

Ritar, A. J., Maxwell, W.M. and Salamon, S. (1984). Ovulation and LH secretion in goats after intravaginal progestogen sponge-PMSG treatment. *J. Reprod. Fert.* **72**, 559-563.

Ritar, A.J., Salamon, S., Ball, P.D. and O'May, P.J. (1989). Ovulation and fertility in goats after intravaginal device-PMSG treatment. *Small Rumin. Res.* **2**, 323-331.

Robertson, H.A. (1977). Reproduction in the ewe and the goat. In: H.H. Cole and P.T. Cupps (eds.), *Reproduction in domestic Animals*. Academic Press, New York, San Fransisco, London, pp. 477-498.

Roberson, M.S., Stumpf, T.T., Wolfe, M.W., Cupp, A.S., Kojima, N., Werth, L.A., Kittok, R.J. and Kinder, J.E. (1992). Circulating gonadotrophinsduring the period of restricted energy intake in relation to body condition in heifers. *J. Reprod. Fert.* **96**, 461-469.

Robinson, J.J. (1986) Nutrition and embryo loss in farm animals. In: Sreenan, J.M. and Diskin, M.E. (ed.), *Embryo Mortality in Farm Animals*. Martinus Nijhoff, Dordretch, Netherlands, pp. 235-248.

Robinson, J.J., MacDonald, I. (1979). Ovine prenatal growth, its mathematical description and the effects of maternal nutrition. *Ann. Biol. Anim. Biochem. Biophys.* **19**, 225-234.

Robinson, J.J., MacDonald, I., Frazer, C. and Crofts, R.M.J. (1977). Studies on reproduction in prolific ewes. 1. Growth of products of the conceptus. *J. Agric. Sci.* **88**, 539-552.

Robinson, T.J. (1954). The production of coincident oestrus and ovulation in the anoestrus ewe with progesterone and pregnant mare serum. *J. endocr.* **10**, 117-124.

Rodgers, R.J., O'Shea, J.D. and Bruce, N.W. (1984). Morphologic analysis of the cellular composition of the ovine corpus luteum. *J. Anat.* **138**, 757-769.

Rodgers, R.J., O'Shea, J.D., Findlay, J.K., Flint, A.P.F. and Sheldrick, E.L. (1983). Large luteal cells the source of oxytocin in sheep. *Endocrinology.* **113**, 2302-2304.

Rogers, L.L.F., Erickson, L.F., Hoversland, A.S., Metcalfe, J. and Clary, D.L. (1969). Management of colony of African pygmy goats for biomedical research. *Lab. Anim. Care,* **19**, 181..

Rolland, R., Hammond, J.M., Schellenken, L.A., Lequin, R.M. and deJong, F.H. (1976). Prolactin and ovarian function. In: V.H.T. James, M. Serio, and G. Giusti (eds). *Endocrine Function of the Human Ovary.* Academic, New York, pp. 305-321.

Rosnina, Y., Jainudeen, M.R. and Nihayah, M. (1992). Superovulation and egg recovery in goats in the tropics. *Vet. Rec.* **130**, 97-99.

Rowson, L.E.A. and Moor, R.M. (1966). Embryo transfer in the sheep: The significance of synchronizing oestrus in the donor and recipient animals. *J. Reprod. Fert.* **11**, 207-222.

Sacco, R.E., Baker, J.F. and Cartwright, T.C. (1987). Production characteristics of primiparous female of five-breed Diallel. *J. Anim. Sci.* **64,** 1612-1618.

Sachdeva, K.K., Sengar, D.P.S., Singh, S.M. and Lindhl, I.L. (1973). Studies on goats. I. Effects of plane of nutrition on the reproductive performance of does. *J. Agric. Sci.* **80**, 375-379.

Sahni, K.L. and Roy, A (1967). A study on the sexual activity of the Barbari goat (*Capri hircus*) and conception rate through A.I. *Ind. J. Vet. Sci.* **37**, 269-276.

Saito, T. and Saxena, B.B. (1975). Specific receptors for prolactin in the ovary. *Acta Endocrinologica* **80**, 126-137.

Sands, M. and Dowell, R.E. (1978). In: The Potential of the Goat for Milk Production in the Tropics. pp. 11.

Salama, A. (1972). Ovarian changes in goats during oestrus. *Ind. Anim. Sci.* **42**, 436-438.

Sauer, M.J., Foulkes, J. A. and O'Neill, P.M. (1982). Use of microtitre plate EIA for direct determination of progesterone in whole milk: application of heterologous systems for improved sensitivity. *Br. Vet. J.* **138**, 522.

Savio, J.D., Keenan, L., Boland, M.P. and Roche, J.F. (1988). Pattern of growth of dominant follicles during the oestrus cycle of heifers. *J. Reprod. Fert.* **83**, 663-671.

Scaramuzzi, R.J. and Radford, H.M. (1983). Factors regulating ovulation rate in ewes. *J. Reprod. Fertil.* **69**, 353-367.

Setaro, F. and Morley, C.G.D. (1976). A modified fluorometric method for the determination of microgram quantities of DNA from cell or tissue cultures. *Analyt. Biochem.* **71**, 313-317.

Shelton, M. (1961). The relationship of size to breeding performance of Angora does. *Agric. Exp. Sta. Res. Bull.* **2339**, 18

Shelton, M. (1978). Reproduction and breeding in goats. *J. Dairy Sci.* **61**, 994-1010.

Simmen, R.C.M., Ko, Y. and Simmen, F.A. (1993). Insulin-like growth factors and blastocyst development. *Theriology,* **39**, 163-175.

Singh, S.N. and Sengar, D.P.S. (1970). Investigation of milk and meat potentialities of goats. *Tech. Rep. Dept. Anim. Husb. and Dairying*. R.B.S. College. Bichpuri Agri. India.

Singh, B.B. and Singh, B.P. (1974). Performances of Jamnapari goats. *Ind. Vet. J.* **51**, 326.

Sittmann, K. (1972). Intrauterine migration and mortality of sheep embryos. *Can. J. Anim. Sci.* **52**, 195-196.

Smeaton, T.C., and Robertson, H.A. (1971). Studies on the growth and atresia of the Graafian follicles in the ovary of the sheep. *J. Reprod. Fertil.* **25**, 245-252.

Smith, J.F. (1988). Influence of nutrition on ovulation rate in the ewe. *Aust. J. Biol. Sci.* **40**, 54-69.

Sonjaya, H. and Driancourt, M.A. (1989). Ovarian morphology and function in 12-week-old lambs from breeds with different ovulation rates as adults. *Reprod Fert. Dev.* **1**, 265-272.

Soules, M.R., Hughes, C.L., Aksel, L., Tyrey, L. and Hammond, C.B. (1981). The function of the corpus luteum in pregnancy, in ovulatory dysfunction, and in luteal phase deficiency. *Fertil. Steril.* **36**, 31.

Spicer, L.J., Tucker, W.B. and Adams, G.D. (1990). Insulin-like growth factor-I in dairy cows: Relationship among energy balance, body condition, ovarian activity, and estrous behaviour. *J. Dairy Sci.* **73**, 929-937.

Staples, L.D. (1989). The development of "regulin" from concept to international market place. *Aust. J. Bioltech.* **3**, 164-175.

Taneja, G.C. (1959). Observations on fetal losses in goats. *Ind. Vet. J.* **36**, 439-441.

Tervit, H.R., Goold, P.G., Mckenzie, R.D., Clakson, D.J., and Drummond, J. (1985). Embryo transfer in Angora and Saanen goats. *N.Z. Vet. J.* **33**, 78-80.

Thimonies, J. and Chemineau, P. (1988). Seasonality of reproduction in female farm animals under tropical environment (cattle, sheep and goats). *Proc 11th. Int. Congr. Anim. Reprod. A.I.* Dublin **5**, 229-237.

Thomas, G.B., Mercer, J.E., Karalis, T. Rao, A., Cummin, J.T. and Clark, I.J. (1990). Effect of restricted feeding on the concentration of growth hormone (GH), gonadotropins and prolactin (PRL) in plasma, and on the amounts of messenger ribonucleic acid for GH, gonadotrophin subunits, and prolactin in the pituitary gland of adult ovariectomized ewes. *Endocrinology,* **126**, 1361-1367.

Thomas, G.B., Oldham, C.M., Hoskinson, R.M., Scaramuzzi, R.J. and Martin G.B. (1987). Effects of immunization against progesterone on oestrus, cycle length, ovulation rate, luteal regression and LH secretion in the ewe. *Aust. J. Biol. Sci.* **40**, 307-313.

Thomford, P.J. and Dziuk, P.J. (1988). Influence of phenobarbital dosage, dietary crude protein level and interval to measurement on hepatic monoxygenase activity in ewes. *J. Anim. Sci.* **66**, 1446-1452.

Trounson, A.O. and Moore, N.W. (1974). Effects of progesterone and oestrogen on the survival and development of fertilized ova in the ovariectomised ewe. *Aust. J. Biol. Sci.* **27**, 511-517.

Turnbull, K.E. Braden, A.W.H. and Mattner, P.E. (1977). The pattern of follicular growth and atresia in the ovine ovary. *Aust. J. Biol. Sci.* **30**, 229-241.

Van der Westhuysen, J.M. (1981). Selection for reproductive efficiency. *Angora Goat and Mohair. J.* **23**, 43.

Veldhius, J.D., Weiss, J., Mauras, N., Rogol, A.D., Evans, W.S. and Johnson, M.L. (1986). Appraising endocrine pulse signals at low circulating hormone concentrations: use of regional coefficients of variation in the experimental series to analyse pulsatile luteinizing hormone release. *Pediat. Res.* **20**, 632-637.

Wallace, L.R. (1948). The growth of lambs before and after birth in relation to the level of nutrition. *J. Agric. Sci.* Camb. **38**, 93-153.

Wathes, D.C., Swann, R.W., Birkett, S.D., Porter, D.G. and Pickering, B.T. (1983). Characterization of oxytocin, vassopressin and neurophysin from the bovine corpus luteum. *Endocrinology*, **113**, 693-698.

Wentzel, D., Botha, L.J. and Viljeon, K.S. (1979). Progesterone levels in the peripheral plasma of the cycling Angora goat doe. *Agroanimalia*, **11**, 27-28.

Wentzel, D. (1987). Effects of nutrition and reproduction in the Angora goat. Goat: *4th International Conference*, Brazil, **14**, 571-575.

Wheaton, J.E., Marche, J.M., Hamra H.A. and Al-Raheem, S.N. (1988). Plasma gonadotrophins and progesterone concentrations during the estrus cycle of Finn, Suffork and Targhee ewes. *Theriogenology*. **30**, 99-108.

Williams, A.H., and Cumming, I.A. (1982). Inverse relationship between concentration of progesterone and nutrition in ewes. *J. Agric. Sci.* Camb. **98**, 517-522.

Wilmut, I., Ashworth, C.J. and Sales, D.I. (1986) The influence of progesterone profile on embryo survival in ewes. In: Sreenum, J.M. and Diskin M.E. (ed), *Embryo survival in farm animals*. Dordretch, Netherlands. pp. 135-141.

Wilmut, I. and Sales, D.I. (1981). Effect of an asynchronous environment on embryonic development in sheep. *J. Reprod. Fertil.* **61**, 179-184.

Wilmut, I. and Sales, D.I. (1982). Does hormonal imbalance cause death of embryos in sheep? *Ann. Report, Anim. Breed. Res. Org*. Edinburgh. pp. 25-30.

Wilmut, I., Sales, D.I. and Ashworth C.J. (1985). The influence of variation in embryo stage and maternal hormone profiles on embryo survival in farm animals. *Theriogenology*. **23,** 107-119.

Wilson, R.T. (1982). Husbandry, nutrition and production of goats and sheep in Africa. In: R.M. Gatenby and J.C.M. Trail (eds.), *Small Ruminant Breed Productivity in Africa.* I.L.C.A., Addis Ababa. pp.

Winters, L.M., Green, W.W. and Comstock, R.E. (1942). Prenatal development of the bovine. *Tech. Bull. Minn. Agric. Exp. Sta.* **151**, 50.

Wooding, F.B.P. and Staples, L.D. (1981). Functions of the trophoblast papillae and binucleated cells in implantation in the sheep. *J. Anat.* **133**, 110-112.

Xu, Z.Z., McDonald, M.F. and McCutcheon, S.N. (1989). The effects of nutritionally-induced liveweight differences on follicular development, ovulation rate, oestrus activity and plasma follicle-stimulating hormone levels in the ewe. *Anim. Reprod. Sci.* **19**, 67-78.