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UNIVERSITY OF PRETORIA
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Reproductive management of semi-intensive Döhne Merino ewes fed with different protein supplements

By

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B.Sc (Agric) Animal Science (Pret.)

Submitted in partial fulfilment of the requirements for the degree of

M.Sc (Agric) Production physiology in the

Department of Animal and Wildlife Sciences

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October 2008

Declaration:

I, Karen Lee, declare that the thesis, which I hereby submit for the degree M.Sc (Agric) Production Physiology at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

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PREFACE

The author wishes to express her sincere appreciation and gratitude to the following persons:

- Prof. E.C. Webb, who acted as promoter and Prof. W.A. van Niekerk, who acted as co-promotor, for their advice, constructive criticism and stimulating discussions during the study
- Prof Johan Terblanche of the Faculty of Veterinary Science, University of Pretoria, who did the laparoscopy.
- Dr. Gretha Snyman, for her valuable guidance and assistance with the statistical analyses of the data.
- My family and friends for their support and encouragement.
- My colleagues from the Grootfontein Agricultural Development Institute for their support.
- My Creator for His unfailing kindness and blessings.

"The important thing in science is not so much to obtain new facts as to discover new ways of thinking about them."

Sir William Bragg 1862-1942

Abstract

Two trial were conducted to determine the possible effects of season, protein supplementation, age and birth status on the reproduction rate (ovulation rate and rate of twinning) of ewes. In Trial 1 the weight, age and birth status if ewes were recorded. 144 ewes were randomly allocated in two treatment groups (urea and mix protein group) synchronised, mated and the number of *corpora lutea*, foetuses observed, lambs born per ewe and the mass of the ewe after lambing were also recorded. Lambing status or the 1-year-old (0.993 ± 0.316) and 2-year-old (1.233 ± 0.134) ewes were lower ($p < 0.05$) than that of the 6-year-old ewes (1.897 ± 0.248). The lambing status and the number of *corpora lutea* of the single born ewes (1.179 ± 0.131; 1.274 ± 0.138) were lower ($p < 0.0001$) than that of the twin born ewes (1.614 ± 0.139; 1.782 ± 0.147), within the urea treatment. In Trial 2, 75 ewes were randomly allocated in four treatment groups (raw lupins, cooked lupins, cottonseed oil-cake and *Fescue* grass), synchronised and the number of *corpora lutea* were recorded. The weight, age and birth status of the ewes were also recorded. The number of *corpora lutea* from the cooked lupin group (1.815 ± 0.184) was significantly higher than that from the cottonseed oil-cake group (1.048 ± 0.209), within the twin born ewe group. It was concluded that season, protein supplementation, age and birth status influenced the reproduction rate of ewes.

Opsomming

Die invloed van seisoen, proteïen aanvulling, ouderdom en geboortestatus van ooie, op hul reproduksie tempo (ovulasie tempo en tweeling tempo) was ondersoek in twee afsonderlike proewe. In Proef 1 was 144 ooie ewekansig verdeel in twee diet groepe (ureum en gemengde proteïen aanvulling). Die massa, ouderdom en geboortestatus van elke ooi was aangeteken. Ooie was gesinkroniseer, gepaar en daarna was die hoeveeldheid *corpora lutea*, fetusse, lamstatus en massa van die ooie na lam aangeteken. Lamstatus van die een jaar oud (0.993 ± 0.316) en twee jaar oud (1.233 ± 0.134) ooie was beduidend laer as die van die ses jaar oud ooie (1.897 ± 0.248) asook die lamstatus en die hoeveelheid *corpora lutea* van die enkel-gebore ooie (1.179 ± 0.131; 1.274 ± 0.138) was laer ($p < 0.0001$) as die van tweeling-gebore ooie (1.614 ± 0.139; 1.782 ± 0.147) binne die ureum diet groep. In Proef 2 was 75 ooie ewekansig verdeel in vier diet groepe (rou lupiene, gekookte lupiene, katoensaadoliekoek, *Fescue* gras), gesinkroniseer en die hoeveelheid *corpora lutea* per ooi was aangeteken. Die ooie se massas, ouderdomme en geboortestatusse was ook aangeteken. Die hoeveelheid *corpora lutea* was beduidend hoer in die gekookte lupiene groep (1.815 ± 0.184) as van die in die katoensaadoliekoek groep (1.048 ± 0.209). Uit die studie is bevind dat seisoen, proteïen aanvulling, ouderdom en geboortestatus van ooie, hul reproduksie tempo beïnvloed.

TABLE OF CONTENTS **Page**

LIST OF TABLES

LIST OF FIGURES **Page**

CHAPTER 1

1.1 GENERAL INTRODUCTION

Nutrition can influence reproductive function in ruminants. However, the relationship between nutrition and reproduction is complex and responses are often quite variable and inconsistent.

Sufficient feeding of the reproducing ewe is of the greatest importance. Underfeeding of the ewe may cause short-term problems like pregnancy toxaemia (Reid, 1963), a reduction in lamb birth weight, resulting in low survival levels (Shinckel, 1963), low ewe milk production (Alexander, McCance and Watson, 1956), poor lamb growth rate (Stephenson, Edwards and Hopkins, 1981) and a reduced wool production of ewes and their lambs (Gunn, 1983). Conversely, high levels of feed may jeopardize lamb survival by greater incidence of dystocia, in addition to being costly (Curll, Davidson and Freer, 1975).

Good nutrition increases ovulation rate in ewes and it appears to do so by increasing the number of selected preovulatory follicles. Both Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) are important in the control of follicle selection and ovulation rate in the ewe (Scaramuzzi, Adams and Campbell, 1993) and it is widely postulated that the effect of nutrition is mediated by central effects on gonadotrophin secretion (Martin, Boukhliq Hotzel and Fisher, 1992). Since the secretion of FSH and LH is under control of Gonadotrophic Releasing Hormone (GnRH) and ovarian hormonal feedback, dietary factors that influence the GnRH neuronal system may have an effect on the secretion of gonadotrophins and ovulation rate.

Early studies suggest an increase in ovulation rate following lupin grain supplementation (Smith and Steward, 1990). This increase in ovulation rate occurred within six days after the feeding of lupin grain (Lindsay, 1976) and the critical period for lupin grain supplementation to increase ovulation rate is four to six days before luteolysis (Steward and Oldham, 1986).

It is necessary to determine an optimum protein intake level for optimum production. In the era we find ourselves, feed costs, especially now that we are open to the global market, are extremely high. Thus to achieve an economical sustainable farming practice, the farmer must be able to predict the production responses of his flock at certain nutritional levels.

There have been many studies, in the past 10 years, on the possible effects worldwide of a high protein diet on ovulation rate, and subsequent embryo survivability but it is necessary to undertake such a study in South Africa because of differing environmental conditions and also management practices.

1.2 PHYSIOLOGY OF REPRODUCTION AND THE INFLUENCE OF NON-NUTRITIONAL FACTORS ON REPRODUCTION

1.2.1 Neuroendocrine regulators of reproduction

It is important to become familiar with the physiological systems most responsible for regulating the natural reproductive processes. The neuroendocrine system, through the hormones that it produces, is responsible for much of this regulation. Even reproductive reflexes that are considered neural reflexes have a hormonal requirement.

Chemically, hormones of reproduction can be divided into two major classes. One class includes the peptide and protein hormones (Table 1). The bonding of a series of amino acids forms these hormones, with a molecular size being the determinant of whether they are called peptide or protein. To be physiologically effective they must be administered systemically rather than orally because they denaturate in strong acids (i.e. stomach acids), strong bases or heat. The second class of hormones is steroids, which are a special class of lipids (Frandson and Spurgeon, 1992)

Table 1 Peptide and protein hormones that regulate reproduction and their function

HORMONE	FUNCTION
Follicle-stimulating hormone (FSH)	Follicle growth Oestrogen release
Luteinizing hormone (LH)	Ovulation *Corpus luteum* formation and function
Adrenocorticotropic hormone (ACTH)	Release of glucocorticoid
Inhibin	Prevents release of FSH
Oxytocin	Parturition
Gonadotrophin-releasing hormone (GnRH)	FSH and LH release
Human chorionic gonadotrophin (hCG)	LH-like
Pregnant mare serum gonadotrophin (PMSG)	FSH-like

(Adapted from Frandson *et al.*, 1992)

1.2.2 Folliculogenesis

The establishment of cyclic ovarian activity at puberty is important for the formation and release of gametes as well as for the establishment of mature sexual capabilities.

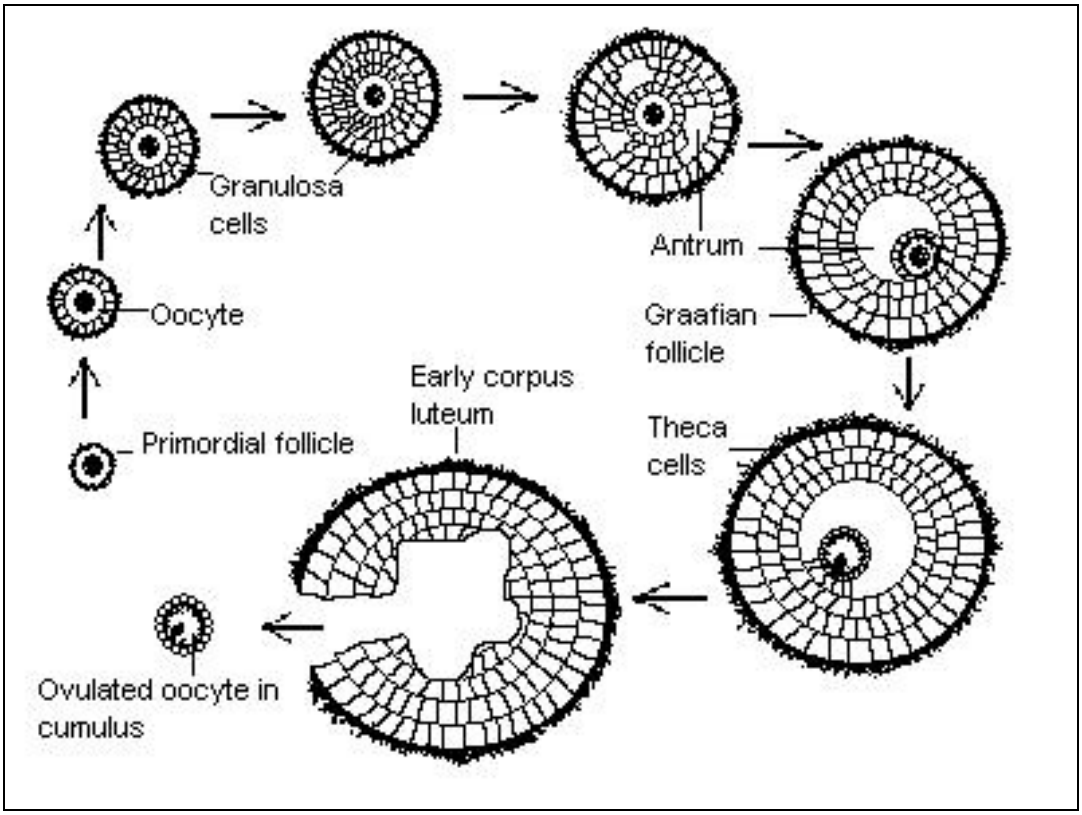
Gamete production proceeds in the embryonic ovary through mitotic division of the primordial germ cells. Mitosis ceases at birth, with the maximum number of oocytes that a female will ever have being present at this time. Meiosis is soon initiated by factors from the *rete ovarii* but is arrested at the resting stage, with resumption of meiosis not occurring until the onset of puberty (Knobil and Neill, 1988).

The control of the reestablishment of growth and development of the primordial follicle is not understood except that it is independent of gonadotropin influence. During the initial hormone-independent phase of follicle development, the oocyte increases in size and activity, which includes the production of RNA and ribosomes. The follicular cells, which initially formed around the oocyte, begin to grow and divide during this period and become granulose cells. These cells produce a glycoproteinaceous substance that forms a layer immediately around the oocyte, called the *zona pellucida*. Granulosa cells maintain contact with oocytes by cytoplasmic processes that form gap junctions with the oocyte. Gap junctions are important for communication among these cells, which lack a direct blood supply. Spindle-shaped cells that organize around the exterior of the basement membrane are called theca cells. Nutrients are supplied to the granulose cells and oocyte from the vascularized theca.

The follicle at the end of the hormone-independent stage is still preantral. The synthesis of receptors for FSH and oestrogen in the granulose and of LH receptors in the theca is required for follicles to enter the hormone-dependent stage. FSH influences oestrogen production by causing the granulose cells to convert androgens, produced in the theca under the influence of LH, to estrogens (Austin and Short, 1972).

FSH also induces its own receptors; this allows the follicle to become increasingly responsive to a relatively steady amount of FSH in the plasma. Estrogens are mitotic and cause growth and division of the granulose. They also induce additional FSH receptors in the granulose. As the granulosa develops under the influence of FSH and oestrogen, it begins to synthesize and release secretions that cause cell separation, resulting in the formation of a space called an antrum. The development sequence of an oocyte and its investments is shown in Figure 1.

Substances, probably of granulose cell origin, that are capable of inhibiting maturation of the follicle appear in follicular fluid. These inhibitors control the growth of the follicle, which consists of both oocyte and surrounding supporting cells, so that oocyte and follicular cell maturation is coordinated. A factor known as the oocyte-maturation inhibiting factor prevents the resumption of meiosis. A luteinization-inhibiting factor prevents the granulose from being luteinized prematurely. Folliculostatin, or inhibin, produced by the granulose is a protein hormone that inhibits follicle growth. Inhibin causes a progressive negative-feedback inhibition of FSH synthesis and release just before ovulation.



(Austin *et al.*, 1972)

Figure 1 Diagrammatic representation of follicular growth

FSH also induces receptors for LH in the granulose. On the other hand, LH decreases the number of FSH receptors on the granulose, especially during the preovulatory surge of LH. These receptor changes are important for the conversion of the granulose from oestrogen secretion in the follicular phase toe progesterone secretion in the luteal phase of the oestrus cycle (Hafez, 1987).

1.2.3 Ovulation

Ewes are spontaneous ovulators rather than having ovulation induced by copulation. With maturation of the oocyte and follicle, the preovulatory surge of LH will initiate a sequence of events that leads to ovulation. Immediately following this surge the concentration of progesterone in follicular fluid increases to be followed by an increase in oestradiol and prostaglandins later. The walls of the follicle will weaken and thin on the point where it will be released. The weakening of the follicle wall permits plasma to escape into spaces between the thecal cells, causing oedema and eventually capillaries penetrate beyond the basement membrane into the granulosa layer, and then the follicle ruptures, thus ovulation has occurred.

LH is released with the start of oestrus and ovulation occurs 30 – 32 hours later. LH release is delayed in ewes that have high ovulation rates. Preovulatory gonadotrophin release ends active follicle growth, and a delay in gonadotrophin secretion in animals with high ovulation rates allows other follicles to mature and develop to the point of ovulation. Luteal production of progesterone is the same regardless of the natural ovulatory rate within a breed. It's only when *corpora lutea* are increased beyond the normal number that increased concentrations of progesterone in the blood are observed. Luteal activity lasts 14 days in the nonpregnant ewe. Spontaneous persistence of the *corpus luteum* occurs at an incidence rate of 2 - 3 % in the ewe (Frandson *et al.*, 1992).

1.2.4 Ovulation rate

A large number of primordial follicles are contained in the ovaries, most of which are resting. Growth is initiated in some of the follicles and they develop slowly through three stages. The transformation of a primordial follicle into an ovulatory follicle takes around six months in the ewe (Cahill and Mauléon, 1980). Once a follicle has entered the final stages, it has two alternatives: it will either degenerate through atresia, or it will ovulate. Of the large number of primordial follicles that initiate growth, only a few survive. Ovulation rate is thus determined more by the number that escapes atresia than by the number of follicles stimulated to grow and ovulate.

1.2.5 Effect of ewe age on reproduction

Records of prolificacy (number of lambs born per ewe lambing) of Targhee, Suffolk and Polypay ewes from flocks participating in the US National Sheep Improvement Program (NSIP) between 1984 and 1994 were used for a study by Notter (2000). These breeds possess the largest numbers of NSIP records. Ewe age in months was calculated for each lambing. Ewes lambing between 6 and 18 months of age were coded as 1-year-old ewes. Ewes lambing between 19 and 30 months of age were coded as 2-year-old ewes. Ewes were also combined into large ewe age groups to compare ewes that were 1, 2, 3 through to 6, or greater than 6 years old at lambing.

Prolificacy differed ($P<0.001$) among ewe age groups in all breeds. Peak prolificacy was generally achieved between 4 and 8 years of age. Exceptions to this generalization include a somewhat sharper peak in prolificacy for the Targhee. Prolificacy of 5- and 6-year-old Targhee ewes averaged 0.06 higher than the prolificacy of 4- or 7-year-old ewes. Also, the prolificacy of Polypay ewes appeared to have begun to decline by 8 years of age. Ewes that were more than 8-years-old at lambing had 0.17 – 0.20 fewer lambs per ewe lambing

than the 3- to 6-year-old ewes (Table 2). Thus, prolificacy did not exhibit consistent declines until 7 years of age.

Table 2 Numbers of observations, least-square constants (LS), and standard errors for ewe effects in prolificacy in Targhee, Suffolk and Polypay ewes[1]

Ewe age group (years)	**Targhee**		**Suffolk**		**Polypay**	
	No	LS	No	LS	No	LS
1	459	-0.61 ± 0.03	2304	-0.47 ± 0.01	2016	-0.69 ± 0.02
2	2784	-0.30 ± 0.01	3331	-0.13 ± 0.01	1795	-0.32 ± 0.02
3	2162	-0.11 ± 0.01	2487	-0.01 ± 0.01	1346	-0.07 ± 0.02
4	1615	0.00 ± 0.02	1725	0.02 ± 0.02	822	0.03 ± 0.02
5	1164	0.05 ± 0.02	1189	0.02 ± 0.02	546	0.04 ± 0.03
6	774	0.07 ± 0.02	757	-0.03 ± 0.02	375	0.00 ± 0.03
7	414	0.00 ± 0.03	490	0.04 ± 0.03	208	-0.01 ± 0.04
8	219	-0.01 ± 0.04	249	-0.05 ± 0.04	70	-0.12 ± 0.07
>8[2]	114	-0.17 ± 0.05	189	-0.19 ± 0.04	53	-0.20 ± 0.08

[1]Constants are expressed relative to the average of the 3- to 6 year old ewes
[2]Ewe age classes of >8 years were combined because of small numbers of observations
(From Notter, 2000)

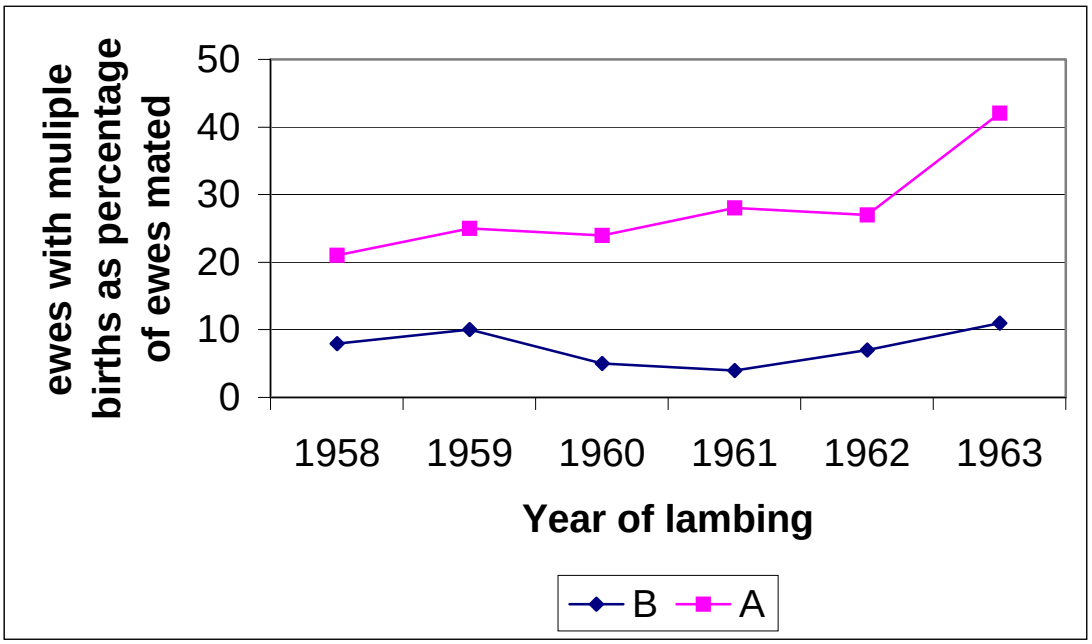
Dickerson and Glimp (1975) used linear and quadratic regression to evaluate ewe age effects on prolificacy in seven US breeds and obtained similar results to those of the previous mentioned study. Across all breeds, prolificacy was a maximum at 5.9 years of age. In another study, Glimp (1971) reported that prolificacy in several US breeds was maximized at 5 years and that 2-year-old ewes produced 0.19 fewer lambs than 3- to 6-year-old ewes.

These analyses suggest that ewe age effects on prolificacy are relative continuous in ewes lambing between 1 and about 9 years of age. Stratification of ewe ages into classes is probably acceptable for purposes of adjusting prolificacy data in animal recording programs, but when sufficient data are available, separate factors for each ewe age are probably superior.

1.2.6 Multiple births

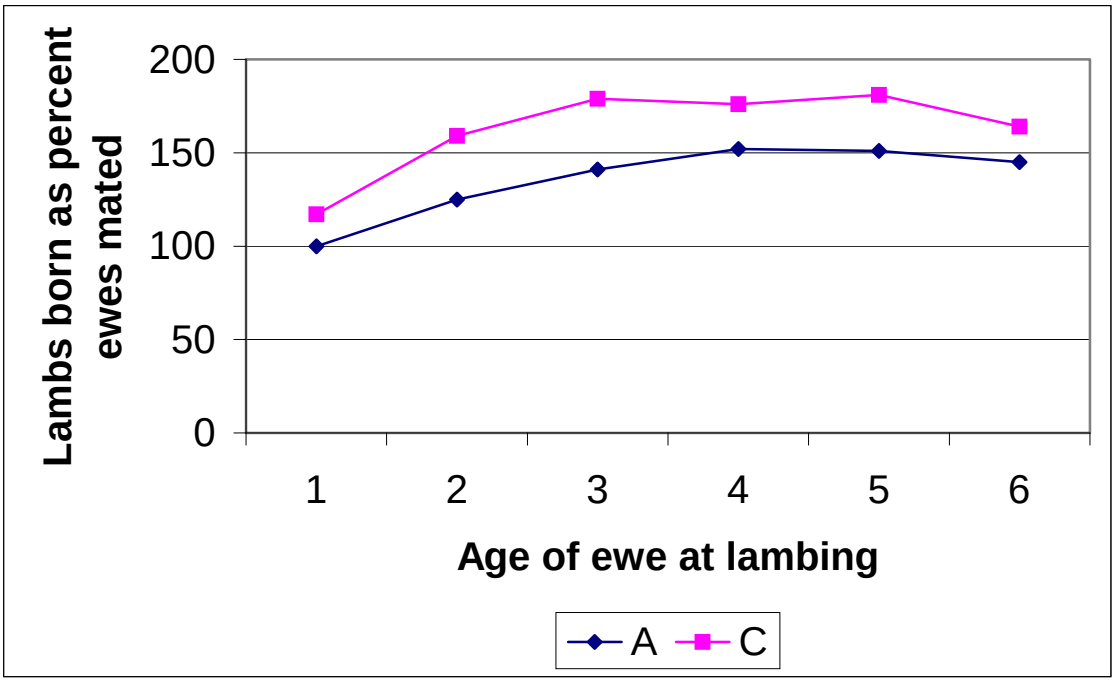
Turner (1966) found that twins could be selected for. The results of selection for multiple births are shown in Figure 2. The graph shows the number of multiple births, as a percentage of ewes joined, in 2 selection groups of medium Peppin Merinos at Deniliquin, Australia. The lower line (B) represents a group in which the sires used were selected as being the rams whose recent female ancestors lambed single lambs only during their own lifetimes. The upper line (A) is of the group in which sires were used whose female ancestors had lambed the greatest number of lambs. All ewes born in each group were joined in that group with no selection being practiced on them. Selection for twin-bearing ability was effective because they found a 3% gain per year that represented a gain in lambs born as a percentage of ewes mated.

The rate of twinning was also investigated within the same study (Turner, 1966). Figure 3 compares the performance of ewes of different ages in the group selected for multiple births at Deniliquin with ewes in a third flock, the original animals of which had been obtained from a commercial breeder who had selected for multiple births among his ewes. Both flocks showed an increase with age of ewe in the number of lambs born per ewe mated. It is suggested that the 'twinning ability genes' were transmitted by both ram and ewe but those in the ewe only were capable of expression; they probably operated by increasing the number of eggs shed (ovulation rate) (Pacham and Triffitt, 1966).



(Turner, 1966)

Figure 2 The percentage of multiple births per ewe mated over a period of six years



(Turner, 1966)

Figure 3 The rate of twinning at different age groups

Turner (1966) and Dolling (1970) found that age also had an influence on the reliability of the ewes' performance as an indication of its genetical potential in terms of multiple births. Heritability values up to 0.40 were observed during the ewes' second and third lambing season. These heritability values were lower in older ewes.

1.2.7 Seasonal breeders and photoperiodism

Most breeds of sheep exhibit seasonal breeding patterns. Sheep are short-day or fall breeders. Their breeding season is initiated as the day length decreases and night time increases and ends when day length and night time is of similar length. However, some breeds like the Merino, Dorset Horn and Rambouillet, have extended breeding seasons, with some individuals being poly-oestrus, if nutrition and climate is favourable.

Reproductive seasonality is characterized by changes at behavioural, endocrine and ovulatory levels giving rise to an annual alteration between two distinct periods; a breeding season, characterized by the succession at regular intervals (mean of 17 days) of oestrus behaviour and ovulation, if pregnancy does not develop, and an anoestrus season characterized by the cessation of sexual activity. The transition from anoestrus to breeding season is gradual, with occurrence of short cycles, because the first *corpus luteum* often regresses prematurely 5 to 6 days after its formation. Both ovulatory activity and oestrus behaviour show parallel seasonal variation but there are some discrepancies at the beginning and at the end of the sexual season when some ovulations are not accompanied by oestrus. It is only after the end of the first ovarian cycle that the behavioural oestrus is exhibited. Silent

ovulations may also occur in some breeds during mid-anoestrus (Ortavant, Bocquier, Peeletier, Ravault, Thimonier and Volland-Nail, 1988).

The role of photoperiod in the regulation of seasonal breeding activities is well known. As breeding season approaches, there is an increase in the frequency and amplitude of episodic surges of LH. The retina of the eye is the photic sensor that transmits light signals by way of the retinohypothalamic tract to the suprachiasmic nuclei. Diurnal signals generated by these nuclei are transmitted to the superior cervical ganglia and then to the pineal gland via sympathetic nerves.

During darkness, the sympathetic activity increases resulting in greater activation of an enzyme needed for the synthesis of melatonin. The pineal gland, through the synthesis and release of melatonin, serves as a mediator between the neural signals induced by changing photoperiod and the endocrine system that regulates cyclic reproductive activity. Through either indirect or direct action on the hypothalamus, melatonin modulates seasonal breeding activity in long-day and short-day breeders. (Reiter, 1974).

At an endocrine level, it is known that during the anoestrus season, follicle growth and regression occur and follicles as large as those found during the luteal phase of the oestrus cycle may be present (Webb and Gauld, 1985). Throughout seasonal anoestrus, the follicles produce steroids, and many of the positive and negative feedback effects of the steroids on secretion of LH continue as in the breeding season (Gordon, 1997). LH continues to be released, episodically, but with lower frequency than during the reproductive season (Thiéry and Martin, 1991). A major difference occurs also in plasma progesterone concentration, which remains virtually at undetectable levels during anoestrus (Karsch, 1984; I'Anson and Legan, 1988). FSH levels seem not to be significantly different from those found during the reproductive season (Walton, McNeilly, McNeilly and Cunningham, 1977).

1.2.8 Live weight and ovulation rate

Analysis of a large volume of data, collected over many years, led to a conclusion that live weight at mating has a significant influence on the reproductive rate of ewes (Coop, 1962), especially the rate of twinning. From a between-flock analysis he estimated that the twinning rose by 5.3% for each additional 4.5kg of live weight. Subsequently these studies extended to include ovulation rate (Kelly, Thompson, Hawker, Crosbie and McEwan, 1983). It was concluded that there was a relationship between live weight and ovulation rate between groups and in some cases within groups of ewes. In most cases, it was reported that for each additional kilogram in live weight there was a 2.0 – 2.5% increase in ovulation rate and a 1.5 – 2.0% increase in lambs born per ewe. However, the relationship varied widely depending upon whether it was measured within flocks of a similar genetic constitution or between flocks and breeds. Cumming (1977) found that, within flocks, ovulation increased from 0 to

0.44 for each additional 10 kg of live weight, the increase for most flocks being 0.25 – 0.30.

The live weight of an ewe is a combination of body size and body condition and as such live weight is not a good measure of an ewe's body nutrient reserves. In a study by Ducker and Boyd (1977), body size had no effect on mean ovulation rate of ewes in the same body condition. In Scottish Blackface ewes, ovulation rate was positively related to body condition at mating (Gunn and Doney, 1975). Live weight *per se* is therefore not a suitable variable to consider in studying ovulation responses in relation to pre-mating nutrition. Body condition, a reflection of the ewe's body tissue reserves, is more appropriate.

1.2.9 Live weight change, feed intake and ovulation rate

Not until the late 1960's was a large effort put into examining the relationship between live weight change and ovulation rate (Gunn, Doney and Russel, 1969). Morley, White, Kenney and Davis (1978) concluded that there is little doubt as to the existence of a "dynamic" effect, related to ovulation rate, although it is far from general even when live weight is changing rapidly at the time of mating.

Flushing includes two processes, firstly an increase in nutrient intake, either by an increase in the level of intake and/or intake of better quality feed and, secondly, a resultant improvement in body condition. Alterations in body condition are generally measured as live weight change once gut fill has been taken into account but body condition can also be measured as a semi-quantitative score (Russel, Doney and Gunn, 1969). In Edinburgh, at the former Hill Farming Research Organization, it was observed that ovulation rate was positively and significantly related to body condition at mating but not significantly related to the level of premating nutrition when ewes were in good condition (score 3.0) or moderately good condition (score 2.5) (Gunn *et al*., 1969; Russel *et al*., 1969; Gunn and Doney, 1975). The work by Gunn *et al*. (1969) suggested that with ewes in poor condition at mating ovulation rate might be related to the level of pre-mating nutrition. In a later study it was found that, in cases of moderate body condition (score 2.0), ovulation rate was positively related to the level of pre-mating nutrition but that this was not the case in ewes of higher body condition (Gunn, Doney and Smith, 1984).

1.2.10 Stress and reproduction

There is little doubt that stress has a detrimental effect on reproductive competence in farm animals. Dobson and Smith (1995) based that there are two main mechanisms by which activation of the hypothalamus-pituitary-adrenal axis reduces the efficiency of the hypothalamus-pituitary-gonad axis. The first mechanism involves interference with correctly timed GnRH secretion controlled by neurotransmitters, and the other is the detrimental

influence of hypothalamus-pituitary-adrenal hormones (especially ACTH) on the action of GnRH at the pituitary.

Most evidence suggests that, although stressors can cause foetal losses in mid-to-late pregnancy, the increased percentage of stress-induced reproductive losses occurs as a result of interference with correct hypothalamus-pituitary function, early embryonic losses result from unsuitable exposure of the ovum to gonadotrophins within the follicle (Staigmiller and Moor, 1984).

1.2.11 Endocrine changes due to heat stress

The sequence of coordinated physiological events that lead to the initiation and maintenance of pregnancy are controlled by an array of hormonal changes that regulate the oestrus cycle. When examining the effects of thermal stress on hormones controlling these processes, it is important to realize that both acute and chronic thermal stress require metabolic adaptations to accommodate altered processes associated with dissipation of heat, water and electrolyte turnover, and altered metabolism. Such hormones as prolactin, growth hormone, thyroxine, glucocorticoids, antidiuretic hormone, epinephrine, norepinephrine and aldosterone have altered secretion rates during acute and chronic periods of adaptation to thermal stress (Thatcher, Collier, Beede and Wilcox, 1986). These hormonal and physiological responses may, in turn, alter control and secretion of the reproductive hormones. It is also important to distinguish between seasonal changes in reproductive hormones and heat stress induced changes within a season.

Heat stress can also directly alter pituitary function. Schillo, Alliston and Malven (1978) demonstrated that patterns of LH secretion in ovariectomized ewes were disrupted by hyperthermia.

Since elevated environmental and uterine temperatures near the time of fertilization are related to pregnancy rate (Gwazduaskas, Thatcher and Wilcox, 1973), factors controlling uterine temperature are important. Greatest rate of uterine blood flow during the oestrus cycle occurs during the peri-oestrus period in association with high ratios of oestradiol/progesterone concentrations due to follicle development and regression of the corpus luteum (Ford, Chenault and Echternkamp, 1979).

1.2.12 Leptin as a metabolic marker affecting reproduction

Leptin is a peptide (secreted by white adipocytes) that plays a role in the regulation of body weight and food intake. It has been implicated in the interaction between nutrition and fertility (Ashworth, Hoggard, Thomas, Mercer, Wallace and Lea, 2000; Clarke and Henry, 1999; Cunningham, Clifton and Steiner, 1999; Gonzalez, Simon, Caballero-Campo, Norman, Chardonnes, Devoto and Bischoff, 2000). Leptin receptors have been identified in many

areas of the brain and in many other tissues, including the ovary.

Administration of leptin has been shown to stimulate GnRH and LH secretion in pituitary cells *in-vitro*, and to a lesser extent, FSH. In mice, leptin increases the number of follicles per ovary (Barash, Cheung, Weigle, Ren, Kabigting, Kuijper, Clifton and Steiner, 1996). A possible mechanism for this regulation would involve leptin binding to the B-endorphin neuron, which impacts on the GnRH neuron. Leptin may also have an effect locally, acting within the ovary to regulate follicle size and possibly oocyte quality.

CHAPTER 2

NUTRITION AND REPRODUCTION

2.1 Influence of energy vs. protein on reproduction

Some of the available evidence suggests that protein rather than energy is involved in acute responses of ewes to nutrition (Knight, Oldham, and Lindsay, 1975). Certainly, Rattray, Jagusch, Smith, Winn, and MacLean. (1980) have shown that in grazing sheep the quality of the diet can determine the response in ovulation rate. However, the partitioning of the components of the diets is complex in the ruminant; in particular, up to 35% of the animal's requirements for glucose can be met by amino acids (Bergman, 1983) and the ultimate amount of amino acids available to the animal from any diet depends in part on the ability of the dietary protein to escape rumen fermentation.

Many experiments in sheep supporting the role of protein have been based on responses to the high protein grain Lupin, which are also high in energy. Hume (1974) and Teleni, King, Rowe, and McDowell (1989) drew to the same conclusion that energy, not protein, provides the important regulatory signal for ovulation after showing that a post-ruminal infusion of energy substrates, including glucose, increased the ovulation rate in ewes.

Table 3 summarises the most common reproductive disorders one may find if the animal is given a feed that has either an excess or deficiency of energy and protein.

Table 3 Nutrient-related abnormalities in reproduction

Nutrient	**Reproductive disorder**
Energy excess	Low conception, abortion, dystocia, retained placenta, reduced libido
Energy deficiency	Delayed puberty, suppressed oestrus and ovulation, suppressed libido and spermatozoa production
Protein excess	Low conception rate
Protein deficiency	Suppressed oestrus, low conception, foetal resorption, premature parturition, weak offspring

(From Bearden and Fuquay, 2000)

There is thus no clear evidence to suggest if either protein or energy has a definite effect on the ovulation rate. For this reason, it is more practical to attribute the effects of diet on ovulation rate to changes in the 'general nutritional status' of the animal, rather than

attempt to partition the effects between protein and energy. This view will probably cover the range of diets normally encountered by the animal.

2.2 Energy metabolism

Dietary carbohydrates provide well over one-half of the energy needed for performance of metabolic work, growth, repair, secretion, absorption, excretion and mechanical work in most warm-blooded animals. Carbohydrate metabolism includes all reactions where carbohydrates are in the forms of polysaccharides, disaccharides and monosaccharides.

In adult ruminants relatively small amounts of dietary carbohydrates escape fermentation. Because ruminants derive the major portion of their energy from volatile fatty acids (VFA), glucose and other monosaccharides, as such, play only a secondary role in the energy metabolism of these animals. Ruminants rely extensively on the production of acetate, propionate, butyrate and valerate by anaerobic fermentation of dietary carbohydrates and other feed constituents within the rumen. Lesser production of the same end products occurs via fermentation in the large intestines. Depending on the diet composition, VFA may contribute up to 80% of the total energy needed by a ruminant.

Other dietary constituents also contribute carbon for VFA synthesis. For example, when cellulose rather than starch is the major dietary carbohydrate for the production of acetate. Increasing the proportion of starch will increase ruminal production of propionate and valerate and decrease production of acetate and butyrate. In addition to production of VFA, the fermentation of dietary constituents by numerous species of bacteria and protozoa in the digestive tracts of animals results in production of CO_2 and methane. These two gasses are lost to the environment, whereas VFA are efficiently absorbed and transported via the portal circulatory system to the liver. The liver efficiently removes the propionate, butyrate and valerate from portal blood, but much acetate passes through the liver to peripheral tissues for subsequent metabolism. Propionate is a major precursor for glucose synthesis in the liver. Approximately half of the butyrate absorbed through the rumen wall is converted to β-hydroxybutyrate, which is metabolised by peripheral tissues rather than by liver (Frandson *et al.*, 1992).

2.3 Influence of energy intake on follicular development

It is clear that extremes of energy intake can alter the growth characteristics of follicles as well as oocyte and embryo development. Dietary restrictions can alter follicle growth characteristics in superovulated sheep (O'Callaghan, Yaakub, Hyttel, Spicer and Boland, 2000). Also poor nutrition, which lowers ovulation rates, is associated with

decreased LH pulse frequency, which is likely due to inadequate hypothalamic GnRH secretion (Rhind, McMillen, Mckelvey, Rodriguez-Herrejon and McNeilly, 1989).

The energy level provided in the diet has important implications for the metabolism of dietary protein. Conversion of rumen degradable protein into microbial protein is dependent on availability of fermentable metabolizable energy. If there is sufficient energy present, microbial protein is formed that is digested further along the gastrointestinal tract. If there is insufficient energy to convert rumen degradable protein to microbial protein, surplus ammonium ions are converted to urea by the liver and removed from the blood by the kidneys. High dietary protein, resulting in high concentrations of urea nitrogen in plasma and milk has been associated with decreased fertility in dairy cattle (Ferguson, Galligan, Blanchard and Reeves, 1993: Butler, Calaman, and Bearn, 1996)

2.4 Influence of energy balance on postpartum follicular dynamics

Lucy, Staples, Michel and Thatcher (1991) examined follicular development in dairy cows by ultrasonography and repeated an effect of energy balance on different populations of ovarian follicle postpartum. The number of class1 (3-5 mm) and class 2 (6-9 mm) follicles decreased and the class 3 (10-15 mm) follicles increased with a positive energy balance before day 25 postpartum. The authors suggested that with an increased energy balance, movement from smaller to larger follicle sizes are enhanced.

Development of dominant follicles postpartum are tolerant to periods of energy deficiency as demonstrated by the selection and growth of follicles over 15 mm in diameter during the second week postpartum, despite the negative energy balance (Beam and Butler, 1997). However, several studies indicate that the ultimate diameter and oestrogen production of dominant follicles are influenced by metabolic factors. In prepubertal heifers (Bergfield, Kojima, Cupp, Wohrman, Peters, Garcia-Winder and Kinder, 1994), postpartum suckled beef cows (Perry, Corch, Cochran, Beal, Stevenson, Minton, Simms and Brethow, 1991) and cyclic lactating dairy cows (Lucy, Beck, Staples, Head, De la Sota and Thatcher, 1992), growth and dominant follicles is decreasing during dietary energy restriction. Dominant follicle diameter and plasma oestradiol increased after energy balance improved from its most negative level in early postpartum cows (Beam *et al.*, 1997).

2.5 Protein nutrition and metabolism

Dietary protein, for the ruminant, can be classified as either “rumen-degradable protein” (RDP) or as “rumen-undegradable protein” (UDP). Certain natural proteins and other processed proteins (e.g. those denaturated by heat treatment or tanned by the application of formaldehyde) escape ruminal degradation but can be readily hydrolysed by the gastrointestinal proteolytic enzymes. These proteins are catabolised to smaller peptide and

amino acids for absorption. The metabolic fate of these amino acids include the synthesis of proteins, hormones and enzymes used for normal body function, and reproduction, and used as fuels in energy metabolism and as precursors of numerous nitrogen-containing compounds such as neurotransmitters, purines and pyrimidines.

The quality of the protein in a feed is dependent on its amino acid profile and digestibility. The protein requirement of an animal is dependent on its physiological status and production level. Essential amino acids must be supplied in the diet of monogastrics, but rumen microbes are the main source of amino acids for ruminants.

Amino acids can be deaminated in the liver, leaving keto-acid, which in turn, goes through the Krebs-cycle and ultimately forms glucose. As a result of deamination in the liver, ammonia is also formed, and this contributes to the formation of urea.

Ruminants are capable of reducing protein loss by recycling urea, normally an excreted product of protein metabolism. Thus, urea can be recycled to the rumen when a diet is low in nitrogen (Frandson *et al.*, 1992).

2.6 Influence of peri-conception nutrition on follicular development

Evidence indicates that embryo/oocyte quality in sheep is affected by nutrient status during the cycle of conception. Peri-conception nutrition plays an important role in determining reproductive outcome in the ewe, particularly in relation to ovulatory performance. Increasing the pasture allowance for more than 3 weeks (Rattray *et al.*, 1980; Smith, Jagusch and Farquhar, 1983) or feeding a high protein diet for 6 days or more (Lindsay, 1976; Oldham and Lindsay, 1984) before the start of oestrus increases the mean ovulation rate in that cycle. However data indicates that the nutritional condition which improves the ovulation rate can be detrimental to oocyte and embryo quality.

The relationship between oocyte/embryo quality and dietary intake is most often studied in the superovulated animal. In the ewe, *ad libitum* feeding for 20 days before mating reduced the number of good quality embryos harvested, as well as the mean ovulation rate compared with ewes that received either 1.5 × maintenance or 0.5 × maintenance energy requirements (Lozano, Lonergan, Boland and O'Callaghan, 2003). In the cow it has been found that with a high intake of either pasture, or dietary crude protein offered as urea or silage and concentrates, adversely affected embryo quality following either superovulation or synchrony of oestrus/AI (Dunne, Diskin, Boland, O'Farrell and Sreenan, 1999). In these studies significant differences in various measures of embryo quality were obtained when the nutritional treatments were applied for as little as 10 days before the commencement of the superovulatory treatment.

Increasing dietary intake for 3 weeks or more before mating (Coop, 1966) or feeding a high protein diet, based on lupin or pea grain, 5-8 days before ovulation (Nottle, Setchell

and Seamark, 1986; Steward *et al.*, 1986) improves ovulation rate, but they can be associated with a disproportionate increase in the number of multiple ovulations that either fail to fertilize or fertilize and fail to develop thereafter (Smith *et al.,* 1983). An explanation for this wastage is that the nutritional conditions required to maximize ovulation rate (i.e. an increase in dietary intake) differ from the conditions required for improved embryo quality (i.e. a relatively low intake, at least immediately after ovulation). This is supported by other studies that indicate high diets before ovulation can have adverse affects on oocyte/embryo quality (Lozano *et al.,* 2003).

Lindsay (1976) suggested that the effect of nutrition on reproductive processes should be thought of in terms of the ewe's "net nutritional status", a term which encompasses both the endogenous and exogenous sources of nutrients available to the ewe. If considered in these terms the changes in reproductive traits associated with nutritional influences can be related to major metabolic changes. These metabolic changes are a consequence of decreasing or increasing feed intake and the associated utilization or storage of nutrients in body reserves.

2.7 Influence of nutrition on ovarian function

The ability of nutrition to alter the ovulation rate and lambing rate of ewes is well known, where a rapid improvement in body condition is usually associated with an increased ovulation rate and litter size (Coop, 1966). Alterations in ovulation rate may be related to the cell entry rate of glucose in animals on a high plane of feeding. Dietary supplements containing high energy and protein have been shown to increase the ovulation rate in ewes (Downing, Joss, Connell and Scaramuzzi, 1995). Similar increases in ovulation rate were reported when glucose was infused directly (Williams, Yaakub, O'Callaghan, Boland and Scaramuzzi, 1997). Thus it is likely that short-term energy supply is directly involved in follicle recruitment (Gutierrez, Oldham, Bramley, Gong, Campbell and Webb, 1997) and perhaps also in follicle growth; however, this effect may be of short duration when the diet level is altered.

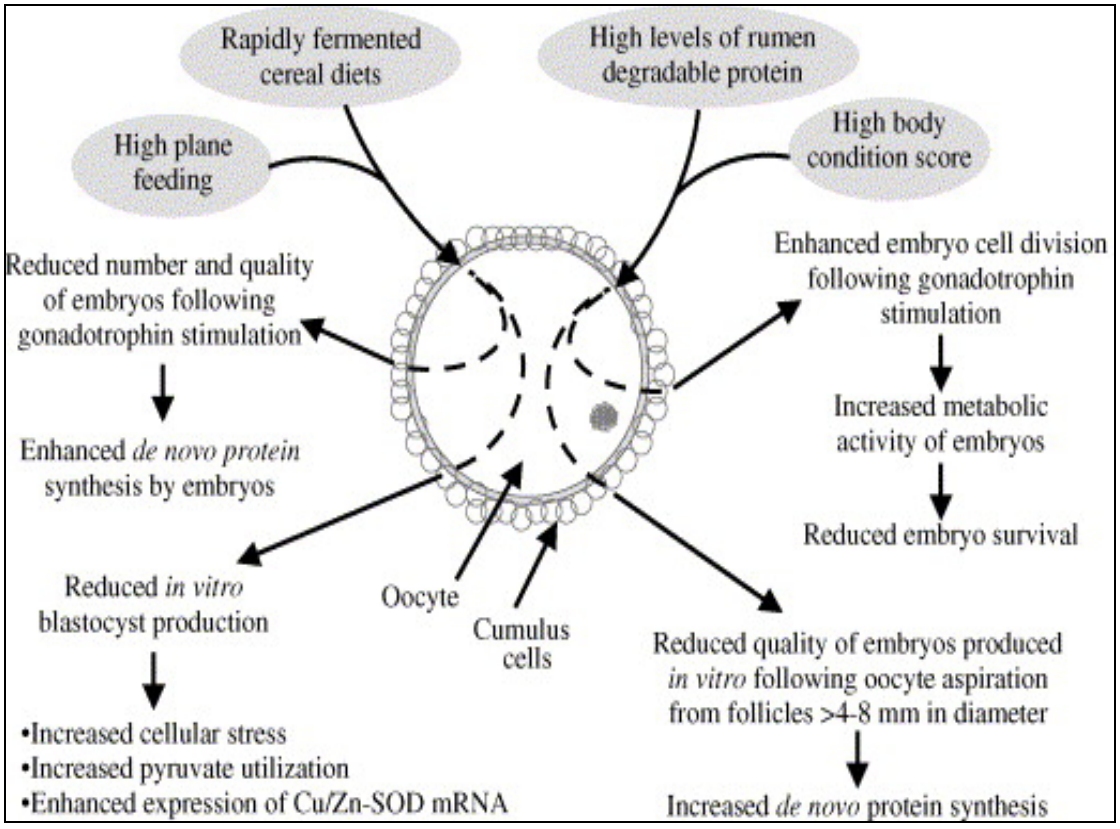
In the case of ewes superovulated with FSH, a lower ovulation rate was recorded in ewes offered diets of half the maintenance energy requirements, compared with ewes offered diets of twice the maintenance energy requirements (Yaakub, O'Callaghan, O'Doherty and Hyttel, 1997).

2.8 Influence of nutrition on the oocyte

Research into methods of improving the efficiency of ruminant multiple ovulation and embryo transfer programmes and *in vitro* systems of embryo production from oocytes

obtained by aspirating ovarian follicle is providing new information on the impact of oocyte-donor nutrition on oocyte quality when using these reproductive technologies.

Figure 4 provides an illustration of the main findings from these studies. Unlike spontaneously ovulating sheep and cattle for which high-plane feeding is beneficial to oocyte quality the opposite is the case in superovulated animals and those donating oocytes for *in-vitro* embryo production. The adverse effect is accentuated in animals in good body condition (Adamiak, Mackie, Powell, Watt, Dolman, Webb and Sinclair, 2003), and those given large amounts of high-starch concentrates that are rapidly fermented in the rumen (Yaakub, O'Callaghan and Boland, 1999).



(from data reviewed by Boland, Lonergan and O'Callaghan (2001) with more recent observations by Adamiak *et al.*, (2003)

Figure 4 Effect of the plane of nutrition and diet type during oocyte maturation on ruminant embryo development following superovulated and/or *in vitro* embryo production.

2.9 Feed intake and progesterone concentrations

Feed intake in sheep can influence the concentration of progesterone, with a strong negative correlation between dietary intake and progesterone concentrations (Rhind, McMillen, Wetherhill, McKelvey and Gunn, 1989). This effect of intake on circulating progesterone concentration may be due to an increase in the rate of catabolism of

progesterone and in hepatic circulation at higher feedback levels (Parr, Davis, Miles and Squires, 1996). Progesterone, through its negative feedback effects, can affect LH pulse frequency and is also thought to play an important role in oocyte maturation and in early embryo development (Kleemann, Walker, and Seamark, 1994; McEvoy, *et al.,* 1989).

In sheep, overfeeding that reduced circulating progesterone concentrations also reduced pregnancy rates (Parr, Davis, Fairclough and Miles, 1987) and decreased both the rate of development and viability of embryos (Creed, McEvoy, Robinson, Aitkin, Palmer and Robertson, 1994).

2.10 Effect of dietary urea on fertility

A manifestation of excess urea in the circulation is the birth of abnormally large offspring (Young, Sinclair and Wilmut, 1998). Ewes fed excess amounts or urea from Day 21 before mating to Day 63 of gestation resulted in oversized lambs at birth (McEvoy, Sinclair, Staines, Robinson, Armstrong and Webb, 1997). It was suggested that this effect occurred through embryonic exposure to high concentrations of ammonia in the reproductive tract *in vivo*. Also links have been established between high levels of ammonia and poor embryo development *in vitro* (Gardner and Lane, 1993).

In sheep, Fahey, Boland and O'Callaghan (2000) reported that despite high levels of dietary urea and blood urea concentrations, there was no effect of dietary urea on ovulation rate in donor or recipient ewes. However, embryo quality in donors was reduced as fewer embryos with more than eight cells were recovered at Day 4 of pregnancy from urea-treated ewes. The diet offered to recipients had no effect on embryo survival. Thus, it was suggested that the effects of urea on embryo quality are likely to be due to alterations in the oviduct environment or deleterious changes in the follicle, rather than changes in the uterine environment. This is supported by data from cattle (Gath, Lonergan, Boland and O'Callaghan, 1999) showing no difference in pregnancy rates at Day 35 after transfer of good quality *in vitro* produced embryos to recipients on different levels of dietary urea.

Links also have been established between high levels of ammonia and poor embryo development *in vitro* in some embryo culture systems (Gardner *et al.*, 1993). The effect seems to be dependent on both the concentration of ammonia and the stage of development at which exposure to ammonia occurs (Hammon, Wang and Holyoak, 2000).

Sinclair, Kuran, Straines, Aubaily, Mackie, Robinson, Webb and McEvoy, (2000) showed that the rate of rumen nitrogen released (slow vs. fast) from isocaloric and isonitrogenous diets can influence the proportions of zygotes produced after i*n vitro* fertilization of oocytes from medium-sized follicles. This suggests that oocyte competency could be undermined by nutritional regimes that lead to high circulating levels of ammonia in plasma.

2.11 Influence of lupin grain supplementation on ovulation rate

Lupin (*Lupinus augustifolius)* is high in digestible protein and energy that may increase ewes' ovulation rate (Lightfoot and Marshall, 1974; Knight *et al.*, 1975). Since these early studies there have been other reports of increased ovulation rate following lupin grain supplementation (Smith *et al.*, 1990). Increase in ovulation rate occur within 6 days after the feeding of lupin grain (Lindsay, 1976) and 4-6 days before luteolysis is the critical period for lupin grain supplementation to increase ovulation rate (Nottle, *et al.*, 1986; Steward *et al.*, 1986).

In an experiment done by Nottle, Kleeman, Grosser and Seamark (1997), feeding lupins increased the number of ovulations from 126 to 146 ovulations per 100 ewes exposed to rams ($P < 0.05$) due to an increase in the number of ewes with twin ovulations (Table 4). This increase is reflected in an improvement in fecundity (lambs born per ewe lambing; $P < 0.05$) but not fertility (ewes lambing per ewe exposed to rams). Net reproductive performance (the product of fertility, fecundity and lamb survival) indicated that 11 extra lambs are weaned per 100 ewes exposed to rams as a result of lupin supplementation at mating.

Table 4 Reproductive performances of Merino ewes synchronized using the ram effect and supplemented with lupin grain for 14 days, commencing 12 days after introduction of vasectomised rams.

Attribute	**Treatment**	
	Control	**Lupins**
Incidence of oestrus (%) 0-14 [1] days	16.1	14.0
15 – 29 days	94.0	93.6
Incidence of non-return to service (%)	83.9	87.2
Live weight day 29 (kg) [2]	51.9 ± 0.5	52.4 ± 0.5
Ovulation rate	1.26	1.46 [a]
Ewes lambing per ewe exposed to rams (%)	94.6	94.8
Lambs born per ewe lambing	1.21	1.36 [a]
Lambs weaned per lamb born (%) single	79.7	78.1
Twin	68.8	73.5
Overall	75.9	75.6
Lambs weaned per ewe exposed to rams (%)	86.9	97.7

1 – Vasectomised rams joined with ewes on day 0 (Adapted from Nottle *et al,* 1997)
2 – Least squares mean ± SE
a – differs significantly ($P < 0.05$)

This experiment was conducted on two commercial farms in a region that experiences a Mediterranean climate. The experiment was conducted in late spring thus the environment was not favourable for the conception of twins. The nutrition was therefore mostly responsible for the results obtained. .

Supplementation with lupins increased ovulation rate by increasing the proportions of ewes with multiple ovulations. This increase is similar to that reported in the past for commercial flocks in southern Australia fed lupins (Knight *et al.,* 1975; Kleeman and Cutten, 1978).

The increase in ovulation rate was shown in 15 extra lambs born per 100 ewes exposed to rams. The effect of lupin grain on the number of lambs born, consists of two components namely, an increase in the number of ewes lambing and an increase in the number of twin births (Knight *et al.,* 1975) as previously mentioned.

Croker, Johns and Johnson (1985) measured the response to lupins in terms of lambs born and found that this response varied from 14 – 21 % between commercial flocks. These workers found that none of the variation observed could be explained by live weight, live weight change, initial blood urea nitrogen or the quantity and quality of dry matter available.

According to Downing, *et al.*(1995) the ovulation rate of ewes supplemented with lupin grain tended to be higher (2.5 $\pm$ 0.2) than that of control fed ewes (1.9 $\pm$ 0.2) (P=0.073). The ovulation rate was increased in lupin-fed ewes in the study reported here without significant alterations in the pattern of gonadotrophin secretion; however insulin, prolactin and growth hormone were significantly altered by lupin grain supplementation.

Ewes that received lupin supplements had higher mean plasma concentrations of FSH before oestrus (Brien, Baxter, Findlay and Cumming, 1979). Similarly, FSH concentrations in plasma of ewes fed a high protein diet that increases ovulation rate are elevated on days 2, 3, 4 and 6 before oestrus (Smith, 1988). It is suggested that an increase in ovulation rate after lupin grain supplementation involve increases in the secretion of FSH at a time in the oestrus cycle when exogenous FSH increases ovulation rate (McNatty, Hudson, Gibb, Ball, Henderson, Health, Lun and Kieboom, 1985). Lupin grain also shown an increase in ovulation rate with no difference in the pattern of release of LH or FSH in either the follicular or luteal phase of the oestrus cycle, suggesting that supplementation may also have local effects on ovarian function (Radford, Donegan and Scaramuzzi, 1980; Ritar and Adams, 1988).

Lupin supplementation of ewes for 7 days did not affect the number of follicles >2 mm in diameter at luteolysis but 48 hours after luteolysis there was a significantly greater number of follicles > 2 mm in diameter (Nottle, Armstrong, Setchell, Seamark, 1985). This was an indication that the increase in the number of follicles was probably the result of decreased atresia in gonadotrophin-sensitive follicles.

There was a rhythm in circulating prolactin concentrations (Karsch, Robinson, Woodfill and Brown, 1989; Jackson and Jansen, 1991). With this rhythm is a response in plasma prolactin between 4 to 8 hours after lupin grain supplementation. Plasma prolactin rises to a peak any hours after supplementation (McAtree and Trenkle, 1971), compared with a fast increase in a stress response (Neill, 1970). Prolactin inhibits androgen synthesis and can suppress *in vivo* production of C19 steroids in the sheep ovary (McNeilly, 1984). The higher concentrations of prolactin in lupin supplemented ewes may inhibit androgen synthesis, as has been reported for gilts given prolactin, in the late luteal phase of the oestrus cycle (Ciereszko and Dusza, 1993) and, through restricting oestradiol secretion, so favour the development of more than a single ovulatory follicle.

The lupin grain supplementation produced changes in the circulating plasma amino acids concentrations (Table 5) (Faichney, 1972). These changes could be derived from an increase in the protein escaping degradation in the rumen or from a significant increase in the amount of microbial protein entering the abomasum from the rumen. The amino acid composition of bacterial and protozoal protein remains relatively constant (Purser and Buechler, 1966; Bergen, Purser and Cline, 1968).

Lupin grain supplementation increases the concentration of the branched chain amino acids. The infusion of the branched chain amino acids, leucine, isoleucine and valine, for 5 days during the luteal phase increases ovulation rate in ewes (Downing, 1994). Therefore, higher plasma concentrations of the branched amino acids found following lupin supplementation may play a role in the increased ovulation rate.

Supplementing lupin grain to ewes increases the digestible organic matter (Lindsay, Purser and Hogan, 1980) because around 35 % of lupin protein escapes degradation in the rumen and passes into the small intestine (Hume, 1974). A higher protein intake increases the metabolic rate (Egan, 1965), therefore, promotes an anabolic state and glucose uptake. Ovulation rate responses to a high protein supplementation are strongly related to the glucose entry rate into the cellular compartment from the extracellular compartment (Teleni *et al.*, 1989). Plasma insulin concentrations were high in the lupin supplemented ewes, because of an increased availability of gluconeogenic amino acids. Therefore, it's highly probable that lupin supplemented ewes have a higher glucose availability and this may be the stimulus responsible for ovulation rate increases in response to high protein supplements. The increased ovulation rate in the lupin supplemented ewes may also involve direct cellular actions of insulin (Poretsky and Kalin, 1987). Insulin has follicular cell functions effects *in vivo* and *in vitro.*

Table 5 The mean (± SEM) plasma concentrations (nmol/ml) of amino acids in ewes fed straw or straw supplemented with lupin grain on days 2 -13 of the oestrus cycle. A blood sample was taken at 23h after feeding on day 11 of the oestrus cycle.

Amino acid	Lupin	Straw	Level of Significance
Phosphorine	4.8 ± 0.2	4.4 ± 0.4	NS
Taurine	32 ± 4	47 ± 8	A
Aspartic acid	1.1 ± 0.1	1.8 ± 0.2	A
Threonine	107 ± 8	37 ± 5	B
Serine	133 ± 9	136 ± 6	NS
Asparagines	56 ± 8	12 ± 6	B
Glutamic acid	38 ± 3	67 ± 6	B
Glutamine	609 ± 83	439 ± 22	NS
Alpha amino adipic acid	14 ± 1	14 ± 1	NS
Glycine	480 ± 20	733 ± 37	B
Alanine	132 ± 45	183 ± 13	A
Citrulline	180 ± 21	59 ± 3	B
Alpha amino butyric acid	26 ± 2	19 ± 1	A
Valine	171 ± 12	120 ± 4	B
Methionine	17 ± 1	10 ± 1	B
Isoleucine	59 ± 6	33 ± 1	B
Leucine	61 ± 7	49 ± 2	NS
Tyrosine	47 ± 5	13 ± 1	B
Phenylalanine	67 ± 7	61 ± 6	NS
Ornithine	90 ± 7	50 ± 3	B
Lysine	116 ± 9	93 ± 6	NS
Histidine	67 ± 3	62 ± 3	NS
1-Methyl-histidine	57 ± 8	60 ± 6	NS
3-Methyl-histidine	70 ± 8	129 ± 4	B
Arginine	124 ± 9	43 ± 2	B

A – ($P < 0.05$), B – ($P<0.01$), NS; not significant

(Adapted from Downing, Joss and Scaramuzzi, 1995a)

2.12 Influence of leucine, isoleucine, valine on ovulation rate

The factors that influence the hypothalamic control of gonadotrophin secretion have important implications for the development of follicles in the ovary. The central catecholaminergic (Karla, Karla, Chen and Clemens, 1978) and serotonergic (Horn and Fink, 1985; Vitale, Parisi, Chiocchio and Tramezzani, 1986) systems influence the secretion of gonadotrophins. Tyrosine and phenylalanine are precursors for the synthesis of

catecholamines while tryptophan is the precursor for serotonin synthesis. The uptake of these and other branched chain amino acid across the blood brain barrier is via a membrane bound transporter, known as the large neutral amino acid transporter. The brain concentrations of phenylalanine, tyrosine and tryptophan are affected by the plasma levels of other amino acids, such as valine, leucine and isoleucine, which compete for uptake into the brain via the large neutral amino acid transporter (Fernstrom and Wurtman, 1972). The Km, of the large neutral amino acid transporter for the sheep, is unknown but, if it is as low as the rat's (Pardrigde, 1977; Pardrigde and Oldendorf, 1977, Fernstrom and Faller, 1978), then competition between the branched chain amino acids may influence the uptake of amino acid precursors for catecholamines and serotonin synthesis.

The infusion of a mixture of the branched chain amino acids, leucine, valine and isoleucine, into ewes for 5 days before luteolysis is associated with a higher ovulation rate (Downing. Joss, and Scaramuzzi, 1997). The total blood concentration of valine, leucine and isoleucine is elevated by approximately 200 nmol/ml while the study of Smith, Clark, Parr and Konlecher, (1992), and this difference is 780 nmol/ml. Positive relationships between the blood concentrations of the branched chain amino acids, nutritional state and ovulation rate, suggest that the levels of branched chain amino acids in the diet are significant factors linked with ovulation rate responses to nutrition (Waghorn, Smith, and Ulyatt, 1990).

The increase in ovulation rate following the amino acid infusion may be a metabolic response to an increased availability of energy substrates. Insulin initiates growth and metabolic response in many cell types including granulosa and thecal cells (Allen, Nilsen-Haminlton, and Hamilton, 1981; Savion, Liu, Laherty, and Gospodarowicz, 1981; Hammond, Mondschein, and Canning, 1989) and insulin has been reported to increase ovulation rate in ewes (Hinch and Roelofs, 1986). However, insulin infusion 3 – 5 days before luteolysis didn't increase ovulation rate (Smith *et al.*, 1992; Downing and Scaramuzzi, 1993). Adequately fed ewes and ewes in a good body condition have high ovulation rates (Cumming, 1977; Morley, *et al.*, 1978; Oldham, Lindsay, and Martin, 1990) and any improvement in their nutrition will not increase energy supply to the ovary as they will be in a positive energy balance. Ewes in a poor body condition and fed a poor quality diet will be in a negative energy balance and thus their ovaries will have a greater response to an improvement in their nutrition. Insulin alone is not likely to be the stimulus to increase ovulation rate but insulin-mediated uptake of glucose may be a link between improved nutrition and a higher ovulation rate.

Increasing the availability of glucose can be achieved directly or indirectly by means of glucogenic substrates. High concentrations of gluconeogenic amino acids increase the insulin secretion, which in turn will increase glucose uptake by the ovaries. Insulin-mediated and insulin glucose uptake promotes cell growth (May and Schomberg, 1981; Savion *et al.*,

1981; Veldhuis, Kolp, Toaff, Strauss, and Demers, 1983; Baranao and Hammond, 1984; Amsterdam, May and Schomberg, 1988) and inhibits differentiated functions, such as steroid secretions, in follicles (Peluso, Delidow, Lunch, and White, 1991).

2.13 Influence of tryptophan, tyrosine, phenylalanine on ovulation rate

Downing, *et al.,* (1997) designed three experiments to determine if there is a link between the secretion of gonadotrophins, ovulation rate in ewes and the supply of some amino acids in the diet particularly, those that are precursors for catecholaminergic and serotonergic neurotransmitter synthesis. Infusions of tryptophan, tyrosine or a mixture of tyrosine and phenylalanine over days 9 to 13 of the oestrus cycle significantly ($P < 0.05$) increased the blood concentrations of the infused amino acids without affecting the secretion of gonadotrophins or increasing ovulation rate. The levels infused produced blood concentrations of amino acids that were 5 to 20 times greater than the levels achieved by lupin grain supplementation (Downing *et al.,* 1995): a treatment that does increase ovulation rate, and much higher than normal circulating concentrations of these amino acids (Waghorn *et al.,* 1990). All three experiments provide no direct evidence based on studies using intravenous infusion of individual amino acids, to suggest that increasing the supply of amino acids in the diet of sheep influences ovulation rate or the secretion of gonadotrophins, growth hormone (GH) and prolactin. The same 23 animals were used during all three experiments. No random design could thus be used for statistical design and analysis. Feed was given *ad lib.* There were thus no means of determining the daily intake of individual animals and therefore its influence if any on the oestrus cycle. The results obtained might not be a true representation of the experiments.

Tyrosine and phenylalanine are the precursors for synthesis of the catecholamines. It is clear that central catecholaminergic systems are involved in the regulation of pituitary hormone secretion (Weiner and Ganong, 1978) and a role for the catecholaminergic systems in the control of LH release in sheep has been suggested by various authors (Jackson, 1977; Deaver and Dailey, 1983). However the infusion of tyrosine or of tyrosine and phenylalanine had no effect on the LH pulse. A possible explanation is that the effects of catecholamines and indolamines on pulsatile LH secretion are dependent on the reproductive state of the ewes (Meyer and Goodman, 1985; Kao, Schaeffer and Jackson, 1992).

The infusion of tyrosine with phenylalanine increases the circulating insulin concentrations on day 3 of the infusion. Intravenous infusion of a large amount of amino acids can stimulate insulin release (Davis, 1972). The metabolism of tyrosine and phenylalanine give rise to fumaric acid and acetoacetic acid (Meister, 1965). Fumaric acid forms part of the Krebs-cycle, therefore the catabolism of tyrosine and phenylalanine will

contribute to the energy status of the ewes and is the probable reason for the increase in the plasma insulin concentration.

2.14 *Influence of Lotus corniculatus* containing condensed tannins on ovulation rate

Improved nutrient supply, for short periods, before and during reproduction and mating are known to affect ovulation rate along with increased follicle size and/or number (Bellows, Pope, Meyer, Chapman and Casida, 1963), reduce related follicular atresia (Haresign, 1981; Downing and Scaramuzzi, 1991), alters plasma gonadotrophin concentration (Smith, 1988) and ovarian sensitivity to gonadotrophins (Downing *et al.*, 1991). These effects probably occur due to changes in live weight and body condition (Allen and Lamming, 1961; Coop, 1962; Knight, 1980), energy and protein intake and protein absorption from the small intestine (Knight, 1980; Smith, 1991; Cruickshank, Smith and Fraser, 1988; Min, Fernandez, Barry, McNabb and Kemp, 2001), plasma concentration of essential amino acids principally branched chain amino acids (Waghorn, 1986; Downing *et al.,* 1995a) and levels of plasma metabolic hormones, especially insulin. Condensed tannins or proanthocyanidins are polyphenolic compounds, which are present in the stems, and leaves of many forage plants, including *Lotus corniculatus* (birdsfoot trefoil), *L. pedunculatus* (big trefoil), *Hedysarum coronarium* (sulla) and *Onobtychis visclifolia* (sainfoin) (McLeod, 1974; Barry, 1989). The reactivity of condensed tannins with proteins is based upon two mechanisms, hydrogen bonding which is reversible and oxidative coupling that is irreversible (McLeod, 1974; Swain, 1979). Most of the positive effects of condensed tannins in ruminant nutrition are associated with its great affinity for leaf protein after mastification (Jones and Mangan, 1977). The condensed tannins protein complexes are stable at rumen pH (6.0 – 7.0) but then release protein at pH < 3.5 in the abomasums and small intestine for hydrolysis and absorption.

Condensed tannins in *L. corniculatus* decreases rumen protein degradability and ammonia formation markedly (Waghorn, Ulyatt, John and Fisher, 1987) and increase both the flux of essential amino acids (52%) through the abomasums and their absorption (62%) from the small intestine. Subsequently grazing experiments with sheep illustrated that condensed tannins in *L. corniculatus* increases both ovulation rate and lambing percentage (20-27%) (Min *et al.,* 2001), without affecting voluntary feed intake.

In an experiment by Ramirez-Restrepo, Barry, Lopez-Villalobos, Kemp and Harvey. (2005), during the first mating cycle, 7 days after the start of mating, there was a linear relationship between ovulation rate and the duration of grazing on *L. corniculatus* before mating (Table 6). A similar trend was evident for lambs born and surviving to weaning, but statistical significance was only obtained for lambs surviving 24 hours after birth. A hundred animals were used in this study and appropriate statistical analysis methods, with a significance level of 95% and 99% were used to interpret the data. In this study many

variables were taken into account and the experiment was conducted in the dry summer period. It seems that the results are an accurate representation of the experiment.

Table 6 The effect of grazing ewes on perennial ryegrass / white clover (*Lolium perenne/ Lolium repens*) pasture or *Lotus corniculatus* on reproductive efficiency of sheep

	Pasture	**Days of *L corniculatus* feeding before mating**			**Level of significance**
Reproductive efficiency	**0 (n = 75)**	**10 (n =75)**	**21 (n =75)**	**42 (n =75)**	
Ovulation rate (*corpora lutea*/100 ewes mated)	173 ± 7.9	182 ± 8.0	189 ± 7.8	200 ± 7.6	P < 0.05
	0 (n = 51)	**10 (n = 42)**	**21 (n = 50)**	**42 (n = 55)**	
Surviving after 24h (lambs/100 ewes lambing)	131 ± 9.0	150 ± 10.0	158 ± 9.1	160 ± 8.7	P < 0.05

n = Number of ewes (adapted from Ramirez-Restrepo *et al*, 2005)

Responses of above mentioned experiment shows that increase in duration of grazing *L. corniculatus* before mating of up to 42 days continued to increase ovulation rate. These results confirm the effects of mating upon *L. corniculatus* to increase ovulation rate summarised by Min, Barry, Attwood and McNabb (2003).

Thus relative to ewes grazing on pasture, the increased reproductive efficiency of ewes mated on *L. corniculatus* suggests that the effect was mediated by the condensed tannins in *L. corniculatus* (Min *et al.,* 2001) which reduces proteolysis of forage protein in the rumen (Jones *et al.*, 1977), reduces rumen and plasma ammonia concentrations, reduce blood plasma urea concentration (Min *et al.,* 2001) and increases the net absorption of essential amino acids (Waghorn *et al.,* 1987) especially branched chain amino acids from the small intestine. These metabolic shifts may promote events such as folliculogenesis, conception, attachment, embryo survival, foetal growth and lamb viability

2.15 Influence of soybean meal on ovulation rate

Molle, Landau, Branca, Sitzia, Fois, Ligios and Casu (1997) found that flushing Sarda ewes with soybean meal, while mated on a mature grassland, was effective in improving reproductive performances.

In particular ovulation rate was increased by 0.40 per ewe ovulating and prolificacy (lambs born per ewe lambing) tended to be higher by 0.30 lambs per ewe lambing in flushed ewes compared to the controls (Table 7). Unsupplemented ewes were unable to reach high ovulation rate and prolificacy levels, even though, in terms of prolificacy, their performance fell well within the range (1.1 – 1.4) regarded as a breed standard (Anonymous, 1987).

Table 7 Conception (%), prolificacy and fecundity rates of the first and second oestrus of ewes that were provided soybean meal flushing supplement during different periods

Reproductive parameters	**Long-term**	**Medium-term**	**Short-term**	**Control**	**Mean ± SEM**
Conception rate %, 1st oestrus	64	87	71	78	75 ± 6.0
Prolificacy, 1st oestrus [a]	1.55	1.77	1.60	1.36	1.58 ± 0.08
Fecundity, 1st oestrus °	1.00	1.53	1.14	1.07	1.19 ± 0.10
Conception rate %, 1st & 2nd oestrus	86	93	71	86	84 ± 5.0
Prolificacy, 1st & 2nd oestrus	1.50	1.71	1.60	1.33	1.54 ± 0.07
Fecundity, 1st & 2nd oestrus°	1.28	1.60	1.14	1.14	1.29 ± 0.09

SEM – standard mean error ° - $P < 0.13$ [a] – $P < 0.15$
(adapted from Molle *et al*, 1997)

Sixty animals were divided into four groups of 15 ewes each on the basis of body weight, body condition and milk yield. All four groups were subjected to the control (unsupplemented) and long-term flushing treatments. The effect of dietary treatments on reproductive performances was evaluated, using the Fisher's Exact Test, and on individual intake, body weight and body condition, plasma metabolites within day of measurement, using a one-way analysis of variance. Least square means were compared, using the *t*-test.

Significance levels differed from 99% to 85%, which is a bit worrisome on the validity of some of the results.

A possible mechanism through which soybean meal increases the ovulation rate, is the direct action of branched amino acids on the ovary, since protein allowance in excess enhances circulating levels of these acids (Downing, Joss, and Scaramuzzi., 1995b)

2.16 Influence of minerals, vitamins on reproduction

Deficiencies in the diet or in the uptake of a number of minor elements may reduce ovulation rate and embryo survival (Lindsay, 1976) but, in general, this is an indirect effect of a primary influence on basic health status.

Both copper (Cu) (Howell, 1968) and selenium (Se) (Hartley and Grant, 1961) inadequacies impair conception by reduced ova implantation, early embryonic loss and foetal death. Segerson and Ganpathy (1981) recorded significantly more fertilized ova, uterine contractions and ewes with large numbers of sperm attached to the zona pellucida due to improved sperm mobility when ewes of marginal blood selenium status (0.05µg/ml) were given injections of 10mg Se and 136 UI vitamin E before mating. Godwin, Kuchel and Buckley (1970) reported significant increases in lambing percentage when ewes with an initial low blood Se concentration (0.026µg/ml) were given oral supplements of 5mg or 25mg Se as sodium selenite or as a Se/Fe powdered metal bolus one month before lambing. Hill, Walker and Taylor (1969) described a significant Cu/Se supplementation interaction resulting in an increased proportion of twins born where inadequacies, described as marginal, of both elements occurred. Scales (1974) reported that in three of four trials where Merino ewes were given 5mg Se as sodium selenate orally 17 days before mating, reduced the proportion of barren ewes by 17%.

The biological role of Cu is exerted through a number of cu-containing proteins including ceruloplasmin and superoxide dismutase (Prohaska, J.R. and Lukasewycz, 1990). When Cu is inadequate in animals, physiological and metabolic functions related to the Cu-enzymes may be impaired and, during clinical deficiency, symptoms will appear.

Cobalt (Co) insufficiencies, by its harmful effects on appetite and body condition score, results in a decline in both oestrus activity and multiple ovulation. In an experimental situation where Co inadequacies were described as marginal because of an absence of clinical symptoms, Fisher and MacPherson (1991) reported that dietary Co supplementation from 16 days before mating resulted in fewer lamb losses due to abortion and probable reabsorption between scanning in mid-pregnancy and birth and significantly fewer neonatal lamb deaths.

Manganese (Mn) is involved in the activities of several enzyme systems including hydrolases, kinases, decarboxylases and transferases as well as iron-containing enzymes which requires Mn for their activity. It is therefore involved in carbohydrate, lipid and

protein metabolism. Mn also plays a role in reproduction. Egan (1972) reported significant increases in lambing percentage when ewes consuming herbage of marginal manganese (45mg Mn/kg dry matter) and zinc (Zn) (20mg Zn/kg dry matter) status were supplemented 45mg Mn and 20mg Zn orally daily. Mn deficiencies in cows results in suppression of conception rates, delayed oestrus in post-partum females and young prepuberal heifers, infertility, abortion, immature ovaries and dystocia (Brown and Casillas, 1986; Maas, 1987; Corah and Ives, 1991).

One of the functions of calcium (Ca) is to facilitate muscle contractions. A reduction in muscle contractility will lead to a decrease in dry matter intake as rumen function decreases, leading to a severe negative energy balance. As a consequence, there is an increase in fat mobilization that may result in fatty liver syndrome and ketosis. An excess of ketone bodies can further suppress appetite (Grummer, 1996). Low Ca concentrations also prevent insulin production, further worsening this situation (Goff, 1999). Ultimately, fertility will suffer and the animals may experience prolonged calvings and retained placenta.

With Iodine (I) deficiencies, a lack of libido and deterioration of semen quality may occur in the adult male but claims of irregular oestrus and a lowered conception rate in females have not been well substantiated (De Groot and Stanbury, 1975).

Vitamin A plays the most important role in reproduction. A deficiency of Vit A may lead to infertility, abortion, a short gestation period, retained placenta and the production of dead/weak or blind young. Vit A deficiency is more common in cattle than sheep (Bearden *et al.*, 2000)

2.17 Period for nutritional treatment

The effective period for treatments was reduced for the ewe, initially by Oldham *et al.,* (1984) and then Steward *et al.,* (1986), who showed that feeding lupins over the last four days of the luteal phase, four to five days before ovulation, is sufficient to evoke the full ovulatory response. Under these circumstances, nutrition can only be affecting the final stages of folliculogenesis. The timing of all the treatments in these studies overlaps only during the final stages of follicle development.

CHAPTER 3

MATERIALS AND METHODS

3.1 TRIAL 1

Hypothesis: Season, urea supplementation, age and birth status influence the reproduction rate of ewes.

3.1.1 Animals

144 Döhne Merino ewes ranging in age from one to seven years, were used. The ewes were allocated randomly to one of two dietary protein treatment groups and then divided into six laparoscopy groups. The ewes were weighed before and after the trial and after lambing. The lambs were also weighed. Birth- and health status of each ewe were also determined before the onset of the trail.

Experimental animals were obtained from the University of Pretoria's Experimental Farm. The Animal Use and Care Committee of the University of Pretoria approved this research project on sheep.

3.1.2 Experimental design

This trail was conducted in and out of the normal breeding season. Season 1 was in the normal breeding season, fall, and season 2 was during seasonal anoestrus, spring. Ovulation points were determined by laparoscopy and the ewes were mated. The number of lambs born per ewe mated was determined and also taken into consideration.

3.1.3 Laparoscopy

Before laparoscopy the animals were deprived of feed and water for at least 12 hours and the wool around the udder and lower belly were shorn. Each ewe was suspended on her sacral and lumbar region within a specially designed steel frame, in order to expose the abdomino-pelvic region for the laproscopy procedure. The trocar-cannula (7 mm) was inserted into the peritoneal cavity through the anaesthetized site. The trocar was removed and the telescope was inserted into the cannula. The peritoneal cavity was inflated with a small amount of Carbondioxide gas, at a pressure of about 200 psi for 3-4 seconds. The ovary was located through the telescope and the *corpora lutea* were counted. The telescope was removed and the gas was allowed to escape to deflate the abdominal cavity before the cannula was removed. An antibiotic spray was applied to the wounds and the animals were given an intramuscular dose of long-acting penicillin. The above-mentioned procedure was used for

both ovaries (Evans and Maxwell, 1986). Prof. J. Terblanche, BVSc. MMed Vet, performed the procedures.

3.1.4 Live mass

The live mass of the ewes was determined on day 0 of the trial as well as after treatment and lambing. The lambs' weights were determined at lambing.

3.1.5 Diet composition and Feeding

The animals were fed *Fescue* grass *ad libitum* and supplemented 250g/ewe/day of the respective treatments. The contents of the two different dietary protein treatments are summarised in Table 8.

Table 8 Contents of the different treatments used in the trial

Treatment 1 (Urea)		**Treatment 2 (Mix protein diet)**	
Components	**Inclusion (%)**	**Components**	**Inclusion (%)**
Yellow Maize	82.4	Yellow Maize	63
Molasses Syrup	12	Molasses Syrup	8
Urea	5.6	Cottonseed Oilcake	23
		Fishmeal	6

The diets were analysed for dry matter (DM), crude protein (CP) and nitrogen content (N) as well as the digestible crude protein content (DCP) described by the AOAC (1985) and Van Soest (1963). Analyses were done at the University of Pretoria Nutrilab, Department of Animal- and Wildlife Sciences. Those analyses are summarised in Table 9.

Table 9 The digestible crude protein content of the maize and the different nitrogen sources

	Sample		**Sample (DM Basis) CP (g/kg)**	**Digestible CP Content (g/kg)**
	N (g/kg)	**CP (g/kg)**		
Yellow Maize	14.22	88.90	101.01	76.253
Urea			2875.00	
Cottonseed Oilcake	57.71	360.69	385.82	308.994
Fishmeal	102.73	642.09	701.35	679.618

Thus the DCP of the urea treatment was 55.708 g/kg and 55.723 g/kg for the mix protein treatment. The assumption was made that the ewes ingested 1500g DM of the *Fescue* grass that had a digestibility of 60 % when the supplement were formulated. The true daily

dry matter, crude protein and digestible crude protein (DCP) intake per ewe was calculated and is summarised in Table 10.

Table 10 The ewe's daily dry matter, crude protein and digestible crude protein intake

TREATMENT	DM (kg/day)	CP (g/day)	DCP (g/day)
Urea	1.12	208.7	161.4
Mix protein diet	1.13	210.3	164.5

3.1.6 Statistical analysis for Trial 1

The effects of age of the ewe, birth status of the ewe, season, treatment, laparoscopy sessions and their interactions were analysed by a repeated measures analysis of variance (ANOVA) of SAS (SAS. 2004). The breeding mass was used as a covariant. Differences between regressions obtained for the different treatment groups were tested for significance by comparing the regression coefficients of the linear regression ($y = a + bc$), using the standard error (SE) of the difference. The difference between regression coefficients was compared against the SE (Significant at 5% level of probability). The least-square means were also calculated and tested with the Proc GLM procedures of SAS (SAS, 2004).

3.2 TRIAL 2

Hypothesis: Protein supplementation influences the reproductive rate of ewes.

3.2.1 Animals

75 Döhne Merino ranging in age from two to six years, were used. The ewes were allocated randomly to one of four treatment groups and then divided into six laparoscopy groups. The division into the laparoscopy groups simplified the managing and handling of the animals. The ewes were weighed. Birth- and health status of each ewe were also determined before the onset of trial 2.

Experimental animals were obtained from the University of Pretoria's Experimental Farm. The Animal Use and Care Committee of the University of Pretoria approved this research project on sheep.

3.2.2 Experimental design

Day 0 of the trial was after a 7-day adaptation period when ewes were fed the basal diet (*Fescue* grass). On day 0, the ewes were synchronised by inserting a progestin sponge ("Ovakron tampons": DNAfrica Anipharm Pty.Ltd., Pretoria, South Africa, containing 40 mg.

flugestone acetate) into the vagina and the live mass of the ewes was determined. On day 9, the ewes were injected with prostaglandin F2α (PGF2α)("Lutalyse", Pharmacia Animal Health – a division of Pfizer Laboratories, Craighall, Johannsesburg, South Africa). The sponges were removed on day 11. Day 13 was the first day of the oestrus cycle. Treatment commenced on day 13. On day 25, the ewes were synchronised again. The ewes were injected with PGF2α on day 37 and the sponges were removed on day 39. The ewes were mated on day 41 and laparoscopies were done on day 51. The experimental design is presented in Table 11.

Table 11 Experimental design

DAY	D.O.W.	LAP 1	LAP 2	LAP 3	LAP 4	LAP 5	LAP 6
0	Mon	SP IN					
1	Tue		SP IN				
2	Wed			SP IN			
3	Thu				SP IN		
4	Fri						
5	Sat					SP IN	
6	Sun						SP IN
7	Mon						
8	Tue						
9	Wed	PGF2					
10	Thu		PGF2				
11	Fri	SP OUT		PGF2			
12	Sat		SP OUT		PGF2		
13	Sun	HEAT, T		SP OUT			
14	Mon	HEAT ,T	HEAT, T		SP OUT	PGF2	
15	Tue	HEAT, T	HEAT, T	HEAT, T			SP OUT
16	Wed	T	HEAT, T	HEAT, T	HEAT, T	SP OUT	
17	Thu	T	T	HEAT, T	HEAT, T		SP OUT
18	Fri	T	T	T	HEAT, T	HEAT,T	
19	Sat	T	T	T	T	HEAT, T	HEAT, T
20	Sun	T	T	T	T	HEAT, T	HEAT, T
21	Mon	T	T	T	T	T	HEAT, T
22	Tue	T	T	T	T	T	T
23	Wed	T	T	T	T	T	T
24	Thu	T	T	T	T	T	T
25	Fri	SP IN, T	T	T	T	T	T
26	Sat	T	SP IN, T	T	T	T	T

Table 11 Experimental design

27	Sun	T	T	SP IN, T	T	T	T
28	Mon	T	T	T	SP IN, T	T	T
29	Tue	T	T	T	T	T	T
30	Wed	T	T	T	T	SP IN, T	T
31	Thu	T	T	T	T	T	SP IN, T
32	Fri	T	T	T	T	T	T
33	Sat	T	T	T	T	T	T
34	Sun	T	T	T	T	T	T
35	Mon	T	T	T	T	T	T
36	Tue	T	T	T	T	T	T
37	Wed	PGF2, T	T	T	T	T	T
38	Thu	T	PGF2, T	T	T	T	T
39	Fri	SP OUT, T	T	PGF2, T	T	T	T
40	Sat	T	SP OUT, T	T	PGF2,T	T	T
41	Sun	HEAT,T,M	T	SP OUT, T	T	T	T
42	Mon	HEAT, M	HEAT,T,M	T	SP OUT, T	PGF2, T	T
43	Tue	HEAT, M	HEAT, M	HEAT,T,M	T	T	PGF2, T
44	Wed		HEAT, M	HEAT, M	HEAT,T,M	SP OUT, T	T
45	Thu			HEAT, M	HEAT, M	T	SP OUT,T
46	Fri				HEAT, M	HEAT,T,M	T
47	Sat					HEAT, M	HEAT,T,M
48	Sun					HEAT,M	HEAT,M
49	Mon						HEAT, M
50	Tue						
51	Wed	LAP 1					
52	Thu		LAP2				
53	Fri			LAP 3			
54	Sat				LAP 4		
55	Sun						
56	Mon					LAP 5	
57	Tue						LAP 6

D.O.W – Day of the week
Lap – Laparoscopy groups
Sp – Progestin sponge
T – Treatment
M – Mated
PGF2 - Prostaglandin F2α injection

3.2.3 Laparoscopy

Before laparoscopy the animals were deprived of feed and water for at least 12 hours and the wool around the udder and lower belly were shorn. Each ewe was suspended on her sacral and lumbar region within a specially designed steel frame, in order to expose the

abdomino-pelvic region fro the laparoscopy procedure. The trocar-cannula (7 mm) was inserted into the peritoneal cavity through the anaesthetized site. The trocar was removed and the telescope was inserted into the cannula. The peritoneal cavity was inflated with a small amount of Carbondioxide gas, at a pressure of about 200 psi for 3-4 seconds. The ovary was located through the telescope and the *corpora lutea* were counted. The telescope was removed and the gas was allowed to escape to deflate the abdominal cavity before the cannula was removed. An antibiotic spray was applied to the wounds and the animals were given an intramuscular dose of long-acting penicillin. The above-mentioned procedure was used for both ovaries (Evans *et al.*, 1986). Prof, J. Terblanche, BVSc. MMed Vet, performed the laparoscopy.

3.2.4 Diet composition and Feeding

Fresh water was available *ad libitum.* During the 7-day adaptation period, the control diet was fed to the animals. The control diet consisted of *Fescue* grass. Treatment started on day 13. Treatment group 1 was fed *Fescue* grass and raw lupins (200g/d/ewe). Treatment group 2 was fed *Fescue* grass and cooked lupins (200g/d/ewe). These lupins were heat treated at 130°C for six hours. Treatment group 3 was fed only *Fescue* grass. Treatment group 4 was fed *Fescue* grass and cottonseed oil-cake (200g/d/ewe).

Feed samples were collected weekly and sealed in polyethylene bags and stored for analysis. The DCP of all treatment groups are similar.

The diets were analysed for dry matter (DM), organic matter (OM), crude protein (CP) and the mineral content as described by the AOAC (1985) and Van Soest (1963). Analyses were done at the University of Pretoria Nutrilab, Department of Animal- and Wildlife Sciences. The analyses of the lupin supplementation and the grass are summarised in Table 12 and Table 13 respectively.

Table 12 Analyses of lupins

As Is Basis					
%DM	**%Moisture**	**%Ash**	**Se(ng/g)**	**Mn(mg/kg)**	**Mg(g/kg)**
92.44	7.56	3.61	153	632	1.99
DM Basis					
%DM	**%Moisture**	**%Ash**	**Se(ng/g)**	**Mn(mg/kg)**	**Mg(g/kg)**
100	0	3.91	166	684	2.15

Table 13 Analyses of grass sample of the control diet

As Is Basis					
%DM	**%Moisture**	**%CP**	**%Ash**	**Mn(mg/kg)**	**Mg(g/kg)**
94.53	5.47	17.51	11.47	41	3.9
DM Basis					
%DM	**%Moisture**	**%CP**	**%Ash**	**Mn(mg/kg)**	**Mg(g/kg)**
100	0	18.53	12.13	43	4.1

3.2.5 Statistical analysis for Trial 2

The effects of breeding mass, age of the ewe, birth status of the ewe, treatment and their interactions were analysed by a repeated measures analysis of variance (ANOVA) of SAS (SAS, 2004). The breeding mass was used as a covariant. Differences between regressions obtained for the different treatment groups were tested for significance by comparing the regression coefficients of the linear regression ($y = a + bc$), using the standard error (SE) of the difference. The difference between regression coefficients was compared against the SE (Significant at 5% level of probability). The least-square means were also calculated and tested with the Proc GLM procedures of SAS (SAS, 2004).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 TRIAL 1

In this trial the weight, age and birth status of ewes were recorded before the onset of the trial. Ewes were synchronised, mated and 10 days after mating the number of *corpora lutea* were determined by means of laparoscopy and 55 days after mating, pregnancy (number of foetuses observed per ewe) was diagnosed by means of an ultra-sound scanner and both were recorded. The lambing status (number of lambs born per ewe) and the mass of the ewes after lambing were also recorded. The total number of observations available for the number of *corpora lutea*, pregnancy diagnosis, lambing status and ewe mass after lambing were 139, 140, 135 and 135 respectively before editing. Records with missing values were removed. The mean, minimum and maximum values for these variables, number of observations (n) used, coefficient of variation, standard deviation and standard error are summarised in Table 14.

Table 14 Description of data set

	Corpora lutea	Pregnancy diagnosis	Lambing status	Ewe mass (kg) after lambing
Mean	1.472	1.292	1.389	52.384
Number of observation	72	72	72	69
Standard Deviation	0.556	0.542	0.571	6.377
Coeff of Variation	37.762	41.979	41.078	12.173
Minimum	1.000	0.000	0.000	37.000
Maximum	3.000	2.000	2.000	69.000
Standard Error	0.066	0.064	0.067	0.768

The significance level of the fixed effects and their interaction effects on the variables was also determined, as mentioned previously in the methodology, and summarised in Table 15.

Significant differences were observed for the number of *corpora lutea* between ewes with different birth statuses and treatment groups, and also in the mass of the ewe after lambing due to several fixed effects (ewe birth status, ewe age X treatment interaction, ewe age X laparoscopy interaction, season X treatment interaction).

Table 15 Significance levels of the fixed effects against the variables (number of *corpora lutea*, pregnancy diagnosis, lambing status and ewe mass after lambing)

Fixed effects	Variables			
	Corpora lutea	Pregnancy diagnosis	Lambing status	Ewe mass after lambing (kg)
Mating mass	NS	NS	NS	NS
Ewe age	NS	NS	NS	NS
Ewe birth status	NS	NS	NS	$P < 0.01$
Season	NS	NS	NS	NS
Treatment	NS	NS	NS	NS
Laparoscopy	NS	NS	NS	NS[a]
Ewe age * Treatment	NS	NS	NS	$P < 0.05$
Ewe age * Laparoscopy	NS	NS	NS	$P < 0.05$
Ewe birth status * Treatment	$P < 0.05$	NS	NS	NS
Ewe age * Season	NS	NS	NS	NS
Ewe birth status * Season	NS	NS	NS	NS
Season * Treatment	NS	NS	NS	$P < 0.05$
Ewe age * Ewe birth status * Treatment	NS	NS	NS	NS

[a] $P < 0.1$

The effects of treatment on the number of *corpora lutea*, number of foetuses observed (pregnancy diagnosis), lambing status and mass of the ewe after lambing, are summarised in Table 16.

Table 16 Effects of dietary protien treatment on the different traits (number of *corpora lutea*, pregnancy diagnosis, lambing status and ewe mass after lambing)(least square means ± standard error).

Treatment	Traits			
	Corpora lutea	Pregnancy diagnosis	Lambing status	Ewe mass after lambing (kg)
Urea	1.517 ± 0.115	1.340 ± 0.107	1.407 ± 0.101	51.728 ± 0.613
Mix	1.497 ± 0.114	1.297 ± 0.106	1.435 ± 0.100	52.925 ± 0.602

As can be seen from Table 16, there were no significant differences between treatments in the different traits. When comparing the lambing status (1.407 ± 0.101; 1.435 ± 0.100) to the number of *corpora lutea* (1.517 ± 0.115; 1.497 ± 0.114) of the urea and mix protein treatment respectively, a negative effect of the urea, although small and not significant, on

embryo development could be observed. The lambing status was lower than the number of *corpora lutea* in the group fed the mix diet, but the difference was smaller compared to the urea treatment group, which suggests that urea had a negative effect on the embryo. Gath *et al.*, (1999) and Fahey *et al.,* (2000), attributed this to alterations in the oviduct environment or deleterious changes in the follicle, rather than changes in the uterine environment. Other researchers confirmed this relationship between high levels of ammonia and poor embryo development *in vitro* (Gardner *et al.*, 1993). The low number of foetuses observed compared to the lambing status, in both treatments, might be ascribed to human error during diagnoses particularly since the first pregnancy diagnosis was performed at a relatively early stage. Figure 5 reflects these non-significant numerical differences between the number of *corpora lutea* and lambing status of the ewes, in the different treatments.

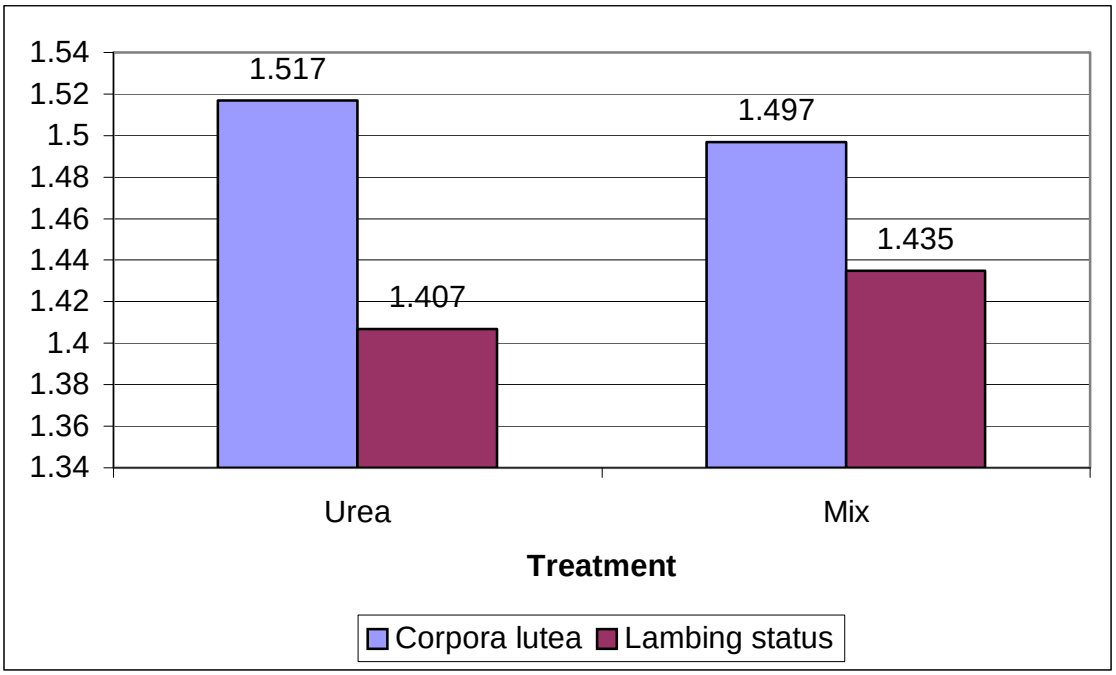


Figure 5 A graphic representation of the comparison between the number of *corpora lutea* and the lambing status (number of lambs born per ewe) of the two respective treatments.

This difference between the number of *corpora lutea* and lambing status could also be due to the rate of rumen nitrogen release, which could have influenced the proportions of zygotes produced (Sinclair, *et al.,* 2000). This suggests that oocyte competency could be undermined by nutritional regimes that lead to high circulating levels of ammonia in plasma.

Table 17 summarises the age of ewe's effect on the different traits. Pregnancy diagnosis of the 2-year-old ewes (1.132 ± 0.143) was significantly lower than that of the 3-year-old ewes (1.682 ± 0.196) and lambing status of the 1-year-old (0.993 ± 0.316) and 2-year-old (1.233 ± 0.134) ewes were significantly lower than that of the 6-year-old ewes (1.897 ±

0.248). As can be seen from Table 17, there was an increase in lambing status, as the age of the ewes increased and at 7 years of age, a decline in lambing status. These fluctuations in lambing status with ewe age are presented in Figure 6. This was the expected result compared to the literature. Glimp (1971) reported that prolificacy in several breeds was maximized at 5 years and that 2-year-old ewes produced 0.19 fewer lambs than 3- to 6 year old ewes. Notter (2000) found that prolificacy differed ($P<0.001$) among ewe age groups in all breeds. Peak prolificacy was generally achieved between 4 and 8 years of age. Exceptions to this generalization included a somewhat sharper peak in prolificacy for the Targhee. Prolificacy of 5- and 6 year old Targhee ewes averaged 0.06 higher than the prolificacy of 4- or 7 year old ewes. Prolificacy did not exhibit consistent declines until 7 years of age.

Table 17 The effect of the age of the ewe on the different traits (number of *corpora lutea*, pregnancy diagnosis, lambing status and ewe mass after lambing)(least square means ± standard error)

Age of the ewe (years)	Traits			
	Corpora lutea	Pregnancy diagnosis	Lambing status	Ewe mass after lambing (kg)
1	1.168 ± 0.362	0.912 ± 0.336	0.993 ± 0.316[a]	53.857 ± 1.904
2	1.541 ± 0.154	1.132 ± 0.143[a]	1.233 ± 0.134[b]	53.185 ± 0.861
3	1.330 ± 0.211	1.682 ± 0.196[a]	1.675 ± 0.184[c]	53.021 ± 1.125
4	1.723 ± 0.255	1.359 ± 0.237	1.688 ± 0.222[d]	51.515 ± 1.346
5	1.617 ± 0.226	1.320 ± 0.210	1.536 ± 0.197	51.695 ± 1.188
6	1.448 ± 0.254	1.535 ± 0.264	1.897 ± 0.248[abe]	51.145 ± 1.496
7	1.723 ± 0.350	1.230 ± 0.325	0.923 ± 0.305[cde]	51.868 ± 1.840

[abcdef] Values with the same superscript differ significantly ($P < 0.05$) within a trait

Figure 6 A representation of the lambing status of ewes at certain ages

The Targhee is a composite breed and is generally viewed as a dual-purpose breed for wool and meat production, which is similar to the Döhne Merino used in this trial. Dickerson *et al.,* (1975) evaluated ewe age effects on prolificacy in seven US breeds and obtained similar results to those mentioned above. Across all breeds, prolificacy was a maximum at 5.9 years of age. In this study the prolificacy was a maximum at 6 years of age, which coincides with the literature.

The lambing status of the single born ewes (1.227 ± 0.104) was significantly lower than that of the twin born ewes (1.615 ± 0.106)(Table 18). The twin born ewes produced 14% more lambs than the single born ewes in this trial. This probably was due to an increase in the number of eggs shed (ovulation rate) (Pacham and Triffitt, 1966).

Turner (1966) used twin- and single born ewes and found that selection for twin-bearing ability was effective because a 3% gain per year was obtained, that represented a gain in lambs born as a percentage of ewes mated, by the twin born ewes. In another study, Olivier, Snyman, van Wyk and Erasmus (1998) used 6,237 records collected over 19 years, to estimate the heritability of the number of lambs born per ewe in Merino's. They determined the estimated heritability as 0.31, thus multiparious births from twin born ewes are highly heritable.

Table 18 Effect of birth status of the ewe on the different traits (number of *corpora lutea*, pregnancy diagnosis, lambing status and ewe mass after lambing)(least square means ± standard error)

Birth status of the ewe	Traits			
	Corpora lutea	Pregnancy diagnosis	Lambing status	Ewe mass after lambing (kg)
Single	1.452 ± 0.119	1.191 ± 0.110	1.227 ± 0.104[a]	52.324 ± 0.638
Twin	1.562 ± 0.122	1.447 ± 0.113	1.615 ± 0.106[a]	52.330 ± 0.646

[a] Values with the same superscript differ significantly ($P < 0.05$) within a trait

As can be seen from Table 19, there was a difference ($P < 0.0001$) between the ewe mass after lambing between the two breeding seasons.

The mass of the autumn breeding group after lambing (48.785 ± 0.625) was significantly lower than that of the spring breeding group (55.868 ± 0.669). These differences could be attributed to the seasonal environmental changes (Lincoln and Short, 1980).

Table 19 Effect of season on the different traits (number of *corpora lutea*, pregnancy diagnosis, lambing status and ewe mass after lambing)(least square means ± standard error)

Season	Traits			
	Corpora lutea	Pregnancy diagnosis	Lambing status	Ewe mass after lambing (kg)
Autumn	1.461 ± 0.117	1.401 ± 0.109	1.431 ± 0.102	48.785 ± 0.625[a]
Spring	1.554 ± 0.122	1.236 ± 0.113	1.411 ± 0.106	55.868 ± 0.669[a]

[a] Values with the same subscript differ significantly ($P < 0.0001$) within a trait

As these changes reached acute levels, the ewes responded by developing a reproductive mechanism involving a 'natural contraceptive method' which restricted the reproductive activity to the best time of the year for assuring that lambings occur at a time that promotes maximal growth and development of the offspring and supports lactation in the mother (Wayne, Malpaux and Karsch, 1989). The autumn breeding group not only had to maintain pregnancy but also generate body heat during the winter, thus having a much higher energy need to fulfil than the spring breeding group. The autumn breeding group's condition 'deteriorated' compared to that of the spring breeding group, due to the energy needed that could not be supplied from the diets and thus the ewes had to mobilize their own body reserves (Frandson *et al*., 1992). Cited studies were done in the Northern hemisphere but can be linked to this study due to the similar results that were obtained.

There were no significant differences between the treatments and age of the ewe interactions effect on the number of *corpora lutea* observed (Table 20).

Table 20 The interaction between the treatments and age of the ewe effects on the number of *corpora lutea* (least-square means ± standard error)

Age of ewe (years)	Treatments	
	Urea (nr of CL's)	Mix(nr of CL's)
1	1.084 ± 0.388	1.252 ± 0.417
2	1.623 ± 0.202	1.459 ± 0.217
3	1.330 ± 0.273	1.329 ± 0.269
4	1.657 ± 0.294	1.790 ± 0.355
5	1.599 ± 0.319	1.635 ± 0.275
6	1.602 ± 0.375	1.294 ± 0.373
7	1.728 ± 0.465	1.719 ± 0.451

CL – *corpora lutea*

Table 21 summarises the treatments and age of ewes' interaction effects on the pregnancy diagnosis. The number of foetuses observed in the 3-year-old, mix diet treatment group (1.763 ± 0.250) was higher ($P < 0.05$) than the 2-year-old urea treatment (1.014 ± 0.188) group. This was expected as urea has a negative effect on embryo development (Fahey, *et al.,* 2000; Gath, *et al.,* 1999; Gardner *et al.*, 1993), and 3-year-old ewes had a higher prolificacy than 2-year-old ewes (Glimp, 1971). The concern of human error during and/or because of early scanning is raised again when one compare the pregnancy diagnosis of the 3 year old ewes fed the mix diet with that of the lambing status (Table 22) of the same group (0.755 ± 0.235).

Table 21 The interaction between the treatments and age of the ewe effects on the pregnancy diagnosis (least-square means ± standard error)

Age of ewe (years)	Treatments	
	Urea (nr of foetuses)	Mix (nr of foetuses)
1	1.154 ± 0.360	0.790 ± 0.387
2	1.014 ± 0.188[a]	1.250 ± 0.201
3	1.601 ± 0.254	1.763 ± 0.250[a]
4	1.430 ± 0.273	1.288 ± 0.329
5	1.283 ± 0.296	1.358 ± 0.255
6	1.405 ± 0.349	1.665 ± 0.346
7	1.494 ± 0.432	0.967 ± 0.419

[a] Values with the same superscript differ significantly ($P < 0.05$)

Table 22 summarises the treatments and age of ewes' interaction effects on the lambing status. The lambing status of the 2-year-old ewes (1.056 ± 0.177) was significantly lower than that of the 4 year old (1.733 ± 0.257) and 6 year old (1.912 ± 0.328) ewes within the urea treatment group. This difference is probably due to the age effect and is supported in the literature (Notter, 2000; Dickerson *et al.,* 1975; Glimp, 1971).

Table 22 The interaction between the treatments and age of the ewe effects on the lambing status (least-square means ± standard error)

Age of ewe (years)	Treatments	
	Urea (nr of lambs)	Mix (nr of lambs)
1	1.026 ± 0.339	0.967 ± 0.364
2	1.056 ± 0.177 [abcd]	1.409 ± 0.189
3	1.595 ± 0.238	0.755 ± 0.235 [a]
4	1.733 ± 0.257 [b]	1.643 ± 0.310
5	1.584 ± 0.279	1.488 ± 0.240
6	1.912 ± 0.328 [c]	1.881 ± 0.326 [d]
7	0.941 ± 0.406	0.905 ± 0.394

[abcd] Values with the same superscript differ significantly ($P < 0.05$)

The lambing status (Table 22) of the 2-year-old ewes, fed the urea treatment (1.056 ± 0.177) was significantly lower than that of the 6-year-old ewes, fed the mix diet treatment (1.881 ± 0.326). This was expected and could be attributed to the age effect mentioned

previously (Notter, 2000; Dickerson *et al.,* 1975; Glimp, 1971) and/ or the negative effect urea has on embryo development (Fahey, *et al.,* 2000; Gath, *et al.,* 1999; Gardner *et al.*, 1993).

Table 23 summarises the treatments and age of ewes' interaction effects on the ewe mass after lambing.

The only significant difference was that between the 2-year-old (54.353 ± 1.196) and 5-year-old (50.241 ± 1.673) ewes within the urea treatment group.

Table 23 The interaction between the treatments and age of the ewe effects on the ewe mass (kg) after lambing (least-square means ± standard error)

Age of ewe (years)	Treatments	
	Urea (ewe mass in kg)	Mix (ewe mass in kg)
1	52.315 ± 2.037	55.400 ± 2.194
2	54.353 ± 1.196[a]	52.018 ± 1.187
3	53.603 ± 1.446	52.439 ± 1.426
4	51.121 ± 1.549	51.908 ± 1.870
5	50.241 ± 1.673[a]	53.175 ± 1.444
6	50.277 ± 1.978	52.013 ± 1.957
7	50.212 ± 2.443	53.525 ± 2.371

[a] Values with the same superscript differ significantly ($P < 0.05$)

Observations in both treatment groups suggest a general decrease in mass as the ewes age (Table 23)

Table 24 summarises the treatments and birth status of ewes' interaction effects on the different traits (number of *corpora lutea*, pregnancy diagnosis, lambing status and ewe mass after lambing). The lambing status of the single born ewes (1.179 ± 0.131) was lower ($P < 0.0001$) than that of the twin born ewes (1.614 ± 0.139) and the number of *corpora lutea* of the single born ewes (1.274 ± 0.138) was lower ($P < 0.0001$) than that of the twin born ewes (1.782 ± 0.147), within the urea treatment. This strongly supports the suggestion that twin born ewes have a higher lambing status, which is also confirmed in the literature (Turner 1966; Pacham *et al.*, 1966; Olivier, *et al.,* 1998). The lambing status of the single born ewes within the urea treatment (1.179 ± 0.131) was lower ($P < 0.0001$) than that of the twin born ewes (1.629 ± 0.132) fed the mix diet treatment. This was expected and could be attributable to the ewe birth status effect, mentioned previously as well as the negative effect urea has on embryo development (Fahey, *et al.,* 2000; Gath, *et al.,* 1999; Gardner *et al.*, 1993).

The number of *corpora lutea* of the urea treatment (1.782 ± 0.147) was higher ($P < 0.0001$) than that of the mix diet treatment (1.375 ± 0.140) within the twin born ewes (Table 24). This difference could only be due to the treatment effect. As already indicated in the literature, urea has no negative effect on ovulation rate (Fahey, *et al.,* 2000). This difference could be a nitrogen-linked effect. Rumen microbes metabolized the available nitrogen to

amino acids that then, in turn, could've been either utilised as is or converted to energy substrates, and then been utilised for ova development (Frandson *et al.*, 1992).

Table 24 The interactions between the treatments and birth status of the ewe effects on the different traits (number of *corpora lutea*, pregnancy diagnosis, lambing status and ewe mass after lambing)(least-square means ± standard error)

Birth status * Treatment interaction	Traits			
	Corpora lutea	Pregnancy diagnosis	Lambing status	Ewe mass after lambing (kg)
Single, Urea	1.274 ± 0.138[a]	1.277 ± 0.140	1.179 ± 0.131[ab]	51.321 ± 0.833
Single, Mix	1.590 ± 0.134	1.152 ± 0.135	1.313 ± 0.126	52.970 ± 0.812
Twins, Urea	1.782 ± 0.147[ab]	1.360 ± 0.148	1.614 ± 0.139[a]	52.544 ± 0.887
Twins, Mix	1.375 ± 0.140[b]	1.533 ± 0.141	1.629 ± 0.132[b]	52.104 ± 0.829

[ab] Values with the same superscript differ significantly ($P < 0.0001$) within a trait

The interactions between season and treatment effects on the different traits are summarised in Table 25. Significant differences were only found between the interactions' effect and mass of the ewes after lambing. Figure 7 illustrates these differences.

Table 25 The interactions between the seasons and treatments effect on the different traits (least-square means ± standard error)

Season * Treatment interaction	Traits			
	Corpora lutea	Pregnancy diagnosis	Lambing status	Ewe mass after lambing (kg)
Autumn, Urea	1.471 ± 0.143	1.452 ± 0.136	1.424 ± 0.126	47.878 ± 0.791[ab]
Autumn, Mix	1.403 ± 0.149	1.387 ± 0.141	1.464 ± 0.131	49.676 ± 0.825[cd]
Spring, Urea	1.539 ± 0.151	1.199 ± 0.143	1.359 ± 0.133	55.980 ± 0.871[ac]
Spring, Mix	1.571 ± 0.152	1.293 ± 0.145	1.478 ± 0.134	55.383 ± 0.859[bd]

[abcd] Values with the same superscript differ significantly ($P < 0.05$) within a trait

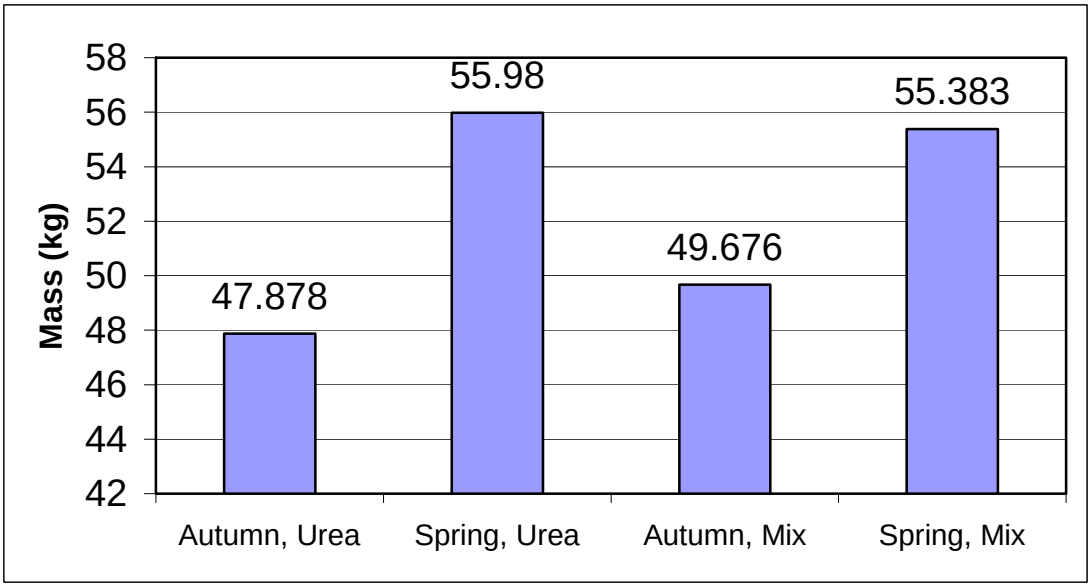


Figure 7 A graphic representation of the interaction between the season and treatment's effect on the average mass of the ewe after lambing

The autumn breeding groups fed the urea (47.878 ± 0.791) and mix (49.676 ± 0.825) diets, mass after lambing were significantly lower than the spring breeding groups fed the same, urea (55.980 ± 0.871) and mix (55.383 ± 0.859), diets. These differences could be attributed to the seasonal environmental changes and availability of optimum feed quality and quantity. This confirms what was previously suggested (Table 19).

Although there was no significant difference between the interaction of the season and treatment's effect on the pregnancy diagnosis, there was a numerical difference that suggest a tendency that autumn breeding season produced a higher number of lambs than the spring breeding season, as can be seen in Figure 8. The role of day length as the determining factor in inducing and suppressing sexual activity in the ewe has been clearly demonstrated in a number of experiments (Yates, 1949; Thwaites, 1965; Wodzicka-Tomaszewska, Hutchinson and Bennett, 1967; Reiter, 1974) and could be one of the mechanisms at work here.

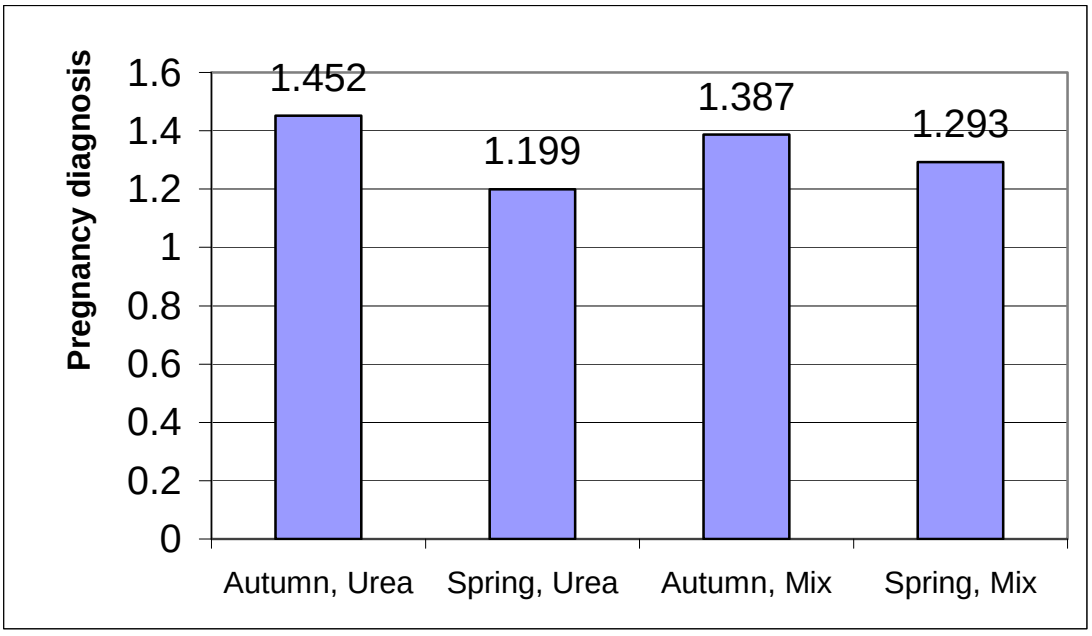


Figure 8 A graphic representation of the interaction between the season and treatment's effect on the pregnancy diagnosis (number of foetuses observed per ewe during scanning).

4.2 TRIAL 2

In this trial the weight, age and birth status of ewes were recorded before the onset of the trial. Ewes were synchronized, mated and 10 days after mating the number of *corpora lutea* were determined by means of laparoscopy and recorded. The total number of observations available before editing was 75. Records with missing values were removed. The mean, minimum and maximum number of *corpora lutea,* number of observations (n)

used, coefficient of variation, standard deviation and standard error are summarised in Table 26.

Table 26 Description of the data set

	Mean	n	Standard Deviation	Coeff of Variation	Minimum	Maximum	Standard Error
Corpora lutea	1.420	69	0.604	42.530	0.000	3.000	0.073

The significance level of the fixed effects and their interactions effect on the number of *corpora lutea* was also determined, as mentioned previously in the methodology, and summarised in Table 27.

Table 27 Significance of the fixed effects on the number of *corpora lutea*

	Fixed effects					
	Starting mass	Ewe age	Ewe birth status	Treatment	Ewe age * Treatment	Ewe birth status * Treatment
Corpora lutea	NS	NS	NS	NS	NS	NS [a]

[a] $P < 0.1$

From Table 27 it is evident that there were no significant differences between the different fixed effects and the total number of *corpora lutea* of all the treatment groups combined. There was a tendency towards a difference ($P < 0.1$) for the interaction effect of birth status of ewes and treatment effect on the total number of *corpora lutea*.

There were numerical differences between the different treatment groups (Table 28) but the difference was not significant. What is odd is the observation that, although not significant, the treatment group fed the basal diet had the highest number of *corpora lutea*.

In previous studies (Lindsay, 1979; Oldham *et al.*, 1984, Steward *et al.*, 1986) it was suggested that feeding a high protein diet increased the mean ovulation rate in the cycle, which didn't seem to be the case here.

Table 28 The treatments effect on the number of *corpora lutea* per ewe

Treatment	Number of *coporalutea* (LSM ± Standard error)
Raw lupins	1.440 ± 0.171
Cooked lupins	1.431 ± 0.145
Basal	1.597 ± 0.179
Cottonseed oil-cake	1.233 ± 0.178

LSM = least square means

The number of *corpora lutea* differs numerically between all the age groups and is summarised in Table 29. The only significant difference was between the ewes at three years of age (1.175 ± 0.173) and those at six years of age (1.804 ± 0.267). This is expected because it has already been suggested that, during many studies in the USA across all breeds, prolificacy was a maximum at 5.9 years of age and that 2-year-old ewes produced 0.19 fewer lambs than 3- to 6-year-old ewes (Dickerson *et al.*, 1975; Glimp 1971).

Table 29 The effect of the age of the ewe on the number of *corpora lutea* per ewe

Age of the ewe (years)	Number of *copora lutea* (LSM ± Standard error)
2	1.375 ± 0.147
3	1.175 ± 0.173[a]
4	1.283 ± 0.170
5	1.489 ± 0.178
6	1.804 ± 0.267[a]

[a] Values with the same superscript differ significantly ($P < 0.05$)
LSM = least square means

As seen in Table 30, there were no significant differences between the birth statuses and the number of *corpora lutea.* There was a numerical difference, showing that there was a higher number of *corpora lutea* in twin born ewes (1.493 ± 0.103) than in the single born ewes (1.358 ± 0.126). This has been found to be the norm and multiple births from twin born ewes are highly heritable (Olivier *et al.,* 1998). A possible explanation for not finding a significant difference could be because the duration of the trial wasn't long enough or too few animals were used for this difference to be expressed.

Table 30 The effect of the birth status of the ewe on the number of *corpora lutea* per ewe

Birth status of the ewe	Number of *corpora lutea* (LSM ± Standard error)
Single	1.358 ± 0.126
Twin	1.493 ± 0.103

LSM = least square means

Table 31 summarises the treatments and age of the ewe interaction effects on the number of *corpora lutea*. The result that stands out immediately, is that of the 6-year-old ewes fed the basal diet (2.860 ± 0.579). Within Table 31, it is the best result found in terms of number of *corpora lutea*, and differs ($P < 0.05$) from various other groups. The differences ($P < 0.05$) between the 6-year-old group and the 2-year-old (1.456 ± 0.239), 3-year-old (0.938 ± 0.394), 4-year-old (1.460 ± 0.346) and 5-year-old (1.273 ± 0.323) ewe groups, respectively within the basal treatment group, is supportive of the evidence found in this trial and trial 1, that prolificacy peaks at 5.9 years of age (Dickerson *et al.*, 1975; Glimp 1971). This could also explain the difference ($P < 0.05$) between the 2-year-old ewes (1.072 ± 0.264) and 6-year-old ewes (2.080 ± 0.395), within the cooked lupins treatment group. The difference ($P <$

0.05) between the 6-year-old ewes fed the basal diet, and the other treatment groups could be attributable that the age effect was so strong it undermined the possible effect the treatments may have had on the number of *corpora lutea*, seeing that all significant differences were from younger age groups.

Table 31 The interaction between the treatments and age of the ewe effects on the number of *corpora lutea* per ewe (least-square means ± standard error)

Age of ewe (years)	Raw lupins (nr of CL's)	Cooked lupins (nr of CL's)	*Fescue* grass (nr of CL's)	Cottonseed oil-cake (nr of CL's)
2	1.831 ± 0.229^{ab}	1.072 ± 0.264^{ac}	1.456 ± 0.239^{d}	1.142 ± 0.276^{e}
3	1.536 ± 0.288^{f}	1.288 ± 0.288^{g}	0.938 ± 0.394^{h}	0.938 ± 0.394^{i}
4	1.553 ± 0.237^{j}	1.137 ± 0.277^{k}	1.460 ± 0.346^{l}	0.982 ± 0.330^{bm}
5	1.491 ± 0.418	1.580 ± 0.395	1.273 ± 0.323^{n}	1.612 ± 0.288
6	0.790 ± 0.586^{o}	2.080 ± 0.395 chim	2.860 ± 0.579 cdefghijklmno	1.488 ± 0.596

abcdefghijklmno Values with the same superscript differ significantly ($P < 0.05$)
CL – *corpora lutea*

Table 32 summarises the treatments and birth status interaction effects on the number of *corpora lutea.* The number of *corpora lutea* observed from the single born ewes (1.048 ± 0.216) was significantly lower than that from the twin born ewes (1.815 ± 0.184), within the cooked lupin treatment group. Because this difference falls within a treatment group, it could only be attributed to the birth status of the ewes. This evidence supports the statement made about Table 30, that twin born ewes have a higher reproduction rate than single born ewes.

Table 32 The interaction between the treatments and birth status of the ewe effects on the number of *corpora lutea* per ewe (least-square means ± standard error)

Treatments	Birth status of the ewe	
	Single (nr of CL's)	Twin (nr of CL's)
Raw lupins	1.418 ± 0.261	1.462 ± 0.181
Cooked lupins	1.048 ± 0.216^{a}	1.815 ± 0.184^{ab}
Basal	1.549 ± 0.233	1.646 ± 0.242^{c}
Cottonseed oil-cake	1.417 ± 0.267	1.048 ± 0.209^{bc}

abc Values with the same superscript differ significantly ($P < 0.05$)
CL – *corpora lutea*

The number of *corpora lutea* from the cooked lupins group (1.815 ± 0.184) was significantly higher than that from the cottonseed oil-cake (1.048 ± 0.209), within the twin born ewes group. This difference was attributable to the treatment.

Lupin is high in digestible protein and energy that may increase ewes' ovulation rate (Lightfoot *et al*, 1974; Knight *et al.*, 1975). Subjecting lupins to heat treatment (cooking) will only increase the Rumen Bypass Protein (RBP). Nottle *et al.,* (1997), Croker, *et al.,* (1985) and Downing, *et al.*(1995), suggested that feeding lupins increased the number of ovulations ($P < 0.05$). This increase was reflected in an improvement in fecundity (lambs born per ewe

lambing; $P < 0.05$). Supplementation with lupins increased ovulation rate by increasing the proportions of ewes with multiple ovulations. This increase was also similar to that reported in the past for commercial flocks in southern Australia fed lupins (Knight *et al.,* 1975; Kleeman and Cutten, 1978).

Brien, *et al.,* (1979) reported that ewes, that received lupin supplements, had higher mean plasma concentrations of FSH before oestrus that increases ovulation rate. It is suggested that an increase in ovulation rate after lupin grain supplementation involve increases in the secretion of FSH at a time in the oestrus cycle when exogenous FSH increases ovulation rate (McNatty, *et al.,* 1985), which could be a possible mechanism here but without the blood assays, it is not possible to confirm that.

Another possible mechanism is that lupin grain supplementation increases the concentration of the branched chain amino acids and Downing (1994) found that the infusion of the branched chain amino acids, increased ovulation rate in ewes . Therefore, higher plasma concentrations of the branched amino acids found following lupin supplementation may play a role in the increased ovulation rate.

Ovulation rate responses to a high protein supplementation are strongly related to the glucose entry rate into the cellular compartment from the extracellular compartment (Teleni *et al.*, 1989). The increase in ovulation rate following the amino acid infusion may be a metabolic response to an increased availability of energy substrates. It's highly probable that lupin supplemented ewes have a higher glucose availability and this may be the stimulus responsible for ovulation rate increases in response to high protein supplements. But, again, it is not possible to confirm in this trail because of the absence of blood assays.

The number of *corpora lutea* from the basal treatment group (1.646 ± 0.242) was significantly higher than that from the cottonseed oil-cake (1.048 ± 0.209), within the twin born ewes group. This result does not fall within expectancies because cottonseed oil-cake is a protein concentrate and thus has a higher CP and RBP content than the basal diet. Thus more protein, and ultimately energy, is available to the animal. This could be a coincidence.

CHAPTER 5

CONCLUSION AND CRITICAL EVALUATION

5.1 Conclusion

One objective of this study was to determine the possible effects of age, birth status and season on the reproductivity of ewes (measured as ovulation rate and rate of twinning).

Reproductivity of ewes was influenced by the age of the animal. Reproductivity of ewes was relatively low at one year of age, but gradually increases as the ewes aged. Maximum reproductivity ranged from ages three to six, and then decreased again from seven years of age.. As the ewes aged, with correct feeding, there was a decline in liveweight. This natural decline seemed to have no negative effect on the reproductivity of the ewes.

The birth status of the ewes had an influence on their lambing status. There was a higher incidence for twin born ewes to produce twins.

Season has also had an effect on the reproductivity of ewes. Day length was the main influence and triggered the ewes to adapt a 'natural contraceptive method' which restricted the reproductive activity to the best time of the year to assure that lambings occurred at a time that promotes maximal growth and development of the offspring and supports lactation in the mother.

The other objective was to determine whether the quality of protein in the diet affected reproductivity (ovulation rate and rate of twinning) of ewes.

Urea inclusion in the diet had no negative effect on ovulation rate. Ovulation rate increased with urea supplementation; a possible mechanism for this was the nitrogen-linked effect on the rumen microbes that increased the ovulation rate. The difficulty of urea usage was that it had a negative effect on zygote formation and embryo development and there was an indication that high circulating levels of ammonia in the plasma could have undermined oocyte competency. Thus urea was a good nitrogen source to increase ovulation rate but not to increase the number of lambs born.

This study showed that supplementation with 200g/day/ewe of cooked lupins increased ovulation rate by increasing the proportions of ewes with multiple ovulations, possibly due to the increased amount of RBP after heat treatment and the availability of amino acids as energy substrates or building blocks for the increased production of gonadotrophins.

This study also showed that supplementing the animals with 200g/day/ewe of raw lupins, had the same effect on ovulation rate as just feeding the *fescue* grass, and supplementing the animals with 200g of cottonseed oil-cake had no positive effect on ovulation rate.

The producer can use this information to construct a management plan and nutritional regimes to increase the reproductivity and productivity of his ewes, which ultimately lead to an increase in income.

5.2 Critical evaluation

5.2.1 Trial 1

A concern was raised about the validity of the pregnancy diagnoses values. It is the author's opinion that the values might not be true due to human error of too early scanning of the ewes. On the overall, this discrepancy does not affect the outcome of the trial because the objectives set could be met. In following trails, if pregnancy diagnosis is one of the measurables, caution should be applied not to schedule the scanning too early, when developing the experimental design and that only one person should do the diagnoses too eliminate variability due to operators' abilities.

In this trial the amount of urea, in the urea treatment group, was included on a maintenance basis. The negative effect of urea on embryo development could just be observed. For better results, if necessary, one could increase the urea inclusion in the treatment group or take blood samples to determine the circulating blood urea or ammonia content.

5.2.2 Trial 2

In this trail the effect of protein supplementation during the normal breeding season was investigated. This effect during the seasonal anoestrus can be further investigated by repeating the same trial during that season. The mechanisms of increased ovulation due to the protein treatments were unclear. These unclarities did not hamper the outcome of the trial for the objective of the effect of protein supplementation on the ovulation rate could be determined. A way to clarify these mechanisms is to take blood samples to determine the circulating gonadotrophins, branched amino acids, gluconeogenic amino acid contents and availability of glucose of the different treatment groups.

Raw lupins and cottonseed oil-cake had no effect on the ovulation rate at the inclusion rate of 200g/day/ewe. To further investigate the effect of these protein concentrates, one can increase the inclusion rate of each.

In this trial the effect of protein supplementation during the normal breeding season were investigated. This effect during the seasonal anoestrus can be further investigated by repeating the same trial during that season.

It is known that twin born ewes will have multiple ovulations. The difference in ovulation rate between twin born and single born ewes was demonstrated in this trial, but sometimes not very clearly (Table 30). Working with reproduction measurables, the method

to get the best results is if the trial is extended over a long period (5 – 10 years) and there is a larger number of animals thus, it is the author's suggestion that for better results the duration of the trial and/or the amount of experimental animals could have been increased.

5.2.3 General

There were no major problems during the study and it is the author's opinion that this study reached the objectives that were set. The hypotheses could be answered namely that season,protein supplementation, age and birth status had an effect on the reproduction rate of ewes.

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