A STUDY OF THE EFFECTS OF POST-MATING PROGESTERONE SUPPLEMENTATION ON THE REPRODUCTIVE PERFORMANCE IN THE EWE

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ABSTRACT

The aim of this study was to investigate the effects of post-mating progesterone supplementation, in the form of CIDRs, on the reproductive performance in recently mated ewes. The effect of two nutrition treatments were also examined by feeding two different pasture allowances to these ewes during a two week period immediately following a synchronized mating.

Two hundred and thirty four Border Leicester X Romney first cross ewes, comprised of 130 mixed-age ewes (3-8 years) and 104 two-tooth (maiden) ewes were flushed on increasing pasture allowances prior to joining. These ewes were naturally mated at a synchronized oestrus following a 13 day CIDR treatment period. During the three days following CIDR withdrawal, 88.0% of the ewes were mated. The mixed-age ewes came into oestrus significantly earlier than the two-tooth ewes ($P < 0.001$). There was a significant linear relationship between liveweight and onset of oestrus ($P < 0.05$), with the heavier two-tooth ewes coming on oestrus earlier than the lighter two-tooth ewes. This was not apparent in the mixed-age ewes.

On Day 2 following mating, ewes were randomly divided into either the high or low pasture allowance levels. Pasture allowance levels were monitored using an Ellinbank Pasture Meter (EPM). Levels of feed intake were estimated for a random sample of 20 ewes both before and after mating through the use of intraruminal chromium releasing devices (CRDs). Sward heights (representing quantity), botanical compositions and *in vitro* digestibilities (representing quality) were recorded for both the flushing and the post-mating period. This information led to the estimation of voluntary herbage intakes during the flushing period (Period I) of 1.2 M, while the intake levels of the ewes following mating (Period II) were calculated to be approximately 1.6 M and 1.0 M for the high and low pasture allowance levels, respectively.

Liveweight changes during Period I indicated that the ewes gained weight. During the differential feeding (Period II) the high fed ewes in Period II tended to continue to gain body weight (4.1% of initial liveweight at mating), while ewes on the low feeding level lost weight over the same period (2.8% of initial liveweight).

Ovulation rates were determined on Days 4 and 5 after mating. The mixed-age ewes had a higher ovulation rate (1.87 ± 0.04) than the two-tooth ewes (1.55 ± 0.06). Both age ($P < 0.001$) and liveweight ($P < 0.01$) of the ewes had significant effects on the ovulation rates.

Post-mating CIDR treatment was randomly administered to half the ewes in each of the two differential pasture allowance levels during Days 8-15 following mating. Forty five animals representing both age groups, feeding levels, ovulation rates and CIDR treatment, chosen at random, were blood sampled over the luteal phase of the oestrous cycle (Days 9 to 14 after mating). The blood samples were collected twice daily both at AM and PM over a six day period. The blood samples were then assayed on a pooled individual basis for progesterone concentration determination.

Analysis of the pooled progesterone data revealed that there were no statistically significant differences between two-tooth versus mixed-age ewes, nor pregnant versus non-pregnant ewes, with respect to peripheral plasma progesterone levels ($P > 0.10$). Progesterone levels however were found to differ significantly ($P < 0.001$) between ewes treated with CIDRs and unsupplemented ewes (1.88 ± 0.10 versus 1.34 ± 0.10 ng/ml), as well as between ewes having a single ovulation and those having twin ovulations (1.49 ± 0.10 versus 1.73 ± 0.09 ng/ml). When the effect of liveweight was corrected for, there was also a significant difference ($P < 0.05$) in pooled progesterone levels between ewes on the high (1.49 ± 0.11 ng/ml) and low pasture allowance levels (1.73 ± 0.09 ng/ml). A significant relationship ($P < 0.001$) was found between ewe liveweight and pooled progesterone concentrations for the two post-mating nutritional levels. The ewes on the high feed level had a constant relationship, while the low fed ewes had a positive relationship between liveweight and pooled progesterone concentrations.

Reproductive performance, as measured by the pregnancy rate, embryo survival rate and lambing percentage, all expressed to the first service, were not significantly affected by either age, feed level or CIDR treatment. The high feeding level (1.6 M) and CIDR treatment were found to slightly improve reproductive performance. The effect of age also appeared to slightly favour the two-tooth ewes

compared to the mixed-age ewes, although this was not found to be statistically significant ($P > 0.10$). There was however a significant effect ($P < 0.05$) of ovulation rate on the resulting pregnancy rate (50.8% for a single CL versus 68.3% for ewes having multiple CLs). The lambing percentage was also significantly affected by the ovulation rate of the ewes ($P < 0.001$), with single ovulating ewes having a lambing percentage of 56.0 ± 11.1%, while those with multiple ovulations had a lambing percentage to the first service of 115.3 ± 6.7%. The mixed-age ewes also had a slightly better percentage of multiple births to the first mating (72.4%) than did the two-tooth ewes (61.6%), although this difference was not statistically significant ($P > 0.10$).

There was a significant interaction between the post-mating feeding level and the CIDR treatment ($P < 0.05$) in the pregnancy and embryo survival rates, as well as for the lambing percentage to the first mating. The post-mating CIDR supplementation had a beneficial effect for the ewes on the 1.0 M pasture allowance level, while the same CIDR treatment for the ewes on the 1.6 M level either had no effect or reduced reproductive performance slightly. The mechanism responsible for the interaction between CIDR treatment and feeding level is not known. It is possible that it a somewhat more complex mechanism than simply a luteal deficiency of progesterone caused by increases in the nutritional level is responsible. An insensitivity to progesterone may be involved, as the low fed ewe tended to respond to post-mating CIDR treatment, while the high fed ewes did not.

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1	Single
≥2	Multiple
AI	Artificial insemination
BPR	Blood production rate
CIDR	Controlled internal drug releaser
CL	Corpus lutea
cm	Centimeter
-C	No CIDR treatment
+C	CIDR treatment
°C	Degree Celcius
CO_2	Carbon dioxide
CPM	Counts per minute
Cr	Chromium
CRD	Controlled releasing device
CV	Coefficient of variation
d	Day
D	Day of oestrous cycle
DM	Dry matter
DMD	Dry matter digestibility
DOMD	Digestibility of the organic matter in the dry matter
DOMI	Digestible organic matter intake
EPM	Ellinbank pasture meter
FO	Faecal output
FSH	Follicle-stimulating hormone
g	Gram
GnRH	Gonadotrophin releasing hormone
H	High feeding or pasture allowance level
ha	Hectare
hCG	Human chorionic gonadotrophin
IM	Intra-muscular
IU	International units
kg	Kilogram
km	Kilometer
L	Low feeding or pasture allowance level
LH	Luteinizing hormone
LSM	Least squares mean
M	Maintenance
MA (>2)	Mixed age ewes

MCR	Metabolic clearance rate
ME	Metabolizable energy
mg	Milligram
MJME	Mega joules of metabolizable energy
ml	Milliliter
m^2	Square meters
n	Number
NA	Non-applicable
ng	Nanogram
NP	Non-pregnant
OM	Organic matter
OMD	Organic matter digestibility
OR	Ovulation rate
P	Pregnant
P-1	Period I
P-2	Period II
PGF 2α	Prostaglandin F 2α
PMSG	Pregnant mare serum gonadotrophin
ppm	Parts per million
SC	Sub-cutaneous
SEM	Standard error of the mean
2T (2)	Two-tooth ewes
ug	Micrograms
ul	Microliter
wt	Weight

CHAPTER I: INTRODUCTION

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The ever-increasing world population has agriculturists, researchers, farmers, as well as politicians and world leaders having to face the enormous task of making sure that there is enough food produced in the world for human survival. One way of dealing with this potential life-threatening situation is by improving the efficiency of current agricultural production methods.

In animal production, several methods have been used to control and enhance fertility in an attempt to keep some balance between supplies and demands of agricultural produce for this exploding world population. These include improved management of herds and flocks, better use of feed reserves and improved nutrition, selection of breeding stock with superior genes and use of various hormonal treatments and drugs to increase reproductive performance.

In the ewe, many ova released from the ovary never develop into viable offspring due to a number of reasons. These include fertilization failure and conception never occurs; early embryonic mortality where the loss occurs early in gestation prior to implantation; prenatal mortality where losses occur after implantation or appear as abortions later on in gestation; and neonatal mortality where the offspring dies at or soon after parturition.

In all domestic species early embryonic mortality accounts for the majority of reproductive failure with much of the loss occurring between conception and implantation. Although the extent of embryonic loss has been established its causes are poorly understood. The variety of reasons why these losses occur include genetic, maternal and environmental factors. By reducing the amount of early embryonic mortality, it is possible to improve reproductive performance which is essential for an increase in livestock production.

For the successful establishment and maintenance of early pregnancy, it is essential that the hormone progesterone, be present in adequate amounts. Although it is secreted from several sources in the female, its main source is the corpus luteum (CL), particularly at certain times of the oestrous cycle and during early pregnancy.

Progesterone concentrations have been used as indicators of pregnancy status and reproductive function - particularly luteal function, since it appears to have a vital role in reproduction. Researchers have used several methods of determining progesterone concentrations and have tried to relate these levels during certain periods in the cycle (particularly after breeding), to embryonic survival and conception rates. Some of these methods use samples collected from the blood plasma or serum and milk. Various factors have been known to influence progesterone levels such as the effects of ovulation rate, season, stress and nutritional level acting through various physiological pathways and complex mechanisms to alter progesterone concentrations.

Inadequate luteal function and hence sub-optimal progesterone concentrations have been implicated in increased incidences of early embryonic mortality. One method of reducing early embryonic mortality is through post-mating progesterone supplementation, particularly if the reproductive wastage is caused through an associated luteal dysfunction. Supplementation of progesterone may be in the direct form where controlled internal drug release devices, sponges, implants, daily injections or oral doses of progesterone are administered. Other methods of progesterone supplementation are the indirect forms where various treatments tend to stimulate luteal function. These treatments include the administration of GnRH, hCG, melatonin, LH and prolactin. These treatments have been shown to increase progesterone levels in both ewes and cows and in some situations they possibly improve the rates of embryo survival. The net result is that more offspring develop and are born leading to an overall improvement in the females reproductive performance.

CHAPTER II: LITERATURE REVIEW

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1 The importance of a high reproductive performance:

The reproductive performance of livestock is recognized as a major determinant of productivity (Land et al., 1986). High reproductive performance in a breeding herd or flock is an important goal for livestock producers to strive for, whether the animals are raised for the production of eggs, meat, milk or fiber. This contributes not only to the biological efficiency of the operation, but also to the economic efficiency of the animal production enterprise (Dickerson, 1970). McGuirk (1976) indicates that maintaining the breeding female is one of the major costs associated with most production systems. By increasing the reproductive rate (the number of offspring produced annually by each breeding female) it is therefore possible to spread this fixed cost over a larger number of offspring available for sale (Piper & Bindon, 1979). The potential to increase reproductive performance in sheep is more feasible than attempting to do the same with cattle as they have a naturally low reproductive rate. Hence, milk production of the cows and growth efficiency of the offspring are very important considerations in cattle production systems (Dickerson, 1970). This increased efficiency results in the livestock producer getting a greater return on his investment by reducing his production costs through increased output.

From the genetic viewpoint, a high reproductive rate is important since it results in a larger number of offspring available for selection, whether these are replacement females or potential herd sires, and this contributes to accelerated genetic gains in other productive traits (Sreenan, 1981; VanVleck, 1981; Rae, 1986).

The efficiency of reproduction may decline for many reasons, including seasonal, genetic, nutritional, anatomic, hormonal, neural, immunological, humoral, or pathological factors. These factors may result in partial or complete reproductive failure. However, those concerned with farm animal production have the continued interest in preventing such failure. Recent advances in reproductive biology appear to promise rapid genetic improvement in farm animals. This improved genetic potential generally means decreased relative cost to

the consumer because, as each livestock unit produces more, the maintenance cost of the unit is a smaller portion of the product (VanVleck, 1981). Hence, better quality animal products at lower prices are made available to the consumer.

2 Determinants of reproductive performance:

In a livestock production system, reproductive performance can be measured in a variety of ways. The reproductive rate is most commonly used to assess reproductive performance. In sheep the reproductive rate is defined as the number of lambs weaned per ewe per year (Rae, 1986) while in beef production it is similarily defined as the number of calves weaned per cow exposed to the bull per year or the average calf crop (Piper & Bindon, 1979). This is a complex trait, with variation resulting from effects contributed by the dam, the sire and the offspring and the interactions among them (Rae, 1986).

The reproductive efficiency in a dairy herd can be assessed by the calving percentage following a defined breeding season. This can be measured in terms of a combination of calving rate, calving date, and the length of the calving season (Sreenan, 1981). Under New Zealand conditions, where the maintenance of a seasonally concentrated calving pattern is an essential requirement for successful dairy farming, obtaining a high herd submission rate is also of impcrtance (Macmillan, 1979).

There are usually three main criteria used to describe reproductive rate. These include (1) fertility, (2) fecundity or prolificacy and (3) survival of the offspring (Piper & Bindon, 1979; Land, 1982; Gunn, 1983; Rae, 1986.) Fertility can be defined as the ability to breed (Gunn, 1983). It may be represented as the proportion of the cows pregnant (Piper & Bindon, 1979) or by whether or not a ewe has a lamb (Rae, 1986). Selection for increased fertility is likely to yield only limited improvement, mainly because the maximum level of performance is 100% of females conceiving to a specific mating. As well, McGuirk (1976) indicates that because of the low estimates of both repeatability and heritability for fertility, the rate of advance towards this upper limit is likely to be slow.

Fecundity is defined as the breeding rate or litter size (Piper & Bindon, 1979; Rae, 1986). In sheep, this is expressed as the number of lambs produced per ewe lambing. This is a consequence of the number of ova shed from the ovaries (ovulation rate) at the oestrus when mating occurred, minus the ova, embryo and foetal wastage expressed as the number of ova not represented by viable lambs at parturition (Gunn, 1983). A reduction in the fecundity or prolificacy results in a smaller litter size and even an increase in ewe barrenness.

Beef production for instance, when compared to other domestic animals, is somewhat inefficient. This is because all cows do not produce a calf each year. It has been estimated that beef production could be increased by 60% in intensively managed herds through twinning (Mapletoft, 1986). Nevertheless, the chance of increasing prolificacy in cattle through an increased selection for twinning, offers little hope for improvement. This is mainly due to the low heritability of the incidence of twinning in cattle (McGuirk, 1976). Because of this low selection potential for increased efficiency, greater emphasis is now being placed on other attempts to increase the reproductive rate in the cow through increasing the proportion of multiple (twin) births. Techniques used include embryo transfer (ET) and steroid immunization which aims to increase the ovulation rate (Piper & Bindon, 1979; Sreenan & Diskin, 1986). It seems logical that a reduction in embryonic mortality will increase prolificacy, not only in cattle, but in other livestock species as well.

Survival rate is decribed as the ability of the offspring to survive to weaning (Rae, 1986) or the proportion of offspring weaned of those born (Piper & Bindon, 1979). However, as litter size increases, survival of the offspring tends to decrease (McGuirk, 1976). Survival rate of the offspring may be reduced because losses due to exposure or starvation, infectious diseases, accidents and predators, and congenital defects being present in the new born, which seriously impairs their viability (Eales et al., 1983). Often the environmental conditions and the level of management available play a major role in affecting offspring survival rates.

In most cases the reproductive rate in sheep is mainly determined by litter size. Both ovulation rate (OR) and embryo survival are major factors influencing litter size with the number of ova shed at any particular heat period being the upper limit for fecundity in the ewe (Allison, 1975). Embryo survival, on the other hand, has less potential for increasing the litter size in the ewe when compared to the OR. There is however speculation that improvement of prolificacy, not only in the ewe and other multiparous species, but in all domestic species will eventually be based on a reduction in embryonic mortality (Bolet, 1986).

3 The impact of prenatal mortality:

Of all the ova that are shed from the sheep ovary, only a proportion actually develop into viable offspring. This 'reproductive wastage', estimated to be 20 to 40%, can be due to failure of fertilization, as well as prenatal mortality (Moore, 1985). Prenatal mortality is the term often used to refer to deaths of the unborn which occur over the entire length of gestation - from conception through to parturition. These deaths can generally be subdivided into embryonic mortality - which include losses of fertilized ova and embryos up to the end of attachment, and foetal deaths - which are losses occurring from the time of attachment until parturition (Chapman, 1980). Attachment or implantation in the sheep occurs during the fourth week of pregnancy (Wilmut et al., 1985b) and at about Day 45 of gestation in the cow (Committee on Bovine Reproductive Nomenclature, 1972). Estimates in the range of 20 to 30% losses occurring from prenatal mortality are often used in describing this type of reproductive wastage in both the ewe (Edey, 1969a & 1979; Moore, 1985) and the cow (Sreenan & Diskin, 1983; Roche, 1986).

The greatest proportion of prenatal mortality appears to be from early embryonic mortality in sheep rather than from foetal death (Quinlivan et al., 1966; Moore, 1985). In cattle, it is estimated that 75 to 80% of the prenatal mortality is manifest in early embryonic death ie. within the first 20 days of fertilization (Sreenan & Diskin, 1983). Foetal deaths in both sheep and cattle are largely due to a number of disease conditions that terminate pregnancy later on in gestation and appear as abortions. Some of these diseases are Campylobacteriosis, Listeriosis, Toxoplasmosis and

Chlamydial abortion in sheep (Rahaley, 1984); and Brucellosis, Vibriosis, Trichomoniasis, Leptospirosis and Infectious Bovine Rhinotracheitis (IBR) in cattle (Anonymous, 1968; Holmes & Wilson, 1984). For reviews on these diseases which primarily cause abortion in sheep and cattle, see Rahaley (1984) and Anonymous (1968), respectively.

It can therefore be stated that the greatest amount of prenatal loss in sheep and cattle occurs primarily as early embryonic mortality. In some cases, the animal will return to service at a regular interval because all of the embryos will have died. In cows when this situation occurs, they are commonly referred to as 'repeat breeders'. These animals return to service at regular intervals and it is often difficult to distinguish whether fertilization failure has occurred, or whether early embryonic mortality has occurred. It appears that most studies clearly indicate that early embryonic mortality is the prominent feature of the repeat breeder syndrome in cattle (Maurer & Chenault, 1983; Ayalon, 1984). Recent information obtained through embryo transfer studies also suggests that fertilization failure may be of considerable importance in repeat breeding animals (Johnson, 1986).

The resultant reproductive wastage in cattle is a major source of economic loss in production through extended calving seasons, shorter lactations, lower production and increased costs of artificial insemination (Roche, 1981). In ewes, partial failure of twin pregnancies also represents a substantial loss in the reproductive performance of the sheep flock. These are losses due to the death of one of two embryos during pregnancy and although estimates vary considerably, Kelly & Allison (1979) estimate that about 32% of ewes with two ovulations lose one embryo during pregnancy. The detrimental effects of embryo mortality to sheep production are not merely restricted to a reduction in the number of lambs born (partial embryo loss) or to the number of barren ewes (total embryo loss), but can also result in the birth of smaller than average lambs when partial embryo loss occurs during implantation (McKelvey & Robinson, 1986). There appears to be a natural tendency for embryos to migrate between the uterine horns in sheep so that a balance in foetal numbers occurs prior to implantation. It has been suggested that this redistribution of embryos occurs so as to

minimize the amount of prenatal mortality occurring (Rhind et al., 1980). If embryonic loss occurs after the implantation process has been initiated, the surviving embryo(s) are unable to utilize the maternal cotyledons vacated by the embryo that died. This results in a reduced number of cotyledon sites per embryo and impairs placental development, since the available placental blood supply and nutrients are not being used to their full potential. Smaller-than-average lambs are born as a result, which may lead to poor neonatal survival (McDonald et al., 1981). If embryonic loss occurs before the initiation of implantation, the embryos can redistribute themselves accordingly and space themselves so that they can take full advantage of the available maternal cotyledon sites for establishing placental development.

4 Factors affecting embryonic mortality:

There are numerous reports indicating the various factors affecting embryonic mortality in both sheep and cattle. See Edey (1969a & 1979), Chapman (1980), Knight (1983), Kelly (1984) and Willingham et al. (1986) for reviews concerning embryonic loss in sheep and Ayalon (1978) and Roche (1986) for articles regarding embryonic mortality in cattle.

The following diagram shows the possible routes of action of various factors as they are thought to influence embryo mortality in the ewe. Even though not all these pathways will be discussed, the diagram may help in understanding the complex associations that exist between the embryo and the uterine environment.

Embryo Survival:

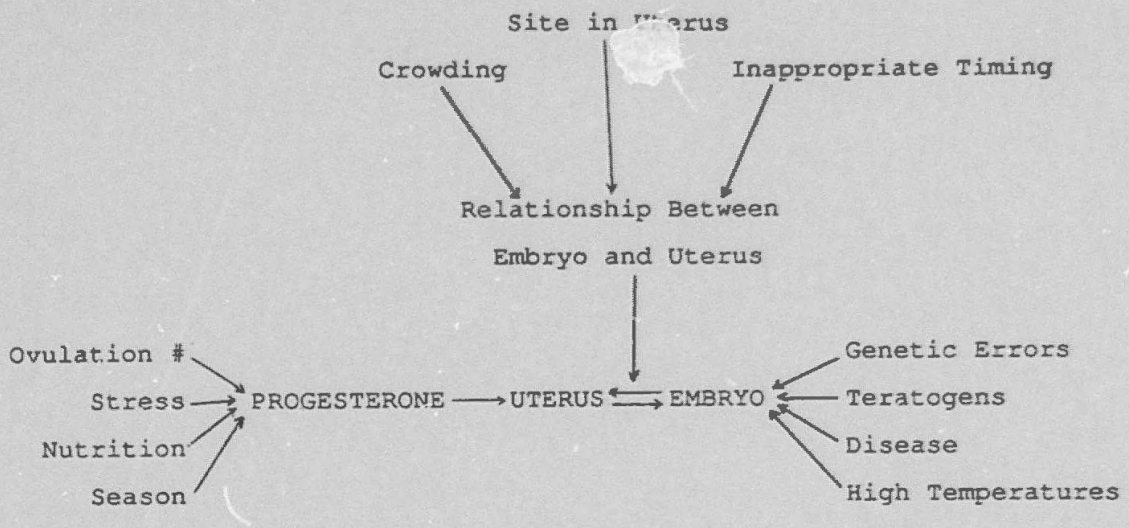

Figure 1: Embryo survival in sheep can be affected in 3 ways:

(1) through the quality of the embryo itself,

(2) the suitability of the uterine environment,

(3) the direct relationship between the embryo and the uterus.

Possible routes of action of environmental factors are shown (Land et al., 1986).

4.1 Embryo vigour:

In order for normal embryonic development to occur after fertilization, it is critical that a normal complement of chromosomes be properly expressed. The presence of any gross chromosomal abnormalities, such as translocations and aneuploidy can affect different stages of reproduction and in particular, the viability of the gametes and embryo (Bolet, 1986). Bishop (1964) proposed that these embryonic losses often occur spontaneously as a result of mutations which are unavoidable and should be regarded as a normal way of eliminating unfit genotypes which would otherwise be poorly suited for survival. It is difficult to say whether these abnormal conditions arise by chance and/or are inherited. It appears that the production of these zygotes with abnormal chromosomes do play some role in early embryonic mortality in domestic animals (Roche, 1986), although this does not solely account for the large embryonic losses in farm animals.

Condition of the gametes at the time of fertilization has also been documented as a factor influencing embryonic mortality in both sheep and cattle (Edey, 1969a & 1979; Ayalon, 1978; Courot & Colas, 1986). The condition of both the ovum and the sperm are of importance here, especially in the case where fertilization occurs but is soon followed by embryonic death. It has been suggested by Courot & Colas (1986) that the sperm, besides contributing to the fertilization process of the ovum, actually plays an important role in contributing to the capacity of the embryo to survive to become a viable offspring. This could depend, not only on genetic factors such as chromosomal abnormalities, but also on environmental factors such as season of semen collection, insemination time, semen handling and processing procedures, and other issues concerning semen quality. When aged sperm and the ovum are involved in the fertilization process, the resulting zygote often dies prematurely (Roche, 1986). Hence it is important to avoid aging of gametes in routine reproductive management programmes for livestock.

4.2 Maternal environment:

The breed or genotype of an animal is a factor that affects embryonic mortality. There appears to be a significant differences in embryonic losses recorded between breeds of sheep (Cumming et al., 1975; Meyer, et al., 1983; Bradford, 1985; Meyer, 1985) and for ewes mated to rams from lines of sheep selected for high versus low prolificacy (Burfening et al., 1977; Kelly, 1984; Wilmut et al., 1985a). In cattle there appears to be no breed differences in the incidence of embryonic death (Ayalon, 1978), however there is evidence of genetic variabitity for conception rate at first service and embryonic mortality among different families of Holstein-Friesians in the U.S.A. (Mares et al., 1961; Menge et al., 1962). Inbreeding in both sheep and cattle appears to influence embryonic mortality (Ayalon, 1978; Doney & Smith, 1968). Meyer (1985) indicates that 'uterine efficiency', which is the genetic variation in uterine ability of the ewes to produce multiple lambs, is another factor involved in explaining breed differences in relationship to embryonic mortality in sheep. Embryonic losses vary between and within sheep breeds according to OR (Bolet, 1986). Valid comparisons between breeds or crosses should therefore only be made at similar ORs using a selected sample.

Ovulation rate in sheep is a major factor affecting embryonic mortality. The prolific breeds of sheep having high ORs appear to have a lower number of lambs born per ovulation than do the less prolific breeds (Edey, 1969a). Sheep with two ovulations therefore have a greater proportion of embryonic mortality than do single ovulating ewes (Edey, 1969a; Wilmut et al., 1985b). Knight (1983) indicates that through partial failure of multiple ovulation (PFMO), embryo mortality exerts a major influence on the reproductive performance of the flock. Embryo transfer studies comparing litter size of ewes of different breeds have shown that OR is the primary factor limiting litter size in breeds characterized by low litter size. Embryonic mortality may be the limiting factor in breeds with both higher OR and litter size (Bradford, 1972).

Embryonic mortality appears to be greater in younger females compared to older ewes (McMillan & McDonald, 1985; Wilmut et al., 1985b). Quirke & Hanrahan (1977) found that even though fertilization rates between ewe lambs and mature ewes were similar, the ova from the younger animals were either of an inherent lower potential for survival and development or that conditions existed within the ewe lambs reproductive tract that were suboptimal, therefore having a deleterious effect on embryonic survival. This significant effect of age on embryo survival was even evident between 1.5 year old maiden ewes having a lower embryo survival rate than the mature ewes, although the difference was less pronounced (Blockey et al., 1975). The same situation appears to exist in cattle where heifers tend to have a higher incidence of embryonic loss than do older cows which have had several calvings (Erb & Holtz, 1958; Spitzer et al., 1978; Macmillan, 1979).

During the period of implantation, the embryo and uterus must develop in a coordinated and synchronous manner. The growing embryo becomes increasingly dependent on the uterine environment for survival and growth. A hostile environment which is unsuitable for the maintenance of the embryo will often lead to an increase in embryo mortality (Edey, 1969a). Therefore, the uterine environment must undergo continual modification in order to cope with the needs of the developing embryo prior to implantation (Findlay, 1984). The maternal hormones act indirectly via the uterine secretions and exert their effect on embryonic survival. Any hormonal imbalances in the

dam leads to a suboptimal or detrimental uterine environment (Fischer & Beier, 1986).

There are a number of physiological processes at which variation in the embryo stage or hormone secretion could occur resulting in asynchrony between the uterus and embryo. This consequently will lead to an increased level of prenatal mortality (Wilmit & Sales, 1981; Wilmut et al., 1985a & 1985b). Recent work using embyro transfer techniques in sheep has focused on the role that hormones play in preconditioning the uterus prior to conception. Hormonal conditions before transfer markedly influence embryonic survival rates (Miller & Moore, 1976; Lawson et al., 1983; Lawson & Cahill, 1983). Differences in uterine secretions including concentrations of ions, energy substrates and proteins, were found to vary between normal females and those animals identified as being repeat breeder cattle (Ayalon, 1984). It therefore becomes apparent that the uterine environment, as mediated primarily through shifts in the hormonal conditions of the dam, influences the incidence of embryonic mortality.

4.3 Environmental conditions:

Embryonic survival tends to vary according to the breeding season of sheep, with maximum levels having been reported in the middle of the breeding season, even though there were a greater number of ovulations at this time (Hulet et al., 1956). Abnormally high embryonic losses have been reported in ewes bred both early and late in the breeding season (Chapman, 1980). For sheep in the U.K., embryo survival rates tend to be lowest in March compared to other times during the year (Ashworth et al., 1984).

The term stress generally can imply many different things, whether they be naturally occurring or induced. Conditions which tend to stress animals have been implicated in causing varying degrees of embryonic mortality in sheep and cattle. Adverse environmental conditions such as high ambient temperatures appear to adversely affect embryo survival by being detrimental to proper embryonic development (Edey, 1969a; Thwaites, 1971; Sawyer, 1979). In cows, a late summer infertility problem exists which has been associated with the effects of high temperatures (Stott & Williams, 1962). Low temperatures combined with wet conditions also tend to increase the

incidence of embryonic mortality in ewes compared to animals which had access to shelter from these adverse environmental conditions (Griffiths et al., 1970).

Man-made stress includes rough and intensive handling of stock, crowding, long and reckless trucking of animals and being chased and worried by dogs. These have all been associated with causing varying degrees of embryonic mortality in sheep (Doney et al., 1976). Chapman (1980) advises to avoid physical stress, fright, heat stress and so forth during mating and pregnancy of the sheep flock and emphasizes that the flock should be left undisturbed, particularily at mating.

There is very little evidence to suggest that specific disease conditions play any significant role in causing embryonic mortality in the sheep (Edey, 1969a & 1979; Chapman, 1980). The disease organisms which cause reproductive losses in sheep occur as abortions in mid to late gestation (Edey, 1979). Hairy shaker disease, also known as border disease, may cause embryonic death, as well as foetal death and abortion in ewes (Chapman, 1980). In cattle, it appears that low grade non-specific infections are not normally a cause of embryonic loss (Sreenan & Diskin, 1983). There are however two infections, Vibrio fetus and Trichomonas fetus, which have been implicated as probable causes of early embryonic mortality in cows (Adler, 1959).

The influence of nutrition as a factor involved in embryonic mortality is well recognized. The subject has been reviewed by Edey (1976), Rattray, (1977), Robinson (1983), McKelvey & Robinson (1986) and Robinson (1986), while others have looked at specific aspects of this relationship. Although there is a vast amount of literature dealing with this topic, there appears to be considerable variation in the reports as to the role that nutrition plays around the time of mating and its effect on embryonic survival in the ewe.

Variation in nutritional level before mating and body condition at the time of mating in the ewe both influence the number of ovulations and embryo survival (Gunn et al., 1969 & 1972). It has been shown that when submaintenance diets were fed in the first few weeks after mating, they were associated with an increased incidence of embryonic mortality (Edey, 1966; Cumming, 1972a & 1972b; Cumming

et al., 1975; Hamra & Bryant, 1982). Other studies however have found that undernutrition had no affect on embryo survival (Parr & Williams, 1982; MacKelvey & Robinson, 1986; Parr et al., 1987a), although embryo growth was retarded in ewes fed submaintenance diets when the embyros were viewed at Days 11 and 21 post-mating (Parr et al., 1982). Other experiments have looked at improved nutrition after mating and found that high feeding levels tended to be detrimental to embryo survival (Edey, 1976; Cumming et al., 1975; Brien et al., 1977; Parr et al., 1987a). These results tend to suggest that extremes in nutrition soon after mating appear to have detrimental effects on embryo survival rates. Robinson (1986) recommends keeping recently mated ewes on maintenance levels of feeding during the first month of pregnancy and to avoid any type of nutritional stress. This tends to keep embryonic wastage to a minimum.

According to Robinson (1986), one can only speculate on the possible mechanisms by which nutrition affects embryo survival and growth. Overnutrition in early pregnancy, has been associated with heat stress, particularly if the females are in good condition at mating. Evidence suggests that during the early cleavage stages the embryos are particularly vulnerable to small increases in maternal body temperature (Edey, 1976). Undernutrition, on the other hand, may result in a spontaneously-arising asynchrony between the uterine environment and the development of the embryo, resulting in an increase in embryonic mortality (Wilmut & Sales, 1981). Conclusive evidence for a modifying effect of nutrition on asynchrony has however not yet been established (Robinson, 1986). It is possible that a disturbance in the amino acid composition, or some other component of the uterine fluid (Fischer & Beier, 1986) and/or a reduction in the availability of glucose (Parr & Williams, 1982) may be responsible for the nutritional effects on embryo survival.

Specific dietary nutrients are known to have a direct effect on embryonic mortality. A deficiency of selenium has been shown to impair embryonic survival in sheep because it is an essential mineral (Hartley, 1963; Piper et al., 1980). Provided there is no concurrent vitamin E deficiency in these selenium-deficient diets, then the supplementation of exogenous selenium to these deficient diets, or by direct administration of selenium will normally rectify the situation and improve the embryo survival rates. Other specific nutrients that

have been implicated in affecting reproductive performance in sheep include deficiencies of protein and some of the trace elements, particularly iron, cobalt, copper, manganese and zinc (Robinson, 1983). In cattle, deficiencies of phosphorus, beta-carotene, selenium and copper have been associated in increased incidences of embryo loss (Roche, 1986). However, Robinson (1986) cautions that for many of these nutrients it is not clear whether their effect is solely on embryo survival or their effect is mediated through some other aspect of reproductive performance.

Several specific types of feed, besides the previously mentioned dietary nutrients have been documented in reducing embryo survival. Oestrogenic forages, particularly red clover has been shown to reduce fertility in both sheep and cattle. This feed is thought to affect fertility by a reduction in fertilization rather than an increase in embryonic mortality (Robinson, 1986). Cruciferous crops such as kale also interfere with embryo survival, particularly if fed during the implantation period (Williams et al., 1965). It is not known whether the increased mortality is due to the result of a goitrogenic effect, anaemia or a reduced copper status (Robinson, 1983).

5 Physiological control of implantation and embryonic survival:

Ovarian function determines reproductive performance. Both the ovary and the ovarian hormones are responsible for ovulation and endocrine control of oestrus, conception and early pregnancy, as well as the development of the reproductive tract through early life and puberty. It therefore becomes necessary to understand and appreciate some of the complex events which occur, particularly at the time of conception and during the following period when a pregnancy is established and successfully maintained.

5.1 The oestrous cycle:

The normal sequence of hormonal changes responsible for the control of the oestrous cycle and ovulation in both the ewe and the cow is governed principally by the hypothalamic-pituitary-ovarian axis. This in turn is modified by hormonal feedback mechanisms involving ovarian steroid hormones, which are themselves produced as a result of stimulation by the gonadotrophins of the developing

follicle(s) and corpus luteum (CL). Regulation of tonic FSH secretion however is not fully explained by the negative feedback effects of the steroids produced by the ovaries, as shown by Goodman et al. (1981). It is thought that the ovary also secretes inhibin, a non-steroid hormone that is produced in developing follicles and tends to control the FSH secretion (Haresign, 1985).

Throughout the entire oestrous cycle of both the ewe and the cow, a similar pattern of hormone secretion exists. Both cycles are divided into a luteal phase (period of an active CL) and a follicular phase (period of a follicle maturation and growth). The main difference occurs in the length of the cycle, with the ewe cycle averaging 17 days, while that of the cow averages 21 days. For reviews on the control of the oestrous cycle in the ewe, see Baird & McNeilly (1981), Smith (1982) and Haresign et al. (1983).

5.2 Processes involved in implantation:

In order for successful implantation and maintenance of pregnancy to occur in both the ewe and the cow, it is essential that biochemical communication between the embryo and the uterine endometrium takes place (Thatcher et al., 1985). There are two lines of evidence supporting the critical nature of this association. The first is known as maternal recognition of pregnancy where the embryo must be present in the uterus by a certain time in order to maintain the function of the CL (Betteridge et al., 1980; Northey & French, 1980). The second is that there needs to be a close synchrony between embryo and the uterine environment (Rowson et al., 1969; Betteridge et al., 1980).

5.2.1 Maternal recognition of pregnancy:

The CL must remain in a functional state with continued progesterone production if a successful pregnancy is to be established. Without continued CL function, or supplemented progesterone from some other source, the embryo would fail to survive and another period of oestrus would occur. It is thought that the fertilized embryo in its prolonged free-living state in the uterus sends out some kind of signal to inform the maternal environment of its presence so that the CL function can still be maintained. This is

a fairly complex sequence of events that must occur by about Day 12 post-mating in the ewe (Moor & Rowson, 1966) and Day 16 post-mating in the cow (Betteridge et al., 1980) if a successful pregnancy is to develop.

The embryo is involved in initiating production of a luteotrophin and/or antiluteolytic substance that acts by suppressing the action of prostaglandin F 2α (PGF 2α) at the uterine or ovarian sites (Cook & Hunter, 1978). PGF 2α is considered to be the main, naturally-occurring luteolytic substance that is secreted by the uterus (Findlay, 1981). The antiluteolytic effect of the early embryo in both the ewe and the cow is due to its presence totally suppressing or inhibiting the release of PGF 2α, at least into the peripheral circulation. In the ewe, Ottobre et al. (1984) showed that the embryo appears to inhibit the luteolytic effect of PGF 2α, rather than to suppress its secretion. It is thought that the blastocyst secretes an antiluteolysin, a protein called trophoblastin, and this in turn acts on the uterus to inhibit the effect of PGF 2α (Findlay, 1984). This signal tends to prolong the lifespan of the CL from one of a cyclic nature to a more permanent CL associated with pregnancy. The luteotrophic effect of the cow embryo is said to be mediated by a similar substance(s) produced by the blastocyst that acts directly on the ovary and in particular the CL to maintain progesterone secretion (Sreenan & Diskin, 1983). Another way the embryo might prevent luteolysis in the ewe is by suppressing the release of luteal oxytocin, since oxytocin is known to be involved in the mechanism responsible for the release of prostaglandin from the endometrium of the uterus (Findlay, 1984).

5.2.2 Synchrony:

A close synchrony between the embryo and the uterine environment is necessary for successful implantation. Several reports (Rowson et al., 1969; Wilmut & Sales, 1981; Wilmut et al., 1985a & 1985b) indicate asynchrony between the embryo and the ewe is a significant factor involved in embryo mortality. Some of this asynchrony has been known to arise spontaneously between the embryos stage of development and that of the dams maternal endocrine environment.

It would be expected that with perfect synchrony the proportion of pregnancies would increase. A modest asynchrony would not affect survival because of the modulating influence of the uterus. However, beyond a certain unknown degree of synchrony, the fall in the proportion of pregnancies would be expected to be dramatic (Rowson & Moor, 1966; Wilmut et al., 1985a). In cattle, synchrony within the range of ± 24 hours is acceptable between donor and recipient (Rowe, 1986), while in ewes the range is within the order of ± 36 to 48 hours (Rowson & Moor, 1966).

Asynchrony between the maternal environment and the embryo usually results from some kind of hormonal imbalance which tends to cause an unsuitable uterine environment for embryonic development (Maurer & Echternkamp, 1982). It has been shown that the timing of the changes in uterine function is determined by the time of the increase in progesterone concentration after oestrus (Miller & Moore, 1976; Wilmut & Sales, 1981; Lawson et al., 1983; Wilmut et al., 1986). The changes in progesterone levels are partly responsible for dictating the synchrony between embryo and uterus. Embryo transfer and other studies with ovariectomized sheep indicate that a relatively small increase in dose of progesterone advanced the 'preparedness' of the uterus to accept embryos by several days (Lawson & Cahill, 1983; Moore, 1985; Moore & Miller, 1985). This role of progesterone could influence the success of embryo transfer as it reveals an opportunity to control the timing of changes in the recipient by administration of progesterone before the time of the rise in endogenous hormone levels (Wilmut et al., 1985b). This would result in a closer synchrony between embryo and uterine environment and lead to an increase in conception rates.

In ewes that have a higher OR, some of the increased embryo mortality associated with these animals is the result of asynchrony between the uterine environment and one of her embryos. As the embryo stages vary within the high ovulating ewe, the chances of that ewe becoming pregnant increase, while the proportion of embryos surviving actually decreases (Wilmut et al., 1985b). When transferring embryos, it is important to consider that a higher proportion of pregnancies will be obtained if the recipients oestrous cycle is matched up with the stage of development of the donors embryo (Wilmut et al., 1985a).

5.3 Hormonal requirements for the establishment of pregnancy:

During the early embryonic period it appears that the first week of development is unaffected by major changes in the endocrine environment (Wilmut et al., 1985b). The early environments only function is to permit development, while the later environment modulates the rate of development.

Oestradiol from the ovaries and adrenals is not required for successful maintenance of the sheep embryo between days 3 and 36 (Cumming et al., 1974; Trounson & Moore, 1974). Miller & Moore (1976) suggest that during the first few days after oestrus, oestradiol may play an important role in governing embryo transport from the oviduct to the uterus. Results from cattle embryo transfer studies show that the ratio of oestrogen to progesterone may be of importance since a higher ratio was found in cows with unfertilized eggs, degenerating embryos, or no recovered oocyte compared to cows that had a normal developing embryo (Maurer & Echternkamp, 1982). This indicates that there must be a necessary combination or balance of hormones present to ensure successful embryonic development.

The timing of the changes in uterine function is determined by the time of the increase in progesterone concentration after oestrus. Progesterone will also increase the rate of cell division during early embryo development (Bindon, 1971; Wilmut & Sales, 1981; Parr et al., 1982). This response is mediated through the influence of the progesterone on uterine function, which in turn influences uterine secretions and ultimately affects the rate of embryonic development (Trounson & Moore, 1974).

Much of the work that has been done in establishing which endocrine changes are necessary for successful pregnancy to occur, have been carried out in ovariectomized animals that have had embryos transferred to them. Through the manipulation of the timing and doses of various hormones, it has been established that there are three phases of hormone treatment necessary for the establishment of pregnancy. These are: (1) the presence of progesterone before mating to mimic a previous luteal phase, (2) oestradiol, and (3) progesterone after mating (Bindon, 1971; Miller & Moore, 1976; Wilmut et al., 1985b). After mating, there are two phases where secretion of

progesterone controls uterine function. The first period is from oestrus until Day 4 or 5 in the ewe oestrous cycle when the supporting level should be low; followed by an increase in progesterone levels to that typical of the luteal phase (Miller & Moore, 1976). Pregnancy will only occur if a certain minimal baseline concentration of progesterone is present during the luteal phase after mating (Wilmut et al., 1986).

A luteal phase prior to oestrus in the ewe is also required if successful pregnancy is to occur (Moore, 1985). This was concluded from experiments involving ewes that were ovariectomized for some months prior to receiving the various hormonal treatments before having embryos transferred to them (Moore & Miller, unpublished). Clearly, ovarian secretory activity during and after oestrus is of major importance in the survival and development of early embryos.

The mechanism by which these ovarian steroid hormones function is thought to be mediated via the uterine environment. It has been suggested by Moore, (1985) that one of their effects is where appropriate secretory activity or replacement therapy is responsible for the presence of substances in the maternal environment that allow the survival and development of embryos. Conversely, they could be responsible for the absence of substances which play an inhibitory role in embryonic development.

5.4 Relationship between progesterone levels and conception rates:

If a normal pregnancy is to be established in both the ewe and the cow, the CL must continue to remain functionally active and secrete progesterone. In the ewe the CL must remain functional up until Day 55 of gestation when the placenta is able to produce adequate amounts of progesterone to maintain pregnancy (Moore & Rowson, 1959; Sachs, 1984). In the cow, a functional CL must be maintained for a much longer time, up to approximately 215 days of gestation before the adrenal gland and/or placenta is able to produce adequate amounts of progesterone for the maintenance of pregnancy (Sachs, 1984).

The concentrations of progesterone in the peripheral blood plasma during the oestrous cycle in ewes have been well documented

(Plotka & Erb, 1967; Stabenfeldt et al., 1969; Obst & Seamark, 1970; Allison & McNatty, 1972; McNatty et al., 1973; Quirke & Gosling, 1975 & 1976; Quirke et al., 1979; Wheaton et al., 1988). There have been several attempts to relate progesterone levels to conception rates both in cows and ewes, but unfortunately the literature has been contradictory. Investigation of the relationship between progesterone concentrations during the oestrous cycle before insemination and the subsequent conception rates has been conducted. Folman et al. (1973), Corah et al. (1974) and Rosenberg et al. (1977) reported that a positive relationship existed between progesterone levels during the cycle prior to mating and the subsequent conception rate, while later studies by Bulman & Lamming (1978) and Diskin & Sreenan (1986) failed to support the existance of this relationship. The appropriate progesterone priming before ovulation could influence the secretory activities of follicles and CLs which exert their effects on the uterine environment, and thus could possibly influence the resulting conception rates (Moore, 1985).

Relationships between conception rates and progesterone levels following mating have also been investigated. Bindon (1971) reported that the mean progesterone concentration for pregnant versus non-pregnant ewes is higher from Days 10-11 onwards, but the difference is not statistically significant until Days 16 to 17. Similar results by Obst & Seamark (1970) indicate that pregnant ewes have higher progesterones levels from as early as Day 12 following mating. Cumming et al. (1971) found that where embryonic loss occurred, plasma progesterone levels fell, but the drop in levels did not become significant until Day 15. Some care should be taken in the assessment, interpretation and comparisons of these findings because of the frequency of blood sampling relative to the frequency of naturally occurring fluctuations in progesterone level. In pregnant cows, even though a higher progesterone level is evident from about Day 13 onwards, it is not until about Day 16 that a significant relationship between progesterone levels and conception rates appeared (Diskin & Sreenan, 1986). At this time, the difference is caused by luteolysis in those animals failing to become pregnant (hence a non-functional CL) rather than a luteal deficiency at an earlier stage.

Considerable variation exists between animals in both basal circulating progesterone concentrations and in the progesterone concentrations associated with the survival of all of the embryos. Partial or total embryo loss in the ewe is associated with lower baseline progesterone levels during the luteal phase of the cycle (Wilmut et al., 1986). Progesterone concentrations in milk, plasma or serum in the early part of gestation can therefore be related to conception rates as a reliable method for diagnosing pregnancy. In the cow Beghelli et al. (1986) indicate that the measurement of progesterone levels in milk can be used as an efficient method of indirectly determining early pregnancy. This has been shown to be reasonably effective if based on two samples, the first one being collected at insemination and the second one 21 to 23 days later. The second sample should be at a level comparable to those of the luteal phase of the cycle, thus indicating pregnancy. Plasma progesterone levels have also been successfully used to determine pregnancy status and to predict litter size in sheep when examined at a latter stage of gestation (Gadsby et al., 1972).

6 Factors affecting progesterone concentrations:

There appear to be four ways whereby progesterone concentration can be altered. (Reference to Figure 1 shows the four factors which have been identified as affecting the hormone levels.) These include CL number, season, stress and nutritional status. These will now be briefly discussed.

6.1 Number of corpora lutea (ovulation rate):

The ovulation rate and hence the number of corpora lutea (CL) will influence the progesterone concentration in the female. This is a situation involving species that are typically litter-bearing, such as the ewe and the sow, rather than the normally single-ovulating cow.

In recently mated, ovariectomized ewes, Bindon (1971) showed that supplementation with varying doses of exogenous progesterone had a clear effect on the number and viability of embryos. From this he hypothesized that the number of CLs, as well as the number of embryos might conceivably influence the level of plasma progesterone. Further

work indicated that ewes with two CLs and no embryonic loss had a higher plasma progesterone concentration than did those with only a single CL, but the difference was evident only after Day 12 post-mating (Cumming et al.,1971).

Lamond et al. (1972) reported that ewes with one or two CLs had similar progesterone levels. Conversely, Eastwood et al. (1976) found ewes with two CLs had greater blood progesterone levels than uniovular sheep. Similar findings were reported by Quirke et al. (1979) and Williams & Cumming (1982) where progesterone levels were found to be slightly higher in ewes with two ovulations than in ewes with only a single ovulation. Others have reported that the level of progesterone during the luteal phase increased as the number of ovulations increased, however each additional ovulation or CL produced less progesterone (Wilmut et al., 1986). This has led to the conclusion that the relationship between the number of CLs and plasma progesterone concentration is not a simple linear function.

6.2 Season:

Season of the year influences the secretion of progesterone in both the ewe and the cow. Progesterone levels and subsequent conception rates were higher in mid-mating season than at either end of the sheep breeding season (Lamond et al., 1973; Wheeler & Land, 1977; Quirke et al., 1979). Similar findings were reported by Kittok et al. (1983) and Wilmut et al. (1986). Ashworth et al. (1984) indicate that there is an increase in embryo mortality in spring mating of sheep and this is due to an associated lower baseline level of progesterone during the luteal phase of the cycle.

Embryo survival of ewes was found to be lower in March (in the U.K.) than any other time of the year (Ashworth et al., 1984). However, when the effects of progesterone were considered, the effect of season was no longer significant, thus indicating that this effect is mediated entirely by progesterone (Wilmut et al., 1986). The resulting infertility caused by the seasonal progesterone secretion difference (or seasonal differences in CRs) is suggested as causing a wide range of uterine environmental conditions that cause asynchrony between uterus and embryo (Ashworth et al., 1984). This results in an increase in embryo mortality and a decline in conception rates.

The mode of action causing this seasonal effect on progesterone secretion and subsequent reproductive performance is believed to depend upon changes in the sensitivity of the hypothalamus to oestradiol which affects subsequent LH secretion (see review by Karsch et al., 1980). These observed differences in progesterone levels may therefore reflect changes in this feedback relationship.

In the cow, seasonal differences in progesterone concentrations also exist. During the summer months, the progesterone concentrations between Days 4 to 15 of the oestrous cycle have been reported to be lower than that of the same portion of the cycle during the winter months (Rosenberg et al., 1977). These lower levels of progesterone in summer correspond to reduced conception rates in the summer versus the winter months. Rosenburg et al. (1977) suggest that this is due to slower CL development during the summer than in the winter. McNatty et al. (1984) reported that the weight of the CL tissue in bovine ovaries was significantly lower in spring than in the autumn-winter period. This difference in CL weight corresponded to a higher mean plasma progesterone concentration from heavier CLs than from the lighter ones. It is reasonable to conclude that seasonal differences in the growth of the preovulatory follicle and size of the CL are a direct consequence of seasonal differences in gonadotrophin secretion.

6.3 Stress:

Stress is another factor that has been shown to influence the embryo survival rate both in sheep and cattle through an association with progesterone concentrations. Much of this stress has an adverse effect on embryo mortality by increasing the activity of the adrenal gland. Treatment with adrenocorticotrophin (ACTH) during the first few weeks of pregnancy in both the ewe and the cow tended to reduce fertility by increasing embyronic mortality. The ACTH treatment mimicks the effect of stress, decreasing embryo survival through increased adrenal gland activity which disturbs subsequent luteal function (Doney et al., 1976; Stoebel & Moberg, 1982). Doney et al. (1976) suggests that the pattern of the ovulatory surge of LH is influenced by both stress and ACTH injection and this could upset oocyte maturation or subsequent luteal function. Cattle injected with ACTH have inhibited progesterone synthesis from the CL, while the

adrenal gland is provoked into releasing more progesterone (Wagner et al., 1972). These results indicate that adrenal hyperactivity (caused by stress-related conditions) is involved in the mechanisms responsible for reduced embryonic survival through altering progesterone secretion.

6.4 Nutrition:

The level of nutrition has been shown to influence the progesterone concentration in the ewe. Cumming et al. (1971) reported that recently mated ewes fed a low level diet, 0.25 maintenance (M), after joining had a significantly higher plasma progesterone concentration than did the better fed ewes on either a 1.0 M or 2.0 M diet. It was concluded that an inverse relationship exists between nutritional level and progesterone concentration within the oestrous cycle of the ewe (Williams & Cumming, 1982). Similar results have been reported by Rhind et al. (1985) who found that progesterone levels were higher in ewes on a low intake after mating compared with those on a high intake. This relationship is of particular significance since undernutrition of ewes in early pregnancy was originally thought to be linked to an increased incidence of embryonic mortality (Edey, 1966).

Parr et al. (1982) showed that when progesterone supplemented ovariectomized ewes were fed differing levels of nutrition during the first three weeks of pregnancy, the underfed (0.25 M) ewes tended to have elevated peripheral progesterone levels compared to the well-fed (1.0 M) ewes. There appeared to be no difference in the incidence of early embryonic mortality between the two feeding levels, however embryos from the undernourished ewes were smaller and less developed than their counterparts from the well-fed ewes when viewed at Day 21 of gestation. This embryonic retardation emphasizes the importance of proper nutrition in early pregnancy.

Overfeeding during early pregnancy in ewes has been shown to reduce the concentration of peripheral progesterone which subsequently has a detrimental effect on the resulting conception rate. Diets in the order of 1.5 M (McKelvey & Robinson, 1986) and 2.0 M (Parr et al., 1987a) resulted in ewes having significantly lower plasma progesterone concentrations and reduced conception rates

than did sub-maintenance level diets of either 0.5 M or 0.25 M respectively. The effects of body condition at mating or the time of embryo transfer appeared to have little effect on the level of embryonic mortality, whereas the level of feeding at the time of embryo transfer and mating had a pronounced effect on the embryo mortality levels ie. ewes receiving the low (0.5 M) compared to the high (1.5 M) level of feeding tended to have significantly less embryonic loss (McKelvey & Robinson, 1986).

These findings have led to re-evaluation of earlier feeding practices which emphasized the importance of a high plane of nutrition, particularly at the time of mating. Good feeding is still essential, however extremes in nutrition can have detrimental consequences. The high levels of feeding, as already mentioned, can decrease peripheral progesterone concentrations and increase embryo losses. The other extreme is undernutrition, which increases progesterone levels and consequently embryonic survival. Cumming et al. (1975) found ewes fed a 1.0 M diet had a higher level of embryo survival than did ewes fed either a 0.25 M or 2.0 M diet. Extended periods of undernutrition however, can cause ewes to have smaller and less developed embryos resulting in both lighter lambs at birth and lower litter sizes (Parr et al., 1982; Robinson, 1986). If developmental events are adversely influenced by maternal undernutrition, then an insidious loss in the form of reduced production throughout adult life could occur (Parr et al., 1982). It is therefore recommended by Robinson (1983) to keep the ewes on maintenance levels of feeding, especially during the first month of pregnancy.

There are few studies which deal specifically with the effects of plane-of-nutrition on embryo survival in the cow, particularly those investigating post-mating feeding levels. However, Lamond (1970) has suggested that the sheep could be used as a convenient model for testing how nutrition influences reproduction. For a comprehensive study looking at the nutritional and hormonal interrelationships in beef cattle and their effects on reproductive performance, see the review by Short & Adams (1988). As well, Gauthier et al. (1984) have reviewed the effects of undernutrition on fertility in ruminants.

7 Physiological basis for nutritional effects on progesterone levels:

The physiological mechanisms responsible for controlling the concentrations of peripheral progesterone in ewes has been an area of interest for some time. Cumming et al. (1971) suggested that the changes in plasma progesterone concentrations observed from nutritional trial work involving sheep were due to either altered metabolic clearance rates (MCR) of progesterone from the body, mobilization of stores of progesterone, or through changes in progesterone secretion rates. Parr et al. (1982) suggested that secretion rates of progesterone are unlikely to be the cause of altered plasma progesterone levels. This was concluded from ovariectomized ewes, where the major endogenous source of progesterone (CL) was replaced with a controlled supply of exogenous progesterone. In these animals, increased plasma progesterone concentrations still occured in the nutritionally-restricted ewes which may have been due to an effect on progesterone catabolism.

The rate of portal blood flow to the liver of ewes was shown to increase as a direct result of feeding, while the fasted ruminant had a reduced blood flow rate (Bensadoun & Reid, 1962). As well, the liver appears to be one of the major sites of progesterone catabolism even though extra-splanchnic organs are also partly involved in progesterone catabolism (Bedford et al., 1974). Recent work by Smith et al. (1986) and Payne et al. (1987) indicate that high feed intake and body weight are associated with an increase in the clearance rate of ovarian steroids from the circulation. These findings tend to support the concept that it is changes in the MCR, rather than changes in the blood production rate (BPR) of peripheral progesterone, which are responsible for differing progesterone concentrations in nutritionally treated ewes.

Parr et al. (1987b) has confimed that increased MCRs of progesterone brought on by high levels of nutrition after mating, reduce peripheral progesterone levels and have been associated with an increased incidence of early embryonic mortality in ewes. It would appear that unless compensatory changes in secreation rate from the ovary occur, then the progesterone levels in some overfed ewes would be altered, resulting in some animals having peripheral progesterone concentrations below the threshold necessary for embryo survival.

8 Effects of post-mating progesterone supplementation on early embryonic mortality:

In order for the establishment and maintenance of pregnancy to occur, a functional CL is essential for the consequent cessation of oestrous cycles. It has been postulated that early embryonic death in a number of species of domestic animals may be due to a deficiency of progesterone (Moore et al., 1960). This deficiency of progesterone is thought to occur during the luteal phase of the oestrous cycle following breeding when a normal CL should be functioning. The rationale for the supplementation of progesterone is to create an environment which allows the embryo to survive to a stage where its effects (antiluteolytic and/or luteotrophic) however mediated, are strong enough to maintain a functional CL (Sreenan & Diskin, 1983; Diskin & Sreenan, 1986). Many of the early studies on conception rate failure, particularly in the cow, attempted to determine if luteal dysfunction was actually the cause of reduced conception rates. Several reports have also looked at the effects of treatment with supplemented progesterone on the conception rates in sub-fertile or repeat breeder cattle and these investigations are indicated in the following tables.

Attempts have been made to supplement progesterone directly, such as in the form of oral administration, injections, implants, sponges and controlled releasing devices. Others have used indirect methods (or luteal stimulation) where certain antiluteolytic or 'luteo-protective' substances have been administered to increase or maintain luteal function. Both direct and indirect treatments have been carried out at varying times following mating in order to increase conception rates. Most of the treatments have been given at the time of initiation or during the luteal phase of the oestrous cycle. Some of the treatments have also been given at mating or immediately after mating. One or more treatments may be involved.

8.1 Direct effects of progesterone supplementation on embryonic survival:

Table 1 summarizes the findings of various studies of direct progesterone supplementation in cows and their effects on increasing the pregnancy rates through increased embryonic survival rates.

Table 1: Summary of studies involving progesterone supplementation in cattle and their effect on pregnancy rate.

Author	Treatment		Pregnancy Rate Cntrl (n)	Pregnancy Rate Trmt (n)	Change (%)
Herrick (1953) [Repeat Breeders]	500mg Repositol Prog. D[A]0 (at service)		5% (1/20)	35% (7/20)	+30%
Dawson (1954) [Repeat Breeders]	100mg Prog. D4-5		17% (3/18)	47% (22/47)	+30%
Wiltbank et al. (1956) [Repeat Breeders]	50mg Prog. D3 to D35		33% (12/36)	44% (16/36)	+11%
[Repeat Breeders]	200mg Prog. D3 to D34		26% (8/31)	39% (12/31)	+13%
Johnson et al. (1958)	500mg Prog. D2,3,4,6,9		42% (29/69)	70% (49/70)	+28%
Sreenan et al. (1979)	100mg Prog. D10 to D20		52% (13/25)	69% (18/26)	+17%
Marcus & Ayalon (1981)	250mg Prog. Sponges D6-7 to D17-18		35% (15/43)	58% (18/31)	+23%
Folman et al. (1983)	PRID[B] D14 to D26	Exp 1	70% (28/40)	82% (31/38)	+12%
		Exp 2	70% (21/30)	73% (22/30)	+ 3%
Sreenan & Diskin (1983)	100mg Prog. D10 to D20	Yr 1	67% (19/28)	79% (19/24)	+12%
		Yr 2	63% (22/35)	68% (21/31)	+ 5%
		Yr 3	65% (24/37)	64% (21/33)	- 1%
		Yr 4	57% (24/42)	55% (23/42)	- 2%
		Total	63% (89/142)	65% (84/130)	+ 2%
Diskin & Sreenan (1986)	100mg Prog. D5 to D35		45% (9/20)	74% (14/19)	+29%
Macmillan & Taufa (1987)	CIDR (12%) D14 to 20		62% (293/472)	76% (92/121)	+14%

A indicates day of oestrous cycle, where D 0 = oestrus.
B Progesterone Releasing Intravaginal Device.

From Table 1 it appears that a trend exists whereby progesterone supplementation increases the pregnancy rates of cows. This is particularly noticeable in the repeat breeder cattle as indicated in the table [See Herrick (1953), Dawson (1954), Wiltbank et al. (1956) and Johnson et al. (1958).]

In sheep the direct supplementation of exogenous progesterone has been used by some workers in an attempt to improve embryo survival rates. Table 2 summarizes the findings of these experiments.

The experiment of Parr et al. (1987a) investigated the effects of post-mating progesterone supplementation on the conception rates of ewes fed three different levels of diet during the first two weeks following mating. When post-mating controlled internal drug releasers (CIDRs) containing progesterone were inserted in some ewes in each of the three feeding levels from Days 8-14 post-mating, a significant increase in the pregnancy rate was seen only in the ewes fed the high ration (2.0 M). The exogenous progesterone supplementation significantly increased the pregnancy rate from 48% (control) to 76% (CIDR-treated). This significant increase in the pregnancy rate for the CIDR-treated ewes on the high feeding level was associated with a dramatic rise in the peripheral progesterone concentration when examined at Day 12 post-mating. Nutritional levels and exogenous progesterone supplementation significantly affected Day 12 plasma progesterone concentrations in sheep, although no interaction was found to exist between these two factors (Parr et al., 1987a).

Table 2: Summary of studies investigating post-mating progesterone supplementation in sheep.

Author	Treatment	Results n	Cntrl	Trmt	Sign	Parameter	Comments
Pearce et al. (1984)	Cronolone (FGA) Sponges D[A]10-13 to D24-27	664	34%	45%	**	Pregnancy Rate	Artificial insemination at a synchronized oestrus and PMSG treatment also used.
			46%	75%	?	Lambing Rate	
Peterson et al. (1984)	CIDR (9%) D8 to D14	220 #1	29%	64%	*	Pregnancy Rate	Natural mating at a synchronized oestrus.
		207 #2	67%	95%	***	Lambing Percentage	
Smith et al. (1985)	CIDR (12%) D10 to D16	450	77%	76%	NS	Lambing Rate	Natural mating on second cycle following synchronized oestrus.
Davis et al. (1986)	CIDR (9%) D8 to D14	283	57%	83%	*	Foetus/Ewe Joined	Artificial insemination at a synchronized oestrus.
			105%	125%	*	Foetus/Ewe Pregnant	
Kerton et al. (1986)	Cronolone (FGA) Sponges D8 to D14	120	50%	50%	NS	Non-Return Rate	Artificial insemination at a synchronized oestrus and PMSG treatment also used.
McMillan (1987)	CIDR (12%) D7-9 to D12-14	74[B]	56%	79%	*	Lambing to 1st Serv	Synchronized oestrus and natural mating for both ewes and hoggets.
			34%	67%	*	NPM/NP[D]	
		140[C]	35%	54%	*	NPM/NP	
Parr et al. (1987)	CIDR (340 mg) D8 to D14 +3 Feed Levels[E] D2 to D14	330 H	48%	76%	*	Ewes Pregnant	Naturally mating at a synchronized oestrus. Nutritional treatments imposed for 2 weeks following mating.
		M	68%	65%	NS	Ewes Pregnant	
		L	67%	60%	NS	Ewes Pregnant	
Murray et al. (1988)	CIDR (9%) D5 to D10, D10 to D15 & D5 to D15	200	79%	100%	NS	Conception Rate	Synchronized mating followed by androstenedione immunization (Fecundin) or injection of 750 IU PMSG at sponge removal.
			1.95	2.32	NS	Litter Size	

A Day of Cycle (Day 0 = Oestrus)
B Hoggets
C Ewes
D Number pregnant with multiple/number pregnant.
E Diets were High (2.0 M), Medium (1.0 M) & Low (0.25 M).

The exogenous progesterone administration in cattle increased the circulating plasma progesterone concentrations, however the increases were not significantly different when compared to the controls (Sreenan et al., 1979). The administration of supplemental progesterone (100 mg/day) to beef heifers from Day 10 to Day 20 post-mating, decreased the weight of luteal tissue, but did not affect plasma progesterone level over this period (Sreenan & Diskin, 1983).

The effect of exogenous progesterone supplementation during the luteal phase of the cycle following mating in both the cow and the ewe tends to increase conception rates, although the results are not statistically significant. The use of progesterone supplementation in repeat breeders therefore may be of some benefit. Sreenan & Diskin (1983) suggest however that sub-fertility problems of greater magnitude are likely to be associated with repeat breeders, rather than simply a deficiency of luteal progesterone. In the case of a nutritionally induced progesterone deficiency, caused by excessive feeding soon after mating, progesterone supplementation is beneficial, as shown by Parr et al. (1987a). This was only evident where the levels of nutrition exceeded twice the normal maintenance requirements. The effect of the progesterone supplementation in this case significantly improved the embryo survival rates.

8.2 The indirect effects of luteal stimulation on embryonic survival:

Indirect supplementation of progesterone can be given in the form of substances such as human chorionic gonadotrophin (hCG,, gonadotrophin releasing hormone (GnRH), melatonin, luteinizing hormone (LH) and prolactin, which cause luteal stimulation and/or result in accessory CL formation. These have been shown to increase peripheral progesterone concentrations during the mid-luteal phase of the oestrous cycle in both the cow and the ewe. In some situations these treatments have tended to result in an improvement of the females reproductive performance.

8.2.1 HCG treatment:

Treatment with hCG has occasionally been used as an alternative to progesterone supplementation. These placental gonadotrophins (hCG) are luteotrophic in the bovine and have therefore been used to stimulate luteal function in the early post-insemination period (Holness et al., 1982). HCG is administered during the early or mid-luteal phases as a luteotrophic substance. This treatment has also been used at around the time of luteolysis to supplement the action of a possible inadequate luteotrophic or antiluteolytic signal from the developing embryo. This hCG treatment might therefore allow the establishment of pregnancy (Diskin & Sreenan, 1986).

Table 3 summarizes the findings of several experiments involving hCG treatment during the luteal phase of the cycle following mating. The majority of this research has investigated the effects of hCG therapy on the conception rates in cattle, including those with sub-optimal reproductive performance (Holness et al., 1982).

Table 3: Summary of studies involving human chorionic gonadotrophin (hCG) treatment in cattle and its effect on pregnancy rates.

Author	Treatment	Pregnancy Rate Cntrl (n)	Pregnancy Rate +hCG (n)	Change (%)
Wiltbank et al. (1961)	1000 IU hCG D15 to D35	63% (26/41)	69% (27/39)	+ 6%
Wagner et al. (1973)	1000 IU hCG D3	50% (18/36)	61% (22/36)	+11%
	2000 IU hCG D3	55% (18/33)	64% (21/33)	+ 9%
Sreenan et al. (1979)	1500 IU hCG D10 to D20	52% (13/25)	65% (17/26)	+13%
Greve & Lehn-Jensen (1982)	1500 IU hCG D13 to D35	75% (44/59)	81% (26/32)	+ 6%
Holness et al. (1982) [Repeat Breeders]	1000 IU hCG D4 to D19	33% (6/18)	41% (9/22)	+ 8%
Santos-Valadez et al. (1982)	5000 IU hCG D15	56% (64/114)	67% (76/114)	+11%
Sreenan & Diskin (1983)	1500 IU hCG D10 to D20	52% (13/25)	65% (17/26)	+13%
	1500 IU hCG D10 to D20	67% (19/28)	57% (13/23)	-10%
	1500 IU hCG D5 to D35	45% (9/20)	56% (9/16)	+11%
Helmer & Britt (1986)	5000 IU hCG D3	66% (34/52)	60% (26/44)	- 6%

Treatment with hCG in cattle produces a small improvement in conception rates, although these increases in reproductive performances have not proven to be significant. These trends with hCG treatment are comparable to the results of progesterone supplementation in cattle, as seen earlier in Table 1.

Kittok et al. (1983) investigated the use of 100 IU of hCG administered on Days 11, 12 and 13 post-mating in seasonally anoestrous, lactating ewes after an artificially induced oestrus.

Treatment with hCG was found to significantly increase the serum plasma progesterone concentrations in the treated ewes. This resulted in a beneficial effect of hCG administration on increasing the conception rate of the treated ewes (58%) compared to the control animals (29%).

It has been reported that administration of hCG in cattle during the first week after oestrus resulted in the formation of larger CLs that contained a greater quantity of progesterone (Veenhuizen et al., 1972). Many studies report significant increases in circulating levels of plasma progesterone following hCG treatment (Sreenan et al., 1979; Greve & Lehn-Jensen, 1982; Holness et al., 1982; Santos-Valadez et al., 1982; Helmer & Britt, 1986). The induction of accessory CLs may be partly responsible for these increases in progesterone concentration (Sreenan et al., 1979; Greve & Lehn-Jensen, 1982). Christie et al. (1979) indicate that a high incidence of accessory CL formation was not found in their work. There is however evidence to suggest that increased synthesis from the current CL also occurs (Santos-Valadez et al., 1982).

The effect of the hCG treatment influences pregnancy rate, either directly or indirectly through an increased production of progesterone. Increased progesterone production could be implicated since the elevation in the serum levels of progesterone occurred during a phase of the oestrous cycle known to be critical for progesterone support (Moor & Rowson, 1966). Administration of hCG may also increase pregnancy rates through supplementing the action of an inadequate luteotrophic or antiluteotrophic signal produced by the developing embryo and therefore allowing more time for its establishment in the uterus (Christie et al., 1979).

8.2.2 GnRH treatment:

The administration of GnRH is another method that has been used to increase conception rates by reducing early embryonic mortality. Tables 4 & 5 summarize the results of work using GnRH treatment in both cattle and sheep. Treatment has been given at the time of mating and after mating. GnRH therapy has also been used to increase fertility in repeat-breeder cows as indicated in Table 4.

Table 4: Summary of studies involving the effects of a single injection of gonadotrophin releasing hormone (GnRH) on pregnancy rate in cows.

Author	Treatment	Pregnancy Rate		Change (%)
		Cntrl (n)	+GnRH (n)	
Lee et al. (1983)	100 ug GnRH at AI	32% (22/69)	48% (31/64)	+16%
[Repeat Breeders]	100 ug GnRH at AI	48% (77/161)	73% (135/185)	+25%
Aboul-Ela & El-Keraby (1986)	100 ug GnRH at AI	55% (17/31)	81% (26/32)	+26%
Macmillan & Taufa (1983)	10 mcg GnRH D7 to D10	64% (53/83)	77% (74/96)	+13%
	10 mcg GnRH D11 to D13	66% (107/161)	75% (107/142)	+ 9%
Macmillan et al. (1986)	10 ug GnRH D11 to D13	61% (168/276)	72% (163/225)	+11%
Phatak et al. (1986) [Repeat Breeders]	111 ug GnRH at AI	38% (177/469)	47% (231/492)	+ 9%

Table 5: Effects of a single injection of gonadotrophin releasing hormone (GnRH) on lambing performance in sheep. (Results summarized from the studies of McMillan et al., 1986).

Age Group	Treatment	Percentage Lambing To First Mating		Change (%)
		Control (n)	Treatment (n)	
Ewes	4 ug GnRH D 11	54% (64/119)	55% (47/85)	+ 1%
Ewes	4 ug GnRH D 12	48% (38/79)	60% (43/71)	+12%
Ewes	4 ug GnRH D 13	48% (32/67)	52% (33/64)	+ 4%
Hoggets	4 ug GnRH D 12	30% (15/49)	53% (26/49)	+23%
Hoggets	4 ug GnRH D 13	48% (45/94)	52% (49/94)	+ 4%

The exact mode of action that GnRH has in increasing fertility is not known. Administration of GnRH has shown to cause dose-related increases in serum concentrations of LH in cattle (Schams et al., 1974; Fernandes et al., 1978; Macmillan et al., 1985b). When GnRH treatment is given at the time of mating (presumably between the endogenous surge of LH and ovulation) it is believed to exert its effect by causing a surge of LH which enhances ovulation and prevents delayed ovum release (Fielden & Moller, 1983; Aboul-Ela & El-Keraby, 1986). It is also possible that the additional surge of LH enhances active luteinization of granulosa cells to ensure adequate progesterone production to maintain pregnancy when successful fertilization occurs (Lee et al., 1983).

The action of GnRH administered during the mid-luteal phase of the oestrous cycle after mating is thought to stimulate the function of the CL either directly or indirectly (Macmillan et al., 1985a; McMillan et al., 1986). Administration of GnRH has been shown to increase the length of the oestrous cycle through prolonging the lifespan of the CL, and therefore progesterone production. This increases the probability that maternal recognition of the presence of a developing embryo will occur (Macmillan & Taufa, 1983; Macmillan

et al., 1985a, 1985b & 1986). The injection of GnRH during dioestrus in cows has also been shown to influence CL and progesterone synthesis, but according to Macmillan et al. (1985a), the precise nature of the drugs action either directly on CL or indirectly through an induced release of gonadotrophins has not been defined.

Low-dose GnRH treatment may produce an effect which modifies the induced luteolysis (Macmillan et al., 1985b). Alternatively, GnRH treatment may induce, via LH, a luteoprotective effect (McMillan et al., 1986). The response on Day 12 is consistent with a luteoprotective effect since the presence of an embryo on Day 12 or Day 13 is necessary for normal CL function in the ewe (Moor & Rowson, 1966).

8.2.3 Melatonin treatment:

Wallace et al. (1988) recently investigated the effects of melatonin treatment on the enhancement of progesterone production and subsequent establishment of pregnancy and embryo survival in seasonally anoestrous ewes. Administration of melatonin, at physiological levels, has been shown to stimulate progesterone production in bovine and human granulosa cell cultures (Webley & Luck, 1986). Exposure to melatonin has also been shown to stimulate progesterone production by the CL in an in vivo perfusion system in the primate (Webley & Hearn, 1987).

Wallace et al. (1988) artificially inseminated ewes and administered melatonin on a daily basis (3 mg/day) for the entire oestrous cycle following AI. When the naturally-ovulating control and induced ewes were compared the results suggested a luteotrophic role for melatonin. The prolonged exposure to melatonin in the treated ewes was associated with higher progesterone concentrations in the luteal phase of the cycle. These changes in plasma progesterone secretions were not accompanied by clear-cut improvements in the conception rate or embryo survival. These findings caused Wallace et al. (1988) to re-evaluate their hypothesis that there is a direct and immediate effect of melatonin on the CL.

9 Purpose and scope of the investigation:

For the successful establishment of pregnancy, the influence of progesterone in adequate amounts is essential. The main source of progesterone secretion at this time is from the CL. In situations where there is inadequate luteal function caused by several factors, post-mating supplementation with progesterone has been shown in some studies to improve the reproductive performance of ewes through increased conception, pregnancy and lambing rates. Without this treatment it is possible that the animal fails to conceive or she may experience an increase in the incidence of early embryonic loss.

Of the several factors affecting progesterone concentration, the nutritional level has been found to be inversely related to the progesterone concentration. Although there has been some research examining this relationship, much of this work has investigated extreme levels of nutrition that would not normally be encountered under normal grazing conditions at mating on New Zealand farms. Most of these reports have come from indoor feeding trials or from ewes fed under feedlot conditions, where preformulated rations have been fed.

The present trial was conducted to examine if post-mating progesterone supplementation (CIDR treatment) has any effect on increasing reproductive performance by reducing the amount of early embryonic mortality. Nutritional intakes which were realistically obtainable under grazing conditions at mating, were used to investigate effects on reproductive performance of ewes, as reflected through altered progesterone levels. In order to accurately assess the progesterone concentrations in the ewes, a randomly selected portion of the flock was blood sampled over the luteal phase of the oestrous cycle following mating to determine the actual hormonal levels present.

To accurately determine the amount of herbage consumed by the ewes during both the flushing period and the two post-mating pasture allowance levels, intraruminal chromium controlled releasing devices (CRDs) were orally administered to a representative portion of the ewes. Treatments of differing pasture sward heights were prepared and offered to the ewes. By controlling the height of swards and hence

the appropriate residual (in terms of kg DM/ha), it was possible to offer the ewes differing amounts of pasture allowance during the experimental period. Faecal as well as pasture samples were collected for the indirect estimation of herbage intake and therefore approximate feeding levels could be determined.

A single flock of sheep, comprised of both two-tooth and mixed age ewes were fed according to standard New Zealand farming conditions where the pasture allowance is gradually increased prior to and during the mating season. The 'flushing' of the ewes was done at this time since increasing ewe intakes is considered to increase the lambing percentage. This is caused primarily through an increase in the OR. Following a synchronized natural mating, the ewes were randomly divided into a 1.0 M (low) level and a 1.8 M (ad libitum or high) level of pasture allowance for a two week period of differential grazing. Ovulation rates of the ewes were determined and blood samples were collected for determination of progesterone concentration.

The overall objective of this experiment was to determine if post-mating CIDR supplementation was effective in improving reproductive performance, by reducing the amount of early embryonic mortality, in ewes grazed on two different pasture allowance levels following mating. Any increase in embryonic survival would be seen in higher conception, pregnancy and lambing rates, which would eventually result in a greater number of lambs at birth.

CHAPTER III: MATERIAL AND METHODS

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1 Experimental design:

The experiment was designed as a 2^2 factorial, involving two levels of post-mating feed allowance (high and low), with and without progesterone supplementation. Two other variables were taken into consideration but were not included as treatment effects when the trial was carried out. These were the effects of two age groups of animals (1.5 year old maiden ewes or two-tooths, compared to mixed-age or mature ewes) and two levels of OR (single versus multiple).

Figure 2 gives an general overview of the experimental design and its relative time schedule. This diagram also illustrates the sections of the experiment investigating voluntary herbage intakes, as well as blood sampling for progesterone concentration determination.

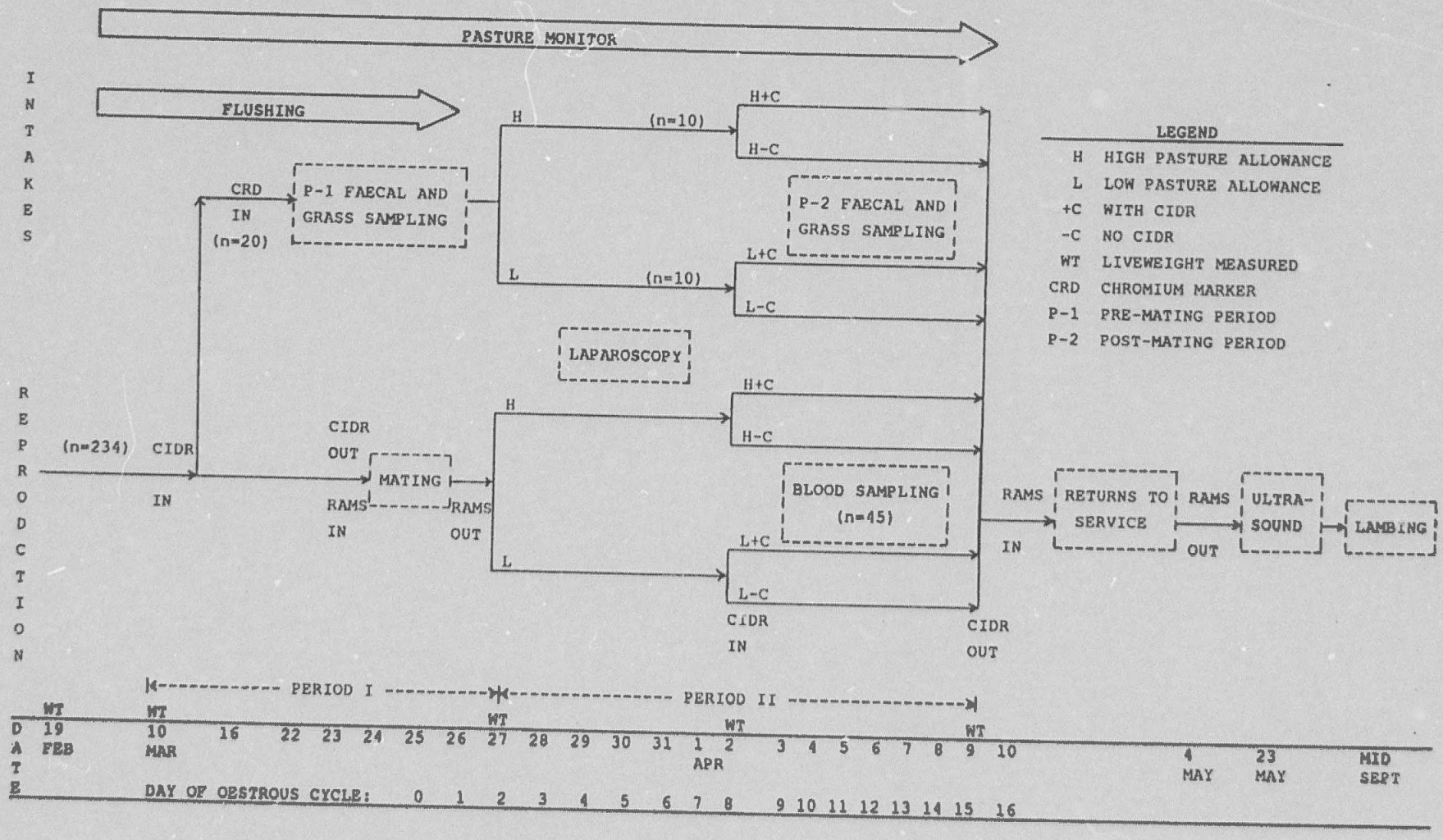

Figure 2: General experimental plan and calendar of events.

2 Animals:

Two hundred and thirty four Border Leicester X Romney first cross ewes were used in this trial. They comprised 104 maiden two-tooth ewes and 130 mixed-age ewes (up to 8 years) that had previously lambed at least once.

The two-tooths ewes were shorn in early February 1988, and the mixed-age ewes the previous November.

All ewes were grazed together from the middle of February 1988 (and flushing of the ewes began about the beginning of March 1988) with increasing amounts of pasture allowance being offered to the ewes so as to provide them with a rising plane of nutrition prior to joining. When it necessitated that the animals be randomly divided into the various treatment groups, an attempt was made to balance for both age and OR, where it was practical to do so.

2.1 Weighing of animals:

The animals were weighed prior to and during the experiment. The animals were weighed directly off pasture without any period of fasting. This meant that some of the liveweight changes may reflect differences in gut-fill, rather than actual differences in weight of body tissues.

2.2 Induction of oestrus and mating:

To induce synchronization of oestrus, all ewes had a Type G controlled internal drug releaser (CIDR) (AHI Plastic Moulding Co., Hamilton, New Zealand) containing 9% progesterone vaginally inserted on 10 March 1988 and removed 13 days later. A total of 18 entire two-tooth Romney rams were introduced to the ewes the day that the synchronizing CIDRs were removed (23 March 1988). This ram:ewe ratio of 1:13 was used so as to provide enough rams to adequately serve the ewes naturally as they came into oestrus. Each ram was individually fitted with a sire sine marking harness.

Tupping marks were observed every 12 hours (06:00 and 18:00 h) over a three day mating period. At the 18:00 h evening observation,

all ewes were yarded and rams removed while the marked ewes were recorded. Any ewes not mated within three days following CIDR removal were drafted from the main mob and removed from the trial.

3 Pasture characteristics and herbage mass:

3.1 Experimental site:

The experiment was conducted at Massey Universitys Sheep and Beef Cattle Research Unit (SBCRU) Haurongo farm, located approximately 3 km west of the Massey campus. The treatment pastures were established on gently rolling farmland, overlaying a mainly Tokomaru Silt Loam soil.

Nine paddocks were randomly allocated between feeding level treatments (low compared to high) to minimize any variation in pasture type between paddocks (see Table 6).

Table 6: Paddock areas, grazing treatments and mean pasture heights.

Paddock Number	Size (ha)	Treatment	Mean Pasture Height[A] (cm)
1	1.0	Low	3.0 - 5.0
2	1.2	Low	4.5 - 6.5
3	1.2	High	7.0 - 9.0
5	1.1	Low	3.0 - 5.0
6	2.0	High	6.5 - 8.5
7	1.3	High	6.0 - 8.0
11	0.2	Low	3.0 - 4.5
12	0.6	High	7.5 - 9.5
13	1.0	High	7.5 - 9.5

[A] Average pasture heights prior to the start of the grazing are given as ranges.

3.2 Determination of pasture cover:

During the early part of March 1988 the paddocks were monitored by taking 50 pasture height readings with an Ellinbank Pasture Meter (EPM) 3 to 4 times per week to assess changes in pasture growth rates. Herbage mass was determined from the paddocks by cutting several random quadrats (0.18 m^2) to ground level with an electric shearing handpiece on 13 March and 1 April. Quadrat sites were selected within paddocks to represent the entire area. Samples were washed to remove soil and oven dried at 80°C for at least 24 hours to determine dry matter (DM) yields. Quadrat herbage yields were related to EPM height readings by regression analysis. Residual pasture mass in each of the paddocks was estimated using the calibration equation.

3.3 Herbage pasture composition and dry matter determination:

Herbage samples were randomly obtained from each of the nine experimental paddocks on 7 April by clipping representative areas of the individual paddocks with handshears. Individual paddock samples were thoroughly mixed before being bulked on a within paddocks basis. A representative sub-sample for each paddock was then sorted into both green and dead material. The green material was further divided into grass, clover and weed components. Fresh weights were obtained prior to the samples being oven dried at 80°C for 24-36 h. After moisture removal, the individual components were then expressed as a proportion of the total dry weight of the sample.

3.4 Pasture rotation:

During the flushing period prior to mating, the experimental animals were used to graze the paddocks to obtain the required sward heights. During this three week period the animals were fed at a flushing level of nutrition (approximately 1.2 to 1.5 M). The ewes were grazed as one group to minimize possible pre-treatment feeding effects. Ewes were shifted between paddocks when the appropriate sward levels (high or low) were reached.

For the post-mating differential feeding period, the ewes were randomly allocated to the high or low feeding groups on Day 2 following the recording of oestrus (where Oestrus = Day 0). To avoid

any paddock interactions, the high and low groups of ewes were grazed together in their groups for the first week of the feeding treatment (Days 2-8 post-mating). The ewes in each feeding group were randomly split into three equal-sized groups for the second week of the differential feeding period. At this time each group was balanced for age and OR.

During the second week following mating, ewes with the chromium controlled release devices (CRDs) (10 per treatment) were grazed with the animals that were randomly selected for the blood sampling part of the experiment. Table 7 shows the paddock rotation that was used during this second week of differential feeding. These 'marker' ewes were run as separate mobs during this time to minimize any stress on the other ewes which might be associated with twice daily handling for sample collection.

Table 7: Pasture rotation used for the post-mating blood sampling and faecal collection during P-2 (Days 9-14).

Feeding Level	Group	n	Days 9-10 (3-4 Apr.88)	Days 11-12 (5-6 Apr.88)	Days 13-14 (7-8 Apr.88)
Low	L-1[A]	36	1	2	5
Pasture	L-2	37	2	5	1
Allowance	L-3	37	5	1	2
High	H-1[A]	37	7	3	13
Pasture	H-2	37	3	13	7
Allowance	H-3	38	13	7	3

[A] Groups containing the randomly selected marker ewes both for blood sampling and faecal collection.

4 Measurements of feed intakes:

4.1 Estimation of voluntary intake:

Voluntary herbage intakes of 20 ewes (10 two-tooths and 10 mixed-age), representative of the flock were estimated using an indigestible marker technique which provides for the continuous slow release of chromium into the rumen (Laby, 1978). Faecal output was calculated from the chromium concentration in faecal samples obtained per rectum. Feed intakes were derived indirectly using an estimate of herbage digestibility using the following formula:

$$DOMI = \frac{\text{Weight of Faeces (FO)}}{(100 - \text{digestibility of herbage grazed})} \times \frac{100}{1}$$

where: DOMI = digestible organic matter intake
FO = faecal output

$$\text{and } FO = \frac{\text{Mean CRD chromium release rate (mg/d)}}{\text{Adjusted atomic absorption reading (mg/g DM)}}$$

(Geenty & Rattray, 1987)

By using this technique, it was possible to indirectly estimate herbage intake of sheep grazing under field conditions.

Intakes were estimated during the pre-mating or flushing period (P-1) for five days, when all the ewes were on the same level of nutrition, and also during the two week post-mating period of differential feeding (P-2). The chromium group of 20 ewes were each drenched with a single intraruminal CRD (3.0 cm core, 65% chromium matrix, 9.00 mm orifice - Captec Pty Ltd., Laverton, Victoria, Australia) on 16 March 1988. These CRDs are also known as chromic oxide sheep capsules. After a five day adjustment period to allow chromium to reach a constant level in the faeces, daily samples were obtained by rectal grab sampling (at 10:00 h) over the five day period (P-1, 22-26 March inclusive). At least 5 gm of wet faecal material was collected from each animal and put into individual containers. The faecal samples were oven dried at 80°C for 36 to 48 h.

During the second collection period (P-2), five two-tooths and five mixed-age CRD treated ewes were randomly allocated to each pasture allowance group. The two pasture allowances were offered from Day 2 to 15 post-mating, (Day 0 = oestrus), but feed intakes were only estimated for the final six days (Days 9-14 inclusive). This ensured that ewe rumen conditions and hence faecal output had reached an equilibrium level for each of the grazing allowances. Any ewes not providing an adequate amount of faecal material at the initial collection were sampled again during the same day. The new faecal collection time was recorded.

Faecal collections were programmed with the daily blood sampling during this post-mating feeding period (P-2), as shown in Table 8.

Table 8: Schedule used for the daily collection of faecal collections and blood sampling during P-2.

Date	Day of oestrous cycle	Time (h)	
		AM	PM
3 Apr 88	9	12:00[A]	20:00
4 Apr 88	10	04:00	18:00[A]
5 Apr 88	11	02:00	22:00[A]
6 Apr 88	12	06:00	14:00[A]
7 Apr 88	13	08:00[A]	24:00
8 Apr 88	14	10:00[A]	16:00

[A] indicates the daily collection of faecal samples.

4.2 Sampling of the grazed sward for <u>in vitro</u> digestibility determinations:

To assess the <u>in vitro</u> digestibilities of the pasture consumed by the ewes during P-1 and P-2, hand plucked samples that characterized the herbage that the ewes were grazing were obtained from each sward. These samples were collected on the same days as faecal sampling and stored at -20°C.

The herbage samples from P-1 and P-2 were freeze dried for four days until all the moisture was removed. The daily samples were then ground through a 1 mm mesh and approximately 20 g of each thoroughly mixed sample was drawn and bulked across pasture allowance levels. This resulted in three pooled samples representing each of the grazing allowances (flushing, high and low post-mating). The *in vitro* digestibility values were then determined from these pooled samples.

4.3 Determinations of *in vitro* digestibility:

Laboratory determination of *in vitro* digestibility of the herbage samples is adapted from the method of Roughan & Holland (1977). Briefly, the procedure involved comparing treatment samples with six standards of known *in vivo* digestibility collected from indoor sheep feeding trials. Known amounts of the freeze dried, ground samples and standards were first extracted with a hot neutral detergent solution to remove soluble cell contents. They were then washed once in hot distilled water and twice in cold distilled water. Cell material was then hydrolyzed with fungal cellulase solution (derived from *Trichoderma* sp.) for 5 h at 50°C initially, then a further 16 h at 50°C. The remaining undigested material was filtered, weighed and ashed. The total percentage ash by weight of the samples and standards was determined simultaneously. Laboratory results for the *in vivo* standards were used to derive a regression relating the laboratory *in vitro* digestibility to their known *in vivo* digestibility values. This regression was then used to estimate *in vivo* digestibility values for the unknown samples. The procedure is able to estimate DMD (Dry Matter Digestibility), DOMD (Digestible Organic Matter in the DM) and OMD (Organic Matter Digestibility). This brief outline of the *in vitro* digestibility procedure is routinely used by the nutrition laboratory at Department of Animal Science, Massey University.

4.4 Faecal chromium analysis:

The analytical procedure for the determination of faecal chromium content follows that of Costigan & Ellis (1987) with some modifications. The basic procedure is as follows:

The oven-dried faeces were bulked intact on an equal weight basis per day (0.5 g DM/ewe/d) for individual sheep within periods. To determine faecal organic matter (OM) content, the composite samples were ashed overnight (12 h) in a muffle furnace at 600°C. The samples were then cooled in a dessicating chamber and the weight of the dry ash was recorded.

The ashed samples were digested by the phosphoric acid-manganese-potassium bromate method of Costigan & Ellis (1987) as modified by Parker (1989). Modifications included the use of 4 ml phosphoric acid and 6 ml potassium bromate (ie. the ratios originally recommended by Williams et al., (1962)).

Briefly, into each of the beakers containing the ashed sample, 0.6 ml of the acid digestion mix was added. (The acid digestion mix is made up of 250 ml concentrated sulphuric acid (98%), 250 ml orthophosphoric acid (85%), 50 ml 10% aqueous $MnSO_4$ $4H_2O$, and 500 ml distilled water.) The beakers were heated to boiling (140 °C) for approximately 90 minutes in an aluminium block placed on a temperature regulated heating plate. (This step ensures a good dispersion of the solids, wetting of the ash, and dissolution of much of the inorganic material.)

The samples were removed from the aluminium block and when the temperature had dropped below 100°C, 0.3 ml of 4.5% potassium bromate (aqueous solution) was added. The beakers were then reheated to 220°C for approximately 90 minutes. After this step, the digested material was transferred quantitatively to a 50 cm^3 volumetric flask and made up to volume using distilled water. Each volumetric flasks was mixed thoroughly and allowed to stand for at least six hours. The supernatent was then decanted off and read at 329 nm on an atomic absorption spectrophotometer (model IL-45, Washington) using a nitrous oxide-acetylene flame. Sample concentrations were read against five standards (0.0, 2.5, 5.0, 10.0 and 20.0 g/ml) made up using chromium (III) nitrate (BHD Chemicals, London) and blank faeces (from sheep not treated with CRDs). Incorporating the blank faeces in the standards provided the correction for background chromium concentrations. A known standard was read after every 10 samples and the standard curve re-entered if drift exceeded 5%. Recovery of chromium oxide added to blank sheep faeces in the range of 10 to 80

ppm was 95.03 ± 5.26% (Mean ± s.d.). Intra- and inter-assay coefficients of variation were 4.54% and 2.54% respectively.

Faecal outputs were estimated by expressing the daily amount of chromium released from the CRDs in ratio to the concentration of chromium in the faeces. Recovery of chromium in the faeces was assumed to be 95%. Corrections were made regarding the soil content of the faeces, even though this value was considered to be almost negligible or non-significant.

4.5 Sheep intake estimations:

Voluntary herbage intakes of sheep during each of the three feeding periods were estimated as the ratio of faecal output (corrected for soil content) to the indigestibility (1 - digestibility), based on *in vitro* determinations of pasture samples taken during each sampling period. This value was then expressed as a proportion of the maintenance requirements of a sheep of a similar body weight.

5 Progesterone concentration:

5.1 Blood sampling:

Forty five ewes of those mated two days after CIDR removal were chosen. These animals represented the two treatment groups and included the two age groups and the two groups separated according to ovulation rate.

To make a standardized evaluation of the plasma pattern of progesterone during the luteal phase of the cycle, twice daily blood samples were taken from Day 9 through to Day 14 inclusive. The samples were collected at different time points each day (0200, 0400, 0600, 0800, 1000, 1200, 1400, 1600, 1800, 2000, 2200 and 2400 hours). The order of the time points for blood sampling was randomized during the sampling period (Jansson *et al.*, 1985). The two blood samples taken each day had to be an AM time and a PM sample time, with a minimum of at least six hours between any two blood sample collections. The sampling times are shown in Table 8 in conjunction with the P-2 faecal collection times.

Previous research has demonstrated considerable variation in plasma progesterone concentrations in ewes sampled intensively over a 24 h period, some of which may be due to diurnal variation (McNatty et al., 1973). For this reason, a single sample is of limited value when used for determination of hormone concentration (Davis et al., 1979). The blood sampling regime used meant that the diurnal variation and daily fluctuations in progesterone concentration were minimized and a more representative value of the concentration of progesterone for the luteal phase of the cycle could be obtained.

Blood samples were collected daily via venipucture to measure plasma progesterone concentrations during the luteal phase of the cycle (defined here to be Days 9-14 post-mating). Two samples per day were collected into heparinized vacutainers and once collected they were immediately transported on ice back to the laboratory. The blood tubes were then centrifuged for 20 minutes at 2500 g and 4°C. The plasma was transferred into duplicate vials and then stored at -12°C until assayed for progesterone concentration.

5.2 Determination of progesterone levels:

A preliminary analysis of individual samples was done with selected samples from all six days (Days 9 AM to Day 14 PM, inclusive). This was carried out to determine if the ewes that failed to conceive to the synchronized mating had reduced progesterone concentrations at any point during the luteal phase of the cycle. Of particular interest was the period around Days 13 and 14, following the time that maternal recognition of pregnancy occurs. For this analysis, six ewes that had returned to oestrus were compared to six ewes that had held to the initial synchronized mating. None of the 12 ewes used in this preliminary analysis received post-mating CIDR treatment and comparisons were made after balancing for similar ORs, ages and feed levels.

The main determination of hormonal levels were made from individually pooled samples (500 ul) for each ewe and consisted of 12 twice daily blood samples that were collected during the blood sampling period.

5.3 Progesterone assay:

Plasma progesterone concentrations were analysed via the method of Kirkwood et al. (1984), with minor alterations to the procedure. This assay has been validated by Dr. K.R. Lapwood of the Department of Anatomy and Physiology, Massey University. Briefly, samples were extracted with 5 ml toluene:hexane (1:2 v/v). The plasma was frozen for 1 hour and solvent was then decanted into clean tubes, dried under air and redissolved in 500 ul ethanol. Duplicate 100 ul samples of ethanol extract were dispensed into plastic tubes and dried, as were duplicate 100 ul samples of standard ethanolic solutions of progesterone (P-1030: Sigma Chemical Co., St Louis, Missouri, U.S.A.) with concentrations corresponding to plasma progesterone levels of 0.625-80 ng/ml. A mixture containing antiserum (courtesy of Dr. J.T. France) at a final dilution of 1:18,000 (Tungsubutra and France, 1978); [1,2,6,7-3H8] progesterone (TRK 413, Amersham, Bucks, U.K.) at 20,000 c.p.m./100 ul; phosphate-buffered saline containing 0.02 m-EDTA and 0.1% gelatin (PBS-EG) in the ratio of 1:1:4 (by vol.) was added (600 ul) to each tube and vortexed. After overnight incubation at 4°C, 600 ul of 2.5% (W/V) charcoal (Norit A; A.H. Thomas Co., Philadelphia, U.S.A.) suspension in PBS-EG were added to the tubes, vortexed and then incubated at 4 C for 10 min. Tubes were then centrifuged at 3000 g for 10 min. at 4°C. The supernatant was decanted into scintillation vials and 5 ml toluene-triton scintillation fluid added before counting for 2 min. in a Beckman LS 7500 scintillation counter. For the eight assays performed, the intra-assay CV was 8.35%, while the inter-assay CV was 20.2%.

6 Reproductive information:

6.1 Determination of ovulation rates:

Ovulation rates of the ewes that were mated 1-3 days after the CIDR removal, were determined by laparoscopy on Days 4 and 5 post-mating (Day 0 = oestrus). Ewes were yarded and fasted overnight to reduce the amount of gut-fill. On 29 March 1988 (Day 4 post-mating), laparoscopies were performed on approximately half the animals. The remaining ewes had their ORs determined the next day following a similar overnight fast.

The laparoscopy procedure used for the determination of ORs is similar to that described by Kelly & Allison (1976). Briefly, ewes were tranquilized with 0.5 ml IM of ACP ("Acepromazine" - Techvet Laboratories Ltd., Tokoroa, N.Z.), approximately 15 minutes prior to laparoscopy. The ewes were shorn around the abdomen and then placed in a specially designed laparotomy cradle. A local anaesthetic (xylocaine - "Lignavet", Techvet Laboratories Ltd., Tokoroa, N.Z.) was administered (3.0 ml SC) in the area where the instruments were to be inserted. This was approximately 3 to 4 cm each side of the mid-ventral line and about 5 cm in front of the udder. This area was washed with an antiseptic mixture (Hibitane - "Savlon", ICI Tasman Ltd., Upper Hutt, N.Z.). The ewes were inverted in the cradles so that the head of the animal was tilted downwards resulting in the cradle being at an angle of about 30 to 40° to the horizontal. Two small incisions were made in the abdominal area to facilitate the puncture of the abdominal wall with the laparoscope trochar and manipulating probe or forceps. The laparoscope was then inserted through this opening into the abdominal area and the ovaries were examined. A probe to manipulate the ovaries allowed all surfaces to be seen. A cannister of CO_2 was available if the abdominal cavity needed to be inflated with gas. After examination of the ovaries, the instruments were removed and rinsed with an antiseptic solution (Savlon) between each animal. Ewes were then injected with 2.0 ml IM of penicillin ("Streptopenicillin" - Ethical Agents Ltd., Auckland, N.Z.). Ewes were removed from the cradles and returned to their respective paddocks upon completion of OR determination.

6.2 Pregnancy status:

Pregnancy was determined initially by observing daily returns to oestrus which occurred approximately two weeks following the synchronized mating. At about 60 days post-mating, real-time ultrasonic scanning was used to confirm pregnancy and to estimate foetal numbers.

6.2.1 Returns to oestrus:

Returns to oestrus of the ewes were recorded from the time that the post-mating progesterone supplementation CIDRs were removed. The ewes were checked daily for mating marks (four rams present) from 10

April to 15 April and then at weekly intervals (two rams present) until 4 May when the rams were removed entirely from the ewes. During this period, the colours of the marking crayons were changed two times to facilitate the recording of the tupping marks.

6.2.2 Ultrasonic scanning for pregnancy determination:

Approximately 60 days from the time of the synchronized mating (23 May 1988), the ewes were subjected to real-time ultrasonic scanning to determine pregnancy and to estimate the foetal numbers. The procedure was used on the ewes fasted overnight, as described by Carter (1987). Fasting of ewes reduced gut-fill which helped obtain a clear image on the monitor, and thus increase the accuracy of the diagnosis of foetal numbers.

Briefly, ewes were held in the dorsal recumbency position for the examination. The hand-held probe was first dipped into a container of lubricant so that when it was placed on the ewes abdominal wall, a good contact could be made between the skin and the probe. The probe was then manipulated both laterally and dorso-ventrally in a systematic routine so that all extremities of the uterus could be viewed. From this image, an estimation of the number of foetuses present and their relative size (hence the probable mating date) was given by the operator and recorded.

6.2.3 Lambing information:

At birth, lambs were tagged and weighed and the date of birth, identification of dam, litter size, and survival rate of lambs were recorded.

7 Statistical information:

The original data was initially subjected to a Bartletts test of homogeneity to see if the variances were common, prior to conducting a test of significance. If the variances were not common, a transformation of the raw data was carried out to adjust the variances so that they would become similar, thus meeting one of the requirements for a test of significance.

In the analysis carried out, both REG (Gilmour, 1985) and SAS (SAS, 1985) statistical analysis packages were used.

7.1 Analysis of variance (ANOVA):

The general linear model procedure (Searle, 1971) was used for the majority of the continuous variables analysed. This analysis included all main effects, as well as the first order interactions. In most models fitted (other than the analysis of the liveweight changes and the pooled progesterone concentrations, which are mentioned below) liveweight was removed as a covariate after the main effects and before the first order interactions were fitted. The most general model fitted was:

$$Y_{ijklm} = \mu + A_i + F_j + C_k + O_l + W_{ijkl} + (AF)_{ij} + (AC)_{ik} + (AO)_{il} + (FC)_{jk} + (FO)_{jl} + (CO)_{kl} + e_{ijklm}$$

where: Y_{ijklm} = an observation of the m^{th} ewe in the i^{th} age group, the j^{th} feeding level, having the k^{th} CIDR effect and the l^{th} ovulation rate.

μ = population mean.

A_i = the i^{th} age effect (i=1...2).

F_j = the j^{th} feeding level effect (j=1...2).

C_k = the k^{th} CIDR treatment effect (k=1...2).

O_l = the l^{th} ovulation rate effect (l=1...2).

W^{ijkl} = the effect of body weight (covariate).

$(AF)_{ij}$ = the interaction between the i^{th} age effect and the j^{th} feeding level effect.

$(AC)_{ik}$ = the interaction between the i^{th} age effect and the k^{th} CIDR treatment effect.

$(AO)_{il}$ = the interaction between the i^{th} age effect and the l^{th} ovulation rate effect.

$(FC)_{jk}$ = the interaction between the j^{th} feeding level effect and the k^{th} CIDR treatment effect.

$(FO)_{jl}$ = the interaction between the j^{th} feeding level effect and the l^{th} ovulation rate effect.

$(CO)_{kl}$ = the interaction between the k^{th} CIDR treatment effect and the l^{th} ovulation rate effect.

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

Higher order interactions were assumed to be negligible. If interactions were found to be non-significant in the initial model fitted, they were removed from subsequent models. The order of fitting in the model depended on the biology of the parameter in question.

Analysis of the liveweight data, for both the CRD ewes (n=20) and the total flock of sheep (n=206), used a model similar to the one above. However, once the differential feeding was started at Week 0, the weighings from Weeks +1 and +2 respectively had the effect of liveweight at Week 0 fitted first into the model as a covariate, followed by the appropriate main effects and interactions.

Analysis of the pooled progesterone concentration data used a similar model to the one previously stated, but the effect of liveweight was taken out prior to the fitting of the feeding level effect. All other main effects were removed first with no adjustment for liveweight. The reason why liveweight was fitted before feeding level was due to considerable variation in the body weights of the 45 animals used for blood sampling, hence the effect of body weight _per se_ may have been masking the effect of feeding level on the pooled progesterone concentrations. It was therefore decided to look at the effect of feeding level on hormonal level at a similar body weight.

7.2 Repeated measures analysis:

A preliminary repeated measures analysis using the REG (Gilmour, 1985) was carried out to compare the progesterone concentrations of the ewes which returned to oestrus with those of the ewes which held to the initial synchronized mating. This involved 12 ewes (six pregnant ewes and six non-pregnant ewes) that did not receive the post-mating CIDR treatment. This procedure involved the repeated testing of the same individuals over a period of time. The individuals are one factor (usually considered as random and serving as replication) and the time dimension is the second factor, a fixed treatment effect (Sokal & Rohlf, 1969). This method is based on the correlation between treatments over blocks and assumes the absence of any treatment by block interactions.

7.3 Discrete variable analysis:

The pregnancy rate data (discrete data) was converted into binomial data, depending on whether the ewe held to the synchronized mating or became pregnant at a subsequent mating, and logit transformation was applied. The transformed data was then analysed by an iterative weighted least square procedure (Gilmour, 1985), which performed maximum likelihood estimates on Logit (P) [Logit (P) = ln (P/1-P), where P is the probability of pregnancy at a subsequent oestrus.]

7.4 Analysis of frequency:

The Chi-square test was used in conjunction with a test of 'goodness of fit' for some of the discrete variables. This test was used to decide whether an observed sample distribution departed significantly from the theoretical one (Sokal & Rohlf, 1969). Included in these analyses were the onset of oestrus, the sex ratio of lambs born and the percentage of multiple births.

7.5 Levels of statistical significance:

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

Symbol	Level of Significance
***	$P < 0.001$
**	$0.001 < P < 0.01$
*	$0.01 < P < 0.05$
+	$0.05 < P < 0.10$
NS	$0.10 < P$

CHAPTER IV: RESULTS

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1 Pasture assessment:

Information regarding the descriptive assessment of the pastures for both the pre-mating and post-mating feeding periods is presented in Table 9. Also included in this table are the results from the *in vitro* digestibility analyses. The figures represented in this table are average values given for the two feeding periods, as well as the ranges.

Table 9: Descriptive assessment of pasture cover, height, composition and *in vitro* digestibilities for the three different pasture allowance levels. Means are given with ranges appearing below in brackets.

	PERIOD I	PERIOD II	
		Group 1	Group 2
Herbage Allowance Level	Flushing	Low	High
Average Pasture Residual[A] (kg DM/ha)	2550 (1900 - 3400)	1450 (1200 - 1700)	2700 (2400 - 2900)
Average Pasture Height (cm)	5.7 (3.5 - 7.5)	3.5 (3.0 - 4.0)	6.0 (5.5 - 6.5)
Pasture Composition (% DM)			
Green Material			
-Grasses	72.7 (60.0 - 75.0)	67.8 (50.0 - 90.0)	76.6 (65.0 - 85.0)
-Clover	2.4 (0.5 - 5.0)	0.6 (0.3 - 1.0)	3.8 (2.5 - 7.0)
-Weeds	0.8 (0.5 - 1.2)	0.6 (0.1 - 1.0)	1.0 (0.5 - 1.5)
Dead Matter	24.2 (15.0 - 35.0)	31.1 (15.0 - 50.0)	18.7 (10.0 - 25.0)
In Vitro Digestibilities:			
Dry Matter Digestibility (%)	67.35	59.43	72.97
Organic Matter: Digestibility (%)	72.94	66.83	76.88
D-Value (DOMD) (%)	63.01	57.25	66.91

[A] The regression equation used to calculate the residual pasture mass was:

kg DM/ha = 245.2 (EPM) - 264.6 (r = 0.838)

It can be seen from Table 9 that there were substantial differences both in the quantity (represented by pasture residual and pasture height) and the quality (both pasture composition and *in vitro* digestibility) of the three different pasture allowance levels offered to the ewes in this experiment. Considerable variation in the range of pasture heights during the flushing period existed, when compared to the two post-mating pasture allowance levels. This was a consequence of the ewes being flushed while they were also being used to prepare the two post-mating pasture allowance levels, where the swards varied considerably in height.

2 Voluntary herbage intake:

The estimated faecal outputs and voluntary herbage intakes, expressed in terms of the digestible organic matter intakes (DOMI), of the ewes during the two feeding periods are presented in Table 10. Ewes in Groups 1 and 2 were grazed together during the flushing period (Period I) and were then divided into the low and high pasture allowances respectively during the post-mating period (Period II). These voluntary herbage intake estimations were calculated using the *in vitro* digestibility information from Table 9, along with the faecal chromium recovery data.

Table 10: Predicted faecal outputs and voluntary herbage intakes of ewes during the two feeding periods (Mean ± SEM).

	PERIOD I (P-1)		PERIOD II (P-2)		Sign. of diff.[C]	
	Group 1	Group 2	Group 1	Group 2	P-1	P-2
Herbage Allowance	Flushing (Med-Low)	Flushing (Med-Low)	Low (Low-Med)	High (High)		
Faecal Chromium (ppm)	6.71±0.31	6.70±0.27	5.39±0.20	6.25±0.24	NS	+
Faecal Ash (% DM) (ppm)	19.76±0.01	19.95±0.01	22.81±0.01	17.87±0.01	NS	**
Faecal Output (g/DM/d)	396±18	395±17	489±17	423±17	NS	+
(g/OM/d)	303±12	301±12	347±16	340±15	NS	NS
DOMI (gOM/d)	1119±44	1113±44	1049±49	1471±63	NS	**
(gOM/kg$^{(0.75)}$)	52.3±1.4	53.4±2.6	50.9±1.8	68.8±2.6	NS	**
DMI (kgDM/d)	1.25±0.49	1.24±0.49	1.17±0.53	1.63±0.70	NS	**
Energy Intake (MJME/d)[A]	12.84	12.73	10.5	17.78		
Maintenance Intake (*M/d)[B]	1.2	1.2	1.0	1.6		

[A] Energy Intake = (DOMD * 16.3 * DMI) (DOMD values from Table 9).

[B] Maintenance Intake = Energy Intake as a proportion of daily maintenance requirements (11 MJME/ewe/d).

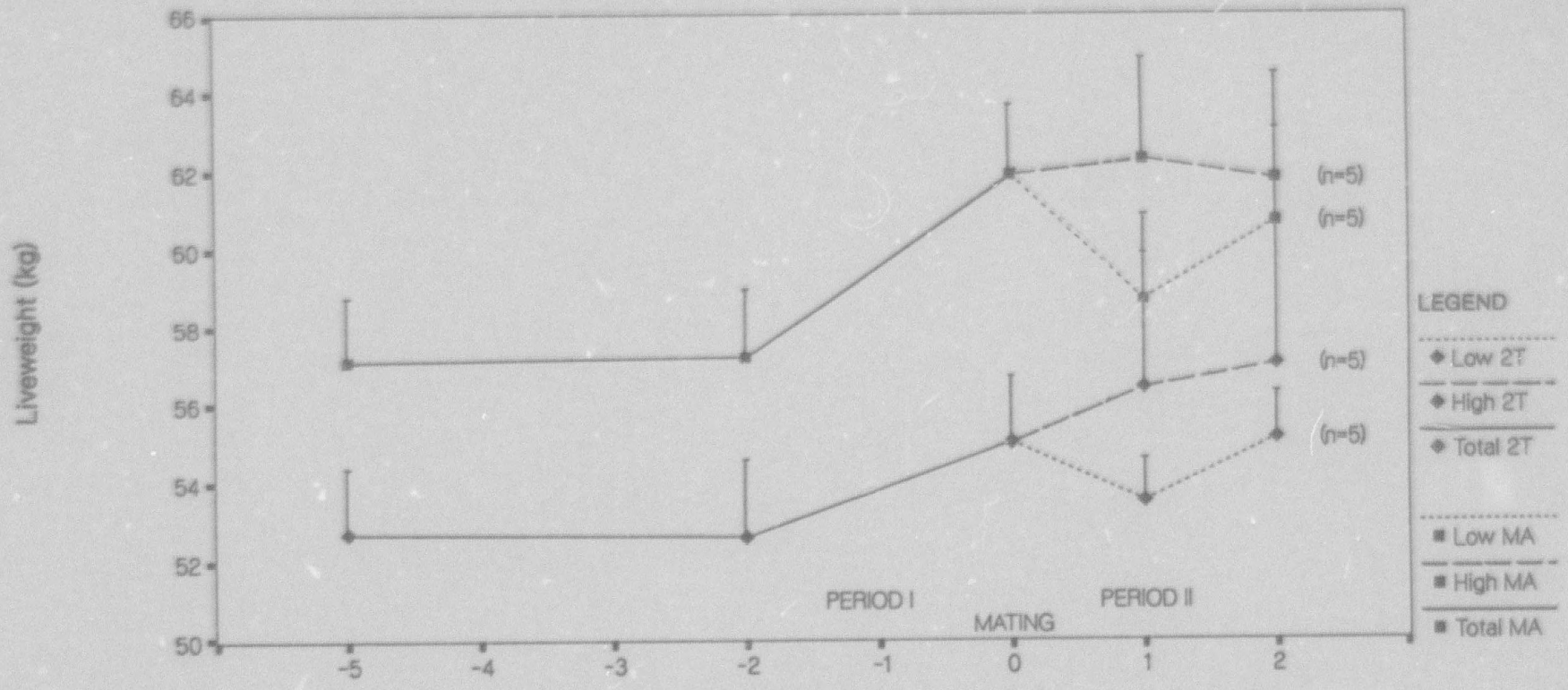
[C] Significance of t-test between groups.

Herbage intakes were similar during the flushing/mating period (P-1) when both groups were grazed together. Intakes increased significantly ($P < 0.01$) in the high (Group 2) pasture allowance group during P-2 to 1.63 kg DM/ewe/day. This is equivalent to 1.6 times the maintenance requirement of a 55 kg ewe (Geenty & Rattray, 1987). At this level of intake and herbage DOMD, liveweight gains of around 100 g/day could be expected. In the low pasture allowance level (Group 1) during P-2, intakes were reduced to levels equivalent to 1.0 times the maintenance requirements of an average, adult ewe.

2.1 Liveweight of CRD ewes:

The mean body weight changes for the 20 CRD ewes (divided into the two age groups of animals) are shown in Figure 3. It can be seen from this diagram that for the first two weighings, there were no factors significantly affecting ewe liveweight. The influence of age however significantly affected the liveweight of the ewes at Week 0, Week +1 and Week +2, respectively. Feeding level was also found to have a significant effect on ewe liveweight at the final two weighings (Weeks +1 and Week +2).

The two post-mating feeding levels produced significant differences in the mean body weights of the ewes on both the low and high feeding levels. The two-tooth ewes on the low post-mating pasture allowance level gained on average 0.1 ± 0.4 kg, while those ewes on the high feeding level gained approximately 2.0 ± 0.5 kg during the same period. The mixed-age ewes on the low feeding level lost 1.2 ± 0.5 kg during the two weeks that they were on this feeding level, while similar ewes on the high feeding level lost 0.1 ± 0.4 kg over the same period.



Significance:					
Age	+	+	**	***	***
Feed Level	NA	NA	NA	***	***
Weight	NA	NA	NA	***	***
CIDR	NA	NA	NA	NA	NS
Feed X CIDR	NA	NA	NA	NA	+

Figure 3: Liveweight changes for the 20 CRD ewes during the experimental period (for the two-tooth (2T) and mixed-age (MA) ewes), as well as the significance levels.

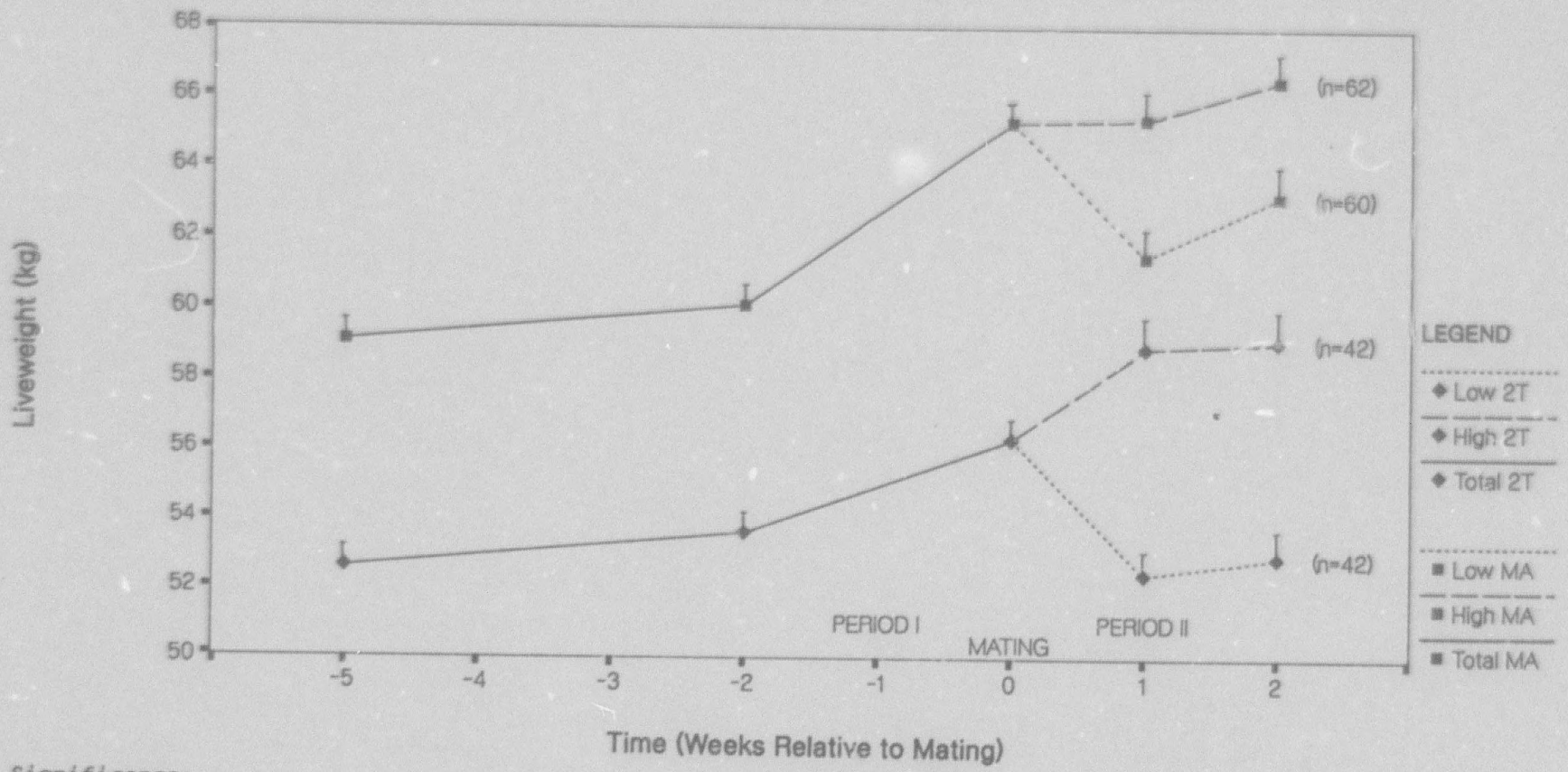
3 Liveweights of all ewes:

Liveweight changes during the two week post-mating period provided additional evidence of whether or not the pasture allowance levels offered during this period were actually approaching those proposed at the beginning of the experiment. The mean body weights changes for the ewes (divided into the two age groups) are shown in Figure 4. Only the data of those 206 animals that were mated over Days 1-3 after the CIDRs were removed were analysed (after square root transformation). All animals that failed to mate at this time or had questionable tupping marks (n=28), were excluded from the analysis.

The two post-mating feeding levels had a highly significant effect on the mean body weight of the ewes, resulting in body weight losses after two weeks of 3.4 ± 0.7 kg for the low feeding level ewes and liveweight gains of 2.8 ± 0.8 kg for the ewes on the high feeding level. The mixed-age ewes on the low feeding level lost an average of 2.1 ± 0.8 kg, while similar ewes on the high feeding level gained an average of 1.2 ± 0.7 kg during the same two week post-mating period of differential feeding.

Figure 4 shows the results of the analysis of the liveweight data. A highly significant ($P < 0.001$) age effect on ewe liveweight existed at all five weighings. Feeding level also had a highly significant effect ($P < 0.001$) on ewe liveweight at the last two weighings (Week +1 and Week +2), during differential feeding.

A highly significant age by feeding level interaction ($P < 0.001$) existed at both the Week +1 and Week +2 weighings. This indicates that the two feeding levels affected the two age groups of ewes differently. Figure 4 shows that the two-tooth ewes have a more extreme liveweight change over this period, than did the mixed-aged ewes. This is indicated by a larger liveweight difference existing between the two feeding levels for the two-tooth ewes versus the mixed-age ewes.



Significance:					
Age	***	***	***	***	***
Feed Level	NA	NA	NA	***	***
Age X Feed	NA	NA	NA	***	***
Weight	NA	NA	NA	***	***
CIDR	NA	NA	NA	NA	NS

Figure 4: Liveweight changes for the total flock (n=206) during the experimental period (for the two tooth (2T) and mixed-age (MA) ewes), as well as the significance levels.

4 Synchrony in onset of oestrus:

Of the original ewes (n=234) initially receiving a CIDR, four had lost them by the time of withdrawal on 23 March 88. This gave a CIDR retention rate of 98.3%.

The incidence and time of oestrus after CIDR removal for the two age groups is summarized in Table 11 and shown graphically (on a daily basis) in Figure 5. Oestrus was observed in 88.0% (n=206) of the ewes during the first three days following CIDR removal. The remaining 28 ewes that failed to mate during this period were excluded from the remainder of this analysis, although some of these ewes were used in other sections of the experiment.

Statistical analysis, using the Chi-square test, revealed that age had a highly significant effect on the onset of oestrus ($P < 0.001$) with the mixed-aged ewes coming into oestrus at an earlier time after CIDR removal than did the two-tooth ewes. Further investigation indicated that in the two-tooth ewes, a significant linear relationship existed between liveweight and onset of oestrus ($P < 0.05$), with the heavier two-tooth ewes coming into oestrus earlier than the lighter two-tooth ewes. In the mixed-aged ewes, there was no significant effect ($P > 0.10$) of liveweight on the onset of oestrus following CIDR withdrawal.

Table 11: Percentage (and numbers) of ewes in each age group showing oestrus at each observation following CIDR removal.

Age Group	Day 1		Day 2		Day 3		Total	Others[A]
	06:00 h	18:00 h	06:00 h	18:00 h	06:00 h	18:00 h		
Two-Tooth	0.0 (0)	13.5 (14)	14.4 (15)	43.3 (45)	2.9 (3)	6.7 (7)	80.8 (84)	19.2 (20)
Mixed Age	7.7 (10)	45.4 (59)	7.7 (10)	20.8 (27)	1.5 (2)	10.8 (14)	93.9 (122)	6.1 (8)
Total	4.3 (10)	31.2 (73)	10.7 (25)	30.8 (72)	2.1 (5)	9.0 (21)	88.0 (206)	12.0 (28)

[A] ewes which had questionable tupping marks or did not mate during the specified 3 day period.

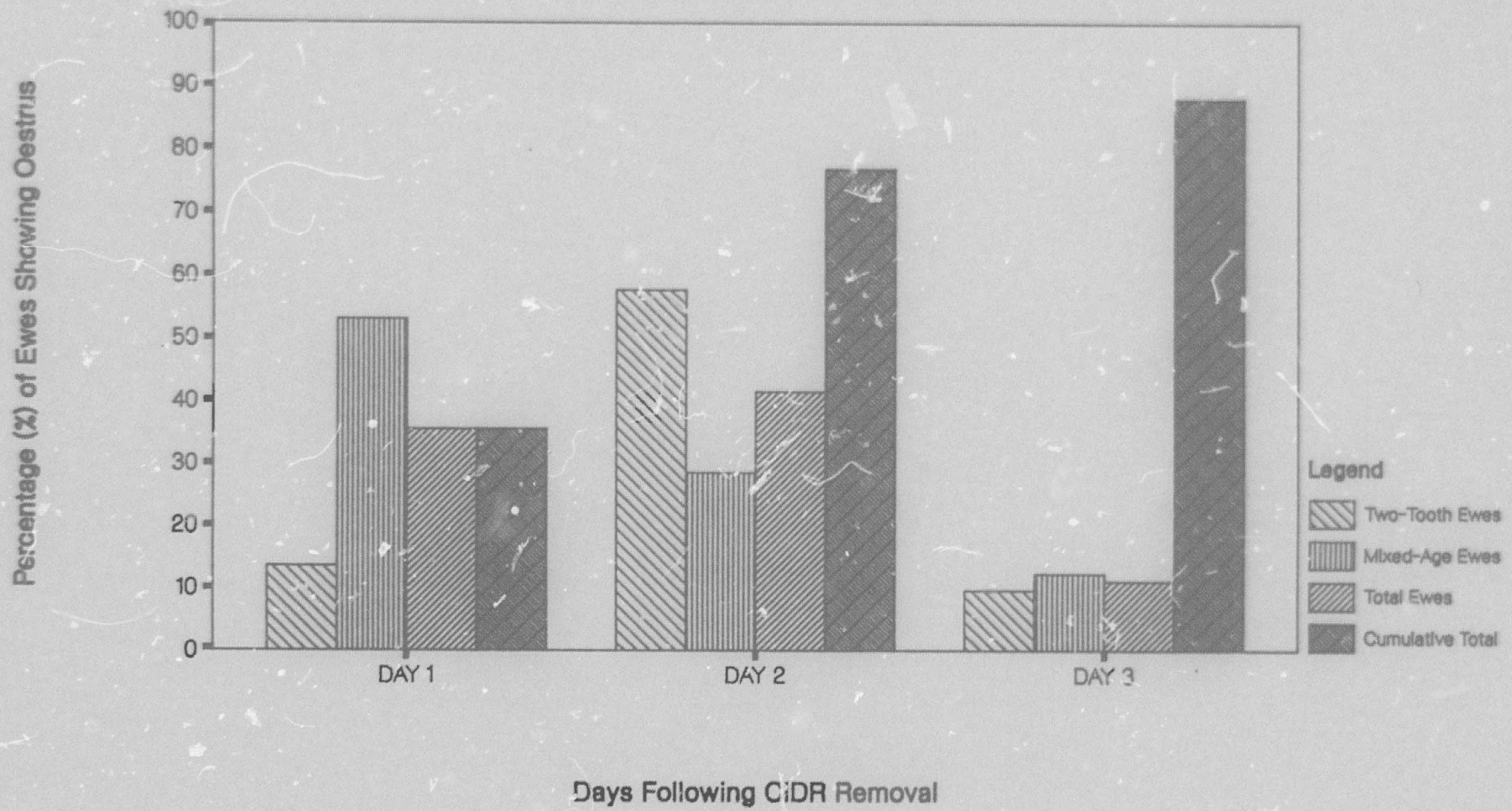


Figure 5: The daily and cumulative incidence of oestrus following CIDR withdrawal (for the two age groups of ewes).

5 Ovulation rate:

There were 61 single (1 CL) ovulations, 138 twin ovulations (2 CLs) and 7 triplet ovulations (3 CLs) recorded at the time of laparoscopy from the 206 ewes which mated. Due to the low number of triple ovulations, the ovulation rate (OR) will be classified in terms of single (1 CL) versus multiple ovulations (two or more CLs), unless otherwise stated.

Analysis of the number of CLs recorded at laparoscopy showed that both age ($P < 0.001$) and liveweight ($P < 0.01$) of the ewes had significant effects on the OR. The two-tooth ewes had a significantly lower OR (1.55 ± 0.06) than the mixed-age ewes (1.87 ± 0.04). Information from Section 3 would suggest that this effect of liveweight on OR is due to the mixed-age ewes being significantly heavier than the two-tooth ewes.

After mating but prior to OR determination, the ewes were allocated at random to either the high or low feeding levels. The mean OR for each of the two feeding groups was later found to be significantly different ($P < 0.05$). The high feeding group had a mean OR of 1.66 ± 0.05, while the low feeding group had a mean OR of 1.81 ± 0.05 - a result entirely due to chance.

6 Progesterone concentration:

6.1 Levels from Days 9 to 14:

The results of the repeated measurement analysis of the daily peripheral progesterone concentration, from Days 9 to 14 inclusive, indicated that there was no significant difference ($P > 0.10$) between the ewes (n=6) that returned to oestrus and those ewes (n=6) that remained pregnant to the first mating. Ewes that returned to oestrus had similar progesterone levels as the ewes which were pregnant, as shown in Figure 6. There was however a significant return by time interaction ($P < 0.01$), thus indicating that this relationship tended to change over time. Liveweight, feeding level and age were found to have no significant effects on the daily progesterone concentrations ($P > 0.10$) during this period.

Ewes with multiple ovulations (2 CLs, n=4) were found to have significantly higher ($P < 0.05$) progesterone concentrations during Days 9-14, than the single ovulating ewes (n=8) as shown in Figure 7. The relationship of progesterone concentration between twin and single ovulating ewes was found to remain constant over time since there was no significant CL by time interaction at the $P > 0.10$ level.

Given these preliminary findings, it was decided to pool the samples as outlined in the materials and methods section, as the sampling regime took into consideration the possible diurnal effects of progesterone secretion. By accounting for the significant effect of CL number in the model used to analyse the pooled progesterone concentration, as well as other main and treatment effects, it was felt that this would not adversely affect the true pooled progesterone concentrations over the 12 sampling times. The samples were then individually pooled over the 12 sampling times and the determination of progesterone concentration was carried out.

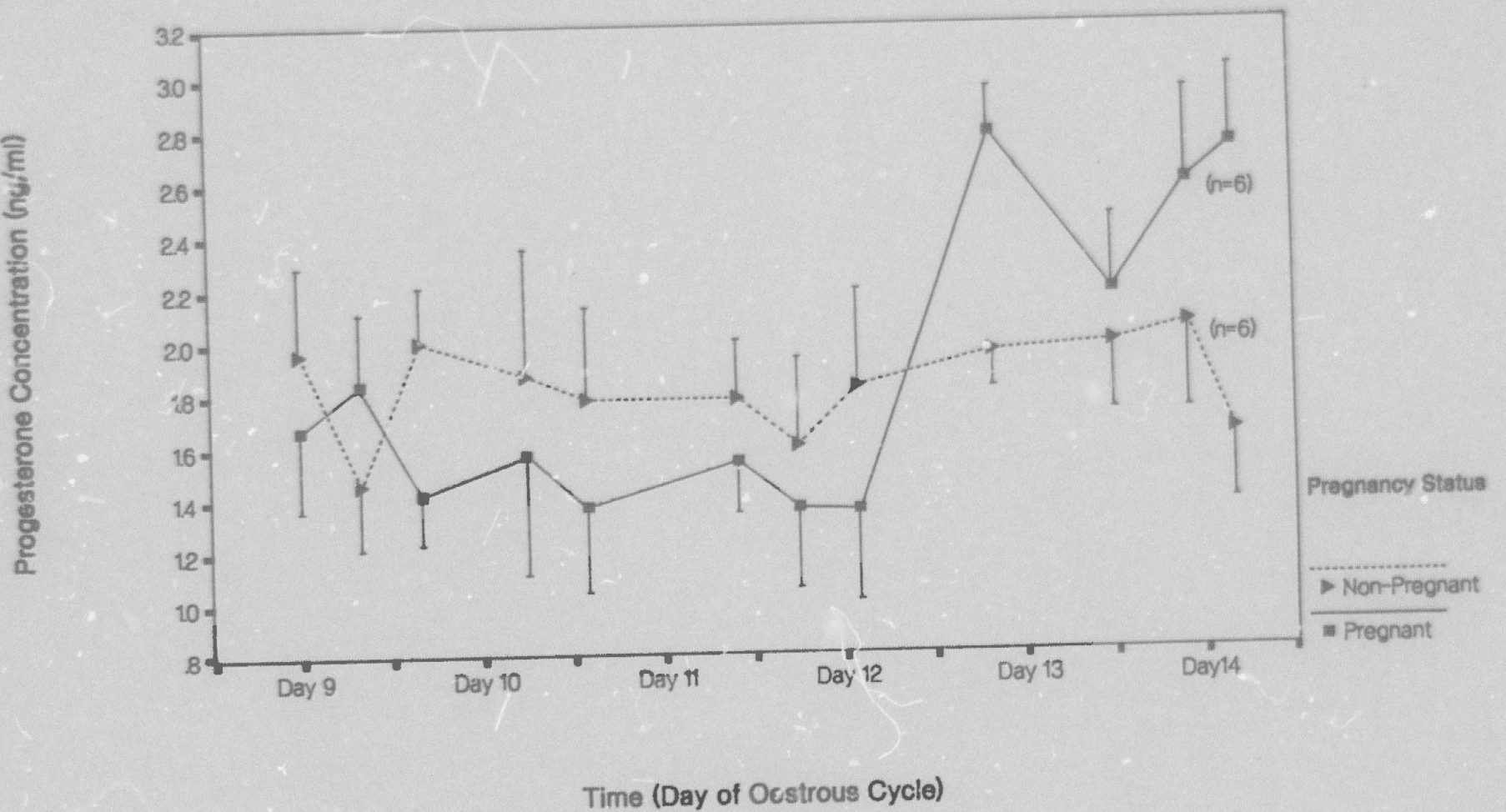


Figure 6: Progesterone concentrations for pregnant versus non-pregnant ewes during Days 9-14 of the oestrous cycle (corrected for common ovulation rate and feed level).

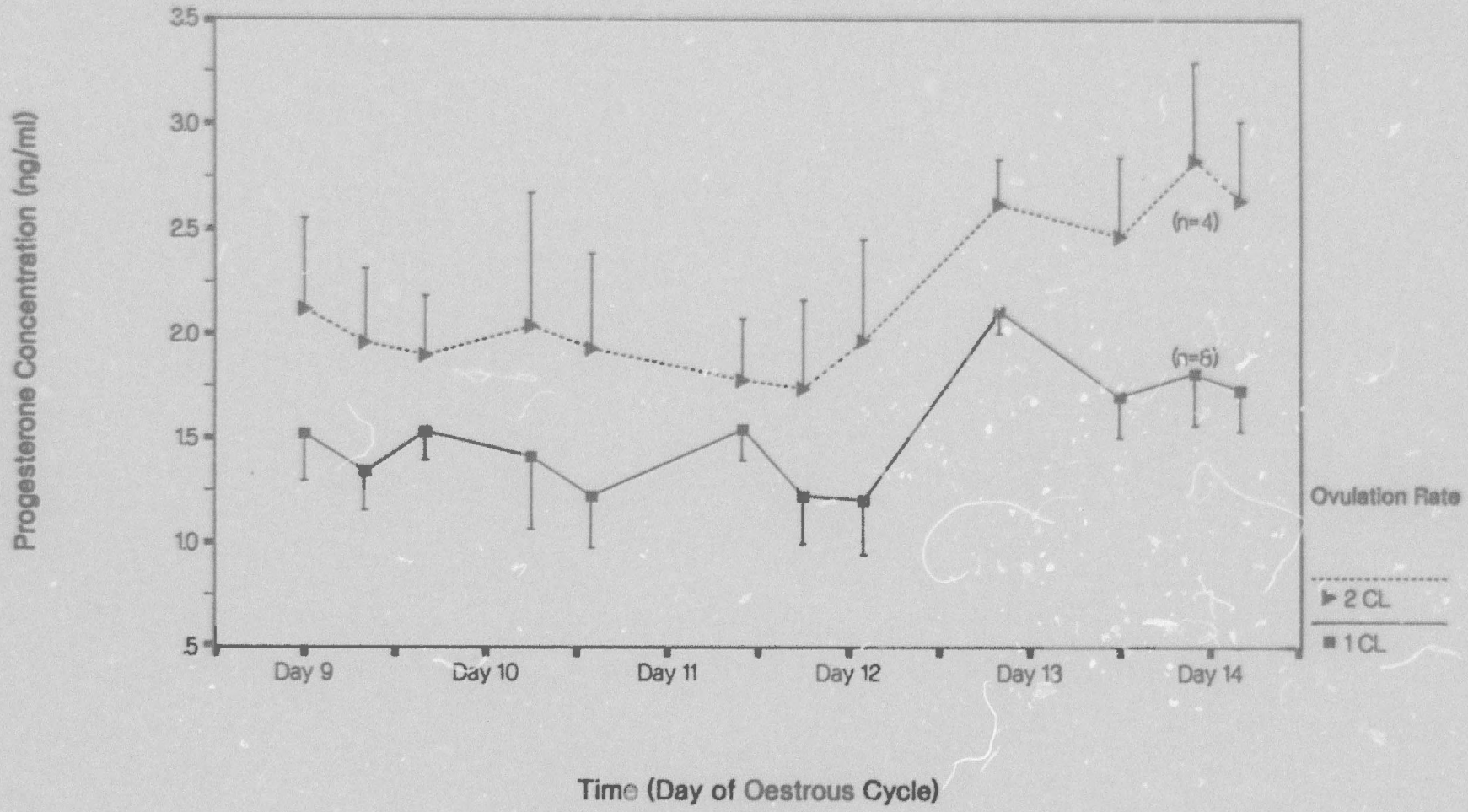


Figure 7: Progesterone concentrations for single versus twin ovulating ewes during Days 9-14 of the oestrous cycle (corrected for common pregnancy status and feed level).

6.2 Pooled progesterone concentration:

The results of the analysis of pooled progesterone concentrations are shown in Table 12.

Table 12: Effect of age, feeding level, CIDR treatment, ovulation rate and pregnancy status on pooled progesterone concentrations.

Parameter	Level	n	Progesterone concentration[A] (ng/ml)	Significance
Age (years)	2	24	1.68 ± 0.10	NS
	>2	21	1.76 ± 0.21	
Feed Level[B]	L	22	1.73 ± 0.09	*
	H	23	1.49 ± 0.11	
CIDR	-	23	1.34 ± 0.10	***
	+	22	1.88 ± 0.10	
Ovulation Rate[C] (OR or CL #)	1	19	1.49 ± 0.10	***
	≥2	26	1.73 ± 0.10	
Pregnancy Status	NP	13	1.63 ± 0.11	NS
	P	32	1.59 ± 0.09	

A LSM ± SEM
B Feeding level effects were first corrected for body weight differences before being analysed.
C The number of ovulations refers to either a single ovulation (1 CL) or multiple ovulations (2 or more CLs).

Initial analysis indicated that there were significant effects of both OR (single versus multiple CL) and CIDR treatment on the pooled progesterone concentrations, at the $P < 0.001$ level. The order of fitting either CIDR treatment or OR in the model did not affect the resulting significance levels. This finding, along with the fact that the interaction between OR and CIDR treatment was non-significant ($P > 0.10$) indicated that there was an additive effect of both OR and CIDR treatment on the pooled progesterone concentrations. The effects of both age and pregnancy status were found to be non-significant ($P > 0.10$). There was a significant body weight effect on the progesterone concentrations ($P < 0.05$) when liveweight was fitted first in the model. The low feeding level had a significantly higher pooled progesterone concentration than did the high feeding level. There was also a significant liveweight by feed level interaction

($P < 0.05$), which indicated that the relationship between pooled progesterone concentration and liveweight differed between the two feeding levels ie. the regression coefficients were found to be heterogeneous.

Figure 8 shows a plot of the individual data points of liveweight and pooled progesterone concentrations for all the animals used in the blood sampling. Also shown in this diagram are the two regression lines indicating the different relationships between the pooled progesterone concentrations and liveweight for both the high and low feeding levels. A second model, using a separate slopes option for the two feeding levels with respect to liveweight, also shows a significant ($P < 0.01$) interaction between liveweight and feeding level.

When the regression coefficients of the two feeding levels were compared, the low feeding level was found to have a slope of $b = 0.0442 \pm 0.0102$ ng/ml. Using the Student t-test, this was found to be significantly different ($P < 0.001$) from zero. The regression coefficient for the high feeding level ($b=0.0145 \pm 0.0129$ ng/ml) was not significantly different from zero ($P > 0.10$), indicating that the relationship existing between progesterone concentration and body weight was constant for the ewes on the high feeding level, irrespective of changing liveweight. The ewes on the low feeding level had a positive relationship between progesterone concentration and liveweight.

Observation of the individual data points ($n=45$) in Figure 8, shows that there are two data points in the low feeding level that could be influencing the slope of the regression line. These two extreme data points were from ewes of both high liveweight and high progesterone concentration. The results of an outlier test (Snedecor & Cochran, 1967) on these two suspect values however indicated that they were not significantly different ($P > 0.10$) from the majority of the individual values for the low feeding level. These two values therefore could not be validly deleted from the original data set.

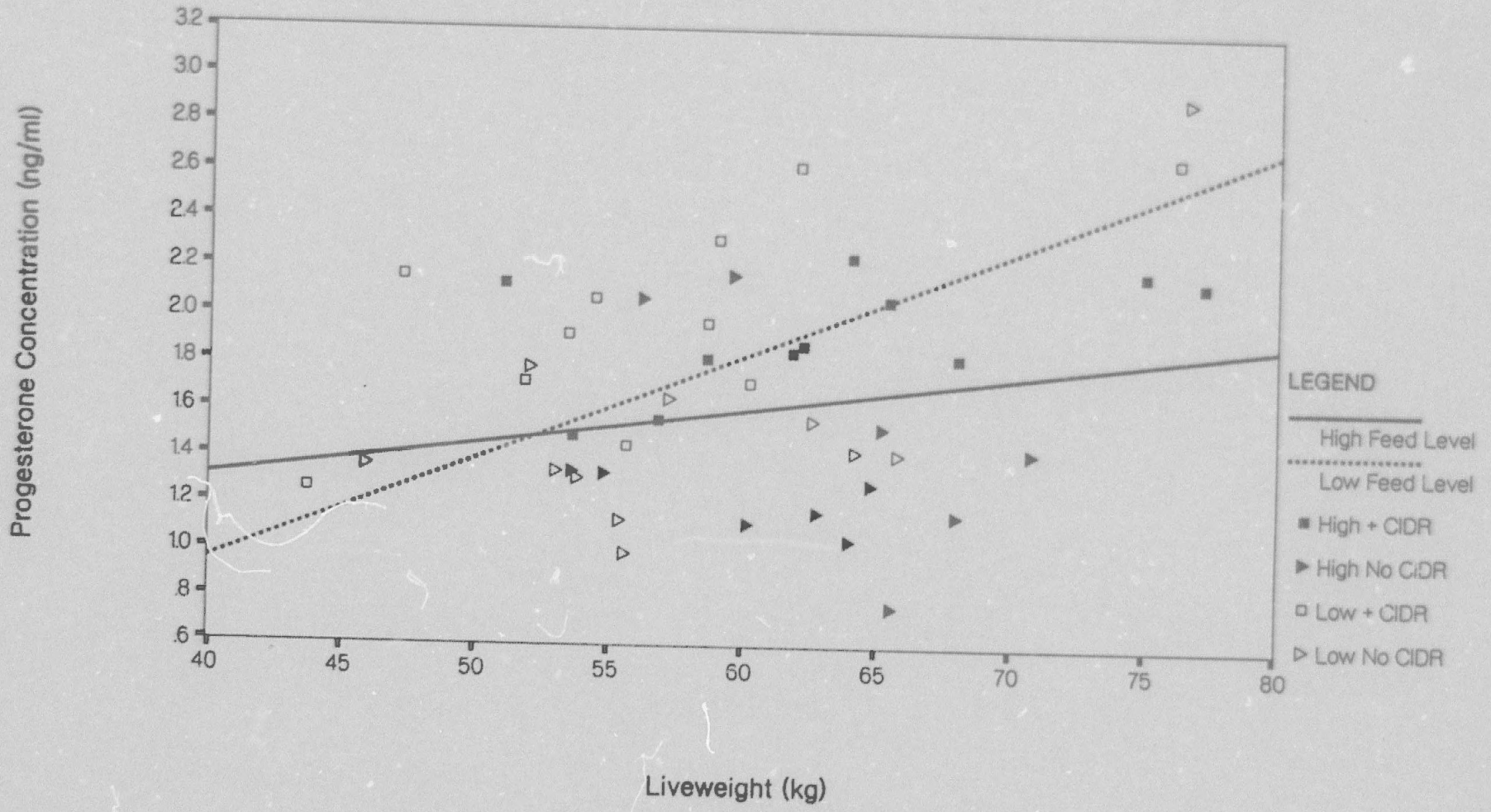


Figure 8: Scatter plot of the individual data points showing the relationship between the pooled plasma progesterone concentrations and liveweight for the two post-mating feeding levels.

7 Pregnancy rate:

The pregnancy rate to the synchronized mating was determined by using data from the returns to service and the ultrasound information. The latter gave an estimate of the approximate time of mating as determined by the relative size of the foetus. The lambing data was also analysed as confirmation of the ultrasound data. Knowledge of the date of lambing was used to estimate the approximate time of conception, using a gestation length of 148 days. Using this method, three errors in the original ultrasound data were detected and the appropriate changes were made and recorded. This analysis used data from 206 mated ewes.

The results of the analysis of the pregnancy rate data (using the logit transformation) are presented in Table 13. A significant ($P < 0.01$) OR effect was found to exist, as well as a significant ($P < 0.01$) feeding level by CIDR interaction on pregnancy rates. All the other effects including age, feeding level and CIDR treatment were found to be non-significant at the $P > 0.10$ level. Presented in Table 13 are the pregnancy rates for the main effects, expressed on the basis of whether the ewe had a single CL or multiple ovulations.

Table 13: Effect of age, feeding level, CIDR treatment, OR and significant interactions on the average pregnancy rate and the average pregnancy rate relative to the number of ovum shed.

	Pregnancy Rate					Pregnancy Rate Relative to the Number of Ovum Shed				
Trait	Level	n	Exp.[A]	Range[B]	Sign	Level	n	Exp.[A]	Range[B]	Sign
Age (yrs)	2	84	.6429	(.5891-.6933)	NS	2 1[C]	39	.5391	(.4689-.6079)	*[C]
						2[C]	45	.7327	(.6721-.7857)	
	>2	122	.6230	(.5782-.6657)		>2 1	122	.4533	(.3739-.5352)	
						2	100	.6603	(.6140-.7037)	
Feed Level	L	102	.6176	(.5685-.6645)	NS	L 1	25	.4790	(.4027-.5562)	*[C]
						2	77	.6627	(.6112-.7106)	
	H	104	.6442	(.5960-.6899)		H 1	36	.5285	(.4577-.5981)	
						2	68	.7055	(.6530-.7531)	
CIDR	-	100	.6100	(.5603-.6575)	NS	- 1	30	.4860	(.4128-.5598)	*[C]
						2	70	.6631	(.6097-.7127)	
	+	106	.6509	(.6033-.6957)		+ 1	31	.5297	(.4563-.6018)	
						2	75	.7011	(.6506-.7471)	
OR	1	61	.5082	(.4444-.5717)	*	NA[D]				
	≥2	145	.6828	(.6429-.7201)						
Feed x CIDR					*					*[C]
	L-	51	.5098	(.4401-.5792)		L- 1	13	.3679	(.2859-.4582)	
						2	38	.5584	(.4850-.6293)	
	L+	51	.7255	(.6588-.7834)		L+ 1	12	.5990	(.5025-.6883)	
						2	39	.7644	(.6997-.8188)	
	H-	49	.7143	(.6457-.7743)		H- 1	17	.6077	(.5165-.6919)	
						2	32	.7709	(.7038-.8266)	
	H+	55	.5818	(.5142-.646)		H+ 1	19	.4579	(.3782-.5439)	
						2	36	.6472	(.5757-.7127)	

A Expected value or LSM.

B Re-calculated from the logit transformed standard errors in the original analysis and is represented as one standard deviation above and below the expected value.

C Ewes having multiple ovulations had a significantly higher ($P < 0.05$) pregnancy rate than did the single ovulating ewes for all traits analysed.

D Non-applicable.

8 Embryo survival (lambs born per ovum shed and lambing percentage):

This analysis used data from 187 animals out of the original 206 ewes mated, as lambing information was missing on 19 ewes. The results from the statistical analysis on lambs born per ovum shed to first service (expressed as a proportion of lambs born to the total number of eggs shed from all ewes mated at the synchronized oestrus), lambing percentage (expressed as the percentage of lambs born to the total number of ewes bred at the synchronized oestrus) and percentage of multiple births to the synchronized mating, are presented in Table 14. Before conducting this analysis, all raw data was first subjected to an arcsine transformation (Snedecor & Cochran, 1967). The findings of this analysis, which represent embryo survival rates, are summarized in Table 14. (The overall mean lambing percentage to the synchronized mating was found to be approximately 102%.)

Table 14: Effect of age, feeding level, CIDR treatment, OR and significant interactions on the embryo survival (represented by average percentage of lambs born per ovum shed and the lambing percentage) and the percentage of multiple births to the first mating.

Trait	Level	n	Lambs Born Per Ovum Shed[A]	Sign	Lambing Percentage[A]	Sign	Percent Multiple Births[B]	(n)	Sign
Age (years)	2	74	.6115 ± 0.05	NS	97.7 ± 9.6	NS	61.6	(23)	NS
	>2	113	.5455 ± 0.04		100.4 ± 7.8		72.4	(42)	
Feed Level	L	95	.5470 ± 0.05	NS	96.4 ± 8.5	NS	69.9	(33)	NS
	H	92	.6099 ± 0.05		101.7 ± 8.7		66.7	(32)	
CIDR	-	94	.5581 ± 0.05	NS	95.5 ± 8.6	NS	67.0	(31)	NS
	+	93	.5988 ± 0.05		102.6 ± 8.7		69.4	(34)	
OR	1	50	.5600 ± 0.06	NS	56.0 ± 11.1	***	NA[C]		
	≥2	137	.5766 ± 0.04		115.3 ± 6.7				
Feed x CIDR				*		*			NS
	L-	47	.4503 ± 0.07		80.7 ± 12.0		41.5	(14)	
	L+	48	.6437 ± 0.07		112.2 ± 12.0		58.5	(19)	
	H-	47	.6659 ± 0.07		110.4 ± 12.1		53.1	(17)	
	H+	45	.5540 ± 0.07		92.9 ± 12.4		46.9	(15)	

A LSM ± SEM

B Chi-square test.

C Non-applicable.

The lambs born per ovum shed at the synchronized mating, was not significantly influenced by age, feeding level, OR, or CIDR treatment ($P > 0.10$). A significant feeding level by CIDR treatment interaction was however found to exist at the $P < 0.05$ level. Likewise, lambing percentage was not significantly influenced by age, feeding level or CIDR treatment ($P > 0.10$). OR was found to have a highly significant effect ($P < 0.001$) on the lambing percentage. A significant interaction between feeding level and CIDR treatment also existed at the $P < 0.05$ level, and this was reflected in the lambing percentage.

Results from the analysis investigating the percent of multiple births to the first mating are also shown in Table 14. There were slightly more multiple births for the mixed-age ewes (72.4%) compared to the two-tooth ewes (61.6%), although this difference was not found to be statistically significant ($P > 0.10$). The feeding level x CIDR interaction produced similar trends to both the lambs born per ovum shed and the lambing percentage, although these results were not significant at the $P > 0.10$ level.

Further investigation into the data on lambs born per ovum shed when analysed with respect to CIDR treatment, revealed that there was a significant effect ($P < 0.01$) of feeding level for ewes not receiving post-mating CIDR treatment. Embryo survival rates were significantly better ($P < 0.05$) for the high feed allowance group (66.6%) compared to the low feed allowance group (45.0%). The effect of age was non-significant. Analysis of the data for CIDR treated ewes showed that the effect of both age and feeding level were non-significant ($P > 0.10$) in influencing the lambs born per ovum shed at the first service, although animals fed at high levels and CIDR treated tended to have the lower embryo survival rates.

When the same data was analysed relative to the two feeding levels, it became evident that at the low feeding allowance CIDR treatment was significant at the $P < 0.05$ level, while the effect of age was non-significant ($P > 0.10$). Ewes on the low feed allowance had significantly better embryo survival rates when treated with CIDRs (64.4%) than those without (45.0%). Ewes on the high feeding level were not significantly effected by either age or CIDR treatment on the proportion of lambs born per ovum shed at the synchronized oestrus. A trend was seen when CIDR treatment was given to high fed

ewes, where embryo survival dropped from 66.6% to 55.4% for CIDR treated animals.

9 Other reproductive traits:

Table 15 in Appendix 1 summarizes the results of an analyses which was performed to see to whether feeding levels or CIDR treatment had any carry-over effects on the subsequent lambing performance. Also included in this table are other main effects which were investigated to determine whether they had any effect on the lambing performance. The reproductive traits examined include mean lamb birth weight, total litter weight, gestation length of ewes, sex ratio of lambs born and the percentage of multiple births. It should be noted that the traits examined in Appendix 1 include all obtainable data from the 1988 breeding season for the flock of ewes used in the present trial, and the data are not limited to the synchronized mating.

CHAPTER V: GENERAL DISCUSSION AND CONCLUSIONS

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1 Voluntary herbage intakes:

The main emphasis of this study was to investigate the effect that post-mating exogenous progesterone (in the form of CIDR treatment) had on the reproductive performance of the ewe, and whether or not the level of post-mating nutrition also affected this trait. In order to achieve this experimental objective, it was necessary to provide two different post-mating pasture allowance levels to the ewes. Although the techniques used to achieve the two pasture allowance levels and the methods of estimating the voluntary herbage intakes of the ewes (using chromium marker in the faeces) were of relative importance, the main aim of this trial was to investigate reproductive performance.

Standard farm stock management, through use of rotational grazing and other techniques, were employed prior to the beginning of the differential feeding period. Sward heights (cm) were regularly monitored and the resulting residual or herbage masses in kg DM/ha were calculated (Earle & McGowan, 1979; Michell, 1982; Matthew & Thompson, 1986). By manipulating the movement and numbers of the stock, it was possible to achieve the pre-determined pasture allowance targets (sward heights) and maintain them throughout the duration of the trial. This resulted in the establishment of two different pasture residuals for the two week period of post-mating nutrition. These pasture allowance levels led to herbage intakes that were considered to be less extreme than those reported in other studies. The sward heights used in this experiment were arbitrarily set (mainly through trial and error) at 3.5 cm and 6.0 cm for the low and high pasture allowance levels, respectively. This technique of controlling sward height in grazing experiments designed to investigate ewe reproductive performance at mating has been used successfully by other researchers (Gunn *et al.*, 1988).

In other similar experiments, the amount of feed offered to ewes has varied widely with ewes often fed specially formulated rations and housed in barns or kept under feedlot conditions. For instance, both Cumming *et al.* (1975) and Parr *et al.* (1987a) used feeding levels of 0.25 M, 1.0 M and 2.0 M. The two extreme feeding levels

would not be encountered under normal farming conditions, particularly in New Zealand where the majority of sheep graze pastures. This experiment has therefore attempted to use pasture allowances (1.0 M and 1.8 M) that would be found under field conditions.

To determine the amount of feed being consumed both before and after mating, techniques such as controlling the release of chromium marker into the faeces (Ellis et al., 1981; 1982), assessing pasture compositions, dry matter determination and in vitro digestibility assessments were used. Through knowing these relative amounts of herbage intakes for these periods (particularly during the post-mating (P-2) period), it is possible to relate the resulting reproductive performance of the ewes, with the grazing intake levels and to have a comparison with other studies where the known intake levels of the ewes have already been determined.

Prior to mating, it is widely recommended to flush ewes for at least three weeks with pasture allowance levels of around 1.5 M being offered to ewes (Rattray, 1986). This level of flushing offers the opportunity to increase the lambing rate through an increase in the OR (Rattray et al., 1980 & 1987). Ewe liveweight is also important at joining as there is an effect of body weight per se on the resulting lambing percentage (Rhind et al., 1986).

The original intention of this trial was to have ewes on a flushing level of nutrition prior to a synchronized mating and then to divide the flock into two different groups for grazing at either maintenance levels or at a level similar to that before joining. The average pasture residual for the pre-mating or flushing period in this trial averaged approximately 2550 kg DM/ha, as shown in Table 9. Rattray et al. (1987) indicate that the most effective flushing is achieved with pastures of around 2500 kg green DM/ha being offered to ewes, which has been met in this experiment.

During the flushing period (P-1) and high allowance post-mating period (P-2) the average pasture sward heights were similar at 5.7 cm and 6.0 cm respectively. However, there were differences in the pasture quality which were represented by both the botanical compositions and in particularly the in vitro digestibilities

(Table 9) of the two grazing treatments. The digestibility of the herbage consumed during the flushing period (72.94 % OMD) was less than that of the high level herbage of P-2 (76.88 % OMD), which led to the estimated feed intake at flushing being calculated to be 1.2 times the maintenance requirement level, whereas the estimated intake on high pasture allowance during the post-mating feeding period was 1.6 M, as shown in Table 10.

The difference in estimated intakes may also have been the consequence of using the flushing period to prepare the pastures for the two different post-mating feeding allowances. The ewes were forced to eat down to low pasture allowance levels, as well as being required only to lightly graze or 'top off' the paddocks in order to maintain appropriate sward heights. This resulted in a considerable range of sward heights existing in P-1 which were not apparent in P-2 (see Table 9).

Following mating, when the differential feeding levels were imposed, feed intake levels increased significantly ($P < 0.01$) in the high allowance group to 1.63 kg DM/ewe/day. This level of feed intake is equivalent to 1.6 times the maintenance requirements of a 55 kg ewe (Geenty & Rattray, 1987). At this level of intake and DOMD, liveweight gains of around 100 g/day could be expected.

The low pasture allowance ewes during P-2 were grazed on pastures that had an average residual of 1450 kg DM/ha, which corresponded to an average sward height of 3.5 cm. Predicted faecal output was interestingly higher in these low fed ewes than in the high pasture allowance ewes ($P < 0.01$). This high faecal output in the low fed ewes can be attributed to the lower digestibility of the herbage consumed (Table 9), and to an increased ingestion of soil, as reflected by a 4.93 % higher faecal ash content (Table 10). The lower digestibility of the herbage can be related to the high proportion of dead matter (31.1% for the low level, compared to 18.7% for the high level). Despite the high dead matter content of the herbage consumed by these low fed ewes, their mean daily intake of 1.17 kg DM/day and the DOMD of the herbage mass means that energy intakes would have been sufficient to maintain liveweights.

The apparent mean daily intakes, as calculated from the in vitro digestibilities and the determination of faecal chromium, indicate that the experimental objective of feeding ewes at either 1.0 M or 1.8 M, during the post-mating period, was basically achieved. The high feeding level however, fell slightly short of that proposed at the beginning of the trial, but it would appear that the ewes were fed to ad libitum conditions. By using the estimated intake information from the 20 CRD ewes, it is possible to extrapolate to the large flock of ewes and assume (with a high degree of accuracy) that the ewes were also on these two respective levels of feed intake during the two week post-mating period. The differences in voluntary herbage intakes are also reflected in the liveweight changes of the 20 CRD ewes, as well as the entire mob of ewes (n=206).

2 Liveweight changes:

The estimated voluntary herbage intakes of the ewes can be compared to the liveweight changes associated with the different levels of nutrition. Liveweight changes have often been used as a measure of the effect of nutritional treatment. Significant body weight x nutrition interactions were detected in the 20 CRD ewes (Figure 3), and when the liveweight changes of the entire mob of sheep were analysed, both of the two post-mating pasture allowance levels were also found to have significantly affected the liveweights at Week +1 ($P < 0.001$) and Week +2 ($P < 0.01$) (Figure 4).

Mean liveweight changes for the total mob of ewes, during the two week differential grazing period immediately following mating, represented a liveweight loss of 2.8% on the low (1.0 M) pasture allowance and a liveweight gain of 4.1% on the high (1.6 M) pasture allowance level. Williams & Cumming (1982) reported losses of 5.7% of initial liveweight when ewes were fed a maintenance level diet over a similar two week feeding period, while ewes on a 2.0 M diet had a gain of 2.1% of liveweight during the same period. A similar study by Parr et al. (1987a) has reported that ewes differentially fed over the same period had losses of 2.6% of initial liveweight on the 1.0 M diet and a slight gain (0.4%) when fed a 2.0 M diet. The report of Williams & Cumming (1982) showed a slightly larger loss of liveweight on the 1.0 M diet, than the values reported in this trial but the work of Parr et al. (1987a) seems to be similar. The ewes on the

2.0 M diet of both Williams & Cumming (1982) and Parr et al. (1987a) did not seem to gain as much weight as the ewes did in this experiment, even though the high level of feeding in this trial was calculated to be 1.6 M. The reason why the ewes in the present study appeared to gain more weight at the 1.6 M pasture allowance level compared to the 2.0 M level of other workers may be due to differences in the way previous workers had calculated the relative feeding levels.

Ewes on the low pasture allowance levels (1.0 M) in both the CRD group and the total mob of ewes, as shown in Figures 3 and 4, appeared to lose weight during the first week following mating. Then during the second week of the differential feeding period these ewes had net gains in liveweight. The rapid and sudden loss in liveweight for the low fed ewes during this first week, may have been due either to the nutritional treatment imposed, or to the stress associated with the large amount of handling or a combination of both. During the two weeks following mating the ewes in this experiment were subjected to considerable handling and movement during pasture rotations, faecal sampling, post-mating CIDR insertion and removal, weighings, blood sampling and laparoscopy. Doney et al., (1976) suggested that the stress associated with minor surgery such as laparoscopy may have detrimental affects on ewe performance However, Kelly & Allison (1976) indicated that a single laparoscopy at mating has no adverse effect on the reproductive performance of ewes.

If in fact a stressful environment existed during the first week following mating (due to laparoscopy and/or excessive handling), this might explain why the low fed ewes tended to gain more liveweight during the second week of differential feeding. However, the ewes on the high pasture allowance level were also subjected to the same management practices and procedures, yet they still had a net gain in body weight over the same two week period. Therefore, it was concluded that the laparoscopy procedure and the frequent handling of the animals did not adversely affect their anticipated changes in liveweight or the subsequent reproductive performance of the ewes. The most likely explanation left therefore, is that a sudden change in the nutritional status is responsible for the initial liveweight changes for both the two feeding levels. In the second week the liveweights seemed to stabilize, as perhaps feeding patterns changed.

As the faecal samples from the CRD ewes were only collected over the second week, changes in feed intake during the entire post-mating period were impossible to determine.

The results of the liveweight changes for the 20 CRD ewes (Figure 3) indicate that age had a slight effect on the liveweight of the ewes at the first two weighings (Week -5 and Week -2), although it did not reach statistical significance ($P > 0.10$). At the Week 0 weighing, there is a significant effect of age on liveweight, at the $P < 0.01$ level. For the final two weighings (Week +1 and Week +2), the effect of age on liveweight is highly significant ($P < 0.001$). For the entire flock, there was a highly significant ($P < 0.001$) age effect at all five weighings (see Figure 4). The effect of age on liveweight has been reported in other studies where the younger ewes tend to have lower body weights (Coop, 1962; Gunn et al., 1986).

3 Incidence of oestrus:

The use of CIDRs was effective in synchronizing oestrus in most of the animals treated, where 206 out of 234 (88.0%) were mated during the first 3 days following CIDR withdrawal. The CIDR retention rate of 98.3% reported in this experiment is comparable to that of McMillan (1986), but slightly better than those reported by both Harvey et al., (1984) and Hamra et al., (1986), which were 95.2% and 87.0% respectively. There was no noticeable incidence of either fluid discharge or unpleasant smell detected with the withdrawal of the CIDRs as reported both Hamra et al. (1986) and Maxwell & Barnes (1986) with sponge removal.

The onset of oestrus was significantly earlier ($P < 0.001$) in the mixed age ewes than that of the two-tooth ewes, as shown in Figure 5. The two-tooth ewes were significantly lighter in body weight than the mixed age ewes and this would explain the difference in oestrus onset between the two age groups. This is in agreement with Welch et al. (1984), who used CIDRs and reported that the best synchrony of oestrus occurred in high body weight and feed allowance ewes. Other studies using progesterone administration as injections or sponges (Lamond, 1963; Allison, 1975; Xu, 1987) had similar results. The majority of the mixed-age ewes in this experiment came on oestrus within the first day following CIDR removal, whereas the

two-tooth ewes were mostly mated on the second day. This finding is in agreement with the work of McMillan (1986), as well as Maxwell & Barnes (1986) who indicate that the majority of the ewes will be in oestrus between 24 and 48 hours following CIDR removal.

4 Ovulation rate:

The significant effect of both age and liveweight on OR was not unexpected, as Coop (1962) has shown that the OR is positively correlated with liveweight at mating. The two-tooth ewes tended to be lighter in body weight and therefore had a lower mean OR (1.55 ± 0.06) than did the mixed-age ewes (1.87 ± 0.04). The low OR in maiden ewes may be due to the low bodyweight of these animals as suggested by Scaramuzzi & Radford (1983). However, the mechanisms by which age influences OR is not clear. Comparison of the reproductive performance of groups of ewes differing in age but of similar body size and condition has revealed that only a very small age effect exists (Gunn et al., 1986). It becomes difficult to avoid the confounding effects of both age and bodyweight on the resulting OR and thus it is quite difficult to say with any certainty that the ORs were due to any one specific factor or combination.

5 Progesterone concentrations:

5.1 Daily progesterone concentration:

The preliminary analysis of the daily progesterone levels of the pregnant versus the non-pregnant ewes showed the relationship remains relatively constant over Days 9-14 of the oestrous cycle (Figure 6). From the results of a repeated measures analysis no significant differences ($P > 0.10$) were found in the progesterone concentrations between the pregnant and the non-pregnant ewes, but the relationship between the two groups of ewes appeared to change over time. The progesterone profile of the pregnant ewes in Figure 6, shows a fairly sharp increase in the progesterone levels at approximately Day 12 to 13 of the oestrous cycle when compared to the non-pregnant ewes. This sudden rise in progesterone concentration may be due to the early embryo signalling via a luteotrophic effect, to stimulate the ewe into producing more progesterone for the establishment of pregnancy (Wilmut et al., 1986).

According to Plotka & Erb (1967), Obst & Seamark (1970) and Allison & McNatty (1972), the progesterone level in ewes after mating normally increases from oestrus to maximum values by Day 9-11. In the ewes which fail to conceive, the progesterone level then drops sharply by Days 14 to 16 of the cycle. Other reports suggest that during the normal oestrous cycle, progesterone levels peak on Day 15 prior to it declining sharply on Day 17 - just before the reoccurrence of oestrus (Stabenfeldt et al., 1969). Brien et al. (1981) have shown that there is a significant difference in progesterone concentrations in the plasma of pregnant compared to non-pregnant ewes as early as 12 days following mating. This is supported by the work of Rhind et al. (1986) who found that ewes which were not pregnant had a rapid decline in progesterone profiles at Day 13 following mating when compared to those ewes which remained pregnant. Although, all the reports differ as far as the absolute levels are concerned, the general progesterone profile patterns are fairly similar with regard to whether or not the ewe conceives to mating or fails to become pregnant.

It appears from these reports that using the peripheral plasma progesterone concentrations as an early indication of the pregnancy status may not be very reliable if the samples were collected prior to Day 12 following oestrus. In the present experiment there may have been a trend towards a difference in hormonal concentrations at Day 12-13 due to an increase in progesterone levels in pregnant ewes, rather than a decrease in non-pregnant ewes. The repeated measures analysis however, indicated that no differences were statistically significant, which may be partly due to the small sample size. If blood sampling were continued after Day 14, it would have been possible to detect a difference in hormonal levels between the pregnant and non-pregnant ewes due to the regression of the CL and not luteal dysfunction (Diskin & Sreenan, 1986).

Other work has indicated that the progesterone levels vary significantly at other times in the oestrous cycle. Rhind et al. (1986) has shown that the mean progesterone profiles of ewes failing to become pregnant after joining were significantly lower than pregnant ewes at Days 4 to 6. Wilmut & Sales (1981) and Parr et al. (1982) suggest that embryo mortality is higher in animals with abnormal progesterone levels up to Day 6 after mating. This may

directly affect embryo survival and implantation, resulting in the ewes failing to establish a pregnancy. It was impossible to verify if progesterone levels actually differed significantly in the pregnant compared to the non-pregnant ewes in this trial, since blood sampling was not carried out prior to Day 9 or beyond Day 14.

5.2 Pooling of blood samples:

For the main progesterone analysis, the blood sampling regime involved the pooling of twice daily samples over the six day collection period for each ewe. This was done to reduce the considerable variation and fluctuations expected in the progesterone concentrations (Stabenfeldt et al., 1969). There is a suggestion that there are diurnal changes in the peripheral plasma progesterone concentrations which occur during the luteal phase of the sheep oestrous cycle (Allison & McNatty, 1972; McNatty et al., 1973). The result is a single sample, representative of the progesterone concentration of each ewe during the luteal phase of the cycle - defined here to be Days 9-14 of the oestrous cycle.

5.3 Age effects on progesterone concentrations:

The effect of age on the pooled progesterone concentrations was demonstrated with the two-tooth ewes having a slightly lower progesterone level (1.68 ng/ml) than did the mixed-age ewes (1.76 ng/ml), although this difference was not significant. The study of Brien et al. (1981) showed the opposite result where the progesterone levels at Day 12 post-mating were significantly higher in maiden (2.75 ng/ml) versus mature ewes (2.36 ng/ml). They have however indicated that other comparisons made by A. Williams (unpublished data) have either shown mature ewes to have higher plasma levels of progesterone in early pregnancy than maiden ewes or there was no significant difference. Other work involving the Romney sheep breed, have shown no differences between immature ewes and mature ewes with regards to peripheral progesterone levels (Smith et al., 1976).

5.4 Effect of CL number on progesterone concentration:

Ewes which had a single CL were found to have a significantly lower progesterone concentration (1.49 ng/ml) than did the ewes which had two CLs (1.73 ng/ml), as depicted graphically in Figure 7. The differences in progesterone level for single versus multiple ovulations was also found to remain constant throughout the blood sampling period. This finding agrees with other studies (Thorburn et al., 1969; Eastwood et al., 1976; Quirke et al., 1979; Brien et al., 1981; Williams & Cumming, 1982). Wilmut et al. (1986) reported that the progesterone concentrations during the luteal phase of the oestrous cycle increased as the number of ovulations increased, although each additional ovulation was found to produce less progesterone.

5.5 Nutrition and progesterone concentration:

In this experiment, the ewes on high feed allowance in this experiment had a significantly lower ($P < 0.05$) pooled progesterone level (1.49 ng/ml) than the ewes on the low feeding level (1.73 ng/ml). These significant differences in progesterone concentrations between the two feeding levels were found after correcting for the effect of body weight in the analysis. This was done because the effect of body weight on progesterone level may have been masking the feeding level effect since the body weights of the 45 ewes used for blood sampling varied considerably from 43.7 kg to 77.2 kg and averaged 60.1 kg. Previous studies have shown that an inverse relationship exists between nutritional level and peripheral progesterone level (Brien et al., 1981; Parr et al., 1982; Williams & Cumming, 1982; Rhind et al., 1985; McKelvey & Robinson, 1986; Parr et al., 1987a). The magnitude of the differences in hormonal levels caused by the two different pasture allowance levels reported in this study are not as large as those in other reports ie. 0.5 M and 1.5 M as reported by McKelvey & Robinson (1986) and 0.25 M, 1.0 M and 2.0 M reported by Cumming et al. (1971), Williams & Cumming (1982) and Parr et al. (1987a).

It has been suggested that modification of the peripheral plasma concentration of progesterone by the current level of feed intake is a result of a change in the rate of progesterone secretion (Cumming

et al., 1971). However, Parr et al. (1982) and Williams & Cumming (1982) thought steroid metabolism or metabolic clearance rate (MCR) brought about by an alteration in hepatic blood flow may have been the mechanism involved. It was been shown that there was a concomitant decrease in hepatic blood flow resulting from undernutrition in the ewe (Bensadoun & Reid, 1962). The liver has been shown to be a major site of progesterone catabolism in the sheep (Bedford et al., 1974).

Recent work by Parr et al. (1987b) has confirmed that alterations in progesterone levels brought about by nutritional treatments have been due to changes in the clearance rates of progesterone and not by changes in secretion rates. This was demonstrated by administering a known amount of progesterone into a group of ovariectomized ewes fed three different levels of feed intake (2.0 M, 1.0 M and 0.25 M) and then measuring the MCR of progesterone from the circulation. The present experiment was not designed to investigate the MCRs of peripheral progesterone, but it was concluded that the differences in pooled progesterone concentrations between the two different feeding levels may be due to the alterations in the metabolic clearance rates.

5.6 Progesterone and liveweight interaction:

Investigation of the relationship between the liveweight and progesterone concentrations (Figure 8) shows that the ewes on the low feeding level had a positive relationship between liveweight and pooled progesterone concentration. This differed significantly from the ewes on the high feeding level, where there was a constant relationship between liveweight and progesterone level, as shown in Figure 8.

Recently, Smith et al. (1986) and Payne et al. (1987) both suggested that both high feed intake and body weight were associated with an increase in the clearance rate of ovarian steroids from the circulation. Presumably, this is due to the heavier animals having a larger liver size and when combined with nutritionally induced alterations in the MCRs, are better able to catabolize the progesterone from the system.

The present analysis showed that a significant liveweight by feed level interaction existed ie. the level of feed intake interacted with the liveweight effect to alter the relationship involving the MCRs of progesterone in the ewes. This appears to agree with the findings of Smith et al. (1986) and Payne et al. (1987).

5.7 Exogenous CIDR treatment on progesterone concentrations:

The exogenous progesterone treatment in this experiment significantly increased pooled progesterone concentrations. The ewes that did not receive any post-mating CIDR treatment had a pooled progesterone level of 1.34 ng/ml, while those ewes that were treated with CIDRs had a significantly higher ($P < 0.001$) progesterone concentration of 1.88 ng/ml. There was no interaction between CIDR treatment and OR with regards to the pooled peripheral progesterone concentrations, thus indicating an additive effect of CIDR treatment and OR existed. This additive effect of the post-mating CIDR administration is in agreement with the findings of Parr et al. (1987a). Other studies have shown CIDR treatment to significantly increase plasma progesterone levels. This was reported by Ainsworth & Downey (1986), Hamra et al. (1986) and Barnes (1987) using ovariectomized ewes and heifers (Macmillan et al., 1987), as well as in entire animals (Parr et al., 1987a; Murray et al., 1988).

6 Reproductive performance:

For ease of discussion, the term 'reproductive performance' will refer to the traits which were of specific interest. These include pregnancy rate, embryo survival rate, lambing percentage, and the percentage of multiple births to the first mating.

6.1 Age:

Both the pregnancy and embryo survival rates in the two-tooth ewes were slightly greater than in the mixed-age ewes; Tables 13 and 14 show the difference to be 64.3% versus 62.3% (pregnancy) and 61.2% versus 54.6% (embryo survival) respectively. These differences were not significant ($P > 0.10$) and so they are unlikely to be real effects. The results for lambing percentage and percent of multiple births to the first mating (Table 14) show the mixed-age ewes to have

a slightly higher, non-significant lambing percentage than the two-tooth ewes (100.4% versus 97.7%). Scaramuzzi & Radford (1983) and Gunn et al. (1986) have reported similar findings. This result can partially be attributed to the significantly higher OR in the mixed-age ewes compared to the two-tooth ewes. This higher OR is also responsible for the slightly greater percentage of multiple births for the mixed-age ewes (72.4%) compared to the two-tooth ewes (61.6%) (Table 14), as shown from the work of Rattray et al. (1980 & 1987).

6.2 Ovulation rate:

The OR of the ewe appears to significantly influence the resulting reproductive performance, as shown in Tables 13 and 14. The pregnancy rate for all the ewes having only a single ovulation (50.8%) is shown in Table 13, while Table 14 shows the embryo survival rate for a ewe with a single ovulation as being 56.0%. In theory, these two values should be the same. The reason why these values differ is partly due to the 206 ewe record used to calculate the value in Table 13, while in Table 14 only 187 records were used. The pregnancy rate value was calculated using the ultrasound data and it is possible that some foetal loss may have occurred after the ultrasound procedure was performed (Quinlivan et al., 1966; Moore, 1985), therefore these values tend to differ slightly.

The resulting pregnancy rate of an ewe can be improved depending on whether the ewes shed a single ovum at oestrus or whether multiple ova were shed, as shown in Table 13. The pregnancy rate improved significantly ($P < 0.05$) from 50.8% to 68.3% for ewes with one CL versus multiple CLs. This is emphasized by the second part of Table 13 which shows significant differences in the pregnancy rate relative to the number of ovum shed for all the main and treatment effects. These results agree with the reports of both Gunn et al. (1972) and Hamra & Bryant (1982) who indicate that the pregnancy rate is markedly improved in sheep with two CLs compared with a single CL.

The results in Table 14 show that there was slightly higher embryonic survival in ewes having shed multiple ova (59.0%) compared to those shedding only a single ovum (54.5%), although this difference was not found to be significant ($P > 0.10$). Although this agrees with Gunn et al. (1972), it does not support the majority of

other studies such as Edey (1969a) or Wilmut et al. (1986) who found that the proportion of individual embryos surviving decreased as the number of ovulations increased. The reason for the conflicting results in this study may be that only two classes of CLs were considered (1 CL versus ≥ 2 CL) and thus it was not possible to verify what actually happened to embryo survival at the higher ORs of three or more.

The lambing percentage to first service has also been reported to be significantly affected by the OR (Cumming, 1972a). Ewes having only a single CL had a reduced lambing rate to first service compared to ewes having multiple ovulations. This observation is confirmed by the results shown in Table 14, where ewes having a single ovulation had a much lower lambing percentage (56.0%) than those ewes with multiple ovulations (115.3%). The reason for the effect of OR on lambing percentage is obvious. The greater the number of eggs being shed, the greater is the likelihood of pregnancy being established and larger litter sizes. As a final point it should be noted that the magnitude of the effect of OR on lambing percentage is considerably greater than the other individual main effects studied ie. age of ewe, post-mating nutrition and progesterone administration.

6.3 Feeding level:

The reproductive performance of ewes on the high pasture allowance levels (1.6 M) and the ewes on maintenance levels of pasture allowance are shown in Tables 13 and 14. Pregnancy rates were 64.4% versus 61.8%, embryo survival rates were 61.0% versus 54.7% and lambing rates were 101.7% versus 96.4% for the high and low feeding levels, respectively. These results were not significantly different in any single case, but they do indicate that the ewes on the high feed intake levels tended to have marginally better reproductive performances. These results would tend to support the findings of Keane (1975) who found that an improved plane nutrition during the breeding period significantly increased the first service conception rates.

In this trial the ewes on the low feeding level had a slightly higher percentage of multiple births (69.9%) than did the high feeding level (66.7%). The reason for this particular trend could be

due to the chance effects of randomization. It was through chance alone that the low fed ewes (1.81 ± 0.05) had a significantly higher OR than the high fed ewes (1.66 ± 0.05). The difference in mean ORs of the two groups may have influenced the results of this trial. Most reports indicate that the ORs significantly affect the pregnancy and embryo survival rates, as well as lambing percentage and the percent of multiple births. Because of these observations, allocation of ewes to the nutritional groups should be based on OR determination soon after mating.

There is considerable debate and lack of consistent findings in the literature concerning optimal levels of nutrition for maximum reproductive performance in the ewe, particularly around the time of mating. Some experiments have looked at improved nutrition after mating and found that high levels tended to be detrimental to embryo survival (Edey, 1976; Cumming et al., 1975; Brien et al., 1977; Parr et al., 1987a). A high plane of nutrition, also combined with high body condition around the time of mating has been associated with high embryonic mortality in ewes (Rhind et al., 1986 & 1988). These reports are obviously in disagreement with the results of this study. Other studies have found that undernutrition had no affect on embryo survival (Gunn et al., 1972; Parr & Williams, 1982; McKelvey & Robinson, 1986; Parr et al., 1987a), although embryo growth was retarded in ewes fed sub-maintenance diets when the embryos were viewed at Days 11 and 21 post-mating (Parr et al., 1982). On the other hand, when sub-maintenance diets were fed during the first few weeks after mating, they were associated with an increased incidence of embryonic mortality (Edey, 1966; Cumming, 1972a & 1972b; Cumming et al., 1975; MacKenzie & Edey, 1975; Hamra & Bryant, 1982).

Most of these results thus suggest that extremes in nutrition soon after mating appear to have detrimental effects on embryo survival rates. It has therefore been recommended by Robinson (1986) that by keeping recently mated ewes on maintenance levels of feeding during the first month of pregnancy and avoiding any type of nutritional stress, embryonic wastage can be kept to a minimum. This recommendation is also supported by the findings of Cumming et al. (1975) who found that embryo survival rate was greatest in ewes fed a maintenance level diet from immediately following mating, compared to ewes fed either a 0.25 M or a 2.0 M level ration. In the preseht

study however, the ewes on the 1.6 M level of pasture allowance appear to have slightly better reproductive performance than the ewes on the 1.0 M intake level, which fails to support Robinsons recommendation concerning feeding levels soon after mating. However, this recommendation assumes that a certain amount of tolerance exists around what is considered to be maintenance levels. Maintenance levels do not mean that ewes must be fed a diet of exactly 1.0 M, although any extreme levels of intake should be avoided. Perhaps there is room to improve embryonic survival in recently mated ewes through feeding higher than maintenance up to a certain level beyond which embryo survival decreases sharply.

6.4 CIDR treatment:

Investigation of the effect of post-mating CIDR supplementation versus no CIDR on the reproductive performance traits shows there were slight improvements in pregnancy rate (65.1% versus 61.0%), embryo survival rate (59.9% versus 55.8%) and lambing rate (102.6% versus 95.5%) respectively (Tables 13 and 14). The percentage of multiple births also shows a similar trend, however all these trends were not statistically significant ($P > 0.10$). Several reports have shown that administration of post-mating CIDRs to ewes significantly improved the reproductive performance of ewes either through improved pregnancy, embryo survival, and/or subsequent lambing rates (Pearce et al., 1984; Peterson et al., 1984; Davis et al., 1986; McMillan, 1987; Parr et al., 1987a). A recent report by Dattena (1989) found that post-transplantation progesterone supplementation, in the form of CIDR treatment, significantly increased the survival rates of transferred embryos and the pregnancy rates in recipient ewes. Others studies however have reported no difference in the resulting ewe reproductive performance (Smith et al., 1985; Kerton et al., 1986; Murray et al., 1988).

In cows, post-mating progesterone supplementation may increase pregnancy rates. The effect of exogenous progesterone supplementation during the luteal phase of the cycle following mating in the cow may tend to increase conception rates, however the results in most individual studies were not statistically significant (Johnson et al., 1958; Sreenan et al., 1979; Marcus & Ayalon, 1981; Folman et al., 1983; Sreenan & Diskin, 1983; Diskin & Sreenan, 1986; Macmillan

& Taufa, 1987). In repeat breeder cattle, the use of progesterone supplementation also seems to be of some benefit (Herrick, 1953; Dawson, 1954; Wiltbank et al., 1956). However, as there are likely sub-fertility problems of greater magnitude associated with these repeat breeder cows, as seen from the abnormally low pregnancy rates of control animals, this might implicate more than just a simple deficiency of luteal progesterone (Sreenan & Diskin, 1983).

The results of the present trial therefore appear to agree with the most of the other reports concerning the beneficial effects of post-mating progesterone supplementation on the resulting reproductive performance. There is a slight trend towards an improvement in the reproductive performance of ewes, although in all cases, none of these findings were statistically significant.

6.5 CIDR x feed level interaction:

Examination of the data on the pregnancy rate to first service (Table 13) indicates that a significant feeding level x CIDR interaction existed ($P < 0.05$). It was found that in the ewes fed at maintenance (1.0 M), supplementation with post-mating progesterone increased the pregnancy rate from 51.0% to 72.6%. Unexpectedly the ewes that were fed a high plane of nutrition (1.6 M) and treated with a post-mating CIDR had decreased pregnancy rates. Table 13 shows that pregnancy rates fell from 71.4% in the untreated group to 58.2% in the CIDR treated group, however the reduction was not statistically significant.

When comparing the results of the embryo survival rates, similar trends to the pregnancy rate data were found. The CIDR treatment increased the embryo survival rate for the low fed ewes, whereas post-mating CIDR treatment given to ewes on the high pasture allowance tended to reduce the proportion of lambs born per ovum shed, as shown in Table 14. Even though this loss was fairly substantial (66.6% to 55.4%), the difference was not statistically significant ($P > 0.10$). This trend was also seen in the percentage of multiple births between treatments, however this was not significant.

Parr et al. (1987a) have shown that progesterone supplementation was only effective in ewes fed a high (2.0 M) feed level, while ewes on either a medium (1.0 M) or low (0.25 M) level of intake did not respond to post-mating CIDR therapy through an improved pregnancy rate. Other studies using exogenous progesterone therapy, often have conflicting results (see Table 2) and in most no mention is made of the nutritional conditions in early pregnancy. Parr et al. (1987a) suggest that the explanation for the opposing reports in the literature which fail to report nutritional status is that an increase in the reproductive performance of ewes given exogenous progesterone treatment will only occur when ewes are fed high rations or are in a rising nutritional state after mating. This was not found to be the situation in this experiment where the ewes on the high feeding allowance level (1.6 M), when supplemented with CIDR treatment following mating, tended to have reduced reproductive performance.

McMillan (1987) however found similar results to those of the present experiment where ewe hoggets treated with CIDRs tended to have reduced reproductive wastage and thus increased pregnancy rates when post-mating nutrition was only adequate for liveweight maintenance. Although the post-mating feeding levels were not monitored in the McMillan (1987) study, they were considered to be consistent with that needed for liveweight maintenance. This however still does not explain why the post-mating CIDR treatment for the high fed ewes (1.6 M) tended to reduce reproductive performance.

Relating these reproductive performance findings to the progesterone concentrations indicates that there was a significant effect of feeding level on the progesterone levels. A higher progesterone concentration existed in the ewes on the low feed allowance level compared to the high fed ewes, once an adjustment had been made for the effect of liveweight. This agrees with the findings of Williams & Cumming (1982) and Parr et al. (1987a) who reported a decline in peripheral progesterone concentration with increasing feed intake. The reasons for this inverse relationship between nutritional level and progesterone concentration was not studied in this present work, but may well be associated with an increase in the metabolic clearance rates (MCR) of progesterone for ewes on a high level of intake, as was recently demonstrated by Parr et al. (1987b).

The mechanism by which post-mating progesterone supplementation affects ewe reproductive performance is not clear. McMillan (1987) suggests that the response may be unrelated to nutritional effects on progesterone levels and that post-fertilization loss may be reduced in multiple ovulating ewes. This conflicts with the conclusions of Parr et al. (1987a), who indicate that an increase in pregnancy rate with exogenous progesterone treatment will only occur when ewes are fed high rations or are in a rising nutritional state after mating. Furthermore, results from the McMillan (1987) study showed that increased multiple pregnancies, rather than more pregnancies, may be a consistent feature in supplemented ewes. In the present study however there were substantial increases in the number of pregnancies, as well as in the embryo survival rates for the ewes on the low pasture allowance level when supplemented with CIDRs following mating.

The reasons for the high fed ewes not responding to post-mating CIDR treatment may involve a change in sensitivity to progesterone, brought about by an alteration in nutritional level. Alternatively, this result could also be entirely a chance occurrence. The question might be asked whether the high fed ewes were insensitive to progesterone supplementation and therefore unable to respond to CIDR treatment, while the low fed ewes were responsive following mating and consequently there was an increase in reproductive performance. It seems somewhat difficult to accept that well fed animals should have shown a decreased reproductive performance when treated with post-mating CIDRs. The question needs to be studied further as to confirm whether high fed ewes have an insensitivity to progesterone and whether the possible mechanisms for controlling it are important.

7 Further research studies:

Results arising from this experiment show that there are several aspects that need further investigation. The determination of whether the effects of CIDR treatment on reproductive performance in well fed and moderately fed ewes occur through either partial failure of multiple ovulation (PFMO) or the factors affecting the pregnancy rate needs to be established. An experimental design including the planned slaughter of a random number of ewes following mating to determine the fertilization rates might be useful in this respect. This may

provide some evidence on the mechanism responsible for the results found in the present experiment. McMillan (1987) has suggested that CIDR treatment may reduce post-fertilization loss in multiple ovulating ewes. The use of other post-mating treatments to stimulate luteal function, such as GnRH or hCG administration to see whether these have any beneficial effect on the reproductive performance of recently mated ewes could also be incorporated into further studies.

The collection of faecal samples for two weeks following mating rather than one week might provide some information as to why the liveweight changes in the post-mating period were not constant. This would have shown whether the level of intake estimated was different for the first week versus the second and if so - maybe these different intakes affected the results. A further refinement in the conduct of this work might be the use of ewe condition scoring to determine the amount of fat cover, rather than merely using the liveweight of the animals as an indication of their nutritional treatments.

Blood sampling of the ewes should also be extended to the entire length of one oestrous cycle following mating. This would enable the determination of the stage of the oestrous cycle when progesterone levels start to differ between pregnant and non-pregnant ewes. Trounson & Moore (1974) indicate that as early as the second day after oestrus, the activity of the CL as assessed by its progesterone secretion, may well influence the success or failure of pregnancy. It needs to be confirmed in animals of various ages, whether abnormally high or low progesterone levels early in the cycle affects the reproductive performance.

The measurement of MCR of progesterone by using ovariectomized ewes treated with CIDRs in a trial such as this, may help in establishing how the present feeding levels interact with CIDR treatment, body weight and other factors to influence progesterone levels.

Determination of ORs prior to the allocation of the ewes to post-mating pasture allowance levels will reduce the chance of OR being different between the two groups. This is important because it was not known what effect the distribution of OR had on the

subsequent reproductive performance of the treatment groups in the present experiment.

Adjusting the feed levels (eg. to 1.5 M and 0.5 M) in order to get levels of herbage allowance on both above and below the maintenance requirements could also be investigated as these were the levels used by McKelvey & Robinson (1986) in their ET studies. Extreme feeding levels however should be avoided, as these are rarely encountered under normal farming conditions. The two different post-mating feeding levels could be extended to include the entire length of the breeding season (4 to 6 weeks) rather than just a two week period as used in this experiment. This would test if the effects of feeding level are important later on in early gestation. It is important to establish what the nutritional requirements of the ewe are in early pregnancy, so the farmer can plan the use of his autumn pastures accordingly. He would thus be able to conserve pasture, particularly if herbage is in short supply and utilize these later during gestation when adequate nutrition of the pregnant ewes becomes essential.

8 Conclusions:

The results of this experiment demonstrate that the techniques of measuring the pasture residual (using the EPM), as well as the CRD capsules to indirectly assess the voluntary herbage intakes were relatively accurate and convenient in estimating the intake levels of ewes on pasture. The flushing level of intake of the ewes prior to mating was estimated to be 1.2 M, while the two post-mating pasture allowance levels offered to the ewes resulted in intake levels estimated to be approximately 1.0 M and 1.6 M for the low and the high fed ewes, respectively. Liveweight changes over the experimental period indicated that ewes were on a rising plane of nutrition (flushed) before mating, which is in accordance with the normal practice of flushing prior to mating. The results from body weights show that the high fed ewes had a net gain of 4.1% of initial liveweight during the two post-mating period, while the low fed ewes had a net loss of 2.8% of the initial liveweight for the same period.

Oestrus synchronization using CIDRs resulted in 88.0% of all ewes (n=234) being mated during the three days following CIDR

withdrawal. The mixed-age ewes appeared to be in oestrus sooner than the two-tooth ewes. Ewe liveweight influenced the onset of oestrus in the two-tooth ewes where the heavier two-tooth were in oestrus sooner than in the lighter two-tooth ewes. There was no effect of liveweight on the onset of oestrus in the mixed-age ewes.

The ORs were significantly higher for the mixed-age ewes (1.87 ± 0.04) than the two-tooth ewes (1.55 ± 0.06). This difference is likely to be associated with the mixed-age ewes being older and heavier ($P < 0.01$) than the two-tooth ewes.

The two-tooth ewes were found to have a slightly better pregnancy rate and embryo survival rate than the mixed-age ewes. The mixed-age ewes however had a slightly better lambing percentage and percentage of multiple births than the two-tooth ewes. This is a reflection of the higher OR of the older animals.

Ewes with multiple ovulations had a significantly better chance of becoming pregnant than did the ewes having only a single ovulation. Multiple ovulations had the effect of doubling the lambing percentage compared to ewes which had shed only a single ovum (115.3% versus 56.0%).

Ewes on the 1.6 M post-mating pasture allowance level with post-mating CIDR supplementation had slightly better pregnancy, embryo survival, and lambing rates than did either the ewes on the 1.0 M level of intake or the untreated CIDR animals. This effect was not additive, instead there was a significant interaction between post-mating feeding level and CIDR treatment.

The effect of high post-mating feed allowance level (1.6 M) decreased the pooled plasma progesterone concentrations (after correcting for the effect of body weight), but no relationship between pregnant versus non-pregnant ewes was found with regard to these hormonal levels from Days 9-14 following mating. Both increased ORs and the addition of post-mating CIDR supplementation significantly increased peripheral progesterone concentrations through an additive effect.

In conclusion, post-mating CIDR treatment appears to improve reproductive performance of ewes on maintenance levels of pasture allowance soon after mating. If ewes have access to ad libitum grazing of good quality pastures, it is recommended not to treat these ewes with post-mating CIDR treatment, as it may have a detrimental effect on the resulting pregnancy, embryo survival rates and lambing rate. Ewes grazing maintenance levels of pasture allowance will only have their reproductive performance improved through either an improvement in nutrition or post-mating progesterone supplementation, but not by both. Only where grazing is limited at mating is post-mating CIDR administration likely to lead to increases in reproductive performance (pregnancy rate, embryo survival rates or lambing performance). It is not known why the CIDR treatment works better at one feeding level and not the other. It however becomes obvious that the mechanism responsible for this decrease in reproductive performance in the better-fed ewes involves a much more complex mechanism than simply a luteal deficiency of progesterone caused by high feed intakes during the post-mating period. Similar studies have led to different conclusions with regard to post-mating CIDR supplementation and its effect on the resulting reproductive performance on ewes. However, the magnitude of the difference between progesterone levels resulting from the two feeding levels in this experiment is relatively small in comparison to the difference between feeding levels in other studies.

APPENDIX

APPENDIX 1

Lamb birth weight and litter size:

The mean birth weights of the lambs were found to be significantly influenced by the number of lambs born ($P < 0.001$), as shown in Table 15. The single-born lambs were heavier (5.02 ± 0.09 kg) than the average weight of multiple-born lambs (4.10 ± 0.09 kg). Similar findings have been reported in other studies (Wallace, 1948; Harrington & Whiteman, 1967; Hulet et al., 1969; Eastwood, 1975; Dalton et al., 1980). Bradford et al. (1974) and Hinch et al. (1983) have shown that an inverse relationship exists between litter size and lamb birth weight.

There was no significant difference in mean birth weight between the two-tooth ewes and the mixed-age ewes in this study. Average birth weights were 4.56 kg for both age groups of ewes. This result differs from the work of Hunter (1956), Karihaloo & Combs (1971) and Dalton et al. (1980) who indicate that mature ewes normally give birth to heavier lambs than do younger ewes. Dalton et al. (1980) found that two year old parous ewes had significantly lower birth weights than did older ewes which had lambed previously.

Total litter weight at birth (Table 15) was significantly influenced by both the age of the ewe ($P < 0.01$) and the number of lambs born ($P < 0.001$). The total litter weight of lambs born to the two-tooth ewes (6.41 ± 0.28 kg) was significantly less than that born to the mixed-age ewes (6.99 ± 0.22 kg). Total litter weights were heavier for ewes with multiple numbers of lambs (8.23 ± 0.13 kg) than with single lambs (5.01 ± 0.14 kg). Hinch et al. (1983) added that there is also a progressively smaller increase in total litter weight for each increase in the number of lambs born per ewe.

Table 15: Effect of age, feeding level, CIDR treatment, cycle and number of lambs born on the mean birth weight, total litter weight, gestation length, lamb sex ratio and percent of multiple births (Data from 187 ewes).

Effect	Level	n	Mean Birth Weight[A] (kg)		Total Litter Weight[A] (kg)		Gestation Length[A] (days)		Lamb Sex Ratio[B] % Ram (n)	% Ewe (n)		Percent Multiple Births (n)	
Age (years)	2	74	4.56 ± 0.12	NS	6.41 ± 0.28	**	148.03 ± 0.29	**	44.4 (48)	55.6 (60)	NS[C]	62.0 (33)	*[C]
	>2	113	4.56 ± 0.09		6.99 ± 0.22		147.41 ± 0.22		51.4 (94)	48.6 (89)		74.9 (67)	
Feed Level	L	95	4.54 ± 0.09	NS	6.65 ± 0.13	NS	147.29 ± 0.20	+	49.0 (72)	51.0 (75)	NS	69.4 (50)	NS
	H	92	4.59 ± 0.09		6.59 ± 0.14		147.67 ± 0.21		48.6 (70)	51.4 (74)		70.8 (51)	
CIDR	-	94	4.55 ± 0.09	NS	6.65 ± 0.13	NS	147.45 ± 0.21	NS	47.9 (69)	52.1 (75)	NS	68.1 (48)	NS
	+	93	4.57 ± 0.09		6.58 ± 0.14		147.50 ± 0.21		49.7 (73)	50.3 (74)		72.1 (52)	
Cycle	1	123	4.64 ± 0.08	NS	6.71 ± 0.11	NS	148.34 ± 0.18	***	51.3 (97)	48.7 (92)	NS	68.2 (63)	NS
	≥2	64	4.49 ± 0.11		6.53 ± 0.16		146.62 ± 0.26		44.1 (45)	55.9 (57)		73.5 (37)	
Litter Size	1	87	5.02 ± 0.09	***	5.01 ± 0.14	***	147.73 ± 0.22	+	49.4 (43)	50.6 (44)	NS	NA[D]	
	≥2	100	4.10 ± 0.09		8.23 ± 0.13		147.22 ± 0.20		48.5 (99)	51.5 (105)			

A LSM ± SEM

B Total number of lambs in this analysis n=291.

C Chi-square test.

D Non-applicable.

Gestation length:

Gestation length was significantly longer in the two-tooth ewes (148.03 ± 0.29 days) than in the mixed-aged ewes (147.41 ± 0.22 days), as shown in Table 15. Even though this difference was statistically significant at the $P < 0.001$ level, the size of the effect was small and of little practical value. This is in agreement with the work of Forbes (1967) who found that as the ewes aged, the length of the gestation period tended to decrease up to the fifth parity and then it started to increase. These results conflicted with those of Bradford et al. (1974) who found that the gestation was shorter in younger ewes than in mature ewes, and stated that parity had no effect other than that associated with age.

The gestation length to the first synchronized mating (148.34 ± 0.18 days) was significantly longer ($P < 0.001$) than that of ewes lambing to subsequent matings (146.62 ± 0.26 days). There appears to be no explanation for this difference, although both Wallace (1948) and Alexander (1956) indicate that poor nutrition during the last two months of pregnancy have been implicated in the reduction of the length of gestation. It was not possible to verify this in the present experiment as the nutritional levels of the ewes were not monitored during the latter stages of gestation.

Ewes carrying a single lamb had a slightly longer gestation length (147.73 ± 0.22 days) than did the ewes which had multiple lambs at birth (147.22 ± 0.20 days) as shown in Table 15, although this difference was not significant ($P > 0.10$). Other workers have also noted that as litter size increased, the length of gestation decreased (Forbes, 1967; Bradford et al., 1974; Eastwood, 1975). However, Boshier et al. (1969) found litter size had no effect on gestation length in sheep.

Sex ratio:

There were no significant differences ($P > 0.10$) between any of the treatments imposed or any of the other factors analysed, with regard to the sex ratio of ram lambs to ewe lambs. The overall sex ratio, including 291 records of lambs born, was 48.8% (142) rams to 51.2% (149) ewes. This ratio is not unexpected since the sex ratio at birth is normally assumed to be 50:50 (Foote & Miller, 1971), however a variety of natural conditions has been reported by Lawrence (1941) to be associated with minor changes in the sex ratio.

Percentage of multiple births:

The two-tooth ewes had a significantly lower ($P < 0.05$) percentage of multiple births when compared to the mixed-age ewes (62.0% versus 74.9%). This appears to be directly associated with the higher ORs in the mixed-age ewes compared to the two-tooth ewes, and is related to both the age and liveweight effects (Rattray et al., 1980 & 1987). The present results are also in agreement with Edey (1969b) who noted that maiden ewes normally have a lower lambing percentage than that of mature ewes.

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