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Effect of immunization against androstenedione on the reproductive performance of ewe lambs: Assessment of dosing regimens

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Abstract

In New Zealand, there are a number of impediments to the adoption of ewe lamb breeding, for example, their poor reproductive performance compared to mature ewes. This low reproductive performance is primarily due to a lower ovulation rate. A series of studies such as improving nutrition pre-breeding and live weight at breeding have been conducted in New Zealand to improve the reproductive performance of ewe lambs, but they have not resulted in significant increases in the ewe lamb ovulation rate. In mature ewes, immunization against androstenedione can increase ovulation rate. Immunized mature ewes release three or more ova in each estrous cycle. To date, little data has been generated for the response to immunization against androstenedione of ewe lambs. One of the most commonly used androstenedione vaccines in New Zealand is Androvax®. A study conducted in Ireland using Fecundin® reported that control ewe lambs showed estrous behaviors at an earlier date than immunized ewes. This thesis, therefore, examines the effect of immunization against androstenedione using Androvax® on the reproductive performance of ewe lambs at two different dosing regimens.

The present study was conducted between February 2017 and January 2018 using 300 Romney ewe lambs. Ewe lambs were immunized with Androvax® using two dosing regimens, 1) ten and six (A10 – 6) weeks and, eight and four (A8 – 4) weeks prior to the introduction of the entire ram. Teaser rams fitted with mating harnesses were introduced to the ewe lamb flock at a ratio of 1:100 and remained with the flock until the start of breeding. During this time, ewe lambs were checked for teaser harness crayon marks as an indicator of the onset of behavioral oestrus. After the removal of the teaser rams, entire rams fitted with mating harnesses were introduced at the ratio of 1: 50 and remained with the flock for two oestrous cycles (34 days). Ewe lambs were also checked for ram harness crayon marks after every 17 days from the introduction of the entire ram to identify the cycle in which they were bred. Pregnancy diagnosis was carried out 90 days after the start of the breeding period. Ewe lambs bearing triplet fetuses were removed from the study. Lambing began in October and ended in November. Lambs were weaned in January at the average of 102 days of age.

The majority of ewe lambs in A10 – 6 and those in a control treatment were marked by the teaser ram prior to the introduction of the entire ram compared with those in A8 – 4 treatment (62 % vs 56 vs 44% respectively, $p<0.05$). Ewe lambs that show signs of behavioral oestrus prior to the introduction of the entire ram have higher ovulation rates leading to a high fecundity rate, however, in this study, ewe lambs in A8 - 4 treatment had greater ($p<0.05$) fecundity rates even though they showed later onset of behavioral oestrus. Ewe lambs in A8 – 4 treatment had higher ($p<0.05$) fecundity rates at pregnancy diagnosis than the control treatment while those in A10 – 6 treatment did not differ from either group (1.66 vs 1.30 vs 1.49 respectively; $p>0.05$). Ewe lambs in A8 – 4 treatment had a greater ($p<0.05$) percentage of twin and triplet fetuses and fewer singles compared with the control and A10 – 6 treatment. At lambing, the number of lambs born did not differ between the three treatments, however, treated groups (A10 – 6 and A8 – 4), had more twins than the control treatment. Generally, immunization against androstenedione increased the fecundity rates but not the itter size and number of lambs weaned and the recommended dosing regimen is eight and four weeks before breeding (A8 – 4). It is, therefore, important for farmers to work on minimizing pregnancy losses and lamb mortality when they used androvax in ewe lambs in order to increase both litter size and number of lambs weaned.

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Chapter 1 : Introduction

Ewe lambs have low and variable reproductive performance when compared with mature ewes (Donald et al., 1968, Forrest and Bichard, 1974, Beck et al., 1996, Corner et al., 2013, Edwards et al., 2015, Edwards et al., 2016). Poor reproductive performance is associated with failure to attain puberty, failure to be mated by the entire ram (McMillan and McDonald, 1983), low ovulation rate (Beck et al., 1996, Edwards et al., 2016) and embryo / fetal losses (Edey, 1967, West et al., 2009, Ridler et al., 2017). Failure to attain puberty is primarily due to low live weight, for a ewe lamb to attain puberty it should achieve 40 – 60% of its expected mature live weight (Ross, 1989). Some ewe lambs, however, reach puberty but still fail to be mated by the entire ram. Kenyon et al. (2005) reported that exposing ewe lambs to teaser ram before introducing the entire ram can improve their receptivity by inducing puberty.

The greatest contributing factor to the poor reproductive performance of ewe lambs is their low ovulation rate (Coop, 1962, Mulvaney et al., 2013, Edwards et al., 2016), resulting in small litter sizes (Beck et al., 1996, Edwards et al., 2016). If ovulation rate could be increased, breeding ewe lambs could be more profitable and attractive to farmers. In mature ewes, the ovulation rate can be increased through various methods including; flushing (Scaramuzzi et al., 2006), increasing live weight at breeding (Kenyon et al., 2004b), genetics (Galloway et al., 2000, Wilson et al., 2001, Davis, 2005, Edwards et al., 2016, Edwards and Juengel, 2017) and immunization against steroids (Henderson et al., 1984, Smith, 1985a). Some of these methods such as flushing and genetics are not applicable in ewe lambs (Juengel et al., 2013b).

In mature ewes, ovulation rate can be increased by immunization against androstenedione. The most commonly used androstenedione-based vaccines in New Zealand are Androvax® and Fecundin®. Androvax® is an anti- androstenedione vaccine that is known for increasing ovulation rate and thus litter size in mature ewes (Henderson et al., 1990, Juengel et al., 2013b). To date no data has been generated for the use of Androvax® in ewe lambs. The dosing regimen for Androvax® has been developed specifically for mature ewes, therefore, it is not known if it is the best regimen for ewe lambs. For mature ewes, the recommended dosing regimen requires two doses, the first, a sensitizing injection is given at eight weeks before breeding and the second, a booster injection, administered four weeks before breeding (West et al., 2009, Juengel et al., 2013b). An Irish study on the effect of immunization with Fecundin found that control ewe lambs showed estrous behaviors at an earlier date than immunized ewes, however, the difference was not significant (Ronayne et al., 1991). This study, therefore, set out to determine the effect of immunization against androstenedione on the reproductive performance of ewe lambs, and to identify the optimum dosing regimen for ewe lambs.

The research questions were:

- Can the Androvax® vaccine increase the reproductive performance of ewe lambs?
- What is the optimal dosing regimen for ewe lambs?

Chapter 2 Literature Review

2.1 Introduction

In New Zealand, sheep farming plays a major role in contributing to the economy of the country (Brito et al., 2017). Despite this, from 2007 to 2017, sheep numbers have declined from 38.48 million to 27.37 million (Beef and Lamb New Zealand, 2018). Of sheep wintered in 2017, 17.7 million were mature breeding ewes while 9.7 million were ewe lambs, dry ewes, weathers and rams (Beef and Lamb New Zealand, 2018).

Since the removal of subsidies in the mid-1980s, New Zealand sheep farmers have been working on improving the profitability of their sheep production systems (Paul et al., 2000). Methods of improving the reproductive performance of mature ewes, and thus the profitability, have been widely discussed. Such methods include; genetic improvements (Davis et al., 1982), flushing (providing ewes with extra feeding before breeding) (Scaramuzzi et al., 2006), increasing live weight and BCS at breeding (Kenyon et al., 2004b), hormonal treatments (Abecia et al., 2012) and immunization (Smith, 1985a). Breeding ewe lambs less than one year of age has been identified as another option for increasing the number of lambs born per year (Kenyon et al., 2014b, Edwards and Juengel, 2017).

In New Zealand, the traditional practice is to breed ewes for the first time when they are 18 to 20 months of age (two tooths) (McMillan and McDonald, 1983, Kenyon et al., 2004c, Kenyon et al., 2014b, Edwards and Juengel, 2017). Currently, 25 to 30% of farmers breed their ewe lambs each year (Kenyon et al., 2004c, Morris and Kenyon, 2014). In a 2004 survey, the major reason cited by farmers for not breeding their ewe lambs was the perception that it can impair their breeding performance as two tooths (Kenyon et al., 2004c). Recent research, however, has shown that if ewe lambs are preferentially fed after they wean their lambs, breeding performance as two tooths was not impaired (Kenyon et al., 2008).

The reproductive success of ewe lambs depends on their ability to complete the reproductive processes such as attain puberty, ovulating viable ova, being mated by a fertile ram, a fully developed reproductive tract and the ability to deliver and raise a lamb(s) to weaning (Edwards et al., 2016, Edwards and Juengel, 2017). Under New Zealand pastoral conditions, the reproductive performance of ewe lambs is lower than that of mature ewes (Corner et al., 2013). Ewe lambs have lower ovulation, conception and fecundity rates and wean fewer lambs compared to mature ewes (Table 2.1). Based on Table 2.1, the ovulation rate of ewe lambs ranges between 1.29 - 1.51, fecundity rates 0.81 – 1.52 lambs born per ewes bred, lamb survival 69 – 89%, number of lambs weaned 0.38 – 0.82 lambs weaned per ewes bred. Collectively, all these can limit the adoption of ewe lamb breeding.

Table 2. 1. Comparison of reproductive performance (ovulation and fecundity rates, lamb survival and number of lambs weaned) of ewe lambs and mature ewes

Dam Age (years)	Ovulation rate	Fecundity (Lambs born/ewe bred)	Lamb survival (%)	Lambs weaned per ewe bred	References
1	NR	1.00 – 1.52	71 – 87	0.38-0.82	(Donald et al., 1968)[1]
2	NR	1.08 – 2.02	83 - 93	0.87-1.65	
3	NR	1.16 – 2.30	95 - 100	1.10 – 2.17	
1	NR	1.5	NR	NR	(Forrest and Bichard, 1974)
2	NR	1.5	NR	NR	
3-8	NR	1.78	NR	NR	
1	1.07	NR	NR	NR	(Beck et al., 1996)
3-5	1.25	NR	NR	NR	
1	NR	NR	89 (single)	NR	(Corner et al., 2013)
	NR	NR	69 (twins)	NR	
Mixed age	NR	NR	96 (single)	NR	
	NR	NR	88 (twins)	NR	
1	1.51	NR	NR	NR	(Ronayne et al., 1991)
1	1.29	NR	NR	NR	
2	2.5	1.61	NR	NR	(Edwards et al., 2015)
3-4	2.4	1.78	NR	NR	
1	1.54	0.81	NR	0.67	(Edwards et al., 2016)
2	2.0	1.48	NR	1.20	

1 Mean from different breeds
NR = not reported

It is likely that methods used to increase the reproductive performance of mature ewes will have a similar effect on ewe lambs. The reproductive performance of ewe lambs has been shown to increase with the use of teaser rams, increasing their live weight at breeding and greater nutrition during pregnancy (Kenyon et al., 2004d, Kenyon et al., 2005, Mulvaney et al., 2010a, Kenyon et al., 2014b, Corner-Thomas et al., 2015). In ewe lambs, these management practices can increase conception and fertility rates (ewe pregnant per ewe bred), lamb birth weights and lamb survival, but have no effect on fecundity rates (Kenyon et al., 2004d, Kenyon et al., 2005, Mulvaney et al., 2010a, Kenyon et al., 2014b, Corner-Thomas et al., 2015). Flushing is virtually impossible in ewe lambs because they require a high level of nutrition after weaning in order to achieve adequate live weights for breeding (Edwards et al., 2016). Hormonal treatments such as the use of progesterone and their analogues (progestagens), prostaglandins and melatonin (Abecia et al., 2012) and immunization against steroids such as androstenedione, testosterone, estrone, and progesterone (Smith, 1985a) have a temporary effect on ovulation rate, however, the use of these treatments has not been investigated in ewe lambs. Edwards et al. (2016) reported that hormonal treatments are expensive, and therefore not cost effective for small scale farmers. Among mature ewes, immunization against androstenedione can increase ovulation rate, leading to a greater percentage of ewes bearing twins and triplet fetuses (Scaramuzzi et al., 1983, Juengel et al., 2013b). An Irish study on the effect of immunization with Fecundin reported that control ewe lambs showed oestrous behaviors at an earlier date than immunized ewes, however, the difference was not significant (Ronayne et al., 1991). Further investigations are, therefore, required to determine the impact of immunization against androstenedione on ewe lamb reproductive performance and the impact of the timing of the doses.

2.2 Ewe lamb breeding

2.2.1 Advantages

There are a number of advantages to breeding ewe lambs to an extensive sheep production system including, more lambs born per year, more lambs produced in a ewe's lifetime and a greater opportunity for genetic gain (Kenyon et al., 2004d, Edwards et al., 2016, Edwards and Juengel, 2017). Ewe lambs that produce a lamb at one year of age will have greater lifetime productivity than a ewe bred at 18 months of age (Kenyon et al., 2004d, Edwards et al., 2016). Edwards and Juengel (2017) reported that a ewe bred as a lambs had an extra 20% lamb production in their lifetime compared with those bred for the first time at 18 months. Similarly, Levine et al. (1978) showed that ewes bred as ewe lambs weaned an average of 3.24 lambs in their lifetime compared with 2.4 for ewes bred for the first time as two tooths. The additional lamb produced results in a greater number of lambs being available for sale, thus increasing farm profits (Corner et al., 2013, Kenyon et al., 2014b).

Ewe lamb breeding can improve the reproductive performance of the ewe when bred at two years of age (McMillan and McDonald, 1983). If a ewe lamb is preferentially fed after weaning their first lamb until the next breeding at 18 months of age, they are more likely to give birth to multiple lambs in their second lambing period (Kenyon et al., 2008, Corner et al., 2013). Two tooth ewes previously bred as ewe lambs have a greater incidence of twin ovulations than those bred for the first time at 18 months of age (McCall and Hight, 1981, McMillan and McDonald, 1983). An additional advantage of breeding ewe lambs is that, two tooth ewes previously bred as a ewe lamb can be run as one mob with mature ewes as they require less assistance during lambing compared to those bred for the first time as two tooths (McMillan and McDonald, 1983, Kenyon et al., 2014a).

Ewe lamb breeding can enable a more efficient utilization of spring feed resources (McCall and Hight, 1981). The New Zealand sheep production system is pasture based, with maximum grass growth occurring in spring, therefore, ewe lamb breeding can enable the efficient use of pasture during this period (McCall and Hight, 1981, Kenyon et al., 2004c).

2.2.2 Disadvantages

There are a number of factors that may limit the success of ewe lamb breeding. These include failure to attain puberty, poor reproductive performance (ovulation, conception, fertility, fecundity rates), high nutritional requirements during pregnancy and lactation, negative impacts on future reproductive performance (two tooths) and increased management requirements (McCall and Hight, 1981, McMillan and McDonald, 1983).

In order to breed ewe lambs, they must first attain puberty (Edwards et al., 2016). The attainment of puberty is influenced by both day length and the ewe’s live weight (Langt and Might, 1967, Ross, 1989, Foster et al., 1985, Bizelis et al., 1990). Edwards et al.

(2016) reported that only 58% of their ewe lambs (45.1 ± 0.6 kg live weight) had reached puberty prior to the introduction of entire rams. Ewe lambs that attain puberty, however, may fail to be mated if they are introduced to the entire ram early in the breeding season before they have had a chance to reach the optimum live weight for breeding (McMillan and McDonald, 1983). Moreover, ewe lambs can ovulate without showing oestrous behaviors such as approaching or standing for the ram which may lead to failure to be mated, ova loss and barrenness (McMillan and McDonald, 1983, Ross, 1989, Edwards et al., 2016). Edwards and Juengel (2017), however, reported that although ewe lambs usually do not show signs of heat during their first ovulation, they can come into heat again after seven to ten days.

The major constraint to ewe lamb breeding is poor reproductive performance (McMillan and McDonald, 1983). Poor reproductive performance is associated with low ovulation rates, failure of the ova to be exposed to sperm, fertilization failure or embryo loss (Edwards et al., 2016) resulting in low fertility and fecundity rates (Corner et al., 2013, Edwards et al., 2016). Ewe lambs are prone to early embryo loss (before day 35 of pregnancy) which may lead to barrenness (Edwards et al., 2016). Generally, ewe lambs wean an average of 0.6 lambs per ewe presented for breeding, while adult ewes wean an average of 1.2 lambs (Kenyon et al., 2004d, Edwards et al., 2016). Ewe lambs that maintain their pregnancies produce lambs that are smaller and lighter at birth, hence have lower survival rates than those born to mature ewes (Corner et al., 2013, Edwards et al., 2016). In addition, ewe lambs have been reported to have poorer mothering ability compared to mature ewes due to a lack of experience which may also limit the survival of their progeny (Corner et al., 2013). The progeny of ewe lambs generally have lower weaning weights than lambs born to mature ewes which can limit their value to a sheep farming system (McMillan and McDonald, 1983, Corner et al., 2013, Edwards et al., 2016).

Ewe lamb breeding can affect the subsequent reproductive performance of the ewe as a two tooth. A study by McMillan and McDonald (1983) showed that ewes bred as lambs had a live weight below 50 kg at their next breeding as a two tooth, while those bred for the first time as a two-tooth weighed more than 55 kg at breeding (McMillan and McDonald, 1983). Low live weights of ewes bred as ewe lambs can be overcome if ewe lambs are provided with preferential feeding between weaning and the next breeding season (Kenyon et al., 2008). Failure to achieve a live weight of at least 50 kg at breeding can negatively affect their breeding performance (Corner et al., 2013), leading to a greater chance of the ewe returning to service and low fecundity rates (Kenyon et al., 2008).

2.3 Factors affecting ewe lamb reproduction

2.3.1 Attainment of puberty and how it can be manipulated

Puberty is defined as the stage at which the ewe lamb has reached sexual maturity and is ready for breeding (Ross, 1989, Nieto et al., 2018) or, alternatively, the time at which the first oestrus occurs (Ensminger, 2002). Before a ewe lamb can attain puberty, the sexual organs and the anterior pituitary gland must be mature allowing for development of sexual characteristics and behavior (Allen and Lamming, 1961, Nieto et al., 2018). Attainment of puberty is primarily driven by luteinizing hormone (LH) (Keisler et al., 1985). During the pubertal transition, the frequency of luteinizing hormone (LH) pulses increases in order to drive follicular development and the production of oestradiol which evokes the gonadotrophin surge and ovulation (Foster et al., 1985).

Ewe lambs will only become reproductively active if they acquire a minimum live weight and are exposed to decreasing photoperiod (Langt and Might, 1967, Ross, 1989, Foster et al., 1985, Bizelis et al., 1990). If this does not occur, the gonadotropin-releasing hormone (GnRH) pulse generator will operate at a low frequency leading to maintenance of pre-pubertal anovulatory condition (Foster et al., 1985, Ross, 1989). The timing of the onset of puberty is influenced by season of birth, age, live weight, BCS, nutrition, and breed of the ewe (Quirke, 1981, Ross, 1989, Edwards et al., 2015, Nieto et al., 2018)

2.3.1.1 Effect of age, live weight, and breed on the onset of puberty

Ewe lambs attain puberty at between six to 18 months of age depending on their breed and live weight (Langt and Might, 1967, Ross, 1989, Bizelis et al., 1990). Ewe lamb live weight at puberty varies widely among breeds but, when expressed as a percentage, ewe lambs generally achieve puberty at 40 to 60% of their expected mature live weight (Ross, 1989). Larger and later maturing breeds, such as Scottish Blackface, reach puberty at a later age than earlier maturing breeds such as Romney (Ross, 1989). Highly fecund breeds such as Finn crosses attain puberty at a younger age and lower live weight compared to less fecund breeds (Table 2.2) (Dickerson and Lasted, 1975, Wickham and McDonald, 1982).

Table 2. 2: The average live weight at puberty of different breeds at puberty

Breed	Live weight at puberty (kg)	References
Dorset	39	(Dickerson and Laster, 1975)
Targhee	41	
Hampshire	42	
Suffolk	50	
Corriedale	44	(Dickerson and Laster, 1975)
	37.1	(Fogarty et al., 2007)
East Friesian crosses	43.1	(Fogarty et al., 2007)
Romney	36	(Kenyon et al., 2005).

2.3.1.2 Effect of post weaning nutrition and growth on the onset of puberty

Postweaning nutrition and growth rate can modify the age at which the ewe lamb attains puberty (Allen and Lamming, 1961, Foster et al., 1985). In order to attain puberty at six to nine months, ewe lambs should grow at 70 to 160 g/d postweaning (Dickerson and Lasted, 1975). *Ad libitum* feeding after weaning can stimulate the growth of ewe lambs thus resulting in earlier onset of puberty (Allen and Lamming, 1961, Wickham and McDonald, 1982, Ensminger, 2002). A high plane of nutrition enhances the formation of luteal tissues leading to greater ovarian weight, thus stimulating the commencement of cyclic ovarian activity (Allen and Lamming, 1961). In addition, rapid lamb growth can increase the luteinizing hormone (LH) pulse rate leading to the pubertal follicular phase and first gonadotropin surge, hence the earlier onset of puberty (Foster et al., 1985). When ewe lambs reach 40 – 60% of their mature body weight, the frequency of LH pulses increases due to a reduction in sensitivity to oestradiol inhibitory feedback and the increase in GnRH pulse generator (Foster et al., 1985). Ewe lambs fed *ad libitum* concentrate mixture attained puberty at a younger age and greater live weight compared to those on pasture alone (moderate feeding) (Allen and Lamming, 1961). A high plane of nutrition, however, can result in attainment of puberty at a younger age, even when the body is still physiologically immature resulting in failure to breed successfully (Allen and Lamming, 1961).

Underfeeding after weaning can result in light ewes at breeding and can impair secretion of LH resulting in insufficient follicular development resulting in ewe lambs failing to attain puberty (Foster et al., 1985). Foster et al. (1985) reported that ewe lambs fed at a maintenance level from weaning to 45 weeks of age (5 – 7 months) failed to attain puberty.

2.3.1.3 Effect of day length on the onset of puberty

Photoperiod influences the onset of puberty in ewe lambs (Foster et al., 1985). Sheep are seasonal breeders with oestrus activity stimulated when they experience decreasing day length following exposure to long days (Langt and Might, 1967, Legan and Karsch, 1980, Yellon and Foster, 1985). Long days increase the responsiveness to negative feedback of oestradiol leading to an anoestrous period, exposure to short days then reduces the effect of oestradiol and hence the onset of the breeding season (Legan and Karsch, 1980). Foster et al. (1985) reported that when lambs were maintained continuously in an artificial photoperiod of 15 hours of light and nine hours of darkness, the onset of the reproductive cycle was disturbed and lambs did not exhibit a luteal phase during the first year after birth (Foster et al., 1985, Yellon and Foster, 1985). In mature ewes, shorter days lead to the onset of the breeding season regardless of the season of the year (oestrus or breeding season) (Legan and Karsch, 1980).

2.3.1.4 Effect of ram effect on the onset of puberty

The ram effect refers to the induction of ewe ovarian activity in response to the introduction of an entire ram or vasectomized rams (teaser) after a period of separation (Rosa and Bryant, 2002). Male sheep secrete pheromones in their wool and wax which stimulates the ovarian activity of ewes if they are run as one mob (Knight and Lynch, 1980). Knight and Lynch, (1980) reported that there was no need for physical contact between ewes and rams for the ram to produce a stimulatory effect. The social interaction of ram and ewes can stimulate the ovarian activity of anoestrus ewes resulting in the initiation of ovulation and oestrous behavior (Rosa and Bryant, 2002). When the ram is introduced to the ewe flock after a period of separation, the pulse frequency of the LH increases, thereby inducing the LH surge and, consequently, the initiation of ovulation (Edgar and Bilkey, 1963, Knight, 1983, Rosa and Bryant, 2002). Stimulation usually occurs within six days of the introduction of the ram and is accompanied by ovulation without signs of oestrus (silent heat; Knight and Lynch, 1980, Schinckel, 1954). Stimulated ewes then come to oestrus again after 17 days (Schinckel, 1954, Rosa and Bryant, 2002).

In ewe lambs, the presence of a teaser ram can result in an earlier onset of puberty compared to those isolated from a ram (Kenyon et al., 2005, Kenyon et al., 2006). The earlier onset of puberty results in more ewe lambs which can be mated early in the breeding season compared to the isolated ewe lambs (Kenyon et al., 2005, Kenyon et al., 2006). Kenyon et al. (2005) reported that 62.6% of the teased ewe lambs were mated in the first 17 days compared to 32.1% of unteased ewe lambs. Ewe lambs mated in the first 17 days of the breeding season lamb earlier in the lambing period thus reducing the length of the lambing period (Kenyon et al., 2005).

2.3.2 Conception rate

Conception rate is defined as the probability of ewes becoming pregnant, or a percentage of ewes that become pregnant of those put to ram (ewes pregnant / ewes put to the ram) (Kleemann and Walker, 2005, Mulvaney et al., 2010a, Kenyon et al., 2014a, Corner-Thomas et al., 2015). For the purpose of this review, the conception rate is defined as the percentage of ewes identified as pregnant of those put to ram. Ewe lambs generally conceive in the second cycle of the breeding period and display a shorter and less intense oestrus compared to mature ewes (Drymundsson, 1973). Moreover, ewe lambs have lower conception rates than mature ewes (Corner et al., 2013). Generally, the conception rate of ewe lambs ranges between 40 to 60% depending on the breed and environmental factors (McCall and Hight, 1981, McMillan and McDonald, 1983, Kenyon et al., 2004d). Conception rates of ewe lambs can be manipulated by a number of factors including ram effect, live weight, BCS and nutrition.

2.3.2.1 Effect of ram effect on conception rate

Introduction of the teaser ram, at least 17 days before mating, stimulates ewe lambs to be receptive to the ram during the breeding period, thus increasing their chances to conceive (Kenyon et al., 2005). Kenyon et al. (2005), reported that 26% of the unteased ewe lambs returned to service compared to 11% of the teased group, which indicates a greater conception rates of teased ewe lambs in the first oestrous cycle (Kenyon et al., 2005). Shorter exposure to a teaser ram (7 days) can increase the receptiveness to the ram but does not improve conception rates (Kenyon et al., 2006).

2.3.2.2 Effect of Live weight and BCS on conception rate

The ewe lamb's live weight and BCS prior to breeding can influence her conception rate (McCall and Hight, 1981, Kenyon et al., 2014a). Ewe lambs that grow slowly after weaning, usually have a low breeding weight leading to poor reproductive performance (McCall and Hight, 1981). The ewe's live weight and body condition score at breeding have the greatest influence in determining the ability of ewe lambs to conceive (Hight et al., 1973, Sejian et al., 2010, Cave et al., 2012). Heavier ewes, with a BCS above 2.5, are more likely to conceive in the first oestrous cycle and maintain a pregnancy (McMillan and McDonald, 1983, Kenyon et al., 2010). Likewise, mature ewes with poor BCS (≤1.5) usually show a delayed oestrus and are more likely to return to service compared with those with good condition (≥2.5; Gunn and Doney, 1975). Cave et al. (2012) reported that 83% of ewe lambs with BCS above 2.5 units conceived compared with 66.1% of those with BCS 1.5 or less. Similarly, Mulvaney et al. (2010a) reported a 72% conception rate in ewe lambs bred at 42 kg compared to 65% of those bred at 36 kg breeding live weight. Stevens (2010) found that, for every 1kg increase in liveweight at breeding, the conception rate increased by 1%. At 40 kg breeding live weight, a ewe has a 40% chance of becoming pregnant, which then increases with greater liveweight gains (Gaskins et al., 2005).

2.3.2.3 Effect of nutrition on conception rate

The nutrition of the ewe lamb, from weaning to breeding, and throughout pregnancy, is important to ensure that those ewes that become pregnant then maintain their pregnancy (Annett and Carson, 2006). Prior to breeding, ewe lambs should be allowed *ad libitum* nutrition which results in improved oocyte quality and, in turn, increases the chances of conception and implantation. Mulvaney et al. (2010b) stated that the conception rate for ewes fed at maintenance and *ad libitum* levels before breeding were 67 and 69% respectively.

The feed allowance should be reduced post-breeding to reduce the incidence of ewe lambs returning to service (Kenyon et al., 2008, Mulvaney et al., 2010b). Returns to service that are on *ad libitum* feed allowance post-breeding are associated with fertilization failure or incompetent implantation (Mulvaney et al., 2010a). Mulvaney et al. (2010b) reported a low conception rate (32% and 28%) in ewe lambs offered maintenance and *ad libitum* feeding levels post-breeding compared with those fed to gain 100 g/d. Rosales Nieto et al. (2015) reported that ewe lambs offered a maintenance feed allowance throughout the breeding period had reduced conception rates by 38% (Rosales Nieto et al., 2015). Liveweight gain post-breeding can account for a 10 – 12% increase in conception rate (Rosales Nieto et al., 2015). In mature ewes, undernutrition prior to breeding can result in the inhibition of GnRH and LH important for ovulation and embryo development (Sejian et al., 2009).

2.3.3 Fecundity rates

Fecundity rates depend on both ovulation rate and embryo survival (Kenyon et al., 2014a). Fecundity rate is influenced by ewe age, level of nutrition, BCS, live weight, and postweaning liveweight gain (Levine et al., 1978, Kenyon et al., 2004b, Gaskins et al., 2005, Rosales Nieto et al., 2015).

2.3.3.1 Effect of age on fecundity rates

The ovulation rates, and, thus, the fecundity rates, of ewe lambs are lower than that of mature ewes (Edwards et al., 2015, Edwards et al., 2016, Edwards and Juengel, 2017) as the ewe lamb may have not fully reached physiological maturity (Meyer and French, 1979). Ewes are at the growing phase up to two years of age and reach full maturity at approximately three years of age (McCall and Hight, 1981, McMillan and McDonald, 1983, Edwards et al., 2016, Edwards and Juengel, 2017). Ewe lambs are prone to low fecundity rates due to low ovulation rate (Drymundsson, 1973, McMillan and McDonald, 1983). Fecundity rates increase sharply between one and two years of age, followed by small increases in the subsequent years after they reach full maturity (Edwards and Juengel, 2017). The fecundity rate of ewe lambs is about 0.87 while, for two tooths, it is around 1.53 fetuses/ewe lambs scanned pregnant (Edwards et al., 2016). The fecundity of ewes of one, two, three and four years of age is around 0.81, 1.55, 1.83, and 1.88

fetuses/ewe pregnant respectively (Edwards et al., 2015, Edwards et al., 2016). Low fecundity rates in ewe lambs can be associated with a small percentage of ewe lambs (4%) bearing multiple fetuses (McCall and Hight, 1981, McMillan and McDonald, 1983).

2.3.3.2 Effect of live weight and nutrition on fecundity rates

Increasing ewe lamb feed intake post-weaning can improve ewe lamb ovulation rates (Rosales Nieto et al., 2015) and the number of fetuses they conceive (Mulvaney et al., 2010a). Heavier ewe lambs begin to cycle earlier than lighter ones which gives them the opportunity to be bred in their second or third cycles when they are likely to shed more ova thus increasing the chances of multiple fetuses (Meyer and French, 1979, Gonzalez et al., 1997, Rosales Nieto et al., 2013, Edwards et al., 2016). Coop (1962) reported that greater live weights of two tooths at breeding result in a higher twinning rate and reduce barrenness. For example, no ewe lambs conceived multiple fetuses at live weights below 36 kg (Coop, 1962). Hight and Jury (1976) found that, for every extra 1kg live weight at breeding there was a 1.2% increase in multiple births.

2.3.4 Pregnancy

2.3.4.1 Nutrition and live weight gain throughout pregnancy

One of the primary determinants of ewe lambs' reproductive success is feed management throughout pregnancy (Kenyon et al., 2014a). Unlike mature ewes, ewe lambs have not yet reached their physiological maturity, therefore, need to gain weight throughout pregnancy (Schreurs et al., 2010). Ewe lambs use available nutrients for both their body growth and fetal growth (Morris et al., 2005, Schreurs et al., 2010). It is, therefore, important for the ewe lamb to achieve live weight above 40 kg prior to breeding as lighter ewes need to partition more nutrients for their growth compared to heavier ones (Schreurs et al., 2010). The nutrient requirements of the fetus in the first trimester of pregnancy, however, are low (Kenyon and Webby, 2007), therefore, this period can be used to maximize ewe lamb growth (Schreurs et al., 2010). Approximately 70% of fetal growth occurs in the last eight weeks of pregnancy (Kenyon and Webby, 2007), therefore more nutrients are diverted for fetal growth at the expense of ewe lamb growth (Schreurs et al., 2010).

In New Zealand, sheep production is primarily pasture based, therefore, ewe performance depends on the pasture allowance provided (Kenyon and Webby, 2007). In the first trimester of pregnancy, ewe lambs should gain 100 to 130 g / d and feed allowance should be increased during mid-pregnancy in order to achieve 200 g/d live weight gain (Kenyon et al., 2008, Mulvaney et al., 2010a). Ewe lambs should be offered pre-and post-herbage masses of 1400 and 1000 kg DM/ha, respectively to achieve liveweight gains of 100 g/d and 1800 and 1200 kg DM/ha to gain 200 g/d (Schreurs et al., 2010). Poor nutrition during pregnancy can lead to lighter ewes at weaning which can have carryover effects on two-tooth breeding (Schreurs et al., 2010).

2.3.4.2 Embryo and fetal loss

Embryo loss refers to the failure of the fertilized ova to develop, implant and progress throughout pregnancy (Wickham and McDonald, 1982, West et al., 2009) or a failure of the ewe to maintain pregnancy (Vanderwall and Newcombe, 2006). Fetal loss can be defined as the in-utero presence of non-viable fetus(es) identified during ultrasonography or when the ewe lamb was confirmed pregnant at initial pregnancy diagnosis but was not pregnant at a subsequent pregnancy diagnosis (Corner-Thomas et al., 2015). Some research collectively describes both embryo and fetal loss as reproductive failure. The embryo becomes a fetus after the development of body parts and organs (Wickham and McDonald, 1982). In New Zealand, reproductive failure accounts for 20 to 30% of the ova shed (Wickham and McDonald, 1982, West et al., 2009). Reproductive failure can tend to occur either during early pregnancy (within 50 days of pregnancy) or during mid-pregnancy (50 to 100 days) rather than the last days of pregnancy (Wickham and McDonald, 1982, West et al., 2009, Ridler et al., 2017).

Early embryo losses generally occur between fertilization and day 30 of pregnancy (West et al., 2009). Early embryo loss is due to a lack of progesterone owing to the failure of the primary corpus luteum (CL) to develop adequately (Vanderwall and Newcombe, 2006). The embryo loss can lead to either return to service or barrenness, depending on when it occurs (Edey, 1967). Early embryo loss prior to day 12 can lead to a return to service while later losses result in barrenness (Edey, 1967, Beck et al., 1996, West et al., 2009). Following early embryo loss (before day 12), the embryonic substances are reabsorbed quickly and the ewe returns to service (Edey, 1967). Embryonic loss before day 12 of pregnancy is, therefore, equivalent to fertilization failure and does not affect the normal length of the oestrous cycle (Edey, 1967, West et al., 2009). Embryo death that occurs after day 12 of pregnancy can prevent the regression of corpus luteum (West et al., 2009) and, therefore, can result in a longer inter-oestrus interval which may prevent re-mating before the end of the breeding season and consequently resulting in barrenness (Beck et al., 1996). For example, embryo loss after day 15 of pregnancy results in an inter-estrous interval of 40 days, therefore, ewes will not be rebred (Beck et al., 1996). In addition, the embryonic loss occurring after day 12 can prevent the regression of corpus luteum (West et al., 2009), therefore, ewes with late embryo loss are identified barren during a pregnancy scan.

Reproductive failure is influenced by a number of factors including; age, genotype, ovulation rate, nutrition, environmental temperature, stress and infection (Wickham and McDonald, 1982, West et al., 2009). Embryonic loss is higher in ewe lambs and ewes more than eight years of age, however, the reasons for this are not clear (Wickham and McDonald, 1982, West et al., 2009). Ridler et al. (2017) examined two commercial flocks of ewe lambs and identified 75% fetal losses which occurred in mid-pregnancy (day 50 – 100). For example, in one flock, 472 ewe lambs were bred and, of these, 11.7% had a reproductive failure, of those 75% were diagnosed at day 80 of pregnancy and 25% at day 110 (Ridler et al., 2017). Moreover, embryonic losses increase with ovulation rate (Wickham and McDonald, 1982, Schoenian and Burfening, 1990). In mature ewes, Schoenian and Burfening (1990) reported embryo survival was 73% in ewes with a single ovulation compared with 61% in twin ovulation and 40% triplet ovulation.

2.3.5 Lambing

2.3.5.1 Birth weight

Lamb birth weight is an important parameter in sheep production as it influences lamb survival, growth rate and weaning weight (Kenyon et al., 2014b). Lamb birth weight is primarily influenced by the age of the dam and the number of fetuses conceived (Everett-Hincks and Dodds, 2008, Mulvaney et al., 2008). Ewe lambs and two tooths usually give birth to lighter lambs compared with mature ewes (Everett-Hincks and Dodds, 2008, Mulvaney et al., 2008, Corner et al., 2013, Pettigrew et al., 2018). The optimum birth weight for high lamb survival of single lambs born to mature ewes is 5.5 to 6 kg (Everett-Hincks and Dodds, 2008, Corner et al., 2013, Pettigrew et al., 2018) whereas, for single lambs born to ewe lambs is 3 to 4 kg (Young et al., 2010, Corner et al., 2013, Pettigrew et al., 2018).

2.3.5.1.1 Effect of birth rank on birth weight

As lamb litter size increases, the birth weight of each lamb decreases (Everett-Hincks and Dodds, 2008, Schreurs et al., 2010, Corner et al., 2013). For example, Everett-Hincks and Dodds (2008) reported that triplets were 1.69 kg lighter than singletons and 0.70 kg lighter than twins (Everett-Hincks and Dodds, 2008). Similarly, Corner et al. (2013) noted that single lambs had 4.2 kg birth weight compared with 3.2 kg for twin lambs. Generally, the birth weight of single lambs born to ewe lambs is similar to the birth weight of twin lambs born to a mature ewe (Corner et al., 2013).

2.3.5.1.2 Effect of ewe lamb live weight on lamb birth weight

The liveweight of a ewe lamb at breeding can influence the birth weight of her lambs (Scales et al., 1986, Kenyon et al., 2008). Kenyon et al. (2008) reported that for every 1kg increase in ewe lamb live weight at breeding, there was an increase in birth weight of 0.046 kg. Similarly, lambs born to ewe lambs weighing 36 kg at breeding were lighter than those born to ewe lambs weighing 42 kg (Mulvaney et al., 2010b). Heavier ewe lambs at breeding, on the other hand, have greater body reserves that can be diverted to fetal growth rather than ewe lamb growth during pregnancy, thus reducing the nutrient competition (Schreurs et al., 2010).

2.3.5.1.3 Effect of ewe lamb nutrition on lamb birth weight

The nutritional management of the ewe lambs before breeding, and throughout pregnancy can affect the birth weight of their lamb(s) (Mulvaney et al., 2008, Schreurs et al., 2010). Preferential feeding of ewe lambs before breeding and during early pregnancy can increase the dam live weight as well as her lambs birth weight (Schreurs et al., 2010). Studies from the UK indicate that lamb birth weight depends on ewe lamb growth in the first 100 days of pregnancy and is a consequence of placental size (Wallace et al., 1996). Adequate placental size enables the fetus to acquire sufficient nutrients during late pregnancy (Kenyon and Webby, 2007).

There is conflicting information between New Zealand and UK studies regarding the effect of feed allowance during pregnancy on lamb birth weight. New Zealand pastoral studies have reported no difference in lamb birth weight of lambs born to ewe lambs offered either *ad libitum* feed allowance or fed to gain 100 g/d throughout pregnancy (Morris et al., 2005, Mulvaney et al., 2008). UK trials that have pen-fed the ewe lambs reported lower birth weights (2.74 kg) of lambs born to ewes fed ad libitum compared with those who gained 75 g/d during pregnancy (4.34 kg) (Wallace et al., 1996). They found that ewe lambs fed *ad libitum* concentrates (gaining 234 g/d) during early and mid-pregnancy had smaller placental size compared with those fed to grow at 75 g/d, hence their lower birth weight (Wallace et al., 1996).

2.3.5.2 Lamb mortality

The ability of the ewe lamb to rear her lamb until weaning is an important component of the ewe lamb breeding system (Schreurs et al., 2010). Ewe lamb breeding shows increased lamb mortality rates compared with mature ewes (Everett-Hincks and Dodds, 2008). The average lamb losses per year ranges between 5 – 30%. The highest rates of death occur in the first three days after birth (McMillan, 1983, Everett-Hincks and Dodds, 2008, Young et al., 2010). Young et al. (2010) reported lamb mortality to weaning of 28%, of which 20% occurred in the first three days after birth (Young et al., 2010), while Everett-Hincks and Dodds (2008), reported 16% overall lamb mortality of which 9% occurred within the first three days of life.

2.3.5.2.1 Causes of lamb mortality

The primary causes of lamb mortality in New Zealand are dystocia and starvation/exposure (McMillan, 1983, Everett-Hincks and Dodds, 2008). Lamb mortality due to dystocia accounts for 4 to 12% of deaths, while starvation/exposure is responsible for 1-5% of deaths (McMillan, 1983, Everett-Hincks and Dodds, 2008, Young et al., 2010). In addition, lamb death due to starvation/exposure is greater in twins 5.3% compared with singletons 1.8% (McMillan, 1983). The lamb mortality due to starvation/exposure is associated with low lamb birth weight (Everett-Hincks and Dodds, 2008), while death due to dystocia is associated with both low and high lamb birth weight. Dystocia is caused by prolonged birth, fetal-pelvic disproportion or fetal malpresentation (McMillan, 1983). Generally, lamb mortality due to starvation decreases as birth weight increases, while death due to dystocia increase with birth weight (Scales et al., 1986).

2.3.5.2.2 Effects of birth weight and birth rank on lamb mortality

Lamb mortality is influenced by both birth weight and birth rank. Birth weight has the greatest influence on mortality rates (Morel et al., 2009, Pettigrew et al., 2018). Pettigrew et al. (2018) predicted that the birth weight of single lambs born to either ewe lambs or mature ewes should be between 3.9 and 5.5 kg and 4.3 to 7.4 kg, respectively in order to achieve 85% survival rates.

Lamb mortality also increases with an increase in litter size and this is associated with a low birth weight of multiple born lambs (McMillan, 1983, Scales et al., 1986, Morel et al., 2009, Young et al., 2010, Pettigrew et al., 2018). In mature ewes, Scales et al. (1986) reported greater lamb mortality in triplets (33%) compared with twins (14.7%) and singles (14.1%). Likewise, in ewe lambs, McMillan (1983), found that a greater proportion of twin lambs (39.2 %) died before weaning compared with single born lambs (33.0%). In twin-born lambs, a lighter sibling is less likely to survive compared with a heavier twin, however, in situations where both siblings have similar birth weights, both lambs have equal chances of survival (Morel et al., 2009).

2.3.5.2.3 Effect of dam age on lamb mortality

Dam age can influence lamb survival (Corner et al., 2013). Lambs born to ewe lambs are less likely to survive (69.0%) compared with those born to mature ewes (84.7 % and 83.3% for single and twins respectively) (Corner et al., 2013). Likewise, Pettigrew et al. (2018) reported that lambs born to mature ewes had greater survival at birth and weaning compared with those born to ewe lambs (96.1 vs 82.2%, respectively) and (83.1 vs 47.3, respectively). Lower survival of lambs born to ewe lambs compared to mature ewes can be due to more assisted lambing in ewe lambs (Everett-Hincks and Dodds, 2008, Young et al., 2010). Indeed, Everett-Hincks and Dodds (2008) found that ewe lambs and ewes above four years of age were more likely to encounter dystocia than two or three-year-old ewes.

2.3.6 Growth to weaning and weaning weight

In New Zealand, lambs are weaned at 10 to 12 weeks of age (Kenyon and Webby, 2007). Lamb weaning weight is an important measure to assess reproductive success of ewe lambs (Kenyon et al., 2008). Lambs with a heavier live weight at weaning are more valuable in sheep farming systems (Schreurs et al., 2010). Heavier lambs have a higher store value, can be weaned at an earlier age or sold directly to slaughter (Kenyon et al., 2006, Kenyon et al., 2008, Schreurs et al., 2010). Early weaning of lambs born to ewe lambs allows the ewe lamb dam more time to recover from liveweight losses as a result of pregnancy and lactation, thus allowing the ewe to achieve a greater liveweight in their next breeding season (Kenyon et al., 2006). Lambs with rapid growth rates and high weaning weights are preferred over lighter lambs at weaning as they increase the selection options for hogget breeding, which are then more likely to breed successfully than lighter lambs at weaning (Kenyon et al., 2004a). The lamb weaning weight is influenced by dam age, liveweight at breeding, nutrition during pregnancy, birth rank and lamb birth weight (Kenyon et al., 2006). Lamb growth in the first four to six weeks of life depends heavily on milk production of the dam (Kenyon and Webby, 2007, Schreurs et al., 2010, Kenyon et al., 2011, Corner et al., 2013).

2.3.6.1 Effect of birth rank on lamb growth to weaning

Lambs born to ewe lambs generally have lower weaning weights than to those born to mature ewes which can limit their value to the farming system (Corner et al., 2013). Moreover, single born lambs grow faster and have greater weaning weight than twin lambs (Young et al., 2010). Morris et al. (2005) reported that single lambs had greater weaning weight (23.80kg) compared with twin lambs (20.49 kg). Single born lambs had 13g/day greater liveweight gain than twins and were 1.5 kg heavier at weaning (Young et al., 2010)

2.3.6.2 Effect of ewe lamb live weight and nutrition on lamb growth

Ewe lamb liveweight at breeding has a positive effect on the growth rate of single lambs to weaning (Kenyon et al., 2006). Ewe lambs that weighed 42 kg at breeding weaned heavier lambs (21.69 kg) than those that weighed 36 kg at breeding (20.11 kg) (Mulvaney et al., 2010b). For every 1kg increase in liveweight at the breeding there was an 0.0016 kg liveweight gain (Kenyon et al., 2006). Moreover, providing additional feed, and thus live weight gain, before breeding and throughout pregnancy, can increase the weaning weight of lambs (Schreurs et al., 2010). Kenyon et al. (2008) reported that lambs born to ewe lambs that were offered *ad libitum* pasture allowance throughout pregnancy were heavier (17.69kg) at weaning compared with those born to ewe lambs that gained 100 g/d (15.05 kg). *Ad libitum* feeding throughout pregnancy can result in ewes producing milk of higher milk yield leading to greater live weight gains compared with those offered 100 g/d (Morris et al., 2005, Mulvaney et al., 2008, Mulvaney et al., 2010b). Lambs that receive an adequate amount of milk grow faster than those which are restricted to milk consumption (Kenyon et al., 2011). Ewe lambs with a greater BCS at late pregnancy can mobilize their body reserves to help support the production of high milk quantity which results in the rapid growth of their lambs to weaning (Mulvaney et al., 2008, Kenyon et al., 2011).

2.4 Methods to manipulate ewe lamb reproductive success

In New Zealand, over the past 20 years, there has been a push to increase the number of lambs born (Meyer and Clarke, 1982, Meyer et al., 1983, West et al., 2009). The number of lambs born depends on the number of ova released during ovulation, therefore sheep breeders use ovulation rate as a selection criterion to increase litter size (Meyer and Clarke, 1982, Meyer et al., 1983, West et al., 2009). About 60% to 70% of the variation in litter size of sheep is due to variation in the ovulation rate (Meyer et al., 1983, West et al., 2009).

Ovulation rate can be increased by various methods including: genetic improvements (Davis et al., 1982), flushing (providing ewes with extra feed four to six weeks before breeding) (Scaramuzzi et al., 2006), increasing live weight and BCS at breeding (Kenyon et al., 2004b), hormonal treatments (Abecia et al., 2012) and immunization against steroids (androstenedione, progesterone) (Smith, 1985a). This review focuses on immunization against androstenedione as a method for improving ovulation rate.

2.4.1 Ovulation rate

Ovulation rate is defined as the number of ova released by a female animal (sheep) in each reproductive cycle (Edwards et al., 2016, Edwards and Juengel, 2017). Ovulation rate can be quantified by counting the number of corpora lutea present after a reproductive cycle (Forcada et al., 1992). Ovulation is primarily controlled by the hypothalamus and pituitary gland through balancing the secretion of hormones such as follicle stimulating hormone (FSH), luteinizing hormone (LH), oestradiol, and inhibin (Figure 1.1) (Henderson et al., 1984, Edwards and Juengel, 2017). FSH and LH are responsible for the development and maturation of follicles (Henderson et al., 1984). In addition, growth factors, such as growth differentiation factor (GDF 9) and borne morphogenic protein (BMP15), are also involved (Scaramuzzi et al., 2011, Juengel et al., 2013a, Edwards and Juengel, 2017). GDF9 and BMP 15 regulate the initial follicular development and the production of ovarian steroids (oestradiol and progesterone) and inhibin which determine ovulation rate (Scaramuzzi et al., 2011, Edwards and Juengel, 2017, de Castro et al., 2016).

Figure 2.1: A schematic diagram showing the relationship between hormones and the secreting organs (Edwards and Juengel, 2017).

2.4.2 Immunization

Immunization involves the use of a vaccine which stimulates the production of antibodies that act against steroids such as androstenedione, testosterone, estrone and progesterone, and inhibin thus changing the endocrine balance (Table 2.3) (Henderson et al., 1984, Smith, 1985a). Steroids are the substances which cannot induce the immune response under normal circumstances due to their low molecular weight (West et al., 2009, Smith, 1985a). When the steroid based vaccine is injected into the ewe's bloodstream, it is linked to the heterologous protein, then a steroid protein conjugate is produced which can stimulate the immune system of the treated ewes (West et al., 2009). Immunization interferes with normal hormonal production allowing more follicles to grow, and regulate follicular atresia, thus allowing more follicles to reach the ovulation maturity (Henderson et al., 1984, Henderson et al., 1989).

2.4.2.1 Immunization against inhibin

Immunization against inhibin works by neutralizing any circulating inhibin, hence allowing continuous secretion of FSH and more follicular growth (Henderson et al., 1984). The commonly used vaccines for immunization against inhibin are purified inhibin and bovine follicular fluids (bFF) (Cummins et al., 1986, Henderson et al., 1989). Purified inhibin neutralizes the endogenous inhibin, increases plasma FSH and, thus, ovulation rate (Cummins et al., 1986). It is difficult and expensive, however, to purify inhibin (Henderson et al., 1989), therefore, small-scale farmers are less likely to adopt this practice. Bovine follicular fluids are composed of the fluids collected from ovaries of the slaughtered cows (Hudson et al., 1985). The immunogen in bFF is inhibin (Henderson et al., 1990). Immunization with bFF advances the onset of the breeding season and increases the growth of the follicles and ovulation rate by 1.3 to 2.0 (Hudson et al., 1985, Henderson et al., 1986, Henderson et al., 1989).

2.4.2.2 Immunization against steroids

Vaccines have been developed to immunize ewes against a number of steroids such as progesterone, oestrogen and androstenedione. Immunization against androgens results in reduced plasma androgen concentration and reduced negative effects of inhibin on FSH secretion, thus allowing more follicles to grow to preovulatory maturity (Henderson et al., 1984). Immunization against progesterone, however, results in increased LH secretion without any effect on FSH (Martensz and Scaramuzzi, 1979). Progesterone is known for reducing LH frequency (Goodman and Karsch, 1980). Ewes immunized against steroids, therefore, ovulate multiple ova, often three or more in each estrous cycle (Smith et al., 1981).

The most commonly used steroid based vaccine is androstenedione protein conjugate. There are a number of androstenedione-based vaccines available including Androvax®, Ovastim®, Fecundin® and Estrone® (Table 1.3) (Van Look et al., 1978, Henderson et al., 1990, Scaramuzzi et al., 1993, O'Connell et al., 2016). Androstenedione is the hormone produced by the ovaries during the anestrous period (period of sexual inactivity) to regulate the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) (Martensz et al., 1976, Martensz and Scaramuzzi, 1979). Androstenedione stimulates the release of FSH and impedes LH production (Martensz and Scaramuzzi, 1979). Immunization with Androvax leads to the production of androgen binding antibodies that bind to androstenedione present in the blood thus deactivating them and reducing the level of biologically available androstenedione (Smith, 1985a, McNatty et al., 1988, West et al., 2009). Owing to a reduced concentration of androstenedione, the hypothalamic-pituitary system becomes less sensitive to oestradiol which regulates the release of FSH (Scaramuzzi et al., 1980, Smith, 1985b, West et al., 2009) resulting in a temporary hormonal imbalance which can take 45 to 50 days (6 to 7 weeks) to be corrected (Scaramuzzi et al., 1980, Smith, 1985b, McNatty et al., 1988, West et al., 2009). Moreover, the presence of androgen binding antibodies in follicular fluids can reduce the rate of androgen-induced atresia thus enhancing the survival of large follicles (Smith, 1985a).

The effects of immunization against androstenedione has been extensively studied in adult ewes (Smith, 1985b, Smith, 1985a, Juengel et al., 2013b). There is conflicting information on changes in the hormonal level following immunization. McNatty et al. (1988) found high (13.8% higher) plasma FSH and LH concentrations during the luteal phase (day 9 – 11), of the estrous cycle (McNatty et al., 1988) whereas Martensz and Scaramuzzi (1979) reported higher levels of LH and progesterone but lower FSH levels. Henderson et al. (1989) noted reduced inhibin concentration after vaccinating with Androvax®. Generally, immunization causes a hormonal imbalance that enables the production, growth and the survival of large follicles and, hence, more follicles will be available for ovulation (Scaramuzzi et al., 1980, Smith, 1985a, Henderson et al., 1989). Scaramuzzi et al. (1980) reported a large ovarian size and weight, and a higher number of preovulatory follicles in treated ewes which they associated with increased gonadotrophic stimulation and a reduced rate of degeneration of larger ovarian follicles in immunized sheep. Generally, immunized ewes have a large number of non-atretic follicles which leads to an increased ovulation rate (Scaramuzzi and Hoskinson, 1984). Immunized sheep have multiple ovulations resulting in a greater number of twinning and triplet born lambs (Gibb et al., 1982, Henderson et al., 1990, Juengel et al., 2013b). Immunization with Androvax® can result in a 31 to 63% increase in ovulation rate compared with Bovine follicular fluid (bFF) which can increase ovulation rate by eight to 100% (Henderson et al., 1989). Henderson et al. (1989) reported a 33-63% increase in ovulation rate in adult ewes immunized with Androvax®. Estrone® and Androvax® resulted in a 40 to 74% greater rate of multiple ovulations (Henderson et al., 1990). Furthermore, immunization against androstenedione can reduce the anovulatory period by stimulating the onset of breeding season or onset of estrous (Gibb et al., 1982, Smith, 1985a, Henderson et al., 1989).

The timing of the administration of steroid vaccines needs to be considered for their effectiveness. When ewes are injected with Androvax® for the first time, they required two injections; the first is a sensitizer and the second is a booster injection (Juengel et al., 2013b). According to the manufacturer's requirements, the sensitizer injection should be administered eight weeks before breeding, allowing four weeks' interval before administering the booster injection (Smith, 1985b, Juengel et al., 2013b). During the four – six weeks following the booster injection, antibody levels are slightly elevated thus limiting the production of oestradiol and exhibition of estrous, therefore, the ram should be introduced after the four weeks after antibody levels fall (West et al., 2009, Juengel et al., 2013b). Allowing a four weeks interval before breeding improves conception rates and reduces ova loss (Juengel et al., 2013b). If ewes are to be treated again in the following year, only one injection is required at four to six weeks before breeding (West et al., 2009).

There is limited data on immunization against androstenedione in ewe lambs. Ronayne et al. (1991) reported that immunization had no effect on the onset of oestrous behaviours and puberty in the two breeds, however, the date for the first oestrous was different. In the Belclare Improver breed, the onset of first oestrous was on the 1st and 2nd November for control and immunized ewes, respectively, while, in Improved Galway breed, control ewe lambs attained the first oestrous on 5th Novevember while immunized ewes showed behavioural estrous on 13th November (Ronayne et al., 1991). Moreover, the ovulation rate varied among breeds; immunization increased ovulation rate by 50% in Belclare Improver and 10% in Improved Galway (Ronayne et al., 1991).

Table 2.3. Different vaccines, target hormones, and ovulation rate

Product name	**Active ingredient**	**Target hormone**	**Ovulation rate**		**Reference**
			Control	**Treatment**	
Androvax		Androstenedione	1.1	1.60	(Henderson et al., 1989)
			1.0	2-3	(Henderson et al., 1990)
			1.61	2.12	(Juengel et al., 2013b)
Estrone		Androstenedione	1.0	2.0	(Henderson et al., 1990)
Ovastim		Androstenedione	1.61	2.24	(Juengel et al., 2013b)
Fecundin		Androstenedione	1.20	1.2	(Campbell et al., 1991)
			1.41	2.09	(Scaramuzzi et al., 1993)
			1.51	1.97	(Ronayne et al., 1991)*
			1.29	1.53	(Ronayne et al., 1991)*
	Protein conjugate	Androstenedione	1.36	2.40	(Van Look et al., 1978)
	BFF	Inhibin		1.30	(Hudson et al., 1985)
			1.40	2.0	(Henderson et al., 1986)
	Purified inhibin	Inhibin	1.32	1.80	(Gibb et al., 1982)
	BMP15	BMP15	1.61	1.79	(Juengel et al., 2013b)

*Ewe lambs

Chapter 3 : Materials and methods

3.1 General information

The current study was undertaken at Massey University's Tuapaka farm, 15 km northwest of Palmerston North, in the North Island of New Zealand (latitude -40.33, longitude 175.73). The experiment was conducted between February 2017 and January 2018 and was approved by Massey University Animal Ethics Committee, Palmerston North, New Zealand.

3.2 Experimental design and Animals

In February 2017, three hundred Romney ewe lambs of about five months of age were selected (a mean of 81 days prior to the start of breeding, P-81). In early March, ewe lambs were allocated to one of three treatment groups: 1) control (n = 100), 2) A10 - 6 (n = 100) and A8 - 4 (n = 100). The A10 - 6 was treated at ten and six weeks before breeding while A8 - 4 was vaccinated at eight and four weeks before breeding. A stratified random allocation was used to ensure that each treatment group had approximately equal numbers of ewe lambs with a similar mean live weight (37 ± 0.27 kg). The treated groups were given Androvax® vaccine (MSD Animal Health New Zealand, Bach no.15.203) subcutaneously. The dose was 2ml and was injected into the anterior half of the neck. The dosing regimen was two doses, the first, a sensitizing injection given at ten or eight weeks before breeding and the second, a booster injection, administered at six or four weeks before breeding. Prior to breeding ewe lambs were also vaccinated against Campylobacter (Campyvax4®, 1ml subcutaneously) and Toxoplasmosis (Toxovax®, 2 ml intramuscularly) in the anterior half of the neck (MSD Animal Health New Zealand). In addition, ewe lambs zinc boluses were administered orally every six weeks (Time capsule® 43g, Agritrade, New Zealand) to prevent facial eczema. The three groups were managed together throughout the study.

At 69 days prior to the start of breeding (P-69), Romney teaser rams (rams rendered infertile by the removal of the small piece of vas deference cords hence restricting the passage of sperm; McGarry, 1960) fitted with mating harnesses (Rurtec Limited, Hamilton New Zealand) were introduced to the ewe lamb flock at a ratio of 1: 100 ewe lambs. Teaser rams remained with the flock until the start of breeding in mid-May. Teaser rams were removed from the flock at the day the entire rams were introduced for breeding (P1). The entire Romney rams were harnessed and introduced at the ratio of 1:50. The entire remained with the ewe lambs for two oestrous cycles (34 days). At the end of the first 17 days period, the mating harness crayon color was changed to allow the identification of the cycle the ewe lambs had been bred. Pregnancy diagnosis was conducted using trans-abdominal ultrasound scan 90 days after the start of the breeding period (P90). Ewe lambs diagnosed with triplet fetuses were removed from the study. At 136 days after the start of the breeding period (P136), pregnant ewe lambs were ear tagged with large plastic tags that allowed identification from a distance (Two-piece ear tags, Allflex, Palmerston North NZ), and then moved to the lambing paddocks. The lambing period began in early October and ended in mid-November. During lambing, ewes were checked twice a day to identify the number of lambs born or which required assistance to deliver their lamb.

3.3 Animal Measurements

Ewe lambs were weighed approximately every two weeks from February until mid-May, (True-Test XR5000 Indicator Datamars New Zealand), and condition scored (BCS scale 1.0–5.0; 1.0 = emaciated and 5.0 = grossly fat; Kenyon et al., 2014a) to ensure that they reached target live weight at breeding (40 kg). During each weighing, ewe lambs were checked for teaser harness crayon marks (Scale 0 to 3; 0=no mark, 1= slight mark at the flank, 2 marked at the rump but the mark is not very clear and 3 clear mark on the rump) were recorded as an indicator of the attainment of behavioural oestrus. Ewe lambs recorded with mark scores of 0 to 1 were deemed to have not started showing behavioural oestrus, while those with scores of 2 and 3 had attained behavioral oestrus. The same score system was used to ram marks in order to identify the cycle during which a ewe lamb had been bred. The crayon marks were recorded at the end of each oestrous cycle. The cycle bred was determined based on the ram harness marks; first cycle had only the color from the first 17 days, second cycle had only the second color, returned to service had both colors and those with no marks were recorded as not bred. At pregnancy diagnosis ewe lambs were identified as either pregnant (yes or no) and for those pregnant, the number of fetuses present (0, 1, 2, 3). At birth, lambs were weighed (Salter Super Samson 10 kg hand-held scale, Salter, Australia) and ear tagged with an EID tag (rapidtag, Allflex, Palmerston North, NZ). Lambing data was recorded using True-test XR52 Portable EID Reader (Datamars, New Zealand). Lambs were weighed again at L46, L74 and L102 at which time they were weaned.

3.4 Statistical analysis

Complete data were collected from 269 of the 300 ewe lambs enrolled (control n= 96, A10 - 6 = 92, A8 - 4 = 81). Of those excluded, five failed to reach the minimum live weight for breeding (40 kg), nine ewe lambs failed to be bred throughout the two cycles, sixteen ewe lambs were diagnosed with triplet fetuses and one was diagnosed with twin fetuses but gave birth to triplet lambs. The data analysis carried out throughout breeding included all ewes presented for breeding (n=295) while the analysis carried out at pregnancy diagnosis excluded the ewe lambs that were not marked by the entire ram (n=286). In addition, the analysis carried out after lambing excluded ewe lambs diagnosed with triplet fetuses and one ewe lamb that was diagnosed with twin fetuses but gave birth to triplet lambs (n=269).

All the data were analyzed using SAS software. The live weight of the ewe lambs was analysed using Proc Mixed. In addition, the live weight gain of ewe lambs throughout the study was analysed using a repeated measures analysis of variance. BCS, number of foetuses, number of lambs born, and a number of lambs weaned were analyzed using Proc Genmod in SAS using Poisson distribution, with logit transformation and the fixed effect of the treatment group. For number of foetuses conceived, number of lambs born, and a number of lambs weaned, live weight at breeding was used as a covariate. The percentage of ewe lambs that attained behavioral oestrus before the introduction of the entire ram, cycle bred (first, second, returned to service or was not bred), that were identified as pregnant, that had a single fetus, multiple fetuses (twins or triplet) at pregnancy diagnosis and at lambing, and ewe and lamb survival to weaning were analysed using Proc Genmod in SAS using a binomial distribution and the fixed effect of treatment group and the covariate of live weight at breeding. The live weight of lambs at birth, L46, L102 and their liveweight gain from birth to L46 and from birth to L102 was analyzed using PROC mixed with a fixed effect of the treatment group and birth rank and sex as a covariate. For live weights post birth, lamb birth weight was added as a covariate.

Chapter 4 : Results

4.1 Live weight and BCS of ewe lambs from P0 to L102

The live weight of the ewe lambs in the three treatment groups did not differ (p>0.05) throughout the study (Figure 4.1). Generally, ewe lamb live weight increased from the beginning of the study and throughout pregnancy until lambing. From L48 to L76, ewe lamb live weight decreased and then increased again to weaning (L102).

Ewe lamb live weights and BCS did not differ (p>0.05) among three treatments at P-81, P0, P90, P136 or L48 (Table 4.1). At L102, however, there was a trend (p = 0.09) for control ewe lambs to be heavier than ewe lambs in the A8 – 4 treatment, although, no differences (p>0.05) in BCS were observed.

4.2 Attainment of behavioral oestrus

Of the 295 ewe lambs exposed to the teaser rams, 160 (54.2%), had harness crayon marks prior to the introduction of the entire ram (Table 4.2). A greater (p<0.05) percentage of ewe lambs in control and A10 – 6 treatments had teaser crayon marks prior to the introduction of the entire ram compared to those in A8 – 4 treatment.

4.3 Pattern of breeding

The live weight of ewe lambs at the start of the breeding period (P0) did not differ (p>0.05) among ewe lambs that were either not marked, or marked in the first cycle, the second cycle or returned to service (Table 4.3). The majority of ewe lambs were marked in the first cycle of the breeding period with a small number that were not marked during the entire breeding period.

The majority of ewe lambs were marked in the first cycle of the breeding season regardless of treatment group (Table 4.4). Treatment had no effect (p>0.05) on the percentage of ewe lambs marked in the first cycle, second cycle or that returned to service. Across the three treatments, more than 95% of ewe lambs were marked by the entire ram.

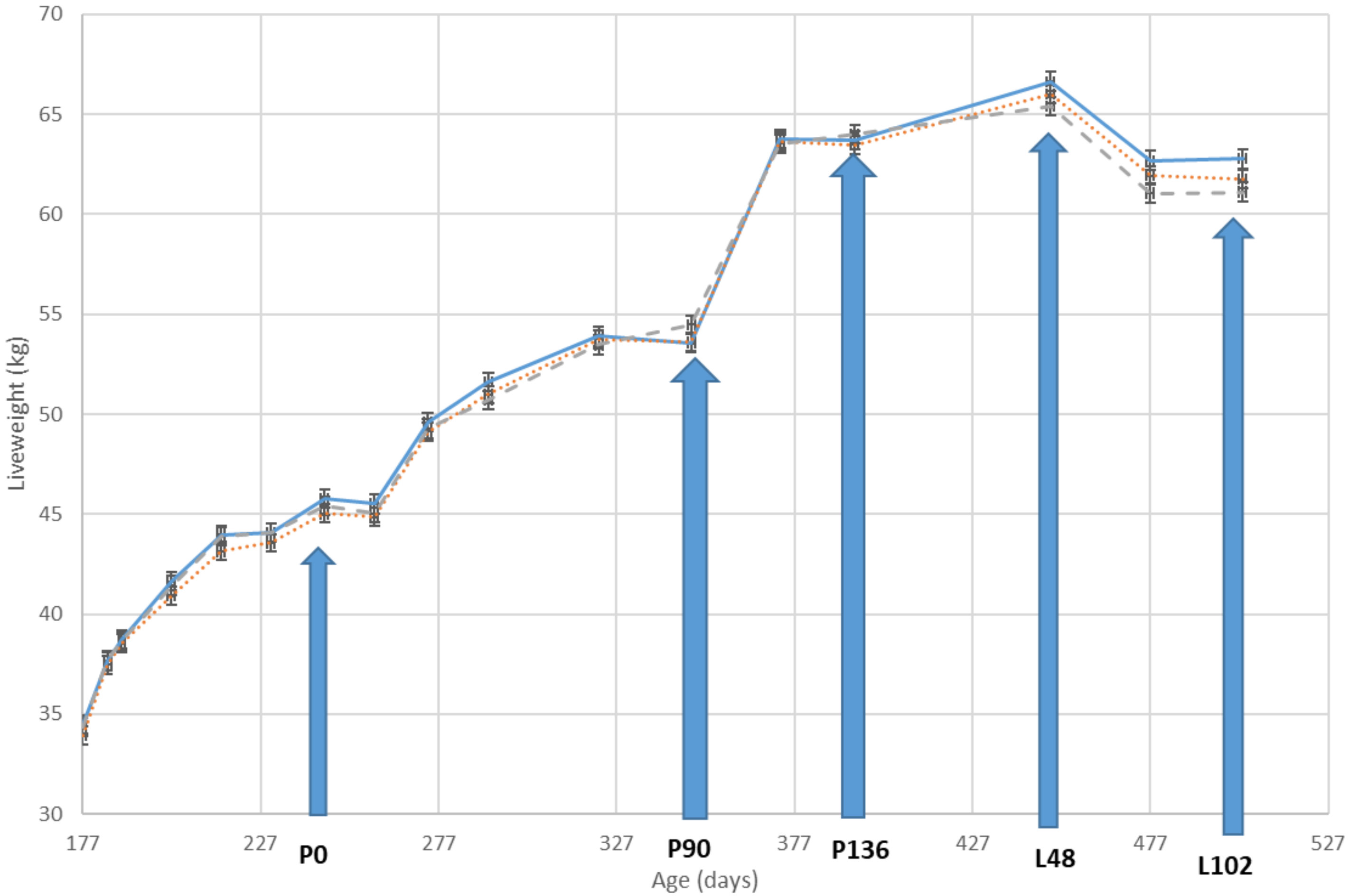

Figure 4.1: Liveweight of ewe lambs in the control (solid line), A10 – 6 (dotted line) and A8 – 4 (dashed line) treatments from the beginning of the study (177 days of age, P-81) until they wean their lambs (503 days of age, L102)

Table 4.1. Live weight (least squares mean ± s.e.) and BCS (back-transformed mean with 95% confidence limits in parentheses) of ewe lambs at P-81,P0, P90, P136, L36 and L102 in the three treatments (control, A10 – 6, A8 - 4)

			Treatment				
Variables	**Time**	**n**	**Control**	**n**	**A10 - 6**	**n**	**A8 – 4**
Live weight	P-81	100	37.5 ± 0.3	100	37.4 ± 0.3	100	37.6 ± 0.3
	P0	99	45.4 ± 0.3	99	44.9 ± 0.3	97	45.0 ± 0.3
	P90	87	53.4 ± 0.4	95	53.5 ± 0.4	90	54.4 ± 0.5
	P136	85	63.5 ± 0.5	86	63.5 ± 0.5	73	63.4 ± 0.6
	L46	82	66.9 ± 0.8	82	66.4 ± 0.8	72	66.1 ± 0.9
	L102	81	62.7 ± 0.7	82	61.7 ± 0.7	71	60.9 ± 0.8
BCS	P0	99	2.94 (2.63 – 3.30)	99	2.94 (2.63 – 3.30)	96	2.99 (2.67 – 3.36)
	P90	87	3.14 (2.77 – 3.53)	89	3.14 (2.79 – 3.53)	83	3.23 (2.87 – 3.65)
	P136	85	3.82 (3.42 – 4.26)	86	3.83 (3.43 – 4.26)	73	3.80 (3.78 – 4.28)
	L46	82	3.11 (2.75 - 3.52)	82	3.05 (2.69 – 3.45)	72	2.99 (2.62 – 3.42)
	L102	80	2.87 (2.52 – 3.27)	82	2.88 (2.53 – 3.27)	71	2.80 (2.44 – 3.22)

Table 4.2 . The number and the proportion (back transformed percentage with 95% confidence limits in parenthesis) of ewe lambs marked by teaser ram in three treatments (Control, A10 - 6 and A8 - 4).

Treatments	**n**	**Percentage marked by a teaser ram (%)**
Control	99	56 (46 - 66) ^{b}
A10 - 6	99	62 (52 - 71) ^{b}
A8 - 4	97	44 (35 - 54) ^{a}

Means within the column with different superscripts differ significantly (P > 0.05).

Ewe lambs that failed to reach minimum live weight at breeding were removed

Table 4.3. The number and percentage of ewe lambs that were recorded with no ram mark or marked by the entire ram during the first or second cycle, or that returned to service and their live weight (kg) prior to breeding (least squares mean ± s.e.)

Entire ram marks	**n**	**Percentage (%)**	**Live weight at P0 (kg)**
No mark	9	3.1	44.4 ± 1.1
Cycle 1 only	231	78.3	45.2 ± 0.2
Cycle 2 only	17	5.8	45.3 ± 0.8
Returned to service	38	12.9	44.6 ± 0.5

Table 4.4. The number and percentage (back transformed percentage with 95% confidence limits) of ewe lambs marked by entire ram or left unmarked and the cycle in which ewe lambs were marked (first or second cycle or returned to service) in the control, A10 - 6 and A8 – 4 treatments

				Cycle		
TRT	n	Entire ram mark (%)	Not marked (%)	First (%)	Second (%)	Returned to service (%)
Control	99	100	0	83 (74 - 89)	3 (1 - 9)	14(9 - 23)
A10 - 6	99	96 (90 -	4 (1 - 10)	79 (70 - 86)	8 (4 - 15)	9 (4 - 16)
A8 - 4	97	99)	5 (2 - 12)	73 (64 - 81)	6 (3 - 13)	16 (9 - 24)

	95 (88 - 98)

4.4 Fertility and fecundity rate at pregnancy diagnosis

Ewe lambs diagnosed with triplet fetuses were heavier (p<0.05) at P0 than those diagnosed as non-pregnant that had a single fetus (Table 4.5). There was a trend (p = 0.06) for ewe lambs that were diagnosed with a single fetus to be lighter at P0 than those diagnosed with twin fetuses. A greater percentage of ewe lambs were diagnosed with a single or twin fetuses than triplet fetuses or non-pregnant.

Table 4.5. The number and percentage of ewe lambs that were not recorded as pregnant (non-pregnant) or that were diagnosed with single or twin or triplet fetuses and their live weight (kg) prior to breeding (least squares mean ± s.e.).

Pregnancy diagnosis	n	Percentage (%)	Live weight at P0
Non-pregnant	25	8.7	44.5 ± 0.6^{a}
Single	114	39.9	44.6 ± 0.3^{a}
Twin	131	45.8	45.4 ± 0.3^{a}
Triplet	16	5.6	46.8 ± 0.8^{b}

Means within the columns with different superscripts differ significantly (p<0.05)

The fertility rate, which was defined as, the number of ewe lambs diagnosed as pregnant per ewe lamb bred, did not differ (p>0.05) among the three treatment groups (Table 4.6). Ewe lambs immunized at eight and four weeks before breeding (A8 – 4) had a greater (p<0.05) fecundity rates, and number of fetuses per ewe lamb bred, than the control group, while those immunized at ten and six weeks did not differ (p>0.05) from either treatment group. In the control treatment, a greater percentage (p>0.05) of ewe lambs were diagnosed as single bearing than those in A8 – 4 treatment. The majority (p>0.05) of ewe lambs in the treated groups were diagnosed as bearing twins. Ewe lambs in A8 – 4 treatment were diagnosed with fewer (p<0.05) single fetuses and more (p<0.05) triplet fetuses than either control or A10 – 6 treatment.

Table 4.6. The fertility rate (ewe lambs diagnosed as pregnant / ewe lambs bred), fecundity rate (number of fetuses /ewe lambs bred), and percentage of ewes diagnosed with single, twin or triple fetuses at pregnancy diagnosis at P90 (back-transformed mean with 95% confidence limits in parenthesis) of ewes in the control, A10 – 6 and A8 – 4 treatments.

Treatments	n	Fertility rate (%)	Non-pregnant	Fecundity rate (Fetuses /ewe lambs bred)	Number of fetuses (%)		
					Single	Twin	Triplet
Control	99	89 (81-94)	11 (6-19)	1.30 (1.09-1.54) ^{a}	50 (40-60) ^{b}	36 (27–46) ^{a}	3(1– 8) ^{a}
A10 - 6	95	94 (87-97)	6 (2-13)	1.49 (1.26-1.75) ^{ab}	42 (32-52) ^{b}	49(38-58) ^{ab}	3(1 -9) ^{a}
A8 – 4	92	91 (84-96)	9 (4-16)	1.66 (1.42- 1.95) ^{b}	27 (18-37) ^{a}	53(43- 63) ^{b}	11(5-18) ^{b}

Means within the columns with different superscripts differ significantly (p<0.05)

Ewe lambs not marked by entire ram were removed

4.5 Lambing performance

The live weight of ewe lambs at the start of breeding period (P0) that were identified as having no lambing records, single or twin fetuses did not differ ($p>0.05$; Table 4.7). A greater percentage of ewe lambs were identified with twin lambs than singles or no lambing records.

Table 4.7. The number and percentage of ewe lambs that were not recorded as lambing (no lamb) or that gave birth to single or twin lambs and their live weight (kg) prior to breeding (least square mean ± s.e).

Lambing outcome	n	Percentage (%)	Live weight at P0
No lamb	65	24.2	45.0 ± 0.5
Singleton	98	36.4	44.7 ± 0.3
Twins	106	39.4	45.3 ± 0.3

A greater ($p<0.05$) percentage of ewe lambs in A10 – 6 lambed than the control treatment, while those in A8 – 4 did not differ from either treatment ($p>0.05$; Table 4.8). Litter size did not differ ($p>0.05$) between the three treatments, however, immunization increased the number of lambs born by 0.30 lambs per ewe joined regardless of dosing regimen. The percentage of ewe lambs that were recorded to give birth to a single lamb did not differ ($p>0.05$) between the three treatment groups, although both immunized treatments (A10 – 6 and A8 - 4) had a greater percentage ($p<0.05$) of ewe lambs that gave birth to twins than the control treatment.

Table 4.8. The percentage of ewe lambs with lambing records, litter size (lambs born / ewe lambs bred) and percentage of ewes that gave birth to single or twin lambs (back transformed percentage with 95% confidence limits in parenthesis) in control, A10 - 6 and A8 - 4 treatments

Treatments	n	Ewes with lambing records (%)	No lambing record (%)	Litter size (lambs born / ewes bred)	Birth rank	
					Single (%)	Twin (%)
Control	96	69 (59 - 77) [a]	31 (23 - 41) [b]	0.96 (0.79 - 1.18)	41 (32 - 51)	28 (18 - 37) [a]
A10 - 6	92	82 (72 - 88) [b]	18 (13 - 30) [a]	1.26 (1.05 -1.52)	37 (27 - 47)	45 (35 - 55) [b]
A8 - 4	81	78 (68 - 86) [ab]	22 (15 - 3 3) [ab]	1.25 (1.03 - 1.52)	31 (22 - 42)	47 (36 - 58) [b]

Means within the column with different superscripts differ significantly ($p<0.05$).

Ewe lambs not marked by entire ram and those with triplet lambs were removed.

4.6 Lamb live weight and liveweight gains

There was a trend (p=0.07) for lambs born to control ewes to be heavier than those born to the A8 – 4 treatment (Table 4.9). When birth rank was included as a covariate, there was also a trend (p=0.07) for lambs born to A8 – 4 treatment to be lighter than those born to A10 – 6 treatment.

Lamb live weights at L48 and L102 did not differ (P<0.05) between the three groups, however, when date of birth was added as a covariate, there was a trend for lambs born to ewe lambs in the control treatment to be heavier at L48 (0.06) than those born to ewe lambs in the A10 – 6 treatment (Table 4.9). Moreover, when lamb birth weight was added as a covariate it showed that for every 1 kg increase in birth weight there was an increase in live weight at L48 and L102 by 1.6 kg and 1.9 kg respectively.

Single born lambs were heavier (P<0.05) at birth, L48, and L102 than twin lambs regardless of their dam's treatment (Table 4.9). The live weight of single lambs did not differ (P>0.05) among treatment groups at birth, L48 or L102. Twin lambs born to ewe lambs in the control group, however, were heavier (P<0.05) at L48 than those born to ewe lambs in the A10 – 6 treatment and while those born to A8 – 4 treatment did not differ (P>0.05) from either group. At L102, however, differences were no longer observed (p>0.05).

There was a trend for lambs born to ewe lambs in the control treatment to have a greater (P=0.06) growth rates from birth to L48 (Table 4.9). The live weight from L0 to L102 did not differ among the three treatments. When lamb birth weight was included in the model of live weight gain from L0 to L48 and L0 to L102 it showed that for every 1 kg increase in birth weight there was a corresponding 13.5 g/d and 9.2 g/d increase in lamb live weight gain, respectively. Single born lambs had a greater liveweight gain from birth until L102 than twins. Within treatments, twin lambs born to ewe lambs in the control gained weight faster (P<0.05) than those in the A10 – 6 group, while those born to A8 – 4 treatment did not differ (P>0.05) from either group.

Table 4.9. Effect treatment (control, A10 - 6 and A8 - 4) and birth rank on lamb birth weight (kg), live weights (kg) at L48 and L102, and liveweight gains (g/day) from L0 to L48, L48 to L102 and L0 – L102 (least square means ± s.e)

Treatments	Birth rank	Live weight (kg)						Liveweight gain (g/day)			
		n	Birth (L0)	n	L48	n	L102	n	L0 - L48	n	L0 – L102
Control		95	4.7 ± 0.1^{ab}	76	14.1 ± 0.2	76	25.0 ± 0.4	75	196.5 ± 4.8	75	199.4 ± 3.8
A10 - 6		110	4.8 ± 0.1^{b}	81	13.5 ± 0.4	82	24.1 ± 0.4	81	183.8 ± 4.8	82	191.5 ± 3.8
A8 - 4		99	4.5 ± 0.1^{a}	76	13.8 ± 0.4	80	24.7 ± 0.4	76	190.0 ± 4.9	80	196.7 ± 3.9
	Single	97	5.2 ± 0.1^{b}	81	14.8 ± 0.2^{b}	84	26.4 ± 0.4^{b}	81	211.3 ± 5.0^{b}	84	213.8 ± 3.9^{b}
	Twin	207	4.2 ± 0.1^{a}	152	12.7 ± 0.2^{a}	154	22.8 ± 0.3^{a}	151	168.9 ± 3.5^{a}	153	177.9 ± 2.8^{a}
Control	Single	39	5.1 ± 0.2^{b}	32	14.8 ± 0.4^{c}	32	26.8 ± 0.6^{b}	32	212.6 ± 7.5^{a}	32	217.6 ± 6.0^{b}
	Twin	56	4.3 ± 0.1^{a}	44	13.2 ± 0.4^{b}	44	23.1 ± 0.5^{a}	44	179.1 ± 6.3^{b}	44	181.3 ± 5.0^{a}
A10 - 6	Single	33	5.4 ± 0.2^{b}	28	14.7 ± 0.4^{c}	29	26.0 ± 0.7^{b}	28	209.1 ± 13.4^{a}	29	209.8 ± 6.5^{b}
	Twin	77	4.3 ± 0.1^{a}	53	12.3 ± 0.3^{a}	53	22.3 ± 0.4^{a}	53	160.6 ± 5.8^{a}	53	173.4 ± 4.6^{a}
A8 - 4	Single	25	5.1 ± 0.2^{b}	21	14.9 ± 0.4^{c}	23	26.4 ± 0.7^{b}	21	214.4 ± 9.2^{a}	23	214.0 ± 7.0^{b}
	Twin	74	4.0 ± 0.1^{a}	55	12.7 ± 0.3^{ab}	57	22.8 ± 0.5^{a}	55	167.5 ± 5.8^{ab}	57	179.0 ± 4.6^{a}

Means within the column with different superscripts differ significantly ($p>0.05$)

4.7 Lamb and ewe survival

There was a trend ($p = 0.098$) for a greater survival rate among lambs born to A8 – 4 treatment compared with born to A10 – 6 treatment (Table 4.10). Single born lambs had a higher survival rate ($p<0.05$) than twin-born lambs.

Ewe lamb survival did not differ ($p>0.05$) among the three treatments. There was a trend for ewe lambs with no lambing record ($p = 0.07$) and those with twin lambs ($p=0.09$) to have lower survival rates than those that gave birth to a single lamb.

There was no difference ($p>0.05$) in the number of lambs weaned among the treatment groups, however, A10 – 6 and A8 – 4 on average weaned 0.13 and 0.2 lambs, respectively compared with control treatment.

Table 3. 10. The lamb survival, ewe survival and number of lambs weaned (back-transformed mean with 95% confidence limits in parenthesis) in the control, A10 – 6 and A8 – 4 treatments and in each birth rank (single or twin)

Treatments	n	Lamb survival (%)	n	Ewe survival (%)	n	No. of lambs weaned per ewe bred
Control	95	82 (73 - 88)	84	96 (88 - 99)	96	0.78 (0.62 - 0.98)
A10 - 6	117	74 (65 - 81)	86	96 (88 - 99)	92	0.91 (0.74 - 1.13)
A8 - 4	101	83 (74 - 89)	72	98 (90 - 100)	81	0.98(0.78 - 1.22)
Birth rank						
No records	-	-	38	93 (79 - 98)	65	-
Single	99	85 (77 - 91) ^{b}	98	99 (94 - 100)	98	0.84 (0.68 - 1.05) ^{b}
Twin	214	73 (66 - 78) ^{a}	106	94 (88 - 98)	106	1.44 (1.23 - 1.69) ^{a}

Means within the column with different superscripts differ significantly ($p<0.05$)

For ewe survival, ewes diagnosed non-pregnant and those with triplet lambs were removed

For lambs weaned, ewes not marked by entire ram and those with triplet lambs were removed.

Chapter 5 : Discussion

5.1 General discussion

The aim of the current study was to determine the effect of immunization with Androvax® on reproductive performance of ewe lambs. This study also assessed the effect of two dosing intervals; ten nd six (A10 - 6) and eight and four (A8 – 4) weeks prior to breeding on reproductive performance. Treatment with Androvax® had no effect on the live weight of ewe lambs throughout the current study. In a previous study of mature ewes, Knight et al. (1985) reported that those immunized with Fecundin had similar live weights to untreated ewes at breeding but at weaning immunized ewes were lighter (49.6 vs 50.3 kg and 50.8 and 51.9 kg). In their study, ewes with triplet lambs remained in the study, whereas, in the current study, ewe lambs with triplet lambs were excluded after pregnancy diagnosis. It has been reported that ewe lambs with a live weight below 50 kg at weaning can have a poor reproductive performance in their following two tooth breeding seasons (McMillan and McDonald, 1983). In the current study, however, all ewe lambs weighed more than 60 kg at weaning, therefore, immunization with Androvax® is unlikely to impair two tooth breeding. Greater ewe weaning weights in the current study, however, may have been due to heavier ewes at breeding, therefore, ewes lambs should be atleast 40 kg at breeding in other to reach 60 kg at weaning.

Ewe lamb live weight immediately prior to breeding in the current study, had no effect on the cycle during which a ewe lamb was bred. Kenyon et al. (2005) reported that ewe lambs with low live weights at breeding (32 kg) are more likely to fail to be bred, however, ewe lambs used in that study were lighter (32 – 36 kg) than those in the present study. This suggests that ewe lambs should weigh at least 40 kg to ensure optimal breeding performance as previously suggested by Corner-Thomas et al. (2015). Increased ewe lamb live weight at breeding is also associated with an increased lambing percentage (Kenyon et al., 2004b, Kenyon et al., 2005). This is consistent with our results, whereby, ewe lambs with greater live weight at breeding were diagnosed with triplet foetuses.

The effect of treatment with Androvax® on behavioral estrous varied depending on the dosing regimen; when administered at eight and four weeks before breeding, ewe lambs showed a delayed onset of behavioral oestrus compared with those immunized at ten and six weeks as measured by teaser ram marks. Ronayne et al. (1991), however, reported no effect of immunization against androstenedione using Fecundin on the onset of behavioral estrous of ewe lambs. In the current study, ewe lambs were exposed to the teaser rams from the beginning of the study, 70 days prior to breeding, while, teaser rams were only introduced twice a day to detect behavioural oestrus in the study by Ronayne et al. (1991). The introduction of a teaser ram after a period of separation is known to advance the onset of behavioral oestrus (Kenyon et al., 2005, Kenyon et al., 2006). It is not known, however, if A8 – 4 treatment negated this effect as the percentage of ewe lambs showing behavioral oestrus was lower than that of the control ewes. The study of Ronayne et al. (1991) used Belclare Improver and Improved Galway breeds and was conducted in Ireland while the current study used Romney ewe lambs, therefore it is not known if the environment and breed differences contributed to this variation.

The delay in the onset of behavioral oestrus in ewe lambs in A8 – 4 treatment may be associated with two factors. Firstly, a strong immune response to the immunization. It has been reported that immunization against BMP 15 or GDF9 can cause a strong immune response leading to anovulation for an extended period (Juengel et al., 2013b, McNatty et al., 2007). Given that Androvax is manufactured specifically for mature ewes, it is possible that it may have caused a strong immune response in ewe lambs, thus delaying the onset of oestrous activity, however, further investigations are required. Secondly, elevated antibody titres following immunization. An Australian study that investigated the effect of immunization against androstenedione in mature ewes using Fecundin reported that the antibody titres remained high after the booster injection had been given, but then decreased over time (by day 25). Ewes with high antibody titres remained in the anoestrous phase and had a shorter oestrous cycle compared with control ewes (Boland et al., 1986). Displaying oestrous behaviors, particularly the willingness to mate, is an indicator of attainment of puberty (Edwards et al., 2016). Ewe lambs displaying estrous early in the breeding season indicates that they had attained puberty and were ready to be bred (Kenyon et al., 2012).

It was expected that variation in the onset of behavioral oestrus would affect the cycle during which the ewe lamb was bred, however, this was not the case in the current study. Kenyon et al. (2012) reported that an earlier onset of behavioral oestrus was associated with a greater proportion of ewe lambs conceiving in the first oestrous cycle. Ronayne et al. (1991), however, reported that immunized ewe lambs can encounter a short luteal phase without showing the behavioral oestrus. It has previously been reported that ewe lambs can attain puberty and ovulate without showing signs of behavioral oestrus (Edwards et al., 2016). It is, therefore, possible that ewe lambs in A8 – 4 treatment had attained sexual maturity before the introduction of the entire ram but showed no signs of oestrus. Moreover, A8 – 4 treatment had delayed the onset of oestrus, it is possible that ewe lambs could have attained puberty immediately after the introduction of the entire ram and, therefore, they were still able to be bred in the first cycle. In mature ewes, immunization had no influence in the cycle during which ewes were bred (Juengel et al., 2013b, Scaramuzzi et al., 1983).

In the current study, the effect of treatment with Androvax® on fecundity rates (number of fetuses / ewe lamb bred) varied depending on the dosing regimen. When administered at eight and four weeks before breeding, there was an increase in fecundity rate compared with the control treatment. When administered at ten and six weeks before breeding, however, the fecundity rate did not differ from either treatment. To date, no studies have reported the effect of dosing intervals on fecundity rates of ewe lambs. Fecundity rates depend primarily on ovulation rate (Edwards et al., 2015, Edwards et al., 2016), therefore, differences in fecundity rates observed suggests there was a variation in ovulation rates. In naturally bred ewe lambs, ovulation rate increases with advancing oestrous cycles (Meyer and French, 1979). It was, therefore, surprising that in the current study ewe lambs in A10 – 6 treatment showed behavioral oestrus prior to breeding but did not have a greater fecundity rate compared with those in A8 – 4 treatment. Knight et al. (1985) reported that immunized mature ewes can maintain their pregnancy but they can encounter a partial failure of multiple ovulations thus reducing the fecundity rates, therefore, it is possible that ewe lambs in A10 – 6 weeks may have had a partial failure of multiple ovulations.

Ewes immunized at 10 and 6 weeks prior to breeding were diagnosed with more singles, were intermediate for twins and had fewer triplets compared with ewes immunized 8 and 4 weeks prior to breeding. Juengel et al. (2013b), reported that mature ewes immunized at eight and four weeks before breeding were diagnosed with a greater percentage of twins (56% vs 49%) and triplets (12% vs 3%) and fewer single fetuses (23% vs 38) compared with control ewes. Among mature ewes, generally only one immunization, a booster, is given four to six weeks prior to breeding. After four weeks of immunization, the majority of follicles should have reached the preovulatory stage and, therefore, are ready to ovulate. Immunized mature ewes commonly ovulate multiple ova, often three or more in each oestrous cycle (Smith et al., 1981) leading to an increased chance of multiple fetuses being conceived (Henderson et al., 1990).

Ewe lambs immunized at eight and four weeks prior to breeding had higher fecundity rates than the control and the A10 – 8 treatment, although, litter sizes at birth did not differ statistically. Generally, immunization increased the number of lambs born by 0.30 lambs per ewe joined, regardless of dosing regimen; this is slightly lower than the 0.39 reported by Geldard et al. (1984) in mature ewes. The difference between studies was likely due to the exclusion of ewe lambs carrying triplets in the current study. In the current study, ewe lambs carrying triplets were removed from the study in order to be offered preferrential feeding. Increased litter size in immunized mature ewes is primarily due to an increased percentage of ewes giving birth to twins and triplets and fewer singles (Scaramuzzi et al., 1993, Juengel et al., 2013b). In the current study, a greater percentage of treated ewe lambs gave birth to twin lambs compared with the control, however, the proportion of single lambs did not differ. Ewe lambs diagnosed with triplet lambs were removed from the study after pregnancy diagnosis, therefore, this reduced the mean litter size of the treated ewe lambs, particularly those in A8 – 4 treatment.

In the current study, it appears that the A10 – 6 treatment reduced fecundity rates at pregnancy diagnosis compared with A8 – 4 treatment, which means that increasing the interval from booster to breeding with six weeks can reduce the fecundity rates of ewe lambs. Scaramuzzi et al. (1983) reported that the interval from the booster to breeding for mature ewes was an important parameter in determining litter size. An interval of less than three weeks, or greater than four weeks can reduce litter size (Scaramuzzi et al., 1983). The maximum reproductive performance was achieved if ewes were bred after 30 days after receiving the booster injection (Scaramuzzi et al., 1983). Knight et al. (1985) reported that it was common for immunized mature ewes to have high ovulation rates and, thus, fecundity rates, but they can experience a partial failure of multiple ovulations leading to low litter sizes. Knight et al. (1985) reported that although there was a 60% and 40% increase in an ovulation rate of immunized ewes in 1983 and 1984, litter size increased by only 26% and 19%, respectively.

The fertility rate (ewes pregnant / ewes bred) in the current study did not differ among the three treatments. This finding is in agreement with Scaramuzzi et al. (1983) and

Geldard et al. (1984) who found that the fertility rate of immunized mature ewes and control ewes did not differ. Fertility in sheep is primarily influenced by anoestrus state as, ewes that are anoestrous cannot be bred (Scaramuzzi et al., 1983). Immunization can only increase ovulation rates, fecundity rates and lambing percentage (Scaramuzzi et al., 1983, Geldard et al., 1984, Boland et al., 1986), therefore, if ewes are naturally infertile immunization cannot change their state. At lambing, however, a greater percentage of ewes in the control treatment had no lambing records compared with ewe lambs in A10 – 6 treatment, which may be associated with reproductive wastage occurring after pregnancy diagnosis. Ridler et al. (2017) reported that ewe lambs can experience reproductive wastage between day 80 and 110 of pregnancy. Fetal loss usually increases with an increase in fecundity rates (Geldard et al., 1984), therefore, it was surprising than fewer treated ewes would have lambing records compared with control ewes. In mature ewes, Smith et al. (1981) reported higher incidences of barrenness in immunized ewes compared with control ewes. It is possible that in the current study, treated ewes had a partial loss of multiple fetuses which can be explained by the increased proportion of single lambs than anticipated, or alternatively poor recording technique.

Lambs born to A8 – 4 treatment, were lighter at birth compared with those in A10 – 6 treatment and lambs born to the control treatment were intermediate. Within birth ranks there was no difference between treatments, however, twins were lighter than single lambs. The overall difference between groups, therefore, was driven by the proportion of twin lambs born. In contrast, Knight et al. (1985) reported that, among mature ewes, lambs born to immunized ewes were lighter than those born to control ewes regardless of birth rank. In that study, Fecundin was used in a dosing regimen at six and three weeks prior to breeding which may have resulted in the differences observed between the two studies.

The live weight of the lambs at L48 and L102 did not differ between the three treatments, however, single lambs were heavier than twin lambs in all treatments. Among twin lambs, those born to ewes in the control treatment were heavier at L48 than those born to ewes in A10 – 6 treatment. Lambs born born to ewes in the A8 – 4 treatment were intermediate in live weight at L48 and L102. The greater liveweight of lambs born to control ewes than those born to A10 – 6 treatment was due to a greater growth rate from birth to L48. It has been reported that lamb liveweight gain from birth to six weeks of age was influenced by ewe milk production (Schreurs et al., 2010, Kenyon et al., 2011, Corner et al., 2013), therefore, it is possible that twin-bearing control ewes produced more milk than treated ewes. At weaning (L102), however, the differences in live weight between twins were no longer observed. Knight et al. (1985) reported that lambs born to immunized ewes had lower live weight at weaning which was associated with their lower birth weight, therefore, it is not surprising that the live weight at weaning in the current study did not differ between the three treatments.

In the current study, lamb birth weight had a positive relationship with live weights at L48 and L102 and growth rates from birth until weaning. Similarly, (Kenyon et al., 2006) reported a positive effect of lamb birth weight on lamb growth to weaning (9 g/d). Heavier lambs at weaning are preferred by farmers as they can be sold directly to slaughter or can be weaned early (Kenyon et al., 2006, Kenyon et al., 2008, Schreurs et al., 2010). Weaning lambs early allows additional time for ewes to gain live weight prior to their next breeding, thus ensuring the ewes achieve adequate live weight (60 kg) (Kenyon et al., 2006).

The live weights of single and twin lambs at L48 were lower, and at weaning, were greater than reported by Morris et al. (2005). In that study, Morris et al. (2005) offered ewe lambs different pasture allowances (maintainance, medium and *ad libitum*) whereas, in the current study ewes were managed under the same nutritional conditions.

There was a trend, in the current study, for lambs born to A8 – 4 treatment, to have greater survival to weaning compared with lambs born to A10 – 6 treatment. The reason for this difference is not known and according to the author's knowledge, no studies have compared lamb survival between dosing regimens. Knight et al. (1985) reported a higher lamb mortality rate among ewes immunized with Fecundin compared with the control. Lamb mortality is to a large degree influenced by lamb birth weight. Knight et al. (1985) reported that single lambs born to immunized mature ewes had lower birth weights than the control group (4.7 vs 5.0 and 4.4 vs 4.6 in 1983 and 1984, respectively). In the current study, lamb birth weights did not differ between the treatments.

5.2 Implications

Immunization against androstenedione has the potential to increase the fecundidty rates of ewe lambs. If it is to be used on commercial farms, however, farmers must have sufficient feed resources to allow the preferential feeding of ewe lambs, particularly those that conceive twin and triplet fetuses. Moreover, increased reproductive rate, particularly multiple fetuses in ewe lambs may be associated with reproductive loss and death of ewe lambs which can affect their welfare.

It has been reported that mature ewes with greater live weights at breeding have a greater response to immunization than those with low live weight, therefore, farmers should aim for greater live weights at breeding in order to add benefit to the additive effect of immunization and liveweight (Scaramuzzi et al., 1983). In their second year, previously immunized ewes should be given a booster injection only, therefore, immunizing ewe lambs at a younger age can be economical, as ewes will receive only one injection for many years, hence, reducing the cost per year (Scaramuzzi et al., 1983). The primary purpose of breeding ewe lambs at a younger age is to improve the profitability of the farm through an increasing number of lambs born, therefore, immunization at eight and four weeks before breeding (A8 – 4) is a method that can achieve this, however, farmers should work on reducing pregnancy losses and lamb mortality in order to wean more lambs.

5.3 Conclusion

Immunization against androstenedione increased the fecundity rates of ewe lambs when dosed at eight and four weeks (A8 – 4) but not at ten and six weeks (A10 – 6) prior to breeding. Immunization at eight and four weeks before breeding reduced the percentage of ewe lambs that were identified as bearing a single fetus and increased the percentage of twin and triplet bearing ewe lambs while the A10 – 6 regime did not reduce singles or increase triplet fetuses. Immunization increased the number of lambs born by increasing the proportion of twin lambs, even though triplet bearing ewe lambs were removed from the study after pregnancy diagnosis. Moreover, there was a tendency for lambs born to A8 – 4 treatment to have greater survival rates than those born to A10 - 6 treatment, but there was no difference in a number of lambs weaned. The live weight of ewe lambs at breeding had no effect on the cycle during which ewes were bred, however, it increased their fecundity rates.

5.4 Limitations and possible further studies

The present study suggests that Androvax® can be used to increase the fecundity rates of ewe lambs. This study also examined the impact of timing of immunization prior to breeding on the reproductive performance of ewe lambs. One of the important parameters identified in this study was the effect of the timing of immunizations on the onset of oestrous activity. Administering a booster injection four weeks before breeding appeared to delay the onset of behavioral oestrus, which is associated with the onset of puberty. In this study, the onset of oestrus was measured using a teaser ram with a mating harness. Marks on the rump of ewe lambs were used to indicate if a ewe lamb had been mounted by a teaser ram. This method is, however, not as effective as other methods because a teaser ram can sometimes miss a ewe lamb showing oestrous behavior or a ewe lamb may undergo silent heat. Attainment of puberty can be determined by measuring the levels of progesterone or LH (Keisler et al., 1985). Ewe lambs are deemed to have attained puberty if they have elevated LH or progesterone concentrations (Keisler et al., 1985, Kenyon et al., 2012). In the current study, the teaser ram method was chosen because it was a practical on-farm method compared with measuring hormone levels.

The onset of behavioral oestrus can also be influenced by levels of antibody titers. High antibody titers can cause a temporary anoestrus in mature ewes (Boland et al., 1986). In the current study, antibody titers were not measured after immunization with Androvax®. It could be worthwhile to determine antibody levels in order to explain differences in the onset of estrous behavior and fecundity rates between the dosing regimens (A10 – 6 and A8 - 4).

The increased fecundity rate of immunized ewes in this study was likely to be associated with an increase in ovulation rate. There was, however, a variation in fecundity rates between the two dosing regimens (A10 – 6 and A8 - 4), therefore, it was not possible to define if differences in fecundity rates between immunized ewes were due to differences in ovulation rate or a partial failure of multiple ovulation or embryo loss as ovulation rates were not determined. Ovulation rate is quantified by counting the number of corpora lutea present after a reproductive cycle (Forcada et al., 1992). This method requires a surgical procedure; therefore, it can cause distress to the ewe lambs and it is not practical on farm. Moreover, some ewe lambs that were confirmed pregnant, or those with twin fetuses, were diagnosed with one lamb, and or no lambing records at lambing, which might be due to embryo loss or poor recording techniques. Further investigations of the effect of immunization on ovulation rate and reproductive wastage are, therefore, required.

During weighing, live weight for some ewes and their lambs was not recorded due to technical problems. To prevent this, the operators should be careful when making records and ensure that all ewes are weighed.

Chapter 6 : References

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