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Effects of Pre-Breeding Management on Ewe Lamb Fertility

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Effects of pre-breeding management on ewe lamb fertility

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**Thesis submitted to the Davis College of Agriculture, Natural Resources and Design
at West Virginia University in partial fulfillment of the requirements for the degree
of**

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Morgantown, West Virginia

2018

**Keywords: Ewe Lamb, Puberty, Feed Supplementation, Weight Change, Breeding Weight,
Average Daily Gain, Fertility**

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Abstract

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Sarah Nancy Carr

Ewe lambs comprise 30% of the breeding flock; however, fertility is 20-30% lower than that of an adult ewe. Lower fertility has been correlated with lower ADG, liveweight, and body composition prior to breeding and research suggests that it can be improved with proper nutritional management. The objective of this research was to evaluate the effects of pre-breeding management practices on ewe lamb fertility.

In replicate 1, 313 Dorset X Texel (DT) and 74 Katahdin (KT) ewe lambs were assigned to a high (0.68 kg/head/day) or low (0.23 kg/head/day) grain supplementation for two months prior to breeding. Liveweights were recorded bi-weekly to calculate ADG and lifetime weight day averages (LWDA) and blood serum was collected by-weekly to determine estrous cyclicity. Half of each treatment group received progesterone pre-treatment prior to ram introduction. Estrous response was observed 96 hours and pregnancy diagnosis was performed 30 days and 60 days after synchronization. Replicate 2 consisted of 68 DT ewe lambs supplemented high (0.91 kg/head/day) or low (0.45 kg/head/day) grain supplementation for four weeks prior to synchronization. All other aspects of the experimental design remained the same as replicate 1. Data from DT ewe lambs in replicates 1 and 2 were pooled for statistical analysis to determine the main effects of nutritional treatment, progesterone pretreatment, breeding weight category, average daily gain category, weight day average category, and each respective interaction. In all replicates, an ANCOVA with breeding age as the covariate was utilized to determine the main effects and interactions, as well as if ADG, breeding weight, and LWDA differed in ewe lambs experiencing a positive or negative reproductive outcome.

There was no significant effect of nutritional treatment or progesterone pretreatment on overall fertility in any replicate. In replicate 1, Higher lambing rates were observed in ewe lambs in the H than in the L ADG category ($P=0.01$). Ewe lambs that were heavier at breeding and had a higher LWDA had greater reproductive outcomes. In replicate 2, ewe lambs in the M but not H ADG category had or tended to have higher conception rate ($P=0.06$), pregnancy rate to the first service ($P=0.02$), proportion lambing ($P=0.03$) and lambing rate ($P=0.06$) than L ADG animal lambs. Reproductive outcomes increased with increasing LWDA for most response variables. In the pooled replicate, most reproductive performance variables were higher ($P<0.01$) in the H compared to the L ADG category. Ewe lambs in the H breeding weight category had higher ($P<0.01$) reproductive responses than ewe lambs in the L and M with ewe lambs in the M category having intermediate values. Ewe lambs in the H and M categories lambed for the first time at a younger age than ewes in the L category ($P<0.01$). Reproductive response variables increased with increasing weight day averages in most response variables, and the age at first lambing was lower in ewe lambs in the H compared to the L LWDA category ($P<0.001$). Breeding weights, ADG, and LWDA were significantly higher ($P<0.05$) in ewe lambs with a positive compared to a negative reproductive outcome for estrous response, conception rate, pregnancy to the first service, overall pregnancy rate, proportion lambing, and lambing to the first service period. Average daily gains (169 ± 44 v 114 ± 12 v 85 ± 5 g), breeding weights (61 ± 5 v 51 ± 1 v 44 ± 1 kg), and LWDA

(227 ± 18 v 189 ± 5 v 167 ± 2 g) were significantly higher ($P < 0.05$) in ewe lambs having triplets and twins compared to singletons.

Increasing the level of nutritional supplementation, ADG, and LWDA can impact fertility, and sufficiently supplementing ewe lambs may allow an advanced estrous response and pregnancy to first service without relying on progesterone pretreatment.

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3.0 Review of Literature

3.1 Introduction

Replacement ewe lambs can compromise up to 20-30% of the breeding animals in sheep production providing an avenue to increase productivity within the flock; however, traditional breeding practices and decreased fertility hinder breeding replacement ewes to lamb at one year of age. Common practice is to breed replacement females as yearlings (16-20 months) allowing them to lamb initially at about two years of age. This practice is done to ensure that ewe lambs would achieve adequate weight and reproductive viability prior to breeding. However, breeding replacement females as ewe lambs (7-9 months) to lamb at one year of age can increase lifetime productivity while decreasing maintenance costs as a result of entering production earlier (Hulet et al, 1969; Jurgens et al, 2012; Kenyon et al, 2014). Additionally, reducing age at first lambing decreases generation interval facilitating faster genetic gains within the flock (Jurgens et al, 2012).

Ovulation, conception and fertility rates in ewe lambs are lower than mature ewes (Beck et al, 1996; Quirke and Hanrahan, 1977), and between 20-40% of ewe lambs exposed to rams do not lamb (Edey et al, 1978). This is likely the result of a short breeding period in ewe lambs. Kenyon et al (2014) noted that after reaching puberty, ewe lambs only experienced 2-3 estrous cycles before entering the anestrous period (Kenyon et al, 2014). Therefore, the pubertal transition is a limiting factor in breeding ewe lambs (Joubert, 1963).

Puberty is not solely a function of chronological age (Gaskins et al, 2005; Rosales Nieto et al, 2013a), but is characterized by the interaction between age, weight, and photoperiod (Foster et al, 1985). Once an animal grows past the inflection point on the growth curve, they will begin to deposit the excess energy as fat which is permissible for the pubertal transition. Average daily gains, breeding weight, body fat composition, and reproductive performance are positively

correlated with reproductive outcomes (Rosales Nieto et al, 2013a). Regardless of breed, heavier females exhibit a higher probability of displaying estrous, becoming pregnant, pregnancy retention, and prolificacy (Corner-Thomas et al, 2015; Kenyon et al, 2006; Kenyon et al, 2009; Kenyon et al, 2010; Rosales Nieto et al, 2013b; Rosales Nieto et al, 2013a). Additionally, as noted by Corner-Thomas et al (2015) and Kenyon et al (2009), an increase in body fat composition increased overall fertility and lifetime productivity.

3.2 Defining Puberty

Puberty is the process of becoming mature enough to successfully reproduce (Bowstead, 1930). In females, this can be defined by three criteria: the age at first estrus, the age at first ovulation, and the age at which a female can competently support pregnancy. Outward signs of sexual receptivity define a female's age at first estrus, but the first estrus does not necessarily mark the time point in which puberty occurs but rather that puberty has been previously initiated. However, the male is necessary for the detection of estrus (Fabre-Nys and Gelez 2007; Fabre-Nys and Martin 1991a). First ovulation in ewe lambs does not coincide with visible estrus (Wettman 1980).

Therefore, tracking an animal's first ovulation is a better predictor of the onset of puberty but doing so requires frequent mapping of follicular growth to determine the exact timing of ovulation. Perhaps the most practical criterion of the occurrence of puberty is whether or not the female can successfully carry, deliver, and rear a neonate (Foster et al, 1985).

From an endocrine perspective, the peripubertal increase in GnRH secretion is permissive of ovulation and estrous cyclicity, in addition to the tonic center's basal level of low-amplitude low frequency secretions. The female's hypothalamic surge center, or medial preoptic area (POA), secretes gonadotropin releasing hormone (GnRH) more frequently and in greater amounts. Enhanced secretion of GnRH stimulates increased secretion of gonadotropins luteinizing hormone

(LH) and follicular stimulating hormone (FSH), which stimulates follicular development and secretion of estrogen resulting in ovulation (Foster et al, 1979; Foster et al, 1986; Ramirez and McCann, 1963).

Various factors such as age, environmental and social cues, genetics and nutrition influence the onset of puberty in ewe lambs. In conventional production systems, puberty is initiated during the fall when photoperiod is shortened and the ewe lamb has reached a threshold age and weight (Ebling and Foster, 1988). Most ewe lambs undergo puberty around 7 months of age once obtaining 40-60% of their mature weight (Kenyon et al, 2014). If a ewe lamb does not reach puberty during the first breeding season, pubertal transition will be halted until the subsequent year (Foster et al, 1985; Foster et al 1986). Because sheep are short day seasonal breeders, estrous cyclicity is only initiated during times of decreasing photoperiod and temperatures (Ebling and Foster 1988).

3.3 Endocrine Mechanisms of Puberty

The ability to successfully reproduce is modulated by endocrine activity from the hypothalamus, anterior pituitary, ovaries, and uterus. Understanding the timing of endocrine activity associated with puberty is critical for developing synchronization protocols and efficiently managing reproduction. This can potentially decrease the generation interval for breeding animals and increase overall productivity.

3.3.1 Timing

The hypothalamus-pituitary-gonadal components that are necessary for puberty and cyclicity are set within a few weeks after birth (Fitzgerald and Butler, 1982; Foster et al, 1975; Huffman et al, 1987; Kennedy et al, 1974; Manning et al, 1993; Mansour, 1959). Prior to puberty, the hypothalamus exhibits an increased hyperresponsiveness to estradiol resulting in inhibition of

GnRH and subsequent gonadotropin secretion and ovulation (Foster et al, 1986). Low doses of exogenous estradiol (<1 pg/ml) have been shown to reduce LH pulses by 50% within 24 hours after initial implant in an ovariectomized ewe lamb, while 1-2 pg/ml of exogenous estradiol will completely suppress LH pulses (Friedman et al, 1992).

As the developing female approaches puberty, the hypothalamic sensitivity to negative feedback by estradiol decreases, allowing for a steady increase in the amount of GnRH secreted (Foster et al, 1986), and an increase in the frequency of LH secretion by the anterior pituitary. In contrast to the pubertal ewe lamb, LH pulse frequency is much lower in the prepubertal ewe (Foster et al, 1979). Pulsatile secretions of LH began at about 11 weeks of age and puberty was reached at about 35 weeks of age in Shropshire and crossbred ewe lambs (Foster et al, 1975). In the same ewe lambs, systemic concentrations of FSH increased from 3-11 weeks, but did not change between 11 and 35 weeks (Foster et al, 1975). Foster et al (1986) used prepubertal ovariectomized ewe lambs chronically treated with estradiol to demonstrate the decreased sensitivity to estradiol inhibition. Prior to puberty, estradiol inhibited the release of LH via GnRH. However, around the typical age of puberty (~30 weeks), the same level of estradiol was unable to suppress LH secretion (Foster et al, 1986). Additionally, ewe lambs ovariectomized at three weeks of age demonstrated frequent LH secretion by six weeks of age further confirming estradiol as the factor inhibiting LH secretion during the prepubertal period (Foster et al, 1988).

However, it is important to note that estradiol does not act on GnRH neurons directly. Herbison et al (1993) showed that GnRH neurons do not contain estrogen receptor alpha (ER α). More recently, Nestor et al (2012) demonstrated that estradiol acts directly on kisspeptin/ neurokinin B/ dynorphin (KNDy) neurons which communicate directly with GnRH neurons through close synapses. It has been shown that exogenous kisspeptin stimulates LH release in prepubertal females (Navarro et al

2004; Kadokawa et al 2008; Lents et al 2008). Nestor et al (2012) further demonstrated that the number of kisspeptin-positive cells increase after puberty compared to the prepubertal period, and increase after ovariectomy in prepubertal ewe lambs providing evidence of the role of kisspeptin and neurokinin B during puberty in female sheep. Similarly, Redmond et al (2011a, 2011b) demonstrated that kisspeptin assumes a significant role in activation of the hypothalamus-pituitary-gonadal axis in prepubertal ewe lambs. Kisspeptin stimulates LH release by acting through the hypophyseal portal system, and kisspeptin positively correlates with both age and LH secretion; whereas, the number of kisspeptin-positive cells increase with age (Redmond et al, 2011a; Redmond et al, 2011b).

Leptin is thought to play a permissive role in the attainment of puberty (Foster and Nagatani, 1999). The amount of leptin in blood is directly related to the amount of adipose tissue in the body (Ahren et al, 1997), and systemic concentrations of leptin increase with growth in prepubertal animals (Rosales Nieto et al, 2014). In the hypothalamus, leptin receptors have been localized in the supraoptic nucleus (SON), paraventricular nucleus (PVN), periventricular nucleus, arcuate nucleus, and lateral hypothalamus (Hakansson et al, 1998). However, GnRH neurons do not contain leptin receptors (Quennel et al, 2009). Rather, kisspeptin and the neuropeptide Y facilitate the communication between leptin and GnRH neurons. Kisspeptin neurons contain leptin receptors, and leptin treatment will increase the expression of *Kiss1* mRNA (Backholer et al, 2010; Luque et al, 2007). Additionally, hypothalamic neuropeptide Y exhibits an inhibitory effect on GnRH activity prior to sexual maturation (Klenke et al, 2010; Li et al, 1999), which decreases during the pubertal transition (Tillet et al, 2010). Furthermore, this was confirmed by delayed puberty onset when Neuropeptide Y was administered (Pierroz et al, 1995). In heifers, increased liveweight prior to puberty was associated with a decrease in neuropeptide Y expression (Allen et al, 2012; Alves et

al, 2015), which has been shown to be associated with increasing levels of systemic leptin (Alves et al, 2015; Cardoso et al, 2014). Collectively, these data suggest that leptin is permissive to the pubertal transition by indicating adequate body weight and condition have been achieved.

3.3.2 Hypothalamus

The endocrine basis for the attainment of puberty is the ability of the hypothalamus to secrete high amplitude and frequent pulses of GnRH that trigger the preovulatory surge of LH and FSH. The hypothalamus is the final constituent to develop that regulates the onset of puberty, and subsequently allows for the pituitary, ovaries, and uterus to function at a mature level (Foster et al, 1975; Foster et al, 1986; Huffman et al, 1987; Manning et al, 1993). However, some studies suggest that the hypothalamus of the sheep has the ability to produce high-frequency GnRH pulses, and respond to estradiol stimulation prior to puberty.

The hypothalamus is sexually differentiated during embryogenesis which is necessary preparation for sexual development at puberty; however, the hypothalamus of both males and female embryos are similar in that they possess a tonic center and a surge center. In utero, testosterone production by the developing gonads passes through the blood brain barrier and is converted to estradiol via aromatase defeminizing the hypothalamus and rendering the surge center inactive in the male. In the female, estradiol secreted by the ovary is bound to alpha-fetoprotein and cannot pass the blood brain barrier allowing the surge center to remain functional (Bakker et al, 2006). The hypothalamic surge center must be fully developed before puberty because the surge center triggers the preovulatory surge of GnRH, which stimulates LH and FSH. The pattern of LH directly mimics that of GnRH secretion (Manning et al, 1993).

3.3.3 Pituitary Gland

The pituitary gland is developed and functional prior to puberty and does not impact the timing of sexual maturation. Luteinizing hormone (LH) and follicular stimulating hormone (FSH) are

present in various amounts preceding the pubertal period, although LH frequency is mostly low and variable (Bindon and Turner, 1974; Echternkamp and Laster, 1976; Foster et al, 1975; Foster et al, 1975b; Huffman et al, 1987). During the week prior to first ovulation in the lamb, Huffman et al (1987) reported a fourfold increase in threshold LH (Huffman et al, 1987). Furthermore, LH amplitude increased with age and was great enough to produce ovulation by 12-20 weeks of age (Manning et al, 1993).

The role of FSH in the pubertal transition is poorly defined; however, by 10-12 weeks of age, systemic FSH levels in the ewe have risen to amounts comparable to mature ewes (Fitzgerald and Butler, 1982; Foster et al, 1975). This evidence indicates that the pubertal transition is limited at the level of the hypothalamus and secretion of adequate amounts and frequency of GnRH.

3.3.4 Ovary

3.3.4.1 *Prepubertal ovarian function*

Germ cells develop during embryogenesis and follicles are present prior to birth; however, they are not responsive to exogenous gonadotropins until 2-4 weeks of age once the granulosa and thecal layers are developed (Kennedy et al, 1974; Mansour, 1959). By 5-6 weeks of age, the ovary becomes responsive to exogenous gonadotropins stimulating ovulation and formation of the corpus luteum (Worthington and Kennedy, 1979). The exogenous gonadotropins pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG), have been shown to stimulate follicular growth and development which results in an increase in systemic estradiol activating the gonadotropin surge mechanism (Foster and Ryan, 1981).

In sexually immature sheep, administration of estradiol can induce an LH surge (Foster, 1984; Foster and Karsch, 1975; Foster and Olster, 1985; Keisler et al, 1985; Land et al, 1970; Squires et al, 1972;; Tran et al, 1979) by stimulating the release of GnRH to act on the anterior pituitary;

however, developing sheep will return to an anovulatory state. Even though exogenous stimulation can induce ovulation and the formation of the CL, puberty and ovarian cyclicity will not be advanced. Prepubertal sheep did not exhibit multiple ovulations after the regression of the CL because the LH pulse frequency was not sustained (Foster et al 1984; Keisler et al, 1985).

3.3.4.2 Progesterone priming

Following initial ovulation, secretion of progesterone from the corpus luteum (CL) moderates the timing of the estrous cycle through controlling prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). In pubertal and seasonally anestrous females, the first ovulation occurs without estrus and is followed by a shortened luteal phase before initiating or resuming normal cyclicity. After ovulation and formation of the CL in females that exhibited a short luteal phase, a premature surge of $PGF_{2\alpha}$ lysed the CL in sheep, goats, and cattle (Menchaca and Rubianes, 2001; Zollers et al, 1993), which is directly related to the number of progesterone receptors in the uterus. In cows, Zollers et al (1993) demonstrated that the endometrium of the uterus of a female experiencing a short cycle possessed significantly less progesterone receptors than a progesterone pretreated female with a normal cycle. In sheep, preovulatory estradiol from growing follicles induced the synthesis of progesterone receptors, and the amount of estradiol secreted was directly related to the number of progesterone receptors on the uterine endometrium (Xiao and Goff, 1999; Zelinski et al, 1980).

LH pulses remain low before the initial ovulation in peripubertal or anovulatory females. In turn, low levels of estradiol are secreted, and the synthesis of endometrial progesterone receptors is decreased. Additionally, up-regulation of the progesterone receptors and the delayed secretion of $PGF_{2\alpha}$ require the exposure of the uterus to progesterone and the subsequent preovulatory rise in estrogen (Kieborz-Loos et al, 2003).

In beef cattle, pretreatment with progestogen resulted in a CL with a normal lifespan (Ramirez-Godinez et al, 1981; Ramirez-Godinez et al, 1982), which increased fertility in postpartum cows by preventing the shortened luteal phase (Ramirez-Godinez et al, 1981). Further research indicates that levels of $\text{PGF}_{2\alpha}$ from the uterus increase steadily during the time of progesterone pretreatment (Cooper et al, 1991); however, progesterone pretreatment is necessary to prevent premature secretion of $\text{PGF}_{2\alpha}$, surpassing the shortened luteal phase. Ovulation followed by the shortened luteal phase prevents the occurrence of a fertile mating during the transition from seasonal anestrus or the pubertal transition.

3.4 Factors Affecting Puberty

Various factors affect the attainment of puberty and the fertility of ewe lambs. Such factors include age, seasonality, nutrition, liveweight, body composition, presence of a male and teasing.

3.4.1 Age

A threshold age must be obtained for females to initiate the pubertal transition; however, chronological age cannot be determined as a function of puberty due to the confounded effects of age, weight, and body composition (Corner-Thomas et al, 2015; Kenyon et al, 2009; Kenyon et al, 2010; Villa-Godoy et al, 1990).

Levine et al (1978) reported that animals bred within their first year of life have an increase in productivity within their initial three years of production. In contrast, Saoud and Hohenboken (1984) reported is no correlation between age at first estrus and reproductive performance within the first 4-5 years of production when the ewe lambs were bred to lamb at about one year of age. Although reports are conflicting, it is logical that breeding replacement females as ewe lambs rather than yearlings will increase their overall productivity regardless of age when bred during the first breeding season.

3.4.2 Seasonality

Sheep are short-day seasonal breeders, meaning that the onset of estrus is stimulated in the fall when photoperiod and temperature decrease (Ebling and Foster 1988). Seasonal polyestrous cycles of sheep occur in autumn and significant variation in length of estrous activity exists among breeds (Foster et al, 1986). Merino breeds have estrous cycles for 200-260 days while black face breeds have estrous cycles for 100-140 days, before anestrus during the spring and summer months (Foster et al, 1986). This allows sheep to undergo parturition in the spring when conditions are ideal for supporting lactation and neonatal growth.

Melatonin from the pineal gland stimulates the hypothalamus via optic stimuli traveling through the central nervous system. During autumn when sheep perceive a decrease in photoperiod, melatonin secretion increases (Yellon and Foster, 1986) down regulating the inhibitory action of RFRP-3 on kisspeptin neurons (Clarke et al, 2008; Smith et al, 2008), and allowing an increase in GnRH pulse frequency and amplitude which stimulates the LH surge and ovulation.

Given adequate nutrition, lambs born in spring will reach puberty in the subsequent fall. In contrast, fall-born ewe lambs will not reach puberty until 10-12 months of age in the next fall (Foster et al, 1986). Irrespective of photoperiod, ewe lambs that have not reached adequate body weight and composition will not undergo the pubertal transition as the development of hypothalamic neurons and their ability to positively respond to stimuli can be impacted by threshold body size and fat composition.

Menassol et al (2012) investigated the interaction between photoperiod and nutrition as it relates to seasonality in the ewe and reported that nutritional status modulates the interpretation of photoperiod stimulating reproduction. Ile-de-France ewes were maintained as well-fed or feed restricted for two complete years. In the second year, half of each group were ovariectomized and fitted with an estrogen implant. Well-fed females had an increase in the duration of ovarian activity (Year 1: 133 ± 5 v 63 ± 12 and Year 2: 176 ± 13 v 81 ± 21 days) due to the fact that restricted females experienced the onset of estrus later and estrous cycles ceased earlier in the breeding season. The time at which ovarian activity was initiated was negatively correlated with body condition (Year 1: $r = -0.69$ and Year 2: $r = -0.73$); whereas, ovarian activity started earlier with each incremental increase in body condition. Additionally, the end timing of estrous activity was positively correlated with body condition (Year 1: $r = 0.42$, and Year 2: $r = 0.74$). With each subsequent increase in body condition score, the timing of transition into the anestrous period was

delayed. During breeding season, serum concentrations of LH remain high (>1 ng/ml) for longer periods in well fed OVX ewes (188 ± 10 v 132 ± 3 days), and the same correlations with BCS were observed with LH for the beginning ($r = -0.62$) and end ($r = 0.71$) of the neuroendocrine activity (Menassol et al, 2012). Hence, adequate nutritional management is necessary to maximize the length of the breeding season in seasonally polyestrous breeders.

3.4.3 Nutrition

Nutrition has the greatest potential to affect puberty onset and subsequent productivity of ewe lambs. The Critical Body Weight Hypothesis describes the phenomenon that heavier females reach puberty at a younger age (Frisch and McArthur, 1974); however, body weight alone is not the best predictor of puberty. Body condition and fat composition more adequately relate to the process of becoming sexually mature.

Nutritional management affects daily gains and has the potential to advance the onset of puberty (Villa-Godoy et al, 1990). In a situation with limited nutritional availability, nonessential processes such as reproduction will cease. With regards to nulliparous animals in a limited nutrition environment, reproductive function would not be initiated due to available nutrients being repartitioned from fat storage and reproduction to supporting physiological processes required for maintenance (Bizelis et al, 1990; Boulanouar et al, 1995; Burfening and Bererdinelli, 1986). Regardless of species, the goal of nutritional management of replacement females is to not delay puberty or reproductive maturity, to reach adequate live weight and composition at breeding, and to maintain acceptable growth during gestation to result in easy parturition (Bearden et al, 2004).

Conventionally, it is recommended to feed ewe lambs 1-1.5 pounds (0.45-0.68 kg) per day of grain plus pasture or hay through the natural breeding season of fall and winter to ensure nutrient demands are met (Jurgens et al, 2012). Replacement ewe lambs are typically fed a lamb finishing

diet until 90-120 days of age (ranging between 36-41 kg; Jurgens et al, 2012). In heifers, feeding a high energy diet (liveweight gain 0.9 kg/day) compared to a low energy diet (liveweight gain of 0.3 kg/day) from 8 months of age allows the female to obtain puberty at an earlier age and have a preovulatory follicle (13mm) present at a younger age (372 ± 0.7 v 435 ± 0.9 days; Bergfeld et al, 1994). A high energy diet also increased the frequency of LH pulses to simulate follicular growth and ovulation at an earlier age. Whereas, the LH pulses are not stimulated to the same degree in the heifers supplemented with a lower energy diet, thus resulting in delayed puberty (Day et al, 1986).

An elevated post-weaning plane of nutrition has been shown to increase average daily gains (ADG), breeding weight, ovulation rate, estrous response, prolificacy, and overall fertility compared to ewe lambs on a restricted diet (Bizelis et al, 1990; Burfening and Berardinelli, 1986). Bizelis et al (1990) split Chios and Karagouniki ewe lambs (85 ± 1 day of age) into two treatment groups: high versus low plane of nutrition. The high plane of nutrition group was supplemented feed to achieve weight gain of 100g/day and to reach 70% mature weight at breeding. The low plane of nutrition group was supplemented to gain 50 g/day and reach 50% mature weight at breeding. The high treatment group grew more rapidly and were 12.5 kg heavier at breeding. In addition, the females in the high plane of nutrition treatment group had a significantly increased ovulation rate (1.51 ± 0.08 v 1.15 ± 0.08), and tended to display estrus at a younger age (322 v 327 days). In a subsequent breeding season, carryover effects related to ovulation rates were observed where the high treatment group maintained a higher ovulation rate during the second year (1.94 ± 0.10 v 1.72 ± 0.11 ; Bizelis et al, 1990). Additionally, Burfening and Berardinelli (1986) fed ewe lambs (5-6 months of age) either a high energy or a low energy diet for three months prior to

breeding and reported that a greater percentage of ewe lambs on the high energy diet lambed (23.4 ± 2.3 v 17.1 ± 2.4).

Restricting dietary protein or energy delays the onset of puberty but not the weight at which puberty is initiated. Ewe lambs averaging 22.1 ± 1.9 kg and 106 ± 1.3 days in experiment one and 35.5 ± 0.6 kg and 131 ± 1.5 days were assigned to be fed a control (18 MJ ME day⁻¹ and 173 g MP day⁻¹), restricted protein (PR: 11 MJ ME day⁻¹ and 66 g MP day⁻¹), or restricted energy diet (ER: 10.2 MJ ME day⁻¹ and 96 g MP day⁻¹). Ewe lambs fed the control diet gained more weight (Experiment 1: 186 ± 6 v 107 ± 6 v 104 ± 6 g/day; Experiment 2: 197 ± 10 v 81 ± 10 v 62 ± 10), were heavier at puberty (Experiment 1: 35.3 ± 1 v 31.5 ± 1 v 30.3 ± 1 kg; Experiment 2: 46.8 ± 1.4 v 44.3 ± 1.4 v 44.1 ± 1.4 kg), and reached puberty at an earlier age (Experiment 1: 169 ± 5.2 v 178 ± 5.2 v 186 ± 5.2 days; Experiment 2: 205 ± 5 v 242 ± 5 v 259 ± 5 days) compared to both the restricted protein and restricted energy dietary treatment groups (Boulanouar et al, 1995). At puberty, restricted groups from experiment 1 were similar in age (186 ± 5.2 v 178 ± 5.2 days). Furthermore, groups that switched from a restricted diet to the control diet showed significant differences in body weight (PR: 33.6 ± 1 v 31.5 ± 1 kg; ER: 32.6 ± 1 v 30.6 ± 1 kg) and weight gained (PR: 177 ± 6 v 104 ± 6 g/day; ER: 155 ± 6 v 107 ± 6 g/day) but did not reach puberty any earlier than lambs fed the restricted diet alone (PR: 179 ± 5.2 v 178 ± 5.2 days; ER: 181 ± 5.2 v 186 ± 5.2 days; Boulanouar et al, 1995). Feeding a diet that meets the energy and protein requirements of replacement ewe lambs is necessary to not delay reproductive maturity. Restricting either dietary protein or energy will delay the onset of puberty to a later age, because it takes longer for the animals to reach the required weight range, composition, amount of adipose tissue, and systemic leptin permissive to the preovulatory surge of gonadotropins.

3.4.4 Liveweight and Body Composition

When ewe lambs fed to achieve a positive energy balance, fat deposition occurs enabling the initiation of puberty. Given that she has reached the threshold age, excess internal energy gets converted into fat stores and the animal begins to prioritize reproduction. Feed restriction can suppress the estrous cycle and reproductive behavior when fat deposits are not available to cover demands (Bronson, 2000; Schneider and Wade, 2000). There is an extended response to negative feedback of estrogen in ewe lambs that lack sufficient dietary energy in the prepubertal period (Foster et al, 1986). In this case, there is not enough blood glucose and fatty acids, nor enough adipose tissue to release leptin to permit pubertal onset. Serum leptin is positively correlated with the amount of body fat stores and is key in signaling the hypothalamus to initiate puberty. Increasing amounts of circulating leptin is permissive of puberty. In mice, exogenous leptin can overcome the delay of puberty observed in feed restricted animals that have not had adequate nutrition to achieve a positive energy balance (Cheung et al, 1997). Additionally, exogenous leptin has been shown to be a trigger puberty in leptin deficient (ob/ob) mice (Chehab et al, 1997), and systemic concentrations of leptin increase with growth as premature animals approach puberty (Foster and Nagatani, 1999; Rosales Nieto et al, 2014). Cardoso et al (2014) showed the relationship between increased body weight gain and puberty in Angus X heifers as it relates to changes in systemic leptin. At 3.5 months of age, heifers were weaned and allocated to either the high gain (1.0 kg/day) or low gain (0.5 kg/day) treatment groups. During the treatment period, blood was collected twice a month to determine serum leptin concentrations and starting at 7.5 months, blood samples were collected two times each week for progesterone quantification. At 8 months of age, LH pulse frequency was greater in the high gain versus the low gain group, and at nine months of age, the proportion of pubertal heifers was greater in the high gain group. Serum concentrations of leptin were greater in the high treatment group at six months of age and remained

higher for the entirety of the experiment (Cardoso et al, 2014). Next, Samadi et al (2014) studied the effects of supplementing an increased plane of nutrition compared to a moderate plane on nutrition on body weight, body condition, and circulating concentrations of leptin in heifers. Leptin was elevated from 11-23 months of age in the increased nutritional treatment group compared to the moderate nutritional group (0.98 ± 0.03 v 0.75 ± 0.02 ng/ml), and this was coupled with heavier body weights and increased body condition scores during this time period.

In addition to leptin, follistatin is thought to be related to body composition and reproduction which is an indicator of the level of muscling. Follistatin is secreted by muscle cells in addition to a variety of other cells and is important for growth and development. Unlike leptin, follistatin appears to have no effect on GnRH secretion in sheep (Padmanabhan et al, 2002), though it is known to act on the pituitary to inhibit FSH secretion in rodents (Ueno et al, 1987). Additionally, follistatin acts on ovarian function in mice. Deletion of follistatin from granulosa cells decrease fertility, decreases litter size, and terminates ovarian activity in mice (Jorgez et al, 2004; Kimura et al, 2010). Rosales Nieto et al (2014) studied the relationships of serum leptin and follistatin to fat and muscle accumulation in pubertal sheep. Circulating leptin is positively correlated with phenotypic values for eye muscle depth, fat, liveweight, post-weaning eye muscle depth, post-weaning fat, and post-weaning liveweight. Follistatin was negatively correlated with eye muscle depth and live weight, post-weaning eye muscle depth, and fat. Furthermore, leptin was positively associated with liveweight at first estrus, but negatively associated with age. Leptin was positively associated with fertility and reproductive rate. However, follistatin was negatively correlated to liveweight at first estrus, fertility, and reproductive rate suggesting an inhibitory role in the pubertal transition (Rosales Nieto et al, 2014).

Regardless of breed, heavier females have a higher probability of becoming pregnant, retaining their pregnancy, and having multiple births (Kenyon et al, 2006; Rosales Nieto et al, 2013b). Research by Rosales Nieto et al (2013b) reported on the positive correlation between average daily gain, breeding weight, body composition, and reproductive performance in Merino ewe lambs. Ewe lambs were more likely to achieve puberty by 251 days if they had higher values for post weaning liveweight, eye muscle depth, and fat. For this study, the average age at first estrus was 197 ± 2 days, and the average weight was 41.1 ± 0.4 kg (67% of mature weight for the Merino breed: 61.6 ± 0.4 kg). Additionally, the proportion of ewe lambs that exhibited an estrous response increased by 4% with each 20g increase in ADG (Rosales Nieto et al, 2013b). The research of Kenyon et al (2006) support these findings showing that heavier (42.1 v 40.8 kg) ewe lambs are more likely to be mated in the first 17 days after ram introduction (39.0 v 37.0 kg), and more likely to be twin-bearing rather than singleton (39.8 v 38.0 kg; Kenyon et al, 2006). Hence, it is imperative to have a proper nutritional plan to get the replacement ewe lambs to an optimal weight before breeding season.

In dairy heifers, the interaction between age and weight relative to puberty were investigated. Heifers fed a high plane of nutrition (ADG=2.0 lbs/day) for one year reached puberty earlier (6-8 months) than those supplemented a moderate or low plane of nutrition. The moderate nutritional plane (ADG=1.5 lbs) displayed estrus between 9 and 11 months while the restricted feed group (ADG=1.2 lbs) reached puberty by 12 months; however, their size was too small to undergo pregnancy and parturition without complication (Villa-Godoy et al, 1990).

Nutrition is the producer's greatest control over the generation interval of the flock (Bearden et al, 2004). Plane of nutrition is directly correlated with body composition in terms of muscling and adiposity. In turn, ewe lambs with a greater body composition have an increase in fertility and

lifetime productivity. Ewe lambs marked in the first 17 days of breeding were heavier at breeding and had a greater BCS (42 ± 0.20 v 39.9 ± 0.44 and 38.7 ± 0.68 kg; 2.4 ± 0.02 v 2.3 ± 0.05 and 2.2 ± 0.7) than ewe lambs marked in the second 17 days of the breeding period or not marked during 34 day breeding period, respectively (Kenyon et al, 2009). In a subsequent study, Kenyon et al (2010) observed the same estrous response; whereas, heavier ewe lambs displayed estrous earlier than lighter weight ewe lambs. Additional findings prove that ewe lambs with a BCS of 2.5 or greater were more likely to be bred in the first 17 days of the breeding period compared to ewe lambs with a body condition of 2.0 or 1.5. Throughout the 34 day breeding period, as body condition score increased, there was an increase in pregnancy rates (BCS 1.5=63.7% v BCS 2.0=76% v BCS 2.5=83%; Kenyon et al, 2010).

Consequently, there is a threshold weight and BCS in which achieving greater gains and condition prior to breeding will not have increasing effects. Corner-Thomas et al (2015) analyzed the relationships between pre-breeding liveweights and BCS on fertility and reproductive rates in crossbred ewe lambs consisting of Romney crossed with Texel, Finnish Landrace, and Coopworth. Fertility was determined as the number of ewe lambs that were pregnant and reproductive rates were determined as the number of fetuses per 100 ewes that were bred. The results showed that fertility rates (conception rates) increased synonymously with liveweights until 47.4 kg of weight, and reproductive rates were highest between 47.5 and 52.2 kg (138%). Additionally, 90% fertility was achieved at the threshold BCS of 3.5, and reproductive rates peaked at 130% with a body condition of 3.0. Given the previous, an increased pre-breeding plane of nutrition increases reproductive outcomes in ewe lambs due to increasing live weights and body composition that are permissive for puberty and fertility responses.

3.4.5 Exogenous Progesterone

Conventionally, exogenous natural progesterone mimics that of the luteal phase of the estrous cycle (Abecia et al, 2012), allowing for the manipulation of the circulating progesterone levels to time the onset of estrus and ovulation (Hansel and Convey, 1983). During the breeding season, removal of exogenous progesterone initiates a cascade of increasing GnRH to increase gonadotropin release. Release of gonadotropins stimulates follicles to grow and release increasing amounts of estrogen to trigger the preovulatory surge of GnRH and LH that stimulate ovulation (Barrell et al, 1992; Goodman, 1994).

Pretreating with progesterone increased the sensitivity of the hypothalamus to estradiol (Fabre-Nys and Martin, 1991b; Robinson, 1955), and prevented the short cycle after ram introduction (Congnie et al, 1982; McLeod and Haresign, 1984; Pearce and Robinson, 1985). Protocols utilizing progesterone during breeding have shown to increase pregnancy rates to the first service by 30% in pubertal ewe lambs (Sawalhah et al, 2011; Stellflug et al, 2001) and have the ability to hasten the onset of puberty in heifers (Hall et al, 1997). Crossbred beef heifers were split into three different age classifications (9.5, 11, and 12.5 months) and half of each group were synchronized using a 10 day implant of Norgestomet (6mg; synthetic progesterone homologue). In the 12.5 month age classification, more progestin pretreated heifers displayed pubertal estrus in the first 5 days following removal compared to the control heifers (82 v 9%). This same result was not observed in the 9.5 or 11 month age groups (progestin x age). Additionally, progestin pretreatment increased the LH pulse frequency in all age classifications, and increased the mean LH concentration in the 12.5 group alone (progestin x age). Finally, progestin pretreatment positively influenced follicular characteristics within the ovaries at the time of implant removal; whereas pretreated females increased the number of follicles with a diameter greater than 8 mm, and increased the size of the largest follicle (Hall et al, 1997).

The ability of progesterone priming to promote the pubertal transition has been further demonstrated in sheep. In seasonally anestrous ewe lambs bred in July (251 days), only lambs that received a progesterone pretreatment (used CIDR device) expressed estrus (Knights et al, 2002), consistent with the requirement of a period of progesterone priming for the induction of estrus at ram introduction (Knights et al, 2001).

3.4.6 Presence of a Male and Teasing

The ram effect is driven primarily by pheromones, and studies have shown that these olfactory cues have the ability to regulate puberty (Knights et al, 2002; Kenyon et al, 2012). The odors and socio-sexual behaviors of the ram activates the main olfactory bulb and the accessory olfactory system to communicate via the amygdala and associated cortices. Signals are sent to kisspeptin neurons in the medial preoptic area of the hypothalamus to simulate GnRH secretion. GnRH activates the release of LH from the anterior pituitary (Fabre-Nys et al, 2015).

Introduction of the male advances the onset of puberty (Ungerfeld et al, 2004) by stimulating an increase in mean LH and LH pulse frequency that simulates follicular development and subsequently ovulation (Knights et al, 2002). Knights et al (2002) assigned mixed breed fall-born ewe lambs (41.8 ± 0.6 kg, and 250.7 ± 1.3 days of age) to one of five treatment groups: 1) the control that received no additional treatment, 2) a group to only be introduced to rams, 3) a group to be treated with only progesterone via a CIDR, 4) a group that was both treated with progesterone and exposed to the ram at CIDR removal, and 5) a group that was progesterone pretreated, exposed to the ram at CIDR removal, and received an injection of estradiol benzoate ($25 \mu\text{g E}_2\beta$). More ewe lambs exposed to rams exhibited an LH surge compared to those that did not have ram introduction (control: 0%, trt 2: 100%, trt 3: 0%, trt 4: 72.7%, and trt 5: 100%). Additionally, ewe lambs exposed to rams displayed a higher LH pulse frequency (pulses/8h, 7.7 ± 0.5 v 2.7 ± 0.8) than those

that were not introduced to a ram (Knights et al, 2002). Kenyon et al (2012) demonstrated a similar response in a consecutive two year study. More ewe lambs achieved puberty when exposed to vasectomized rams for 17 days compared to control females not exposed (48 v 24% and 80 v 18%; Kenyon et al, 2012). However, it must be noted that puberty cannot be accelerated via exogenous hormones or the ram effect in animals that have not achieved adequate age, liveweight, or body condition for the hypothalamus to respond positively to estradiol.

The use of a teaser ram also positively enhances fertility response in ewe lambs. The use of a teaser has been shown to advance the number of females mated during the first 17 days of breeding (62.6 v 32.1%) and increase the overall pregnancy rate (88.8 v 82.2%; Kenyon et al, 2006). Additionally, ewe lambs exposed to vasectomized rams exhibited a higher conception rate to the first 17 days (18.2 v 6.8%; 27 v 17%; Cave et al, 2012). Exposure to vasectomized rams through either direct contact or fence line contact during the peripubertal period increased the proportion of ewe lambs that initiated estrous cyclicity prior to breeding compared to ewe lambs that did not have contact with vasectomized rams (40.0 ± 4.8 and 31.7 ± 4.8 v 8.8 ± 4.8 , respectively; Hudgens et al, 1987). The exposure of ewe lambs to rams increased the growth rate of the largest follicle (between 0 and 36 hours), increased the diameter of the largest follicle at 36 hours, and increased ovulation rate compared to control animals, as well as animals treated with progesterone or estrogen (Knights et al, 2002). This increase in follicle diameter has been reported on day 9 (6.9 ± 0.4 v 5.8 ± 0.4 mm) and day 12 (7.1 ± 0.4 v 5.4 ± 0.4 mm) in the Romney breed (Kenyon et al, 2012).

Furthermore, the length of time of teasing has an effect on fertility. In studying the effects of teasing for 17 days or 8 days before breeding, Kenyon et al (2006) found that teasing for 8 days would increase the estrous response (88.6 v 75.1%) but not the pregnancy rates during the first 17 days period (61.4 v 58.0%). However, teasing for 17 days increased both the estrous response

(92.3 v 75.1%) and the pregnancy rate (70.7 v 58.0%) to the first 17 day period when compared to the unteased ewe lambs. Additionally, ewe lambs teased for 17 days are more likely to be twin bearing rather than singleton compared to unteased or ewe lambs teased for 8 days (Kenyon et al, 2006).

3.5 Ewe Lamb Fertility

Overall fertility is significantly reduced in ewe lambs compared to mature ewes. To understand the mechanisms behind this reduced fertility, it is important to first understand fertility in mature animals. As previously discussed, sheep are seasonally polyestrous breeders meaning that they initiate cyclicity in the fall and undergo seasonal anestrus in the spring and summer months. The length of time a female cycles varies by breed. Merino breeds cycle for 200-260 days allowing for 11-15 breeding opportunities during the season. Black face breeds cycle for 100-140 days giving only 6-8 opportunities for successful breeding. Furthermore, ovulation rates vary significantly; whereas, highly prolific breeds such as the Barbados black belly and polypay breeds can ovulate multiple ova during one cycle compared to less prolific females such as the Columbia breed.

Although, heritability of reproductive traits is low (Safari et al, 2007). Heritability for lamb survival and litter size are estimated to be 0.3 0.19, respectively (Afolayan et al, 2008). Additionally, Fogarty et al (1994) heritability of number of lambs born and the number of lambs weaned as 0.17 and 0.08, respectively. Therefore, it is believed that epigenetic factors and management practices modulate the decreased fertility in ewe lambs.

A thorough understanding of gestation in sheep is necessary to understand the mechanisms for decreased fertility in ewe lambs compared to mature ewes. After ovulation, spermatozoa will connect with the ovum in the ampullary-isthmic junction of the oviduct where fertilization will occur. Initially, a single celled zygote with two pronuclei will form, and within 24 hours the cell will begin to undergo division. About day 3-4, the embryo will enter the morula stage where individual cells can no longer be distinguished. Within days 4-10 differentiation will begin and a blastocyst will form with an inner cell mass that will become the fetus, a blastocoele cavity, and trophoblast cells. Additionally, within this time period the blastocyst will hatch out of the zona

pellucida and prepare for implantation. Implantation will occur about day 16. Gestation in sheep lasts 145-150 days.

Post fertilization day 12-15, trophoblast cells from the blastocyst signal the ewe's pregnancy via the action of interferon-tau (INF- τ) inhibiting the release of PGF2 α prolonging the life of the CL (Bazer et al, 1989). This paracrine action is dependent on the presence of progesterone receptors on the uterine endometrium (Ott et al, 1992).

Progesterone is the primary hormone for the maintenance of pregnancy during gestation. Throughout pregnancy, plasma progesterone increases gradually during the first 90 days of gestation and then peaks at day 125 prior to falling before parturition (Bassett et al, 1969); however, the source of progesterone changes during this time period. In the first third of the pregnancy, progesterone is secreted from the CL in high enough quantities to prevent cyclicity and prepare the uterus for implantation. Around day 50, the placenta is secreting progesterone in high enough quantities to maintain pregnancy and the CL regresses (Casida and Warwick, 1945).

3.5.1 Ovulation Rates

Ewe lambs experience a decrease in overall fertility attributed to lower ovulation rates and higher embryonic mortality rates compared to mature ewes. Ewe lambs have a lower ovulation rate compared to mature ewes as demonstrated in Romney and Romney cross (1.14 v 1.82 and 1.44 v 1.84; Mulvaney et al, 2013) Clun forest (1.07 v 1.25; Beck et al, 1996), and Galway (1.51 v 3.07) breeds (Quirke and Hanrahan, 1977). Additionally, lowland Clun Forest ewes experience a subsequent increase in ovulation rate as they increase in age (ewe lambs= 1.15 v yearlings= 1.55 v two year olds= 1.75; Forrest and Bichard, 1974).

When analyzing the ovulation rate within each individual ewe lamb, the ovulation rate does not change between cycles in the first breeding season following puberty. However, there are fewer

viable embryos that result from mating the first cycle versus the second or third (Hare and Bryant, 1985).

3.5.2 Conception and Pregnancy

Pregnancy rates in ewe lambs are lower than yearlings and mature ewes (56 v 93 v 95%) as demonstrated in Clun Forest lowland sheep (Forrest and Bichard, 1974), and a significant decrease in pregnancy rate for ewe lambs compared to mature ewe has been demonstrated in a composite breed ($\frac{1}{2}$ Romney, $\frac{1}{4}$ Texel, $\frac{1}{4}$ Finn; 7 v 86%; Mulvaney et al, 2013).

During the first two months after conception, progesterone is supplied from the corpora lutea (CL; Wilmut et al, 1995), and is essential for maintaining pregnancy. Lower serum progesterone has been observed in ewe lambs compared to mature ewes between 14 and 30 days of gestation (Davies and Beck, 1993) and has been related to increased incidence of embryonic loss. In contrast, administering hCG (100 IU) or progesterone (12 mg) to ewe lambs did not affect blastocyst development (Nephew et al, 1994); however, administering a higher dosage of hCG (150 IU) at mating increased embryo viability in the ewe lamb by increasing the crown-rump length (12.7 mm v 11.9 mm), the amniotic sac width (12.0 mm v 11.4 mm), and the number of placental placentomes (122.4 v 90.8) compared to the control group (Khan et al, 2003). These findings lead to the suggestion that administering hCG at mating can increase embryonic development and overall fertility of ewe lambs. Research demonstrates that injecting 200 IU of hCG 12 days post mating did not improve lambing outcome compared to the control (41 v 48%; Khan et al, 2009). Therefore, the timing and dosage of administration are critical.

Quirke and Hanrahan (1977) observed that Galway ewe lambs have a high ovum cleavage rate (80%) but a low implantation rate (<40%), and ova from ewe lambs have a lower capacity for

survival compared to mature ewes (Quirke and Hanrahan, 1977). In addition, mature oocytes derived from prepubertal Merino ewe lambs (4-6 months) are less likely to reach the blastocyst stage after cleavage when compared to mature ewes (3-5 years, 7.4 v 24.6% respectively) suggesting that mature oocytes from prepubertal females do not possess the same potential for development (O'Brien et al, 1996). Further, fertilized oocytes derived from ewe lambs and mature ewes have similar cleavage rates, but there is a significant difference in the number of cleaved zygotes that develop to the blastocyst stage (mature ewes: 26.8% v lambs: 13.9 and 17.8 %; Kochhar et al, 2002). The embryos of peri-pubertal ewe lambs do not possess the same pre-implantation developmental potential as embryos derived from mature ewes.

3.5.3 Prenatal and Perinatal Mortality

Prenatal mortality rates are elevated in ewe lambs compared to mature ewes due to a decrease in ova viability. Research has shown that there is no difference in embryonic survivability between ewe lambs and mature ewes (79 v 80%) on embryonic day 15, but by embryonic day 30, the amount of ewe lambs containing viable fetuses decreased significantly compared to mature ewes (69 v 88% respectively; Beck et al, 1996). This indicates that there is a higher rate of embryonic loss by 30 days of pregnancy in ewe lambs. This is thought to be associated with insufficient levels of progesterone; though, the mechanism for embryonic loss is not clearly defined.

Perinatal and postnatal mortality is elevated in neonates born to ewe lambs and this is predominately attributed to small size and low birthweights. Neonates of ewe lambs have the greatest probably of survival when born above 4.1 kg, but the probability of mortality increases when lambs born to ewe lambs weigh between 3.3 and 4.1 kg at birth (McMillan and McDonald, 1983). Furthermore, ewe lambs have a decreased percentage of lambs that survive until weaning compared to mature ewes with singleton or twins (69 v 84.7 v 83.3% respectively); whereas, the

lowest survivability of any neonate has been observed in twins born to ewe lambs (Corner-Thomas et al, 2013).

Nutrition during pregnancy is critical in maintaining productivity and a limited plane of nutrition during the early stages of gestation may contribute significantly to the weight and survivability of neonates. Mulvaney et al (2008) researched the effects of nutritional supplementation to ewe lambs during early pregnancy and birthweights. Ewe lambs were allocated to one of three different treatment groups: low, medium, or high. The low treatment group was fed pasture during the first 100 days of pregnancy to maintain liveweight. Then their nutritional plane was increased to gain 180g/day until parturition. The medium nutritional treatment group was fed to maintain steady growth of 100g/day throughout the entire gestational period. The high treatment group was fed *ad libitum*. The results show that lambs born to ewe lambs in the low treatment group had lighter birthweights (18.1 v 20.6 v 21.8 lbs) and had lower survival rates (36 v 53 v 85%) compared to the medium and high nutritional groups (Mulvaney et al, 2008). A subsequent study found that late gestational supplementation (medium-pre-grazing herbage mass of 1400 kg DM/ha v *ad libitum* pre-grazing herbage with a minimum herbage mass of 1800 kg DM/ha) after day 85 of pregnancy has no effect on lamb survival (80 v 78% respectively; Mulvaney et al, 2012), indicating that a high level of nutritional supplementation during early pregnancy is crucial for neonatal survivability and overall fertility of the ewe.

3.5.4 Prolificacy

Prolificacy varies significantly by breed whereas as Polypay is a highly prolific breed with ewe lambs having a 47% chance of multiple births. Conversely, the Columbia breed has an elevated likelihood of singles (1% chance of multiple births), and breeds such as Targhee and Rambouillet are intermediate (13 and 14% chance of multiple births respectively; Gaskins et al, 2005).

However, ewe lambs have lower prolificacy compared to mature ewes (1.38 v 2.28) as demonstrated in texel crossbred breed (Annett and Carson, 2006), and is likely due to the decreased ovulation rate and the increased prenatal mortality rate as previously described.

4.0 Problem Statement

In many sheep production systems, replacement females are bred between 16 and 20 months of age to lamb at about two years; however, ewe lambs reach puberty between seven and nine months, almost one year prior to first breeding. This extended development period between puberty and breeding increases costs and decreases lifetime productivity of each replacement ewe, hindering profitability of the enterprise. Moreover, conventionally developed replacements that fail to conceive can no longer be classified as slaughter lambs and are sold as culls at a discounted price. Therefore, breeding replacement females to lamb at one year of age presents as an opportunity to decrease production costs and increase lifetime productivity.

Fertility in ewe lambs is 20-30% lower than that of mature ewes, and is related to weight gain, nutritional status, and timing of estrous cyclicity. Heavier females reach puberty earlier, have a higher probability of becoming pregnant, greater pregnancy retention, and are more prolific. However, attainment of puberty is not directly correlated with age suggesting that various factors including breed, pre-breeding management, and breeding practices may have a greater impact on ewe lamb fertility. Genotypic and phenotypic variation among breeds also affects fertility outcome of replacement females and is particularly evident for breeds compared to breeds differing in prolificacy.

Failure of ewe lambs to conceive has been associated with an altered endocrine environment in the peripubertal animal. This period is marked by a transient rise in progesterone important for modulating ovarian activity. Breeding protocols utilizing exogenous progesterone or an analogue have been shown to increase estrous response, pregnancy to first service, and conception rates. Evidence in heifers suggests this is because progesterone treatment hastens the onset of puberty in helping overcome silent ovulations and the short lived corpus luteum.

Furthermore, higher nutritional regimens resulting in increased liveweight gains and body condition have been positively correlated with increases in ovulation rates, estrous response, and prolificacy. The level and duration of supplementation and the exact amount of pre-breeding gains necessary for pubertal initiation and a positive fertility outcome are loosely defined. Therefore, the objective of this research is to evaluate the effects of pre-breeding management on ewe lamb fertility.

5.0 Introduction

In many sheep production systems replacement ewe lambs comprise 30% of the breeding flock. Therefore, their individual productivity and fertility have a significant impact on overall productivity and profitability of the enterprise. Fertility of replacement females is 20-30% lower than that of adult ewes which can lower the overall profitability of the flock (Quirke et al, 1978). Between 20% and 40% of ewe lambs mated fail to lamb (Edey et al, 1978) due to decreased ovulation rates (Forrest and Bichard, 1974; Mulvaney et al 2013; Quirke and Hanrahan, 1977), decreased serum progesterone resulting in increased embryonic loss (Davies and Beck, 1993), low implantation rates (Quirke and Hanrahan, 1977), decreased pregnancy rates (Forrest and Bichard, 1974; Mulvaney et al 2013), and decreased prolificacy (Annett and Carson, 2006; Gaskins et al, 2005). Failure of ewe lambs to conceive during the breeding season have been associated with lighter live weights at breeding and lower weight gains between weaning and breeding (Gaskins et al, 2005; Hudgens et al, 1987; Nieto et al, 2013). Interestingly, failure of ewe lambs to conceive is not correlated with age between 160-230 day of age (Gaskins et al, 2005; Rosales Nieto et al, 2013b). The ability for ewe lambs to conceive and lamb is dependent on puberty; however, puberty alone does not equate high fertility (Rosales Nieto et al, 2013b). This was demonstrated by Rosales Nieto et al (2013b) which showed that almost 90% of ewe lambs had attained puberty prior to breeding but only 36% of the ewe lambs conceived during two 21 day breeding periods.

Failure of ewe lamb to conceive has been associated with an altered endocrine environment and a transient rise in progesterone in the peripubertal animal, and breeding protocols using exogenous progesterone or a progesterone analogue have been utilized in an attempt to increase fertility in ewe lambs (Knights et al, 2002; Sawalha et al, 2011; Stellflug et al, 2001). The analogue medroxyprogesterone acetate (MAP, 60 mg) has been shown to increase pregnancy rate by 30%

in progesterone pretreated ewe lambs compared to the control group (31 v 61%, control and progesterone pretreated respectively; Stellflug et al, 2001). Additionally, in an out-of-season breeding study, fall-born ewe lambs pretreated with progesterone via a Controlled Internal Drug Releasing (CIDR; 0.3 g progesterone) device were able to display estrus and ovulate following ram introduction (Knights et al, 2002). In heifers, progestin treatment hastened the onset of puberty (Hall et al, 1997) and reduces the occurrence of silent ovulation and the short lived CL in animals induced to ovulate during the anestrous period (Ramirez-Godinez et al, 1981; Ramirez-Godinez et al, 1982).

Further, nutritional manipulations have been shown to affect the onset of puberty, fertility, and subsequently overall productivity of ewe lambs. Nutritional management affects daily gains and has the potential to advance the onset of puberty (Villa-Godoy et al, 1990). Regardless of breed, heavier females have a higher probability of becoming pregnant, retaining their pregnancy, and having multiple births. In addition, there is a positive correlation between average daily gains, breeding weight, body composition, and reproductive performance in Merino ewe lambs (Neito et al, 2013). Further, heavier females are more likely to be mated to the first estrous cycle following ram introduction, and have a greater chance of bearing twins rather than singles (Kenyon et al, 2006). However, there are breed variations associated with nutritional supplementation and fertility outcomes that can affect the outcome of nutritional supplementation. Prolificacy is one of the greatest breed difference associated with fertility with ewe lambs of the Polypay breed having a 47% chance of having multiple births compared to the Columbia breed having an elevated likelihood of singles with a 1% chance of multiple births. Breeds such as Targhee and Rambouillet have intermediate prolificacy with a 13% and a 14% chance of multiple births respectively (Gaskins et al, 2005).

Additionally requirements for growth can vary significantly by breed and is associated with differences in mature weights. Large wool breeds such as Dorset and Texel ewes have a mature weight ranging from 67-91 kg; whereas, smaller wool breeds including Merino and Cheviot as well as tropical hair sheep such as Katahdin ewes have a mature range between 55-85 kg. Therefore, it is essential to apply an adequate level of supplementation to obtain optimal weight gains and weight for breeding replacement females of each respective breed. Therefore, the objective of this research is to evaluate the effects of pre-breeding management on ewe lamb fertility.

6.0 Materials and Methods

6.0.1 Materials

The procedures used in these studies were approved by the West Virginia University Animal Care and Use Committee AICUC #13-1101. The Controlled Internal Drug Release Device (CIDR) was obtained from Zoetis Animal Health, Kalamazoo, MI.

6.1 Replicate 1

6.1.1 Farm and animals

Research was conducted during the breeding seasons of 2014 and 2015 at West Virginia University's Reymann Memorial Farm in eastern West Virginia and at Wherry Farm in Scenery Hill, Pennsylvania.

A total of 313 Dorset X Texel (DT) ewe lambs and 74 Katahdin (KD) ewe lambs were utilized. All animals were reared on pasture with *ad libitum* forage and water, and supplemented concentrate.

6.1.2 Experimental protocol

Ewe lambs were balanced across nutritional treatments by birth type and age prior to being allocated to receive either high (0.68 kg/head/day) or low (0.23 kg/head/day) grain supplementation containing 14% crude protein for 2 months prior to breeding to achieve weight gains (5 and 10 kgs, respectively). Respective diets were supplemented for two months prior to breeding and live weights were recorded biweekly. Weight data were used to calculate changes in weight for the supplementation period, average daily gains (ADG) for the treatment period, and lifetime weight day averages (LWDA).

Prior to ram introduction (d-5), half of each treatment group were randomly selected to receive a progesterone pretreatment using an intravaginal CIDR (ZOETIS EAZI-BREED™ CIDR® 0.3g progesterone). On d0 progesterone treatment was removed and rams were introduced. All rams

had fertility previously proven and were equipped with marking harnesses to determine estrous response.

Figure 1: Experimental design (a) and timeline (b) of replicate 1.

6.2 Replicate 2

6.2.1 Farm and animals

Research was conducted during the breeding season of 2016 at Wherry Farm in Scenery Hill, Pennsylvania.

A total of 68 Dorset X Texel (DT) ewe lambs were utilized for experimentation. All animals were reared on pasture with *ad libitum* forage and water, and supplemented concentrate according to the respective treatment group.

6.2.2 Experimental protocol

Replicate 2 was conducted to evaluate the effect of providing higher levels of supplementation for a shorter duration on fertility of ewe lambs. Lambs were balanced based on age and birth type prior to receiving either high (0.91 kg/head/day) or low (0.45 kg/head/day) grain supplementation (14% crude protein). Respective diets were supplemented for four weeks prior to estrous synchronization and liveweights were recorded biweekly. Weight data was utilized to calculate changes in liveweight, average daily gains, and lifetime weight day averages (LWDA).

Figure 2: Experimental design (A) and timeline (B) of replicate 2.

6.3 Blood collection and progesterone quantification

Blood samples were collected bi-weekly via jugular venipuncture at the start of the feeding period until CIDR insertion to determine if the ewe lambs were ovulating by the beginning of the breeding period. Blood samples were collected into heparinized tubes (EDTA; Monoject, 15% EDTA K3 liquid, Tyco Healthcare Group, Mansfield, Massachusetts, USA) and centrifuged for 20 minutes at 3000 rotations per minute. Plasma was harvested and stored at -20°C until quantification of progesterone via I-125 radioimmunoassay (*ImmuChem*[™] Double Antibody Progesterone 125-I RIA Kit, MP Biomedicals, Costa Mesa, CA). Progesterone concentration was measured in individual plasma samples and expressed as nanograms per milliliter (ng/ml). Values of greater than 1 ng/ml indicated puberty and were used to determine ovulatory status of each female.

6.4 Estrous detection and ultrasonography

Estrous response was determined by observation of raddle marks 96 hours after ram introduction. Following synchronization of estrus, pregnancy diagnosis using transrectal ultrasonography (Aloka 500 Corometrics Medical Systems, Wallingford, CT, USA with a 7.5 MHz linear transducer) was conducted one month after ram introduction and overall pregnancy was determined two months after ram introduction. Lambing data was recorded. This data was used to determine conception rates, pregnancy to first service, overall pregnancy rates, and lambing rates.

6.5 Breeding weight category, average daily gains category, and weight day average category

Animals within each respective breed were classified by breeding weight, average daily gains for the treatment period, and weight day averages to determine their effects on reproductive responses. Groups were divided by calculating the mean $\pm$ one half of the standard deviation (low: $L < \mu - \frac{1}{2} \text{STD}$, medium: $\mu - \frac{1}{2} \text{STD} < M < \mu + \frac{1}{2} \text{STD}$, high: $H > \mu + \frac{1}{2} \text{STD}$). Characteristics of each breed

for breeding weight category, average daily gain category, and weight day average category are reported in Table 1.

Table 1 Description of ewe lamb characteristics

	Replicate 1		Replicate 2	Pooled DT Replicates
	Dorset X	Katahdin	Dorset X	Dorset X
	Average Daily Gain Category (g)			
<i>High</i>	138±3	163±10	99±6	99±6
<i>Medium</i>	67±3	100±7	43±5	43±5
<i>Low</i>	-27±5	53±7	-34±6	-34±6
	Breeding Weight Category (kg)			
<i>High</i>	51.4±0.4	36.6±1.0	47.1±0.4	51.4±0.4
<i>Medium</i>	40.1±0.4	30.1±0.9	41.6±0.4	40.1±0.5
<i>Low</i>	32.8±0.6	23.4±0.9	35.4±0.4	32.8±0.6
	Lifetime Weight Day Average Category (g)			
<i>High</i>	198±2	176±4	186±2	198±2
<i>Medium</i>	159±2	148±4	146±2	159±2
<i>Low</i>	117±2	116±2	98±2	117±2

6.6 Pooled DT Replicates:

Data from Dorset X Texel ewe lambs from replicates 1 and 2 were pooled for statistical analysis to determine the main effects of nutritional treatment, progesterone pretreatment, breeding weight category, average daily gain category, weight day average category, and each respective interaction on this specific breed.

6.7 Statistical analysis

A two-way Analysis of Covariance (ANCOVA) was conducted using the PROC MIX procedure of SAS (Statistical Analysis System version 9.4 for Windows; SAS Institute, Cary, NC, USA) to determine the effect of nutritional treatment group, breed, progesterone pretreatment, breeding weight category, average daily gain category, weight day average category, and each respective interaction. Although nutritional treatment groups were balanced for age, breeding age was used as a covariate in the statistical model to increase the power to detecting treatment effects of breed,

progesterone pretreatment, and each weight category. Additionally, an ANCOVA using the PROC MIXED procedure with breeding age as a covariate was used on the pooled replicate to determine if breeding weight, average daily gain for the treatment period, and weight day average differed in lambs experiencing a positive or negative reproductive outcome. All one degree comparisons were conducted using Tukey's LSD. All results are presented as least squares means $\pm$ the standard error of the mean (LS Means $\pm$ SEM) to account for unbalanced treatments. Results were considered significant at a confidence level of $P \leq 0.05$, and a tendency when $0.05 \leq P \leq 0.1$.

7.0 Results

7.1 Replicate 1

7.1.1 Effect of Breed, Nutritional Treatment, and Progesterone Pretreatment on Growth and Reproductive Response of Ewe Lambs

Katahdin (KT) ewe lambs grew faster during the nutritional supplementation period, but Dorset X Texel (DT) ewe lambs were older and heavier at breeding, ($P < 0.0001$, Table 2). More DT than KT ewe lambs ovulated prior to breeding ($P < 0.01$), and DT ewe lambs showed a higher estrous response and more conceived to the 1st service period ($P < 0.01$, Table 2). Overall, animals on the high plane of nutrition grew at a greater rate, but no effect of nutritional supplementation on reproductive response or overall fertility was observed (Table 2).

Progesterone pretreatment did not impact reproductive response (Table 2), although more females that were not pre-treated with progesterone ovulated prior to breeding.

In ewe lambs that did not receive progesterone pre-treatment, more DT than KT conceived ($P < 0.01$, 85 ± 5 v $53 \pm 10\%$ respectively); however, a similar effect between breeds was not seen in ewe lambs receiving progesterone pre-treatment (Progesterone X Breed, $P = 0.05$).

Significantly more DT ewe lambs that were not pre-treated with progesterone ($P = 0.02$, 68 ± 5 v 54 ± 3 , respectively) were pregnant to the first estrous cycle compared to those that received progesterone treatment; however, no difference ($P = 0.22$) in pregnancy to first service was observed among progesterone treatments in KT ewe lambs (Progesterone X Breed, $P = 0.03$).

7.1.2 Effect of Average Daily Gain (ADG) Prior to Breeding on Reproductive Response of Ewe Lambs

The reproductive response of ewe lambs in low (L), medium (M) and high (H) ADG categories is presented in Table 3. Compared to animals in the L ADG category, animals in the H and M tended to have higher conception rates ($P = 0.09$ and $P = 0.06$ L v M and L v H, respectively), and more

lambled ($P=0.05$ and $P=0.02$ L v M and L v H, respectively). Higher lambing rates were observed in ewe lambs in the H than in the L ADG category ($P=0.02$).

7.1.3 Effect of Weight at Breeding on Reproductive Response of Ewe Lambs

The reproductive response of ewe lambs in low (L), medium (M) and high (H) breeding weight categories is presented in Table 4.

Ewe lambs in the M and H breeding weight category showed a higher estrous response, pregnancy rate, proportion lambing, lambing rate, and a lower average age at first lambing than lambs in the L breeding weight category (Table 4; $P<0.05$). However, no effect of breeding weight category on conception rate and number born was detected, and reproductive responses did not differ between ewe lambs in the H and M breeding weight categories.

DT ewe lambs had a more pronounced conception rate, pregnancy to first service, and age at first lambing (Table 4) in the H, M, and L breeding weight categories compared to KT ewe lambs in each respective category (Breed X Breeding Weight, $P<0.05$).

7.1.4 Effect of Lifetime Weight Day Average (LWDA) Prior to Breeding on Reproductive Response of Ewe Lambs

Compared to the low (L) LWDA category, ewe lambs in the high (H) and medium (M) LWDA category showed a greater estrous response, pregnancy to first service, overall pregnancy rate, proportion lambing, lambing rate, and proportion achieve puberty by breeding (Table 5). This effect was more pronounced in DT than KT ewe lambs for most response variables (Breed X LWDA, $P<0.05$) except for overall pregnancy and proportion lambing.

Table 2 Effects of breed (DT v KT), nutritional treatment (H: 0.68 kg/head/day, L: 0.23 kg/head/day), and progesterone pretreatment on growth and reproductive response of ewe lambs.

Reproductive Response	Breed			Nutritional Treatment			Progesterone Pretreatment		
	Dorset	Katahdin	p-value	High	Low	p-value	Yes	No	p-value
n	313	74	-	193	194	-	259	128	-
Age at breeding (months)	9.1±0.1	6.9±0.2	<0.0001	8.0±0.2	8.0±0.2	NS	8.7±0.1	8.7±0.02	NS
Weight at breeding (kg)	43.2±0.5	29.5±0.9	<0.0001	42.9±0.7	41.2±0.7	NS	-	-	-
Average daily gain (g)	77±4	97±8	<0.0001	160±7	135±7	<0.01	-	-	-
Estrous response (%)	74±3	57±6	<0.01	62±4	69±4	NS	66±4	66±4	NS
Conception rate (%)	77±3	63±8	0.09	66±6	74±5	NS	73±5	69±6	NS
Pregnancy 1st service (%)^a	57±3	38±6	<0.01	43±4	52±5	NS	49±5	49±5	NS
Pregnancy overall (%)	75±3	65±6	NS	70±4	70±5	NS	75±4	65±4	<0.1
Proportion lambing (%)	60±3	54±6	NS	56±4	58±5	NS	60±5	56±5	NS
Lambing rate (%)^b	69±4	60±8	NS	67±6	63±7	NS	68±6	63±6	NS
Age 1st lambing (days)	420±1	423±2	NS	422±1	421±1	NS	421±1	422±1	NS
Number born	1.18±0.03	1.10±0.06	NS	1.2±0.046	1.1±0.5	0.09	1.1±0.05	1.1±0.05	NS
Ovulated prior to breeding (%)	26±2	8±5	<0.01	16±4	18±4	NS	13±4	26±4	0.01

a. Number of ewes diagnosed pregnant on day 30-35 as a percentage of all ewes exposed to rams.

b. Lambs born per ewe exposed to ram.

Values are least square means ± SEM (number of animals).

Table 3 Effect of average daily gain (DT, H: 138±3 g, M: 67±3 g, L: -27±5 g; KT, H: 163±10 g, M: 100±7 g, L: 53±7 g) prior to breeding on reproductive response of ewe lambs.

Reproductive Response	Average Daily Gain Category			H v M	M v L	H v L
	High	Medium	Low			
n	143	152	83			
Estrous response (%)	73±6	66±4	61±	NS	NS	NS
Conception rate (%)	76±7	72±6	57±7	NS	0.09	0.06
Pregnancy 1st service (%)^a	56±6	49±5	36±6	NS	0.08	0.02
Pregnancy overall (%)	70±6	72±4	63±6	NS	NS	NS
Proportion lambing (%)	65±7	60±5	45±6	NS	0.05	0.02
Lambing rate (%)^b	79±9	64±6	51±8	NS	NS	0.02
Age 1st lambing (days)	420±2	423±1	423±2	NS	NS	NS
Number born	1.2±0.06	1.1±0.04	1.1±0.07	0.06	NS	NS
Ovulated prior to breeding (%)	18±5	19±4	12±5	NS	NS	NS

a. Number of ewes diagnosed pregnant on day 30-35 as a percentage of all ewes exposed to rams.

b. Lambs born per ewe exposed to ram.

Values are least square means ± SEM (number of animals).

Table 4 Effect of weight at breeding (DT, H: 51.4±0.4 kg, M: 40.1±0.4 kg, L: 32.8±0.6 kg; KT, H: 36.6±1.0 kg, M: 30.1±0.9 kg, L: 23.4±0.9 kg) on reproductive responses of DT and KT ewe lambs.

Reproductive Response	Breeding Weight Category						P-Value			
	Dorset			Katahdin			H v M	M v L	H v L	Interaction
	High	Medium	Low	High	Medium	Low				
n	129	118	64	22	27	22	-	-	-	-
Estrous response (%)	90±4	72±4	49±5	59±9	68±8	41±9	NS	<0.001	<0.0001	<0.1
Conception rate (%)	89±4	74±4	46±7	59±12	60±10	76±15	NS	NS	NS	0.01
Pregnancy 1st service (%)^a	80±4	53±4	23±6	35±10	40±9	31±10	0.1	<0.01	<0.0001	<0.01
Pregnancy overall (%)	86±4	76±4	50±5	71±9	75±8	43±9	NS	<0.0001	<0.0001	NS
Proportion lambing (%)	71±4	62±4	42±6	58±10	62±10	35±11	NS	<0.01	<0.01	NS
Lambing rate (%)^b	90±6	67±6	43±8	61±14	69±12	39±14	NS	<0.01	<0.01	NS
Age 1st lambing (days)	418±1	419±1	432±2	426±3	423±3	421±4	NS	0.03	0.07	<0.01
Number born	1.29±0.04	1.1±0.04	1.03±0.08	1.0±0.10	1.1±0.09	1.0±0.10	NS	NS	NS	NS
Ovulated prior to breeding (%)	39±4	21±4	11±5	10±9	6±8	6±9	0.08	NS	0.02	NS

a. Number of ewes diagnosed pregnant on day 30-35 as a percentage of all ewes exposed to rams.

b. Lambs born per ewe exposed to ram.

Values are least square means ± SEM (number of animals).

Table 5 Effect of lifetime weight day average (DT, H: 198±2 g, M: 159±2 g, L: 117±2 g; KT, H: 176±4 g, M: 148±4 g, L: 116±2 g) prior to breeding on reproductive response of ewe lambs.

Reproductive Response	Lifetime Average Daily Gain Category						H v M	M v L	H v L	Interaction
	Dorset			Katahdin						
	High	Medium	Low	High	Medium	Low				
n	135	92	84	21	23	28	-	-	-	-
Estrous response (%)	89±4	78±4	39±5	67±9	80±9	55±8	NS	<0.0001	<0.0001	0.02
Conception rate (%)	90±4	71±5	54±8	64±13	69±11	81±13	NS	NS	NS	0.03
Pregnancy 1 st service (%) ^a	77±4	56±5	19±6	44±10	56±10	45±9	NS	<0.01	0.0001	<0.001
Pregnancy overall (%)	87±4	73±5	52±6	76±10	83±9	57±9	NS	<0.001	<0.001	NS
Proportion lambing (%)	73±4	62±5	36±6	63±11	76±10	44±10	NS	<0.001	<0.001	NS
Lambing rate (%) ^b	89±6	75±7	28±8	71±14	85±13	56±12	NS	<0.001	<0.001	<0.1
Age 1 st lambing (days)	418±1	421±1	427±2	424±3	421±3	419±3	NS	NS	NS	0.02
Number born	1.3±0.04	1.2±0.05	0.9±0.07	1.1±0.11	1.3±0.10	1.2±0.11	NS	NS	NS	0.02
Ovulated prior to breeding (%)	32±3	42±4	-3±5	18±9	14±8	14±8	NS	<0.001	<0.01	<0.01

a. Number of ewes diagnosed pregnant on day 30-35 as a percentage of all ewes exposed to rams.

b. Lambs born per ewe exposed to ram.

Values are least square means ± SEM (number of animals).

7.2 Replicate 2

7.2.1 Effect of Nutritional Treatment and Progesterone Pretreatment on Growth and Reproductive Response of Ewe Lambs

Average daily gain and weight at breeding tended to be higher in ewe lambs receiving the high level of nutrition supplementation (0.91 kg/head/day v 0.45 kg/head/day; $P=0.09$, 51 ± 9 v 28 ± 9 g and $P=0.07$, 42 ± 2 v 40 ± 1 kg, respectively). More ewe lambs receiving the high than low level of supplementation showed estrus ($P<0.01$, 100 ± 5 v $77\pm7\%$); however, no effect of level of supplementation on other reproductive response variables was observed.

No effect of progesterone pretreatment on reproductive responses was observed in replicate 2. Ewe lambs averaged 10.5 ± 2.8 months of age at breeding.

7.2.2 Effect of Average Daily Gain (ADG) Prior to Breeding on Reproductive Response of Ewe Lambs

Ewe lambs in the M ADG category had or tended to have higher conception rate ($P=0.06$), pregnancy rate to the first service period ($P=0.02$), overall pregnancy rate ($P=0.08$), proportion lambing ($P=0.03$) and lambing rate ($P=0.06$) than lambs in the L ADG category (Table 6).

7.2.3 Effect of Weight at Breeding on Reproductive Response of Ewe Lambs

In replicate 2, breeding weight category had no significant effect on reproductive response variables (data not shown).

7.2.4 Weight day average category

Reproductive outcomes increased with increasing weight day averages for most response variables (Table 7); however, there was no difference in the age at first lambing or prolificacy between the weight day average categories.

Table 6 Effect of average daily gain (H: 99±6 g, M: 43±5 g, L: -34±6 g) prior to breeding on reproductive response of ewe lambs.

Reproductive Responses	Average Daily Gain Category			H v M	M v L	H v L
	High	Medium	Low			
n	24	25	19	-	-	-
Estrous response (%)	88±7	92±7	83±8	NS	NS	NS
Conception rate (%)	67±10	78±10	49±12	NS	0.06	NS
Pregnancy 1st service (%)^a	59±10	75±10	40±11	NS	0.02	NS
Pregnancy overall (%)	51±10	67±10	40±12	NS	0.08	NS
Proportion lambing (%)	49±10	62±11	24±13	NS	0.03	NS
Lambing rate (%)^b	50±12	67±12	30±15	NS	0.06	NS
Age 1st lambing (days)	459±1	458±1	454±2	NS	NS	0.08
Number born	1.0±0.08	1.08±0.07	1.24±0.13	NS	NS	NS
Ovulated prior to breeding (%)	29±10	40±10	37±11	NS	NS	NS

a. Number of ewes diagnosed pregnant on day 30-35 as a percentage of all ewes exposed to rams.

b. Lambs born per ewe exposed to ram.

Values are least square means ± SEM (number of animals).

Table 7 Effect of lifetime weight day average (H: 186±2 g, M: 146±2 g, L: 98±2 g) prior to breeding on reproductive response of ewe lambs.

Reproductive Responses	Lifetime Weight Day Average Category			H v M	M v L	H v L
	High	Medium	Low			
n	21	21	26	-	-	-
Estrous response (%)	104±19	91±13	74±24	NS	NS	NS
Conception rate (%)	145±26	92±18	-26±35	<0.01	0.02	<0.01
Pregnancy 1st service (%)^a	140±26	85±18	-30±35	<0.01	0.03	<0.01
Pregnancy overall (%)	94±28	62±20	13±37	0.08	NS	NS
Proportion lambing (%)	113±31	61±20	-17±38	0.01	NS	0.06
Lambing rate (%)^b	124±35	70±23	-24±43	0.02	NS	0.06
Age 1st lambing (days)	457±4	456±2	459±7	NS	NS	NS
Number born	1.08±0.23	1.17±0.13	0.99±0.41	NS	NS	NS
Ovulated prior to breeding (%)	-8±28	20±20	83±4	NS	NS	NS

a. Number of ewes diagnosed pregnant on day 30-35 as a percentage of all ewes exposed to rams.

b. Lambs born per ewe exposed to ram.

Values are least square means ± SEM (number of animals).

7.3 Pooled DT Ewe Lambs:

7.3.1 Effect of Nutritional Treatment and Progesterone on Growth and Reproductive Response of Ewe Lambs

The average daily gain ($P < 0.001$), was higher in ewe lambs receiving the high compared to the low level of supplementation; however, weight at breeding did not differ (Table 8).

Lambing rate tended to be higher ($P = 0.08$) and prolificacy was significantly higher ($P < 0.01$) in ewe lambs receiving the high compared to the low level of nutritional supplementation. More animals ($P = 0.02$) receiving the low level of supplementation had ovulated prior to the breeding period. No effect of level of nutrition was observed for other reproductive response variables.

Compared to females that received progesterone pre-treatment, more females that did not receive pretreatment ovulated prior to breeding ($P = 0.0001$) and were diagnosed pregnant to the first breeding period ($P = 0.03$). Females that did not receive progesterone pre-treatment were heavier at the beginning of the breeding period ($P < 0.001$).

In ewe lambs that did not receive progesterone pretreatment, more ewe lambs supplemented the low level of nutritional supplementation ovulated prior to breeding ($P < 0.01$, 56 ± 6 v 30 ± 7 , respectively) compared to the H level of nutritional supplementation (Progesterone X Nutritional Treatment, $P = 0.02$).

7.3.2 Effect of Average Daily Gain (ADG) Prior to Breeding on Reproductive Response of Ewe Lambs

With the exception of estrous response, number born and age at first lambing, all other reproductive performance variables were higher ($P < 0.01$) ewe lambs in the H compared to the L ADG category. Ewe lambs in the M category had intermediate values were not significantly different from H and L categories (Table 9).

7.3.3 Effect of Weight at Breeding on Reproductive Response of DT Ewe Lambs (Pooled)

More H ewe lambs ovulated before the start of the breeding period than in the L ($P<0.0001$) or M breeding weight category ($P<0.01$).

Ewe lambs in the H breeding weight category had greater ($P<0.01$) reproductive responses than ewe lambs in the L and M with ewe lambs in the M category having intermediate values, except for proportion lambing and number born where there was no difference between the H and M and M and L categories respectively. Additionally, ewe lambs in the H and M categories lambed for the first time at a younger age than ewes in the L category ($P<0.01$; Table 10).

7.3.4 Effect of Lifetime Weight Day Average (LWDA) Prior to Breeding on Reproductive Response of Ewe Lambs

High and medium LWDA categories were significantly younger at breeding than the low LWDA category ($P<0.0001$, 7.9 ± 1.5 v 9.1 ± 1.7 v 11 ± 2.5 respectively).

Reproductive response variables increased with increasing weight day averages in most response variables ($P\leq0.05$, $P<0.0001$ and $P<0.05$ for H v M, H v L and M v L, respectively; Table 11). Additionally, the age at first lambing was lower in ewe lambs in the H compared to the L LWDA category ($P=0.0002$) with M LWDA ewe lambs being intermediate (H v M, $P<0.05$; M v L, $P=0.0004$).

7.3.5 Average Breeding Weight, ADG, and WDA for positive and negative reproductive outcomes

Breeding weights, ADG, and LWDA were significantly higher ($P<0.05$) in ewe lambs with a positive compared to a negative reproductive outcome for estrous response, conception rate, pregnancy to the first service, overall pregnancy rate, proportion lambing, and lambing to the first service period (Table 12). Additionally, ADG (169 ± 44 v 114 ± 12 v 85 ± 5 g respectively) breeding weights (61 ± 5 v 51 ± 1 v 44 ± 1 kg respectively), and LWDA (227 ± 18 v 189 ± 5 v 167 ± 2 g

respectively) were significantly higher ($P<0.05$) in ewe lambs having triplets and twins compared to those having singletons (Data not shown).

Table 8: Effects of nutritional treatment (H: 0.68-0.91 kg/head/day, L: 0.23-0.45 kg/head/day), and progesterone pretreatment on reproductive responses of DT ewe lambs. Data was derived by pooling the data for DT ewe lambs in replicate 1 and replicate 2.

Reproductive Responses	Nutritional Treatment			Progesterone Pretreatment		
	High	Low	p-value	Yes	No	p-value
n	151	162	-	226	87	-
Age at breeding (months)	9.3±0.2	9.2±0.2	NS	9.2±0.2	9.5±0.2	0.02
Weight at breeding (kg)	45±1	43±1	NS	42±1	46±1	<0.001
Average daily gain (g)	94±6	65±6	<0.0001	74±5	86±7	0.1
Estrous response (%)	77±3	75±3	NS	74±3	81±5	NS
Conception rate (%)	76±4	79±4	NS	74±3	84±5	<0.1
Pregnancy 1st service (%)^a	58±4	59±4	NS	55±3	68±5	0.03
Pregnancy overall (%)	76±4	73±3	NS	76±3	70±5	NS
Proportion lambing (%)	63±4	60±4	NS	60±3	65±5	NS
Lambing rate (%)^b	79±5	66±5	0.08	71±5	75±7	NS
Age 1st lambing (days)	429±1	431±1	NS	430±1	430±2	NS
Number born	1.3±0.04	1.1±0.04	<0.01	-	-	-
Ovulated prior to breeding (%)	26±4	39±4	0.02	21±3	43±5	0.0001

a. Number of ewes diagnosed pregnant on day 30-35 as a percentage of all ewes exposed to rams.

b. Lambs born per ewe exposed to ram.

Values are least square means ± SEM (number of animals).

Table 9 Effect of average daily gain (H: 99±6 g, M: 45±5 g, L: -36±6 g) prior to breeding on reproductive response of DT ewe lambs. Data was derived by pooling the data for DT ewe lambs in replicate 1 and replicate 2.

Reproductive Responses	Average Daily Gain Category			H v M	M v L	H v L
	High	Medium	Low			
Estrous response (%)	78±4	77±4	68±5	NS	NS	NS
Conception rate (%)	82±4	82±4	59±6	NS	<0.01	<0.01
Pregnancy 1st service (%)^a	64±4	63±4	40±6	NS	<0.01	<0.01
Pregnancy overall (%)	80±4	74±4	64±6	NS	NS	0.02
Proportion lambing (%)	68±4	61±4	49±6	NS	NS	0.01
Lambing rate (%)^b	86±6	66±6	58±9	0.02	NS	<0.01
Age 1st lambing (days)	430±1	431±1	433±1	NS	NS	NS
Number born	1.3±0.04	1.1±0.048	1.2±0.08	<0.01	NS	NS
Ovulated prior to breeding (%)	31±4	29±4	19±6	NS	NS	0.09

a. Number of ewes diagnosed pregnant on day 30-35 as a percentage of all ewes exposed to rams.

b. Lambs born per ewe exposed to ram.

Values are least square means ± SEM (number of animals).

Table 10 Effect of weight at breeding (H: 51.4±0.4 kg, M: 40.1±0.5 kg, L: 32.8±0.6 kg) on reproductive response of DT ewe lambs. Data was derived by pooling the data for DT ewe lambs in replicate 1 and replicate 2.

Reproductive Responses	Breeding Weight Category			H v M	M v L	H v L
	High	Medium	Low			
Estrous response (%)	91±4	73±4	50±5	<0.001	<0.001	<0.0001
Conception rate (%)	89±4	74±4	47±7	0.01	<0.01	<0.0001
Pregnancy 1st service (%)^a	81±4	54±4	24±6	<0.0001	<0.0001	<0.0001
Pregnancy overall (%)	85±4	75±4	49±5	0.07	<0.0001	<0.0001
Proportion lambing (%)	71±4	61±4	41±6	NS	<0.01	0.0001
Lambing rate (%)^b	91±6	67±6	43±8	<0.01	0.02	<0.0001
Age 1st lambing (days)	427±1	429±1	441±2	NS	<0.0001	<0.0001
Number born	1.3±0.04	1.1±0.05	1.0±0.08	<0.01	NS	<0.01
Ovulated prior to breeding (%)	41±4	23±4	13±5	<0.01	NS	<0.0001

a. Number of ewes diagnosed pregnant on day 30-35 as a percentage of all ewes exposed to rams.

b. Lambs born per ewe exposed to ram.

Values are least square means ± SEM (number of animals).

Table 11 Effect of lifetime weight day average (H: 198±2 g, M: 159±2 g, L:117±2 g) prior to breeding on reproductive response of DT ewe lambs. Data was derived by pooling the data for DT ewe lambs in replicate 1 and replicate 2.

Reproductive Responses	Weight Day Average Category			H v M	M v L	H v L
	High	Medium	Low			
n	135	92	84	-	-	-
Estrous response (%)	92±4	81±4	43±5	0.05	<0.0001	<0.0001
Conception rate (%)	91±4	73±5	55±7	<0.01	0.04	0.0001
Pregnancy 1st service (%)^a	81±4	60±5	23±6	<0.001	<0.0001	<0.0001
Pregnancy overall (%)	87±4	74±4	53±5	0.02	<0.01	<0.0001
Proportion lambing (%)	75±4	63±5	38±6	0.09	<0.01	<0.0001
Lambing rate (%)^b	92±6	79±7	32±8	NS	<0.0001	<0.0001
Age 1st lambing (days)	427±1	431±1	436±2	0.05	0.02	<0.001
Number born	1.3±0.04	1.2±0.05	0.9±0.07	NS	<0.001	<0.0001
Ovulated prior to breeding (%)	41±4	23±4	13±5	<0.01	NS	<0.0001

a. Number of ewes diagnosed pregnant on day 30-35 as a percentage of all ewes exposed to rams.

b. Lambs born per ewe exposed to ram.

Values are least square means ± SEM (number of animals).

Table 12 Average breeding weight, ADG, and LWDA for positive and negative reproductive outcomes in DT ewe lambs. Data was derived by pooling the data for DT ewe lambs in replicate 1 and replicate 2.

Reproductive response	Average daily gain (g)			Breeding weight (kg)			Lifetime weight day average (g)		
	Reproductive outcome			Reproductive outcome			Reproductive outcome		
	Yes	No	p-value	Yes	No	p-value	Yes	No	p-value
Estrous response	83±5	64±8	0.03	45±1	36±1	P<0.0001	171±2	144±3	P<0.0001
Conception rate	91±5	58±9	<0.01	47±1	40±1	P<0.0001	174±2	151±3	P<0.0001
Pregnancy 1st service^a	91±5	62±6	<0.001	47±1	39±1	P<0.0001	177±2	148±2	P<0.0001
Pregnancy overall	87±4	54±8	<0.001	45±1	39±1	P<0.0001	170±2	149±3	P<0.0001
Proportion lambing	91±5	64±6	<0.001	45±1	40±1	P<0.0001	172±2	155±3	P<0.0001
Lambing to the 1st service^b	96±6	72±5	<0.01	47±1	41±1	P<0.0001	178±3	158±2	P<0.0001
Ovulated prior to breeding	92±8	72±5	0.03	48±1	42±1	P<0.0001	177±	159±2	P<0.0001

a. Number of ewes diagnosed pregnant on day 30-35.

b. Number of ewes lambing by day 14 of the lambing period.

Values are least square means ± SEM (number of animals)

8.0 Discussion

The primary objective of this study was to evaluate the effects of pre-breeding overall fertility in nulliparous sheep. This was done with the intent to apply research findings to lamb production in the North Eastern United States. The present study demonstrated that (i) ewe lambs with heavier breeding weights have an increased reproductive response, (ii) progesterone pretreatment may not be necessary to advance estrous response at breeding in replacement females that have achieved adequate growth to undergo the pubertal transition, and (iii) achieving higher weight day averages rather than focusing on gains during the pre-breeding period allows for greater control of the reproductive response of ewe lambs.

8.1 Breed effects

Large variation and confounding effects of breed, environment, and farming system limit the ability to compare the difference in reproductive responses between Dorset X Texel (DT) and Katahdin (KT) ewe lambs. Even so, more DT than KT ewe lambs attained puberty (ovulated) prior to breeding, and DT ewes showed a higher fertility at first service; however, there was no significant effect of breed on overall fertility or lambing rate. The increase in the percentage of DT ewe lambs cyclic prior to breeding could be attributed to the differences in age and weight at breeding (9 v 7 months and 43 v 29 kg for DT and KT ewe lambs, respectively). Despite KT ewe lambs being younger and lighter at breeding, they grew at a faster rate during the pre-breeding period. Given additional growth during the breeding period, more KT lambs could have been bred at subsequent service periods potentially resulting in similar overall lambing rate in the two breeds.

8.2 Nutritional treatment

An elevated plane of nutrition had no effect on fertility in the immediate pre-breeding period which is contradictory to current literature in ewe lambs (Bizelis et al, 1990; Burfening and Bererdinelli, 1986) and heifers (Mackey et al, 2000; Schillo et al, 1992; Villa-Godoy et al, 1990). In ewe lambs, elevated nutrition has been shown to advance puberty, stimulate a higher ovulation rate, and stimulate the estrous response (Bizelis et al, 1990; Burfening and Bererdinelli, 1986). Additionally, in dairy heifers, an increased plane of nutrition has been shown to advance puberty; whereas a restricted nutritional plane delayed puberty and resulted in the inability of the heifers to competently support pregnancy (Villa-Godoy et al, 1990). Acute nutritional restriction induced anestrus in cycling heifers (Mackey et al, 2000) and delayed puberty in prepubertal heifers (Day et al, 1986). Feed restriction can suppress the estrous cycle and reproductive behavior when fat deposits are not available to cover demands (Bronson, 2000; Schneider and Wade, 2000). Ewe lambs that lack sufficient dietary energy in the prepubertal period exhibit an extended response to negative feedback of estrogen (Foster et al, 1986). This is associated with concentrations of systemic leptin signaling body condition and being permissive of the pubertal transition (Cordoso et al, 2014; Foster and Nagatani, 1999; Rosales Nieto et al, 2014; Samadi et al, 2014).

In the present study the lack of observed differences in reproductive responses in ewe lambs fed different levels of supplementation is presumably due to the type and length of supplemental feeding. The present study used a diet containing 77.3% total digestible nutrients (TDN) and 15.6% crude protein (CP), which is higher than the recommended diet for 44 kg ewe lamb (64.5% TDN and 12.6% CP). When ewe lambs were fed a control (75% TDN and 15% CP) or nutrient restricted diet (60% TDN and 11.4% CP) for one month prior to breeding, it was observed that dietary

restriction delayed age but not weight at puberty (Boulanouar et al, 1995). This suggests that weight is a limiting factor in the attainment of puberty.

Bizelis et al (1990) supplemented ewe lambs with a high plane of nutrition starting at 85 days of age and observed increased ovulation rate and estrous responses. Additionally, Burfening and Berardinelli (1986) supplemented replacement ewes with either a high or a low energy supplement for three months prior to breeding and reported a significant effect on proportion lambing.

Finally, the system of feeding could have resulted in significant within treatment variation in weight gains which would have precluded the detection of differences in reproductive responses across nutrition supplementation treatments.

8.3 Breeding weight category

Due to the expected within nutrition treatment variation in weight gains, ewe lambs within breed were classified based on observed weight gains and breeding weight. A greater percentage of ewe lambs classified as having high breeding weights attained puberty compared to those classified as low weight ewe lambs. This finding is consistent with the Critical Body Weight Hypothesis which suggest that heavier females reach puberty at a younger age (Frisch and McArthur, 1974).

Females that were heavier at breeding were more likely to express estrus, conceive and become pregnant to the first service. This positive impact of weight at breeding on fertility is consistent with current literature in ewe lambs and heifers, and has been associated with an increased body composition, which allows for a greater proportion of ewe lambs to have initiated cyclicity prior to breeding.

Several authors report body condition is positively related to liveweight (Geisler and Fenlon et al 1979; Kenyon et al 2004a; Russel et al, 1969; and Sanson et al 1993), and a positive correlation

between breeding weights and reproductive performance in ewe lambs has also been observed (Rosales Nieto et al, 2013b). Kenyon et al (2009 and 2010) demonstrated an advanced estrous response in heavier and higher conditioned females compared to lighter weight females and those with a lower body condition score. Additionally, as body condition increases, a successive increase in pregnancy rates is observed (BCS 1.5=63.7% v BCS 2.0=76% v BCS 2.5=83%; Kenyon et al, 2010). Rosales Nieto et al (2013b) quantified this effect demonstrating that a 4% increase in estrous response with each 20 g increase in average daily gain in the pre-breeding period (70 days prior to breeding). Consequently, there is a threshold weight and body condition beyond which achieving greater gains and conditioning prior to breeding will cease to have an effect on fertility. In composite ewe lambs, a bodyweight threshold showed no benefit to additional liveweight gains greater than 47.5 kg, but significant differences in fertility and reproductive rates were observed prior to 47.5 kg (Corner-Thomas et al, 2015). This is in agreement with the findings of Kenyon et al (2008, 2009, 2010), and Rosales Nieto et al (2013b). Additionally, a 90% fertility (number of ewe lambs pregnant/ 100 ewes bred) was achieved at a threshold body condition score of 3.5 and reproductive rate (number of fetuses/ 100 ewes bred) peaked at 130% with a 3.0 body condition score (Corner-Thomas et al, 2015).

In the present study the lambing rate increased with increasing body weight. This finding is supported by the work on Kenyon et al (2006, 2008, 2009) and demonstrates that heavier ewe lambs are more likely to become pregnant and carry multiple lambs. Liveweights are the most significant factor affecting the number of fetuses present one month after ram introduction (Kenyon et al, 2004b).

Interestingly, heavier females were younger at their first lambing. This suggest that beyond a threshold age, weight gain and weight at breeding may be the major determinants of fertility in ewe lambs.

It is beneficial to know the specific weights associated with positive reproductive outcome. Therefore, an ANCOVA with breeding age as the covariate was utilized to evaluate each reproductive outcome as the dependent variable. Data suggests that for DT ewe lambs, a breeding weight of 45 kg is ideal for replacement females to express estrus, become pregnant, and lamb. However, to advance the estrous response, become pregnant, and lamb to the first service, ewe lambs should average 47 kg at breeding. A liveweight of 40 kg was suggested for Merino ewe lambs to be bred to the first estrous cycle (Kenyon et al, 2005; Kenyon et al, 2006; Kenyon et al, 2009), and 45 kg for Merino ewe lambs to have high reproductive rates (Rosales Neito et al. 2013a). Though within flock, the variation in pubertal weight can vary significantly (Dyrmundsson et al, 1972); it is suggested that 50-70% of the mature liveweight is the critical range for ewe lambs to undergo the pubertal transition (Hafez, 1952; Dyrmundsson, 1973). In the Merino breed, there is a linear response between liveweight and the pregnancy response in ewe lambs between 30 and 45 kg (Rosales Nieto et al, 2013a). This threshold effect is similar to that observed in mature ewes (Ferguson et al, 2011). In the present study, breeding weights of 51 kg and 61 kg were necessary for increased prolificacy. Presumably, this is to ensure that ewe lambs have a great enough liveweight and body composition for not only high ovulation rates but maturity to support multiple fetuses. In Merino ewe lambs, each additional kg liveweight gain prior to the suggested threshold of 47.5 kg resulted in 4.8 more fetuses per 100 Merino ewe lambs (Rosales Nieto, 2013a).

Regardless of breed, heavier females have a higher probability of becoming pregnant, retaining their pregnancy and having multiple births. Elevated liveweight and body condition are permissive

of puberty and subsequently enhance fertility response in nulliparous females. Overfeeding to achieve a breeding weight that is substantially higher than threshold is not economically beneficial. Therefore, efficient nutritional management is necessary to optimize reproductive performance in the first breeding season.

8.4 Progesterone pretreatment

Progesterone pretreatment was shown to advance the first estrous response in nulliparous females (Knights et al., 2002; Sawalhah et al., 2011; Stellflug et al. 2001). However, in the present study ewe lambs that were not pre-treated with progesterone showed higher reproductive rates. This is likely due to the greater proportion of non-progesterone pretreated ewes that attained puberty prior to the breeding season resulting in a high estrous and pregnancy rate to the first service period.

These findings indicate that if lambs can be fed to grow at a sufficient rate and reach the threshold weight and body condition score to attain puberty prior to the breeding season a significant proportion will conceive during the first service period.

8.5 Average daily gains in the immediate pre-breeding period

Ewe lambs achieving high daily gains in the period prior to the breeding season showed improved reproductive outcomes. Increasing the average daily gains in the pre-breeding period was also previously shown to improve reproductive responses and overall fertility in ewe lambs (Bizelis et al, 1990; Burfening and Bererdinelli, 1986), and heifers (Cardoso et al, 2014). In contrast Rosales Nieto et al (2013a) found no effect of increased average daily gains during the prebreeding period in Merino ewe lambs and suggested this was due to the already elevated average daily gains being

obtained (> 200 grams/day). Similarly, heifers experiencing a rapid rate of gain during this period exhibited a lower estrous response than slower growing heifers (Ferrel et al, 1982).

Data from this experiment indicates that ewe lambs should gain > 91 g/d in the immediate pre-breeding period to express estrous, become pregnant, and lamb. Further, to support multiple fetuses, ewe lambs would need to gain 114 g/d for multiple births.

The present study is in agreement with the work of Boulanouar et al (1995) who suggested that short term increases in the plane of nutrition to achieve greater gains during this period may not be always be sufficient in overcoming long term nutrient restrictions. Ewe lambs that switched from a restricted protein or restricted energy diet to a control diet were heavier at breeding and gained more weight during the treatment period, but were unable to achieve puberty earlier than lambs fed solely a restricted diet (Boulanouar et al, 1995).

8.6 Weight day average

Elevated lifetime weight day average gains significantly enhanced fertility in replacement females. There was a successive increase in estrous response, conception rate, pregnancy outcomes, proportion lambing, lambing rate, and prolificacy as weight day averages increases. Faster growth results in ewe lambs achieving puberty at a younger age (Rosales Nieto et al 2013a, Hawker and Kennedy, 1978); thus, allowing ewe lambs to be mated at a younger age. It is important to note that the animals with higher weight day averages were younger at breeding and were younger at first lambing than animals that grew at a slower rate.

Replacement females that conceived and lambled to their first breeding season achieved weight day averages > 172 g per day. Additionally, ewe lambs that produced multiple progeny achieved weight day averages > 189 g per day.

9.0 Conclusion

There is some evidence that increasing the level of nutritional supplementation and increasing average daily gain in the immediate pre-breeding period can impact fertility; however, a short-term increase in supplementation may not be enough to overcome previous nutrient restrictions or light weight gains. Therefore, achieving higher gains over the animal's lifetime rather than focusing on the pre-breeding period may be of greater concern. Increasing weight day averages has the greatest impact on fertility responses in nulliparous females. Furthermore, sufficiently supplementing ewe lambs may provide a way to advance the estrous response and pregnancy to first service without having to rely on the use of progesterone pretreatment in the synchronization protocol for breeding replacement females.

10.0 References

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