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**Effect of Nutrition, Lactation and Gonadotropin Stimulation on Ram-Induced LH Surge
and Subsequent Fertility in Early Post-Partum Ewes during Seasonal Anestrous**

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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
Master of Science in Reproductive Physiology**

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2015

Keywords: Ewe, Anestrous, Lactation, Gonadotropin, Feed Supplementation, Fertility

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ABSTRACT

Effect of Nutrition, Lactation and Gonadotropin Stimulation on Ram-Induced LH Surge and Subsequent Fertility in Early Post-Partum Ewes during Seasonal Anestrous.

Abiodun Emmanuel Adebisi

This study evaluated the effect of lactation, exogenous gonadotropin (GS), and nutritional supplementation (SUPP) on fertility of Katahdin ewes during seasonal anestrous. Experiment 1 evaluated effects of lactation and GS on ram-induced LH surge. Lactating (65 to 80 d postpartum; pp) and dry ewes were randomly assigned to be treated with an estrus induction protocol (EIP) consisting of a 5-d treatment with a CIDR device with an injection of a gonadotropin mixture (240 IU eCG and 120 IU hCG; P.G. 600®; 3 ml/animal i.m.; n = 16) or no further treatment (n=16) at removal of CIDRs. Experiment 2 was similar to experiment 1 and evaluated the specific effects of lactation and GS on the ram-induced LH surge and subsequent fertility. Lactating (approximately 68.7 ± 1.3 days pp) and dry ewes were treated with CIDR device with (n = 42) or without (n = 38) an injection of the gonadotropin mixture. In Experiment 3, effects of both levels of nutritional supplementation and GS on fertility of lactating ewes were evaluated. Lactating ewes (n = 87) on pasture, were assigned randomly and then balanced for litter size to receive either a low or high amount of grain supplementation (13.3% Crude Protein; CP; 0.68 kg or 1.14 kg/d/animal; equivalent to 30 and 50% of projected Dry matter intake; DMI, respectively) from 1.5 to 2.5 months postpartum and then treated with CIDR for 5 days with or without GS at CIDR removal. Analysis of Variance (ANOVA) was used to evaluate the main effects and their interactions on response variables. In experiment 1, the proportion of ewes with a detectable LH surge was greater ($P = 0.0001$) in ewes treated with EIP with an injection of gonadotropin mixture, but there was no effect of lactation status. In experiment 2, the mean proportion of ewes with detectable LH surge tended to be higher in ewes receiving GS than in ewes not receiving GS ($P = 0.07$; 87.1% vs 57.2%, respectively). Estrous response was higher in ewes receiving than in ewes not receiving GS ($P = 0.0045$; 63.9% vs 38.6%, respectively) with no effect of lactation status. GS tended to increase estrous response in lactating but not dry ewes (LACT x GS, $P = 0.1$). Conception and pregnancy rates were lower in lactating ewes compared to dry ewes ($P = 0.0172$, 0.0131). In Experiment 3, estrous response was greater in ewes receiving than in those not receiving GS ($P = 0.009$; 67.4 ± 7 and $33.6 \pm 7\%$, respectively) and tended to be greater in ewes receiving the high vs. low level of supplementation ($P = 0.10$; 58.6 ± 8 and $42.3 \pm 6\%$, respectively). The pregnancy rate (PR) was increased by GS ($P = 0.003$; 49 ± 7 and $19.9 \pm 7\%$, for ewes receiving and not receiving GS, respectively) and by increasing the amount of supplement ($P = 0.05$; 43.8 ± 8 and $25.1 \pm 6\%$, for ewes receiving the high and low level of supplementation, respectively). The interaction of GS and nutritional supplementation was not significant for any of the variables measured ($P > 0.1$). The mean peak concentration of LH and time to peak concentration in ewes with detectable LH surge were not affected by any treatment in this study. In conclusion, during anestrous, early postpartum fertility is reduced by lactation in Katahdin ewes, but fertility in lactating ewes can be restored to that of dry ewes with nutritional supplementation and by providing exogenous gonadotropin as part of an EIP.

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REVIEW OF LITERATURE

I. Sheep Industry in the United States

Over the last 5 decades, the prevalent feature of the sheep industry in the United States (US) has been a steady decline in sheep and lamb inventories. This has been a major issue of concern and focus for the producers and policymakers alike. The decline in sheep and lamb numbers has created difficulties in the flow of sheep and lamb products through a shrinking marketing system as producers have struggled to respond to market signals while maintaining profitability (BANR, 2008).

The market for sheep products has shifted from wool to meat so as to meet the increasing consumption of lamb and as a response to low wool prices and lower cost of synthetic fibers which made them more attractive to the consumers (Jones, 2004). Also, the increase in demand for lamb has been inelastic as a result of low supply which is due to decreased imports and expansion in the cultural and ethnic market, and buying habits which have shifted from its dependency on price to the quality of the product (Dumas, 2011).

However, several factors have contributed to the decline in the sheep inventory. These factors include but are not limited to low lamb prices, low lamb consumption when compared to other meats, limited success of attempts to promote and differentiate U.S lambs from other meats and, increasing disease and predator losses (Jones, 2004).

In an attempt to address the declining sheep inventory, the American Sheep Industry (ASI) Association developed and implemented the “Let’s grow program”. The goal of this initiative is

to encourage sheep producers to improve production efficiencies in light of the high cost of feed, fuel and other costs of production in order to sustain and expand the sheep industry.

In a recent report by National agricultural Statistics Service (NASS), the inventory of all sheep and lambs in the US (5.28 million) increased by 1% (35,000 head increase) from 2014-2015. This increase in breeding animals included a 15,000 head increase in replacement lambs that are under 1 year of age. This is expected to maintain a tight lamb and mutton supply and consequent inflation in the price (Mathews and Haley, 2015) since these sheep that can be sent to slaughter need to be recruited for breeding. As a result, there is still a need for increasing production efficiency in the sheep industry.

In summation, the United States sheep industry has experienced a significant decline in sheep inventory. Presumably, the decline in production is due to a number of factors, such as low profitability of sheep enterprises, increased competition in demand between markets, loss of animals due to predation and parasitism, and low productivity of ewes. Attempts to increase productivity of the ewe and in particular, increase the frequency of lambing are hampered by the seasonal nature of reproduction in the ewe. Attempts to shift the seasonal pattern of reproduction have been partially successful, but the lambing rate is still lower than is observed during the breeding season, and limited success has been realized from attempts to breed lactating ewes during anestrus. Approaches that enhance the fertility of ewes bred out-of-season can further increase profitability of producers, leading to increased production and revitalization of the sheep industry (Reviewed by D'Souza, 2013).

II. Seasonality of Sheep Reproduction

The seasonality of reproduction is an adaptive physiological process utilized by wild animals to cope with seasonal changes in temperature and food availability, that is, breeding is initiated when the environment will allow for the best survival of the young. The reproductive patterns of an animal are a consequence of the interaction of the endogenous regulatory mechanisms - mainly endocrine- and environmental signals. These signals may affect reproductive mechanisms, that is, estrous cycles can cease to occur in some periods of the year in females or they can ovulate and come into estrus during the seasonal non-cycling period due to the acute stimulus of male presence (Ungerfeld and Bielli, 2012).

Although seasonality is almost completely lost in cattle and pigs due to domestication, it is retained in most breeds of sheep, goats and horses originating from temperate latitudes (Malpaux et al., 1996). The annual cycle of daily photoperiod is the determinant factor of onset and duration of breeding seasons while environmental temperature, nutritional status, social interactions, lambing date and lactation are considered as modulating factors (Rosa and Bryant, 2003). The timing of the breeding season is dependent on the length of the gestation period so that parturition occurs in the spring, which is the optimal season for nourishment and survival of the young. Thus, sheep and goats with 5 month gestations are short-day breeders with conception occurring in autumn and winter, whereas horses which have an 11 month pregnancy, are most fertile during the long days of spring (Ortavant et al., 1985).

III. Endocrine Regulation of the Estrous Cycle

The estrous cycle is defined as the number of days between the start of two different periods of estrus, or heat (Kennedy, 2012). A complex regulatory system involving a number of hormones controls the estrous cycle (see figure 1). According to Karsch and Moenter (1990), the hypothalamus secretes GnRH into blood vessels that flow to the anterior pituitary (AP). GnRH acts on specific cells of AP to release FSH and LH which are transported to the ovaries to prompt follicular development. As the follicles grow, LH and FSH act synergistically on the follicles to stimulate secretion of estradiol (E_2), which increases as the maturing follicles get larger. The E_2 has a positive and negative feedback on hypothalamus and AP to control release of FSH and LH (Legan and Karsch, 1980, Karsch et al., 1984) and a peak in the blood concentration of E_2 results in the secretion of large quantity of LH (pre-ovulatory LH surge), which is responsible for rupture of follicle and release of ovum; the ovulation (Thomas et al., 1988). The principal peaks of FSH in plasma have also been shown to coincide with the pre-ovulatory rise the level of LH (Foster et al., 1975). After ovulation, LH transforms follicle into a corpus luteum (CL) which secretes progesterone (P_4). As LH stimulates the CL to secrete P_4 , the increasing P_4 has negative feedback on hypothalamus to decrease GnRH production and consequently on AP to decrease FSH and LH secretions (Skinner et al., 1998). This may reduce follicular growth and suppresses estrus and ovulation as long as the progesterone level is high.

Progesterone is the major hormone that supports and maintains gestation as it plays a major role in the preparation of the reproductive tract for pregnancy. If there is no conception, $PGF_{2\alpha}$ produced by the uterus then causes the regression of the corpus luteum and lowers the level of plasma P_4 (McCracken et al., 1970; McCracken et al, 1972; Inskeep and Murdoch, 1980). The decrease in P_4 signals the end of luteal phase and the start of the new follicular phase as the

hypothalamus begins to produce GnRH. However, if conception occurs, the CL persists and continues the secretion of P₄ to maintain pregnancy throughout the period of gestation (Spencer et al., 2004; 2007).

Figure 1. Estrous cycle. *Sheep Production Handbook* (2002).

IV. Expression of Seasonality in the Ewe

Reproductive seasonality in the ewe is characterized by behavioral, endocrine and ovulatory changes which give rise to an annual alternation between two distinct periods; a breeding season which is characterized by the succession at regular intervals (mean of 17 days) of estrous behavior and ovulation in the non-pregnant ewe, and an anestrus season characterized by the cessation of sexual activity. The transition from anestrus to breeding season is gradual, with the occurrence of shortened estrous cycles. These short estrous cycles occur due to premature regression of the corpus luteum (CL), 5–6 days after its formation. Both ovulatory activity and estrous behavior show parallel seasonal variation but there are some discrepancies at the beginning and at the end of the sexual season when some ovulations are not accompanied by estrus. It is only after the end of the first ovarian cycle that the behavioral estrus is exhibited (Rosa and Bryant, 2003). In some breeds, silent ovulations, which are not related either to the onset or the end of the sexual season, may also occur during mid-anestrus (Land et al., 1973; Ortavant et al., 1988).

It is clearly evident that the difference between breeding and non-breeding season is brought about by the frequency of GnRH pulses, which is substantially reduced during anestrus season as compared to the breeding season (Goodman et al., 1982; Robinson et al., 1985; Barell et al., 1992; Karsch et al., 1993). During the last oestrus cycle of the breeding season, the regression of the CL is not followed by a GnRH surge and ovulation (Legan and Karsch, 1979), and there is an increase in the frequency of GnRH pulses during the transition from anoestrus to the breeding season (Legan et al., 1977).

The control of seasonal breeding is thought to be caused by a marked seasonal shift in the response of the hypothalamus to estradiol, such that estradiol has negative feedback action on the hypothalamus and anterior pituitary during anestrus and positive feedback during the breeding season (Goodman et al, 1982; Legan and Karsch, 1979; Platt et al., 1983). In ovariectomized ewes that were treated with estradiol (released at constant levels via implant) at mid-luteal phase, LH levels were constantly high during the breeding season ($\text{LH} > 5\text{-}10\text{ng/ml}$) but maintained basal levels during the anoestrus season (Karsch et al., 1980). This is further supported by other studies in which continuous release of LH was observed, but with lower frequency during the anestrus than during the reproductive season. For example, one pulse every 8-12 hours is observed during anestrus compared to one pulse per 3-4 hours in the mid-luteal phase, one pulse per 1-2 hours immediately prior to the preovulatory LH surge and one pulse every 20 minutes during the preovulatory LH surge (Martin, 1984; Thiéry and Martin, 1991).

Follicle growth and regression continue during anestrus season and follicles as large as those found during the luteal phase of the oestrus cycle can be observed (Hutchinson and Robertson, 1966; Matton et al., 1977; Webb and Gauld, 1985). The follicles produce steroids throughout seasonal anoestrus and many of the positive and negative feedback effects of the steroid on secretion of luteinizing hormone (LH) continue as in the breeding season (Gordon, 1997).

The plasma concentration of progesterone remains virtually undetectable during anoestrus (Roche et al., 1970; Karsch, 1984) and there seems to be no significant change in the level of follicle stimulating hormone (FSH) from those found during the reproductive season (Goding et al., 1969; Roche et al., 1970; Walton et al., 1977). According to Gonzalez-Bulnes et al. (2000), in the ewe, seasonality can be measured by the change in weight of the ovaries all through the

year as a result of the presence or absence of corpora lutea and the variation in the number of follicles which modify the types and levels of steroid hormones secreted.

V. Expression of Seasonality in the Ram

In Rams, seasonal variation has been reported in sexual behavior, testicular weight and volume, hormonal activity and gametogenesis (Schanbacher and Lunstra, 1976; Lincoln and Davidson, 1977; Ortavant et al., 1985; Gundogan et al., 2002). Behavioral and physiological variations are less pronounced in the ram than in the ewe. Continuous spermatogenesis and sexual activity are observed in the ram while ovulation and estrus is arrested in the ewe. Spermatogenesis and sexual activity are low at the end of winter and during spring but high at the end of the summer and during fall (Lincoln and Short, 1980; Haynes and Schanbacher, 1983; Pelletier and Almeida, 1987).

Sexual activity in Rams is stimulated 1- 1.5 months earlier than in ewes to allow rams to achieve a high level of sexual activity at the time when the cyclic period of the ewes starts. This is said to be important because while anestrous ewes can ovulate within a few days after hormonal stimulation due to the ever-present large-sized follicles, the ram needs approximately 45 days to complete spermatogenesis (Rosa and Bryant, 2003).

Lincoln and Davidson (1977) reported an increase in LH and FSH concentration in Soay rams 2-4 weeks after the abrupt change from long (16h light : 8h darkness) to short days (8h light: 16h darkness) followed almost immediately by a rise in plasma testosterone levels and testicular growth.

VI. ENVIRONMENTAL FACTORS CONTROLLING SEASONALITY

i. Photoperiod as a Principal Factor

Photoperiod is the duration of light exposure within a 24-hour day. A long day photoperiod (LDPP) consists of a 16- to 18-hours of light exposure and 6- to 8-hours of darkness while the short day photoperiod (SDPP) is characterized by 8 hours of light exposure followed by 16 hours of darkness. Photoperiodicity is the physiological reaction of organisms to the length of day or night.

Photoperiod regulates cyclicity in seasonal breeders (Hamadani et al., 2013). There is transduction of photoperiodic information into neuroendocrine changes through variations in melatonin secretion from the pineal gland. In ewes, melatonin triggers variations in the secretion of GnRH responsible for seasonal changes in their reproductive activity (Thiery et al, 2002). Dahl et al. (2000) reported that there is a correlation between photoperiod and secretion of melatonin from the pineal gland, such that circulating melatonin is nearly absent during exposure to light but rapidly increases upon exposure to darkness.

Melatonin is a modified amino acid which is synthesized from serotonin and its secretion is mainly by the pineal gland, with secondary secretions by the retina, skin, bone marrow, blood platelets, gut and probably other structures, with little or no significant systemic contribution (Liu et al., 2004). The solubility of melatonin in lipid and water allows its passage across cell membranes (Pardridge, 1980). It therefore gains access to bodily fluids, tissues and cellular compartments (cerebrospinal fluid, urine, saliva, preovulatory follicle, amniotic fluid, semen and milk) after its release in the circulation, and passes across information about the lighting of the environment to various parts of the body (Hamadani et al., 2013).

The melatonin rhythm is associated with the dark hours of the day with the photic information transmitted to the central pacemaker through the retino-hypothalamic fibers. During the day, the photic information from the retino-hypothalamic tract inhibits melatonin synthesis due to the presence of light (Hamadani et al., 2013). In an earlier study, Lewy et al., 1980 reported that melatonin production was suppressed when artificial light of sufficient intensity and duration was administered at night.

The melatonin signal was reported to be a viable regulator of neuroendocrine state that can turn on or off the reproductive axis of pinealectomized animals treated with infusion of melatonin repeatedly administered in the night. This mimics the pattern of its secretion typical of long or short photoperiods. The same melatonin signal leads to opposite neuroendocrine responses in spring-breeding species, since a progressive decrease in the duration of the night-time melatonin signal results in reproductive activation as days lengthen in the spring (Johnson, 2007).

Melatonin enhances GnRH synthesis in sheep, so sheep are short day breeders (fall breeders). Rams are also affected by photoperiod but to a lesser extent than in the ewes. In ram, there is decrease in sperm production, increase in abnormal spermatozoa and decreased fertility as photoperiod increases in the spring and early summer. In ewe, there is inactivity of the ovaries, absence of both dominant follicles and behavioral estrus (Senger, 2003).

The site or pathway of action of the effect of melatonin is still unknown. Melatonin binding sites are preferentially localized in the pars tuberalis (PT) of the adenohypophysis, which suggests that this may be the site of action; however the direct delivery of melatonin into the PT does not influence LH secretion. In contrast, melatonin delivered in the mediobasal hypothalamus mimics the effects on LH secretion of peripherally administered melatonin. This suggests that, despite

the presence of high melatonin binding in the PT, the mediobasal hypothalamus contains the physiological target site of melatonin actions on LH release. The action of melatonin on LHRH secretion appears to involve a complex circuit of interneurons and does not seem to be directly on LHRH neurons (Malpaux et al., 1996).

Dardente (2011) reported evidence for a circadian-based molecular mechanism within the pars tuberalis of the pituitary, which links the short duration melatonin signal of the long day length to the hypothalamic increase of triiodothyronine (T3) through a thyroid-stimulating hormone/deiodinase-2 paracrine mechanism. This implied that the mechanisms of T3 action within the hypothalamus are pertinent to seasonal timing.

Irrespective of the mechanism and site of action of melatonin, its administration either by daily dosing or through constant-release devices allows short days to be mimicked and therefore it can be used to control the seasonal reproduction in both the ram and the ewe (Rosa and Bryant, 2003). However, Chemineau et al. (1992) reported that for the treatment to be effective the animals must have been exposed to long days prior to receiving the melatonin. The duration of treatment with melatonin necessary to induce ovulatory cycles was reported as 14-21 and 40-60 days for rams and ewes, respectively (Haresign, 1992).

ii. Environmental Temperature as a modulating Factor

Environmental temperature plays an important role in reproductive activity as one of the modulators of the photoperiod effect on seasonality. However, environmental temperature cannot impose seasonality on reproductive activity of the ewe. Wodzicka-Tomaszewska et al. (1967) found that the annual rhythm of reproduction persisted in ewes kept under a constant photoperiodic schedule of 12 hours of light-12 hours of dark daily despite the dramatic changes

in temperature. Jansen and Jackson (1993) reported that photoperiod length does not influence the expression of circannual reproductive rhythm where episodic variations in ovarian cycle density were observed in 2 groups of ewes (ewes kept under 12 hours of light – 12 hours of dark and in those kept under 8 hours of light-16 hours of dark in a photo-chambers) under restricted temperatures. The period of circannual rhythm was similar in both groups and episodic variations in ovarian cycle density were observed in most ewes in both groups despite temperature changes within the photo-chambers where the ewes were housed. Therefore, the likely explanation is that temperature changes play a permissive role in the expression of circannual reproductive rhythms in the sheep. Temperature fluctuations may therefore serve to complete the series of stages necessary for full expression of a circannual rhythm of breeding and nonbreeding seasons in the ewe.

However, temperature might modify the onset of breeding season. Gundogan et al., (2003) reported that lowering the environmental temperature for sheep has given mixed results- earlier onset of breeding activity is favored by general protection of the ewe from high summer temperature while extremely low temperature may delay or shorten the breeding season. Ewes maintained under low temperatures during the summer period began their reproductive season earlier than those kept under temperatures typical of that season (Dutt and Bush, 1955; Godley et al., 1966), and a positive correlation was also found between mean temperature in July and the date of the onset of the breeding season in Clun ewes (Lees, 1966).

High temperature has been known to also affect fertilization, embryo survival and fetal size in sheep. Dutt (1954) and Hulet et al. (1956) reported frequently occurring morphological abnormalities in ova early in the breeding season of sheep. The primary ovum abnormalities

noted with exposure to high temperatures have been shrunken cytoplasm, enlarged cytoplasmic globules or vacuoles, ruptured vitelline membranes or ruptured zona pellucida.

Heat stress can affect fertility in ewes. Dobson et al. (2012) reported that heat stress can have a disruptive effect on endocrine interactions to cause a suppression of the GnRH and consequently the frequency and amplitude of LH pulse. Their study showed that heat stress can delay the onset of estrous behavior and increase the interval between estrus and onset of the pre-ovulatory LH surge. In rams, prolonged exposure to high temperatures can result in lack of libido and further lower sperm quality (Schoenian, 2011). As the anestrus period coincides with the summer, the high temperatures at this time and associated heat stress can lower fertility in ewes that might have spontaneous ovulations at this time or in ewes induced to breed out of season.

iii. Nutritional Status as a modulating Factor

Nutrition is considered a very important factor influencing reproductive function and the effects of nutrition is regulated through several hormonal signals such as leptin, insulin and growth hormone (Rosa and Bryant, 2003). In the Mediterranean or tropical climates, nutritional level is probably responsible for some seasonal acyclicity. However, Gordon (1997) concluded that nutritional level appears to have little effect on the onset and duration of the breeding season in both temperate and tropical breeds.

The mechanisms involved in the regulation of reproductive processes by nutrition are complex and not clearly known (Smith, 1991; Robinson, 1996). However, some studies supported the hypotheses that nutrition affects the release or clearance of gonadotropins, the clearance of progesterone, and changing the degree of response of ewes to the inhibition of estradiol and inhibin by balancing FSH secretion and gonadal feedback; these reports are based on its effect on

ovulation rate (Adams et al., 1997), pregnancy rate (Parr, 1987) and seasonality of reproduction (Boukhliq et al., 1996) respectively.

Live weight is influenced by nutrition and it has been established that ewes with higher live weights have increased fertility, higher ovulation rate, earlier onset of puberty and higher rate of embryo survival (Smith 1991; Robinson, 1996; Scaramuzzi et al., 2006; Ferguson et al., 2011). Poor performance in ewe lambs is said to occur at a live weight below 35kg at breeding while a better performance is seen above 40-45kg (Allison et al., 1975; Gaskin et al., 2005; Kenyon et al., 2005). In a study to explore the effects of nutrition on birthweight and survival of merino lambs, Oldham et al. (2011) reported an increased birthweight and survival rate of the progeny as a result of improved feeding regimen during pregnancy.

In a study carried out by Forcada et al., (1992) in Rasa Aragonesa ewes, it was reported that there is a consistent reduction in the duration of seasonal anoestrus in ewes maintained at a constant and moderately high body condition score of 2.7 and higher compared to ewes maintained at a constant lower body condition score of 2.5 and lower for two consecutive years. Also, body condition score of 2.0 to 4.0 has been said to increase the proportion of single-bearing to multiple-bearing ewes and increase conception rate in ewes during seasonal anestrus so as to achieve a higher fecundity (Gonzalez et al., 1997). These findings suggest that the duration and or depth of seasonal anestrus can be reduced in ewes that have a moderately high level of fat reserves (Forcada and Abecia, 2006).

Nutrition has been reported to influence the onset of ovarian cyclicity in postpartum ewes (Restall and Starr, 1977). The effect of nutrition on sexual activity is minimal compared to its effect on ovulation rate but severe malnutrition can cause a significant extension in the length of

seasonal anoestrous period, attributable to an earlier end of the reproductive season (Knight et al., 1983a).

In a study to determine if there is a central interaction between photoperiod and nutrition, Île-de-France ewes maintained on a restricted state (low nutritional status) had a shorter season of ovarian activity than those maintained in a well fed state (high nutritional status) due to modification in the timing of seasonal transitions between periods of ovarian activity and anestrus (Menassol et al., 2012).

Brown (1994) reported that reproductive function in young male animals seems to be more susceptible to dietary restrictions of energy and protein as severe feed restriction may cause permanent gonadal and neural tissue damage while in adults, restriction in feed intake can cause a reduction in androgen secretion and semen quality which is restorable by re-feeding previously underfed adults.

In rams, changes in nutrition lead to profound responses in testicular size; therefore the rate of sperm production largely due to changes in the size of the seminiferous tubules and the efficiency of spermatogenesis. Gonadotropin secretion is affected for only a few weeks in case of a moderate change of nutrition but testicular growth is affected for several months (Martin and Walkden-Brown, 1995).

Ram on higher planes of nutrition reach puberty at a younger age and heavier body weight as compared to those on lower plane of nutrition (Ragab et al., 1966) with delayed testicular descent, penile development and appearance of spermatozoa in the ejaculate in the latter (Pretorius and Marincowitz, 1968). According to Hotzel et al. (1997) part of the effect of nutrition on testicular growth in ram is independent of changes in GnRH secretion as differences

in the testicular growth induced by dietary treatment (lupin grain supplementation) in rams immunized against GnRH were not associated with changes in gonadotrophin or testosterone secretion.

iv. Lambing Date as a modulating Factor

Lambing date can also influence the seasonality of reproduction in sheep. An early lambing date is associated with an early onset of the subsequent breeding season. In a study to determine the effect of lambing date on the subsequent breeding season, Mitchell et al. (1997) reported that the onset and duration of ovarian activity but not its cessation were significantly affected by the previous lambing date,. According to this author, the mean dates of onset of ovarian activity for January and May lambing ewes were 5 September and 25 September while those of cessation were 5 April and 10 April, with the number of the ovarian cycles been 12.4 (s.e. 0.29) and 11.5 (s.e. 0.38), respectively. Lactating ewes that lambed in August, September and October recorded higher estrus rate than those that lambed in November and December when exposed to teaser rams for estrus detection 1-2 hours daily throughout the year (Hashem et al., 2011)

v. Social Interactions as modulating Factor.

The social interaction within a group of animals of the same species can affect various reproductive processes. In sheep, social interactions have been found between and within sexes such as male to male, female to female and male to female interactions.

In a male to male interaction, the sexual performance of future mature rams can be dependent on the social environment under which the ram lambs are reared. Rearing of rams in all male groups may increase the likelihood of male-male interaction and hinder or cause a delay in the development of sexual interest in females (Hulet et al., 1964, Price et al., 1988). Further,

separation of ram lambs from the ewe lambs between 3 and 6 months of age causes a delay in the occurrence of first copulation (Orgeur and Signoret, 1984). Maina and Katz (1997) showed that the sexual performance of rams may be enhanced by exposing them to a ram that has recently mated a ewe in estrus. It is thought that this effect is due to olfactory cues associated with mating. However observing courtship mating behaviors of other rams does not improve the sexual performance of the observing ram (Price et al., 1991).

In female-female interaction, the sudden introduction of ewes in estrus to seasonally anoestrus ewes can induce and synchronize ovulation in the anoestrus ewes and thereby bring about an early onset of the breeding season (Muir et al., 1989; Zarco et al., 1995), so also the continuous presence of cyclic ewes will elucidate same effect (O'Callaghan et al., 1994; Nugent and Notter, 1990). On the other hand, the exposure to non-cyclic female flockmates has no effect on the reproductive state of anoestrous ewes (Donovan et al., 1991).

In male-female interaction, Rosa et al., (2000) reported that exposure of rams to both oestrus and anoestrus ewes brought about an increased level of sexual activity and elevated serum testosterone concentration in the rams, with highest response found in experienced rams stimulated by sexually receptive ewes (Gonzalez et al., 1991). This phenomenon is referred to as the 'female effect'. The 'female effect' is more evident during the anestrus season and when ewes are introduced to the rams at sunrise as compared to noon (Gonzalez et al., 1988).

The ram lambs reared with the ewes during the 'adolescence phase' of development and learning, which is the period between weaning and puberty as defined by Orgeur and Signoret (1984) exhibited enhanced sexual performance while ram lambs that were isolated from ewes during this developmental phase showed abnormal and delayed male mating behavior as adults.

Also, according to Illius et al. (1976), yearling rams exposed to oestrus ewes for a period of 6 months have larger testes, higher plasma testosterone and greater sexual activity in contrast to rams isolated from ewes. However, the fact that contact with ewes by the ram lambs during rearing resulted in an improved sexual performance does not imply a successful elimination of rams with sexual orientation directed solely towards other rams (Zenchak et al., 1981, Price et al., 1988).

On the other hand, the continuous exposure of rams to ewes can have a regulatory effect on the ovarian activity in the ewes. Ewe lambs kept close to rams for 1.5 to 2 months before ovulation had their first ovulation earlier than ewes isolated from the rams (Al-Maully et al., 1991).

The acute effect of ram introduction on the seasonally anovular ewes which brings the ewes into ovulation and estrous during late anestrous or early oestrous season is referred to as 'Ram effect' (Rosa and Bryant, 2002). The effect of rams to stimulate ewes is primarily mediated by pheromones which are released by the rams (Signoret, 1991) and acts in conjunction with other behavioral stimuli exhibited by the rams. On the other hand, the ewes have sensory cues such as smell, sight, sound and touch which can be used individually for optimum effect or synergistically to achieve a maximum effect in the perception of the stimuli (Rosa and Bryant, 2002).

Watson and Radford (1960) initially documented the effect of various level of relationship between the rams and ewes and concluded that odor and sound from the rams were enough to stimulate the ewes while sight and touch were not of necessity in inducing the response. Furthermore, a study on ewes with different sensory cues ablated showed a reduction in the oestrous response of the group of ewes with impaired sense of smell as compared to other

sensory cues which suggested that oestrous response is stimulated by the rams via olfactory cues (Morgan et al.,1972).

Knight and Lynch (1980) demonstrated the presence of pheromones in the wool and wax of the rams but not in the urine by smearing wax from around the eyes and bare skin of the flanks of the rams on the nostrils of ewes in a group, applying recently clipped wool over the ewes' muzzle in another group, and spraying urine from the rams on the last group. Ovulation was stimulated in the group of ewes treated with wool and wax relative to those exposed to rams but no significant stimulatory response in ewes treated with rams' urine. These observations were further confirmed by Signoret and Lindsay (1982) and Knight et al. (1983b).

It seems that the pheromones are secreted with the suint as a mixture of oily secretion from the sudoriferous glands and viscous secretion of the odoriferous glands of the skin (Martin et al., 1986). Based on the information presented above, full physical contact with ram is not necessary for induction of a reproductive response but it is required to maximize the response of the ewes to stimuli from rams (Pearce and Oldham, 1988).

There is also a behavioral component of the ram effect. Signoret et al. (1982) reported that castrated males (wethers) with intense sexual activity, which were treated with androgen, induced ovulation more potently than sexually inactive rams treated with the same dosage of androgen.

VII. Means of overcoming Seasonality in Ewes.

i. Ram Effect

As previously noted, ram effect has been the most patent and best known phenomenon among all reproductive effects that are socially mediated in sheep reproduction and still the most investigated based on its considerable economic benefit (Rosa and Bryant, 2002).

An increase in basal LH secretion was documented in a group of ewes within 10 minutes (Martin et al. 1980), and an LH surge within 38 hours of ram introduction during anestrus (Knights et al., 2002) after a pre-conditioning period of isolation from the rams. The exact period of isolation from rams adequate to elicit the ram effect has not been established but varied with each report from as little as 2 weeks to as much as a month, and this also varied with breeds, location and season of the year (Oldham 1980; Martin et al., 1986).

An increase in the frequency of LH pulses due to maintained contact of ewes with rams during anestrus culminated in an LH surge within 54 hours after ram introduction (Chanvallon et al., 2011) and induced synchronized ovulation in anestrus ewes within 40 hours after the LH surge (Oldham et al., 1979; Martin et al., 1986; Scaramuzzi et al., 2014). This first ovulation is referred to as 'silent' ovulation because it is not accompanied by an estrous behavior. In some ewes, silent ovulation usually results in abnormal development of the first corpus luteum followed by regression after 6-7 days. Following premature regression of the CL, the ewes start a new cycle without estrus display but a normal luteal function (Oldham and Martin, 1978). The third wave of ovulation will then be accompanied by estrous behavior around 22- 28 days after ram introduction. Ewes that have normal luteal phase which last for about 10 days after the first

silent ovulation can exhibit sexual behavior around 17-20 days after initial introduction of rams (Fabre-Nys et al., 2015).

Despite the fact that secretion of LH occurred a few minutes after ram introduction, the presence of the rams with the ewes must be maintained until ovulation so as to achieve a higher percentage of ewes ovulating (Lindsay et al., 1975; Oldman and Cognie, 1980). Signoret et al. (1982) reported that there is reduction in the percentage of ewes that ovulated when the rams were withdrawn within 24 hours. However, some ewes can still return to anoestrus after one or 2 ovulations, in the presence of rams (Rosa and Bryant, 2002).

Continuous exposure of ewes to rams can affect the onset and the end of the breeding season, the duration of oestrus in mature ewes and accelerate the attainment of puberty in ewe lambs (Rosa and Bryant, 2002). According to Donovan et al. (1991), the breeding season ended 3 weeks later and the onset of the next breeding season was earlier by a month in ewes that were continuously exposed to vasectomized rams during the breeding season.

Some breeds of ewes that are less seasonal can be stimulated by rams some months before the onset of the breeding season but if they are introduced to rams in mid-anoestrous season, the ewes can easily return to the anovular condition after the first ovulations (Goodman et al., 1982). This is supported by the findings of Knight (1983) in Australia who observed 22% and 42% of Merino ewes exposed to rams in October returned into anoestrus 24 days and 50 days after ram introduction respectively. In breeds of ewes that are more seasonal, where stimulation by rams is only achievable 2 weeks to a month prior to the start of the reproductive season, cyclic activity can be maintained throughout the breeding season (Rosa and Bryant, 2002).

The effect of ram introduction has also been reported in lactational anoestrous ewes. In a study conducted by Poindron et al. (1980), most of the lactational anoestrous ewes responded with increased LH secretion and ovulation when introduced to the rams 21 days after parturition during breeding season. Also, Wright et al. (1989) discovered that an abrupt ram introduction can bring about oestrus in lactational anoestrous ewes during the breeding season as compared to the ewes that have been in continuous contact with rams for a period of 3 months.

The age and level of experience of the ewes can also play a role in the effectiveness of the ram effect. Ewes that are young in age with no prior sexual experience exhibited lower ovarian response when compared with older ewes with an initial sexual experience (Chanvallon et al., 2010). In pre-pubertal ewes exposed to rams, it was found that there is reduction by 16 days in their mean age at first oestrus and ovulation (Kassem et al., 1989; Al mauly et al., 1991), and reduction by 19 days in the mean age to conception (Kassem et al., 1989).

The effect of rams on induction of ovarian activity might be dependent on sexual performance of the rams. For example, exposure of ewes to rams with high level of sexual performance significantly increased the display of oestrus (95%) as compared with rams with low level of sexual performance (78%) (Perkins and Fitzgerald, 1994).

Knights et al. (2002) reported that ram introduction alone can induce increased LH pulse frequency, follicular development, and ovulation in fall-born ewe lambs during seasonal anestrus. Although the use of ram introduction alone to induce fertile estrus in anestrus ewes bred out-of-season has been effective, reproductive responses may be low when compared with ewes that are bred in season (Silva and Ungerfeld, 2006). Therefore, additional treatments along with the ram effect have been used to increase fertility in ewes bred during seasonal anestrus.

ii. Use of exogenous hormones

Several studies have been carried out using exogenous hormones to induce fertility in seasonally anestrous ewes. Progesterone for an example has been used as a management tool to effectively synchronize and induce estrus in ewes (Welch et al., 1984; Hamra et al., 1986; Wheaton et al., 1992).

Progesterone can be delivered orally, intravaginally, via implant or injection but intravaginal delivery of progesterone via controlled internal drug releasing devices (CIDRs) is the only approved method for use in the US. Ewes that were pretreated with progesterone via CIDRs displayed estrus behavior earlier and in a more synchronized way when compared with the ewes treated orally with Melengestrol acetate (MGA) (Daniel et al., 2001) or sponges (Welch, 1983; Welch et al., 1984). Although, the retention rates of sponges and CIDRs are similar, CIDRs are easily removed while sponges seemed to get stuck to the vaginal constriction (Welch, 1983). The mean concentrations of progesterone produced by the 2 different CIDR types (9% vs 12% progesterone), were similar but almost double the mean concentrations produced by the sponges and implants throughout the treatment period in ovariectomized ewes (Hamra et al., 1986).

Three thresholds of progestogen treatment were suggested to be required in stimulating adequate response in the ewes. First threshold is the dose of progestogen necessary for the inhibition of ovulation, followed by a second threshold of higher dose which is required for oestrus and a third with increased dose necessary for optimum fertility (Robinson, 1967; Robinson and Smith, 1967; Robinson et al., 1967).

A rapid and adequate absorption of the progestogen at the initial stage appeared to be necessary for optimum fertility, and this was achieved at the higher doses of progesterone. An increase in

progesterone dose from 500 to 1000 mg resulted in a linear increase in fertility with similar quantity of progesterone (250mg) absorbed by the ewes regardless of the dose administered within the range of 500-1200 mg (Mac Donnell and Crowley, 1978).

Exogenous hormones can also be used in conjunction with ram effect to achieve an increased fertility in anestrus ewes. Treatment with progesterone (P₄) just before ram introduction during anestrus period inhibited the untimely regression of corpus luteum in all ewes that ovulated in response to the ram (Pearce et al. 1985) and also increased estrous activity and mating in seasonally anovular ewes at the synchronized ovulation (Jabbar et al., 1994).

Injection of Barbarine ewes with 20 mg of exogenous progesterone at the exact time of ram introduction significantly shortened the number of short estrous cycles and resulted in a more synchronized estrus. Progesterone treatment increased the time from ram introduction to ovulation by about 38- 40 hours causing a delay in the preovulatory surge of LH (Lassoued et al., 1995) and ensuring that only corpora lutea that are responsive to prostaglandin are present in ewes that are undergoing estrous cycles at regular intervals upon withdrawal of progesterone (Acritopoulou and Haresign, 1980). This pretreatment with progesterone facilitates the expression of estrus at the time of the ram-induced ovulation during non-breeding season (Ungerfeld et al., 1999; Knights et al., 2001ab).

In a study to determine the effectiveness of CIDRs (polycaprolactone [PCL] insert with 0.82 g P₄) to induce fertile estrus and if CIDRs with FSH would increase prolificacy in anestrus ewes introduced to rams, Knights et al. (2000) reported that CIDRs in combination with ram introduction at CIDR removal was more effective than ram introduction alone to induce synchronized estrus and ovulation, yielded higher pregnancy rates after one or 2 service periods.

Treatment with progesterone (CIDRs) for 5 days was also reported to be as effective as for 12 days. Progesterone pretreatment in combination with estrogen and ram introduction also resulted in a high estrous response. Estrous response was greater in ewe lambs treated with estrogen after a P₄ pretreatment (81.8%) than in ewe lambs that were treated with P₄ and introduced to rams alone (45.5%; Knights et al., 2002).

Co-treatments using gonadotropin is commonly used to successfully establish tight synchrony and provide acceptable level of fertility upon natural mating (Wildeus, 2000). Most of the ewes treated with progestogens and then equine chorionic gonadotropin (eCG) which was formerly referred to as pregnant mare serum gonadotropin (PMSG) displayed estrous behavior but with an average conception rates (Gordon, 1963; Hulet and Foote, 1967; Laster and Glimp, 1974).

Equine chorionic gonadotropin (eCG) acts only like LH in equids but has FSH-like activity in non-equids. According to Lunstra and Christenson (1981), ewes synchronized with progestogen followed by eCG in both in-season and out-of-season breeding showed increased ovulation rate relative to untreated ewes that were bred in season but with marked variability and decreased lambing response. The reduced fertility was suggested to be due to low semen quality or sperm transport of rams, which is attributed to out of season, and possibility of asynchronies of timing of onset of estrus, ovulation and fertilization in the ewes (Lunstra and Christenson, 1981).

Follicular dynamics was also measured in anestrus ewes treated with medroxyprogesterone acetate (MAP) sponges for 12 days after which 750 UI eCG was given at the sponge removal. The MAP and eCG treatments resulted in the recruitment of more ovulatory follicles without any change in the rate of follicular growth, and also increased the size of the ovulatory follicles by delaying ovulation and the LH surge (Leyva et al., 1998).

In another study by Burfening and Van Horn (1970), pretreatment with 1mg of oestradiol-17 β one day prior to a 13-16 day progesterone treatment with eCG at progesterone removal brought an additional 30% of ewes into oestrus. There was also a 15% increase in pregnancy to first service in ewes pretreated with estradiol as compare to those without estradiol pretreatment. However, the long-acting biological activity of eCG can result in large number of non-ovulated follicles due to continual recruitment of antra follicles (Armstrong et al., 1983).

The effect of eCG dose on fertility ranging from 250 UI – 750 UI was investigated in both in season and out of season breeding. It was reported that there is no significant improvement on fertility by increasing the dosage of eCG administered (Ainsworth and Shrestha, 1985; Smith et al., 1988; Knight et al., 1989; Zarkawi et al, 1999, Zeleke et al., 2005). For example, Zaiem et al. (1996) treated ewes during anestrus with 3 doses of eCG (300, 450, 600 IU) following 14 day treatment with 40mg of flurogestone acetate (FGA) and reported that the fertility rates for all the 3 doses of eCG were similar (81.2 to 84.3%) but higher ($P < 0.05$) than ewes in the control group treated with FGA only (57.5%). There was also increased level of prolificacy at 450 UI (155.5%) and 600 UI (176.9%) when compared to those in the control group (130.4%) which suggested that optimum effect of eCG can be achieved between 450 to 600 IU.

Also, in a review by Baril et al. (1998), it was reported that there is declining fertility in ewes treated with eCG after a prolonged use.

Injection of Merino ewes with 100 μ g of GnRH 24 hours after the removal of MAP sponge inserted in the ewes for 12 days caused advancement in the time of ovulation during breeding season but showed no effect on the timing of ovulation during anestrus season (Ryan et al., 1992). Also, Jabbour and Evans (1991) reported a reduction in the time to estrus in cyclic Merino

ewes when they were injected with 100µg of GnRH 12 hours after the removal of MAP sponge inserted in the ewes for 12 days. Injecting GnRH to induce premature ovulations in seasonally anovulatory resulted in high frequency (58%) of corpora lutea (CL) with a short life span. However the lifespan of CL induced in the ewes injected with P₄ alone was normal (Pearce et al., 1985). Therefore, progesterone when used as part of an estrous induction protocol not only facilitate the display of estrus but it has a luteal protection role as well

P.G. 600^(R) is a product that contains 400 UI and 200 UI of eCG and human chorionic gonadotropin (hCG) per 5ml, respectively and it is approved for use in inducing estrus in pubertal gilt. A few studies have examined its use as part of the protocols for progesterone-based estrus induction system in sheep at the 5ml-dose level prescribed for swine (Wildeus, 2000). In a study conducted by Morrical et al (1995) to evaluate the influence of individual or combined use of MGA and P.G.600^(R) on the reproductive performance of ewes out of season, a 20% increase in conception rate was reported when ewes were injected with 5ml of P.G. 600^(R) at 10 days after treatment with MGA. Safranski et al. (1992) however reported that there is no significant effect on conception rate and prolificacy when P.G. 600^(R) was given after 10 days of MGA treatment but there was increased ovulation rate when compared to MGA treatment only. In another study, Windorski et al. (2009) also reported a level of recruitment, growth and subsequent ovulation of the follicles in anestrus ewes treated with P.G. 600^(R) and MGA i.e., there was an increased level of recruitment, growth and subsequent ovulation of the follicles when compared with MGA treatment alone but no difference in the ewe prolificacy or fertility between the 2 groups.

More recently, D'Souza et al. (2014) used a lower dose of P.G. 600^(R) of 3ml in non-lactating anestrus ewes as estrus induction protocol rather than the 5ml recommended in swine in an attempt to keep the level of estradiol secreted as the follicles grow from being too high and

thereby reduce fertility. The authors reported a higher estrous response, conception and pregnancy rates in the treated ewes. Additionally, a similar treatment improved fertility in fall-born ewe lambs bred during mid-anestrous but did not improve fertility in yearling females (Knights et al, 2015). Therefore, the combination of exogenous hormones and “ram effect” can act to improve reproductive performance of seasonally anestrous ewes.

VIII. Effect of Lactation on Ewe Fertility

Several studies reported that lactation increases the interval between parturition and resumption of estrous activity in spring lambing ewes (Pope et al., 1989) and also reduces fertility (Hulet and Foote, 1967). Further, lactating ewes tend to resume reproductive activity later than ewes that have their lambs weaned off earlier after lambing (Mallampati et al. 1971, Restall, 1971, Sefidbakht et al., 1971).

A slow increase in the LH levels was reported after lambing and this is indicative of a gradual physiological recovery of the pituitary (Restall and Starr, 1977) but suckling prevents the secretion of LH at early postpartum (Stellflug et al., 2001) via the secretion of endogenous opioids (Lozano et al., 1998) that are partially responsible for the negative feedback of estradiol on GnRH-LH pathway (McNeilly, 1994). There is an increase in the frequency and the duration of suckling as the lambs grow older with a 3 fold increase in suckling intensity of lambs that were unrestricted to suckle compared to those that were restricted to 3 times daily (Mandiki et al., 1990) which may be the reason for suppression of ovulation in ewes that have lactated longer.

In a study carried out in Winter and Spring lambing ewes, Restall and Starr (1977) reported that more ewes ovulated from the group of ewes that had their lambs removed at birth (72%) than in

group of ewes that were lactating for 40 days postpartum (40%) with more elevations of LH levels in the former (64%) than the latter (43.8%). Also in an experiment carried out by Pevsner et al. (2010), the presence of lambs were reported to be a depressing factor of both ovulation and estrous responses to the ram effect in lactating ewes in the spring and removing lambs from the ewes for 24 hours before stimulation may increase ovulation response.

In lactating ewes, uterine involution can be a limitation aside from the interval between parturition and resumption of cyclicity. When fall lambing ewes are maintained at high nutritional plane, uterine involution is completed by third week after lambing (Rubianes and Ungerfeld, 1993) and according to Rubianes et al. (1996), the first oestrus is seen in the ewes around 50days postpartum. In a study conducted on 50 ewes that lactated for 1, 24 and 56 days, Shevah et al. (1974) reported that 8 out of 17 ewes that suckled for 1 day displayed spontaneous oestrus 2 - 20days postpartum compared to only one out of the 33 that lactated for 24 and 56 days. All the ewes displayed estrus behavior after 12days treatment with progestagen vaginal sponges beginning from 20-24 days postpartum. The mean intervals between sponge removal and onset of estrus for ewes that were suckled lambs for 1, 24 and 56 days were 35.3 ± 1.5 , 33.2 ± 1.6 and 41.0 ± 1.5 hours respectively ($P < 0.01$).

Nearly all the lactating ewes that were treated from 3 days after lambing with various combinations of progestogen (MAP) and eCG ovulated before 15 days postpartum irrespective of the season of lambing with certain proportion (29%) showing oestrus after further treatment with MAP and eCG between days 15-26 postpartum (Restall et al., 1978). The authors further reported low fertility (17%) in spring lambing crossbred ewes when mating was induced up to 30days postpartum which improved in later mating, (30%).

Another major limitation to oestrus and ovarian resumption after parturition is the body weight loss (Mbayahaga et al., 1998) which may be suckling-induced. Haresign (1981) reported that under-nutrition resulted in reduction in body condition of ewes, reduction in the LH pulse frequency and consequently the reproductive performance of ewes.

In a study to evaluate the effect of different levels of body condition on reproductive performance of North Country Cheviot ewes, Gunn et al. (1991) reported a lower ovulation rate in ewes with low body condition scores of less or equal to 2.5 at 5 weeks prior to mating but a significant increase in ovulation, conception and lambing rates was observed in ewes with higher body condition scores. According to Molina et al. (1994), the body condition score at mating affected the fertility of the Manchega ewes ($P < 0.05$) with fertility rate exceeding 80% in the ewes with high body condition score even in the least fertile mating season (spring- summer months).

Weaning may be a useful tool in improving reproductive performance in postpartum ewes. (Ungerfeld and Sanchez-Davila, 2012). The combination of hormonal treatment with weaning at 7 or 40 days after parturition, or restricted suckling improves reproductive efficiency in Pelibuey ewes (Ronquillo et al., 2008). However, in some other studies, weaning has been reported to be unbeneficial (Warren et al., 1989; deNicolo et al., 2006) to the reproductive responses of ewes treated with exogenous hormones.

Ungerfeld and Sanchez-Davila (2012) evaluated the effect of lamb weaning on the response of postpartum ewes to oestrus synchronization or induction treatments during breeding season and reported that there is possibility of inducing fertile oestrus in lactating ewes during early postpartum and that weaning lambs early before stimulation did not improve the response of the

ewes. This was also supported by the experiment carried out by Pope et al. (1989) who recorded that ewes that suckled for 40 days during the spring had higher conception rates than ewes whose lambs were weaned at birth. A beneficial effect of lactation was reported by Hashem et al. (2011) as higher number of follicles was recorded in lactating ewes than in dry ewes during non-breeding season. This may be responsible for an increased ovulation rate, pregnancy rate and litter size in ewes bred while lactating (Pope et al., 1989; Forgarty et al., 1992).

Hashem et al. (2011) concluded that the reduction in estrus behavior was mainly due to seasonal effect rather than lactational stress. The authors reported that incidence of estrus, which was significantly affected by the season, was higher in ewes that lambed during the breeding season than those that lambed during non-breeding season. Highest proportion of estrus rate and lowest postpartum anestrus interval were achieved during the first lactation stage which represents the highest lactational strength (40-60 days) in ewes that lambed earlier in the breeding season (August, September and October) than later in the breeding season (November and December).

IX. Effect of Weaning on Lamb Growth and Survival

Total profitability in sheep enterprises is basically evaluated by sheep productivity and high productivity is achieved through optimization of reproduction in ewes, as well as lamb growth and survival (Boujenane et al., 1998). While weaning is often used to enhance early rebreeding of ewes in accelerated lambing programs and early resumption of cyclicity in lactating ewes by suppressing the effect of suckling and reducing the nutrient demand on the ewe, it involves physical separation of the dam and lamb and modification to the feeding habit of the lambs, both of which can induce stress in both the mother and the young (Orgeur et al., 1998).

Stress, such as the separation of mother and young causes the activation of the hypothalamic-pituitary-adrenal system and the autonomic nervous system so as to make necessary adjustment in response to the social perturbation (Mormede, 1995). Stress can have a deleterious effect on animal health and welfare by inducing a malfunctioning of the immune system. In a study to measure the consequences of 2 weaning protocols; progressive weaning- daily separation which increased in duration starting when lambs are 3.5 weeks old till definitive weaning at 3 months, and sudden weaning- weaning abruptly at 3 months, Orgeur et al (1998) reported that none of the procedures seemed highly stressful in sheep, with the most striking difference been the vocalizations of the animals which was suppressed after 2 days in sudden weaning as compared to its persistence throughout all the periods of temporary separation in progressive weaning. Lambs showed higher level of sensitivity to parasite infestation in the progressive weaning procedure which may be explained to be as a result of greater sensitivity to parasite at a younger age. This may also be due to compromised immune system consequential to stress from repetitive daily dam-lamb separation until weaning in the progressive weaning procedure (Karakus, 2014).

Watson and Gill (1991) reported that lambs suckled for 3 months have much better prospects than weaned lambs when faced with a substantial parasite challenge. In a study carried out by these authors, the mean fecal egg count for weaned lambs at 12 weeks was twice that for controls when lambs were experimentally infected with 5,000 *haemonchus contortus* and 10,000 *Trichostrongylus colubriformis* larvae at 8 weeks. This was attributed to the possibility of a better immune response in the suckled lambs as a result of milk feeding. In another study, Iposu (2007) concluded that suckling may lower the establishment of nematode larvae through direct effect of

milk and also enhance development of host immunity to infection. This was further supported by Zeng et al. (2001) who established that worm burdens were lower in milk-fed lambs.

In a study conducted by Vecihi et al. (2009) to investigate the effect of 45, 60 and 75 days of weaning on growth and survival rates in Awassi lambs, it was reported that survival rate was significantly lower in the group of lambs weaned at 45 days old when calculated on days 120 and 210 compared to the other 2 groups, and also the live weight gain was lower in lambs weaned at 45 days when calculated on day 210. According to Knights et al., (2012) higher levels of mortality observed in lambs that were weaned early may be partly due to reduced immune function.

High growth rate in lambs is dependent on an adequate supply of milk and availability of high quality forage (Geenty and Sykes, 1983). Dikmen et al. (2007) reported that there is a tendency for a higher live weight in lambs kept on their mother to suckle for the first 60 days of lactation compared to those kept on their mother for only 9 hours daily from 3 days postpartum. Early weaning between 7 -8 weeks caused about 25% reduction in the live-weight gain of the lambs compared to weaning at 14-16 weeks which grew 30% faster (Dynes et al., 2006). Lambs that were weaned early showed a continuous reduction in their growth rate which persists for the duration of about 2 months while an increased growth rate has been reported in lambs that were suckled continuously or un-weaned as compared to lambs weaned early (Simpson-Rastle et al., 2012; Knights et al., 2012).

PROBLEM STATEMENT

Increasing the frequency of lambing can increase lifetime productivity of the ewe and profitability of sheep operations. However, the seasonality of reproduction in sheep limits lambing to once per year which contributes to the low productivity of the ewe in traditional production systems. Attempts to increase the frequency of lambing by inducing fertile estrus in dry anestrous ewes with use of progesterone pre-treatment prior to ram introduction have been attempted (Knights et al., 2001ab; D'Souza et al., 2014). These approaches capitalize on the ram-induced secretion of gonadotropins and ovulation, the “ram-effect”. However, early weaning of lambs have resulted in reduced growth and productivity of the weaned lambs (Schichowski et al., 2010; Knights et al., 2011; Simpson-Rastle et al., 2012; Karakus, 2014;), which negatively impacts any gains in productivity earned through increase frequency of lambing.

While early re-breeding of lactating ewes is desirable and can be achieved in ewes that lamb in the fall, attempts to rebreed lactating ewes during the non-breeding season has not been successful. Suckling has been known to reduce secretion of gonadotropin and might impact the ram-induced release of these hormones. Whether the poor reproductive response of lactating ewes during anestrus is due to a failure of rams to induce the ram effect response, and whether reproductive outcome can be improved by providing exogenous gonadotropins have not been fully evaluated.

Loss of body weight (lower body condition) irrespective of the season is associated with lactation due to the nutrient demand placed on the ewe by the suckling lambs. Loss of body weight and low body condition score are also known to suppress LH pulse frequency and

reproductive performance. The effect of nutritional supplementation on its own and in combination with gonadotropin supplementation is yet to be fully evaluated.

Tropical hair sheep breeds are capable of breeding year round under tropical conditions and are less seasonal in temperate regions. The Katahdin breed of sheep was developed with the inclusion of tropical hair sheep genetics. However, little data exist on the ability of this breed to conceive during the anestrus period and in particular while lactating.

Therefore, the overall objective of this study was to evaluate the effect of lactation status, and gonadotropin and nutritional supplementation on the induction of LH surge and subsequent fertility on early rebreeding of Katahdin ewes during the seasonal anestrus period.

INTRODUCTION

Increasing the frequency of lambing using accelerated lambing programs holds great potential for increasing the productivity and profitability of sheep operations. Accelerated lambing programs often involve rebreeding of ewes by 3 months after lambing so as to achieve a lambing interval of 8 instead of 12 months. To rebreed ewes in the early postpartum period, lambs are typically weaned from the dams as part of the management strategy (Pevsner et al., 2010). However, lambs separated from their dams before 3 months of age may be more susceptible to diseases due to underdeveloped immune system and may experience stress which results in reduced feed intake and growth rate (Karakus, 2014; Dwyer, 2008; Schichowski et al., 2010; Knights et al., 2011). Therefore, developing reproductive management strategies to successfully rebreed ewes while they are still suckling their lambs would increase the frequency of lambing without compromising growth and health of lambs.

The abrupt introduction of rams during anestrus induces the “ram effect” response, which is characterized by increased secretion of the gonadotropins, follicle growth and ovulation in dry (non-lactating) ewes. The ram effect can also induce fertile estrus if ewes are pretreated with progesterone only (Knights et al., 2001ab). Further, small improvements in reproductive outcome in dry mature (D’Souza et al., 2014) and nulliparous (Knights et al., 2015) ewes bred during anestrus have been achieved by providing exogenous gonadotropins at the time of progesterone withdrawal and ram introduction. The reproductive outcomes following attempts to rebreed lactating ewes during seasonal anestrus have largely been unsuccessful (Whiteman et al., 1972; Mandiki et al., 1990; Cognie et al., 1975; Silva and Ungerfeld, 2006; Martemucci and

D'Alessandro, 2010). Whether the poor reproductive performance in lactating ewes bred during seasonal anestrus is due to a muted “ram effect” response and whether supplementation with gonadotropins can improve reproductive outcome in lactating ewes have not been fully investigated.

Significant loss of body weight of ewes can occur during lactation, and loss in body weight can delay the resumption of estrous cycles and early rebreeding of females (Mbayahaga et al., 1988). Feed supplementation can be used to minimize the loss in body weight during lactation. Ewes maintained on a higher plane of nutrition had a shorter duration of anestrus and resumed ovarian activity earlier compared to those maintained on a lower plane of nutrition (Menassol et al., 2012). The effect of feed supplementation on the “ram-effect” response and fertility of lactating ewes bred during anestrus has not been fully established.

Therefore, the objectives of this study were: 1. To evaluate the effect of progesterone gonadotropin supplementation and lactation status on the ram-induced LH surge. 2. To evaluate the specific effect of gonadotropin supplementation and lactation on the ram-induced LH surge, and fertility. 3. To compare the independent and combined effects of gonadotropin and nutritional supplementation on the ram-induced LH surge and fertility in lactating ewes.

MATERIALS AND METHODS

Location and Animals

This study was carried out at the West Virginia University Farm in Wardensville, WV located at 39.10°N, 78.58°W, using mature Katahdin ewes during Summer (June) of 2014 and 2015. Rams and ewes were separated for at least a month prior to the start of the experiment. Rams were introduced to the ewes in a confined pasture to ensure proper stimulation at a ram to ewe ratio of not less than of 1:9. The rams were joined with the ewes for about 40 days into the experiment starting from the day of controlled internal drug releasing device (CIDR, containing 300 mg of progesterone; InterAg; Hamilton, New Zealand) removal.

Experimental Approach

All procedures were approved by the West Virginia University Animal Care and Use Committee **(PROTOCOL # 14-0604)**.

Experiment 1

A 2x2 factorial arrangement of treatments was used to evaluate the effects of an estrus induction protocol and lactation status on the ability of rams to induce an LH surge in ewes during the anestrus period. Ewes (N =32) that were either separated from their lambs at least 3 months earlier and were non-lactating/dry (n =16; DRY) or were being suckled by their lambs (lactating; n=16; LACT) were randomly assigned to receive the estrus induction protocol (EIP) or no further treatment (CON). The estrus induction protocol was previously shown to be effective in getting dry ewes to breed out-of-season (D'Souza et al., 2014; Knights et al., 2015) and consisted of a 5 day pre-treatment with progesterone delivered by a controlled internal drug releasing device (CIDR, 0.3 g progesterone; InterAg; Hamilton, New Zealand) and gonadotropin stimulation (GS) using a single injection of a gonadotropin mixture PG 600 (3 mls i.m., 240 IU

PMSG + 120 IU hCG). Ewes in the four treatment groups (DRYEIP, DRYCON, LACTEIP and LACTCON, n = 8 each) were introduced to rams with marking harnesses when progesterone/CIDRs were removed from the EIP treated ewes.

Experiment 2

Experiment 2 was similar to experiment 1 and was designed to evaluate the effects of lactation status and gonadotropin supplementation on the ram-induced LH surge and subsequent fertility. Briefly, lactating (LACT; n = 58; approximately 68.7 ± 1.3 days postpartum) and dry ewes (DRY; n = 22) were treated with CIDR devices for 5 days. On the day of insert removal, ewes in each lactation status were randomly selected to receive GS (DRYGS; n = 11, LACTGS; n = 31) or no further treatment (DRYCON; n = 11, LACTCON; n = 27). Lactating ewes were balanced for birth type. All ewes were then exposed to rams. Blood samples were collected from a subset of ewes (N = 33) for analysis of the LH profile and pregnancy diagnosis was conducted on all ewes by ultrasonography 30 days later.

Experiment 3

In experiment 3, the effects of level of nutritional supplementation and GS on ability of rams to induce an LH surge and on the subsequent fertility of ewes during the anestrus period was evaluated. At approximately 38 ± 1.3 days postpartum ewes (N = 87) were randomly assigned within rearing type to be given a high or low level of grain supplementation for 30 days (HIGH; 1.14kg and LOW; 0.68 kg, of a 13.3% crude protein (CP) ration; equivalent to 50% and 30% CP respectively). At the end of the 30 day supplementation period all ewes were treated with CIDR devices for 5 days and half of the ewes assigned to each grain supplementation level were randomly assigned to receive GS or no further treatment prior to exposure to rams. Blood

samples were collected from a subset of ewes (N =22) for analysis of the LH profile and pregnancy diagnosis were conducted on all ewes as described in experiment 2.

Estrus Detection

Rams were fitted with marking harnesses when introduced to the ewes so as to monitor sexual activity. The occurrence of estrus was determined by observation of ewes for raddle marks from day 0- 4 after the introduction of ram.

Blood Collection and LH Quantification

Blood samples were taken via jugular venipuncture. In the first experiment, blood samples were taken every 4 hours for 40 hours beginning at ram introduction (0 h). In experiments 2 and 3 blood samples were taken 2 h prior to ram introduction (-2h), at ram introduction (0h) and at 2, 4, 6, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48 hours after ram introduction. All 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 kept frozen at -20⁰C until used for quantification of LH. Using a radioimmunoassay that has previously been validated in sheep (Goodman et al., 2004), LH was measured in duplicate using 100 µl of plasma and expressed in terms of NIH-LH-S12. The minimal detectable concentration of LH in these assays averaged 0.10 ng/tube; intra- and inter-assay coefficients of variation were 15.0% and 18.2%, respectively.

Pregnancy Diagnosis

The ewes were examined via trans-rectal ultrasonography using an Aloka 500 (Corometrics medical Systems, Wallingford, CT, USA) with a 7.5MHz linear trans-rectal probe, 30 - 35days after ram introduction to determine pregnancy to the first service period.

Statistical analysis

An analysis of variance (ANOVA) was conducted using the GLM procedure of SAS (Statistical Analysis System version 9.4 for Windows; SAS Institute, Cary, NC, USA) with fixed effects of lactation status (LS) and EIP (Experiment 1), LS and GS (Experiment 2), level of grain supplementation and GS (Experiment 3) and their interactions. Response variables included proportion of animals with a detectable LH surge, time to peak of the LH surge (hours from introduction of rams to the highest LH values in animals in which an LH surge was detected), and peak concentration of LH (highest LH values in animals in which an LH surge was detected). Chi-square analysis using the PROC FREQ procedure of SAS was used to evaluate the following response variables; proportion of ewes with a detectable LH surge, proportion of ewes detected in estrus; proportion of ewes in which an LH surge (the first sample in which concentration of LH exceeded the base line after introduction of rams ; mean of first 2 samples, by two standard deviations) was detected, pregnancy and conception rates to the first service period (number of ewes pregnant at pregnancy diagnosis as a percentage all ewes exposed to rams and of ewes detected in estrus, respectively). When appropriate, Fisher's exact test of independence was used. Least square means were calculated for all variables and differences were considered significant at $P \leq 0.05$ and tending to be different at $0.05 < P \leq 0.1$.

RESULTS

Experiment 1

In experiment 1, the proportion of ewes with a detectable LH surge was greater ($P = 0.0001$; 93.75% vs. 18.75%; figure 2) in ewes receiving the EIP than in ewes exposed to rams only, but was not affected by lactation status. In animals with a detectable LH surge, the mean peak concentration of LH and time to peak concentration (68.1 ± 25.5 ng/ml and 35.5 ± 2.7 h, respectively) was not affected by lactation status, the use of an EIP or their interaction.

Experiment 2

In Experiment 2 the mean proportion of ewes with a detectable LH surge was 72.7% and tended to be significantly higher in ewes receiving (87.1%) than in ewes not receiving gonadotropin stimulation (57.2%; $P = 0.07$; Table 1; figure 3). There was no effect of treatments on the peak or time to peak concentration of LH in ewes with a detectable LH surge (Table 1).

The estrous response was not affected by lactation status but was higher in ewes receiving (63.9 ± 8.7) than in ewes not receiving gonadotropin stimulation (38.6 ± 8.7 ; $P = 0.0450$; Table 1; figure 4). Gonadotropin stimulation tended to increase estrous response in lactating but not in dry ewes (LACT X GS, $P = 0.1$; figure 4). Conception and pregnancy rates were lower in lactating than in dry ewes ($P = 0.0172$, 0.0131 , respectively) but were not affected by gonadotropin stimulation (figures 5 and 6, respectively).

Experiment 3

Experiment 3 was conducted with only lactating ewes. The proportion of ewes with a detectable LH surge was not affected by level of nutritional supplementation but tended to be higher in ewes receiving than in ewes not receiving gonadotropin stimulation ($P = 0.06$; 91.7 ± 31 vs $55 \pm 13\%$; Table 2; figure 7). In ewes with a detectable LH surge, neither the peak concentration nor the time to peak LH concentration was affected by treatments (Table 2). The pattern of LH secretion in ewes with a detectable LH surge and ewes in which an LH surge was not detected is shown in figures 8(a, b, c) and 8(d, e), respectively. Figures 9 (a, b) show the pattern of LH secretion of ewes that were detected in estrus and diagnosed pregnant but without a detectable LH surge.

The estrous response was higher in ewes receiving than in ewes not receiving gonadotropin stimulation ($P=0.009$) and tended to be higher ($P=0.1$) in ewes on the high compared to the low level of nutritional supplementation (figure 10). There was no effect of treatments on conception rate, however, the pregnancy rate was higher in ewes receiving the high compared to the low level of supplementation ($P = 0.05$) and in ewes receiving compared to those not receiving gonadotropin stimulation ($P = 0.003$; figure 11).

Table 1 (Experiment 2): Effect of Lactation and Gonadotropin Stimulation on Characteristics of the Ram-Induced LH Surge and Pregnancy; Means $\pm$ SEM.

Variable	Lactating			Dry			P-Value		
	Gonadotropin +	Gonadotropin -	Mean	Gonadotropin +	Gonadotropin -	Mean	Lactation (L)	Gonadotropin (G)	Interaction (L X G)
N(*)	31 (6)	27 (5)	58 (11)	11 (11)	11 (11)	22	-	-	-
LH Surge (%)	83.3 $\pm$ 18	60 $\pm$ 20	71.7 $\pm$ 13	90.9 $\pm$ 13	54.5 $\pm$ 13.3	72.7 $\pm$ 9	NS	0.07	NS
LH Surge Peak Time (h)	38.4 $\pm$ 7.7	40 $\pm$ 6	39.2 $\pm$ 3.8	34.4 $\pm$ 3.3	38.7 $\pm$ 4.3	36.5 $\pm$ 2.7	NS	NS	NS
LH Surge Peak Conc. (ng/ml)	97.4 $\pm$ 26	95.4 $\pm$ 34	96.4 $\pm$ 21	82.2 $\pm$ 18	93.9 $\pm$ 24	88 $\pm$ 15	NS	NS	NS
Estrus	67.9 $\pm$ 9	22.2 $\pm$ 10	45.3 $\pm$ 7	60.0 $\pm$ 15.0	54.5 $\pm$ 14.3	57.3 $\pm$ 10	NS	0.0450	0.1
Conception rate (%) ^a	57.9 $\pm$ 9.6	60 $\pm$ 19	58.9 $\pm$ 10	100 $\pm$ 18	100 $\pm$ 18	100 $\pm$ 12	0.0172	NS	NS
Pregnancy rate (%) ^b	39.3 $\pm$ 9	13.6 $\pm$ 10	26.5 $\pm$ 7	60.0 $\pm$ 14.4	54.5 $\pm$ 13.8	57.3 $\pm$ 10	0.0131	NS	NS

(*) Number in parenthesis represents the number of animals that were bled for determination of LH concentration.

a Number of ewes diagnosed as pregnant on day 30 as a percentage of the ewes that were observed to be in estrus

b Number of ewes diagnosed pregnant on day 30 as a percentage of all ewes exposed to rams

Table 2 (Experiment 3): Effect of Level of Supplementation and Gonadotropin Stimulation on Characteristics of the Ram-Induced LH Surge and Pregnancy; Means $\pm$ SEM.

Variable	Low Supplement			High Supplement			P-Value		
	Gonadotropin	Gonadotropin	Mean	Gonadotropin	Gonadotropin	Mean	Supplement	Gonadotropi	Interactio
	+	-		+	-		(S)	n	n (S X G)
N	28 (6)	22 (5)	50 (11)	18 (5)	19 (6)	37 (11)	-	-	-
(*)								(G)	
LH Surge (%)	83.3 $\pm$ 18	50 $\pm$ 18	71.7 $\pm$ 13	100 $\pm$ 18	60.0 $\pm$ 20	75 $\pm$ 13	NS	0.06	NS
LH Surge Peak Time (h)	38.4 $\pm$ 2.6	40 $\pm$ 3.3	39.2 $\pm$ 2.1	34.4 $\pm$ 2.6	38.7 $\pm$ 3.3	36.5 $\pm$ 2.1	NS	NS	NS
LH Surge Peak Conc. (ng/ml)	97.4 $\pm$ 25	95.4 $\pm$ 33	96 $\pm$ 21	101 $\pm$ 25	64.7 $\pm$ 33	83 $\pm$ 21	NS	NS	NS
Days Postpartum	65.9 $\pm$ 2.3	69.5 $\pm$ 2.6	67.7 $\pm$ 1.7	70.5 $\pm$ 3	70.1 $\pm$ 2.8	70.3 $\pm$ 2	NS	NS	NS
Estrus	62.5 $\pm$ 8.3	22.2 $\pm$ 9.1	42.4 $\pm$ 6.2	72.2 $\pm$ 11.1	45.0 $\pm$ 10.6	58.6 $\pm$ 7.7	0.1	0.0009	NS
Conception rate (%)	55 $\pm$ 11	66.7 $\pm$ 20	60.8 $\pm$ 11	83.3 $\pm$ 14	55.5 $\pm$ 16	69.4 $\pm$ 11	NS	NS	NS
Pregnancy rate (%)	35.5 $\pm$ 8	14.8 $\pm$ 8.6	25.1 $\pm$ 5.9	62.5 $\pm$ 11.2	25 $\pm$ 10	43.8 $\pm$ 7.5	0.05	0.003	NS

(*) *Number in parenthesis represents the number of animals that were bled for determination of LH concentration.*

a *Number of ewes diagnosed as pregnant on day 30 as a percentage of the ewes that were observed to be in estrus*

b *Number of ewes diagnosed pregnant on day 30 as a percentage of all ewes exposed to rams*

Figure 2. The proportion of dry (DRY) and lactating (LACT) ewes treated with (EIP) or without (CON) an estrus induction protocol during anestrus showing a LH surge (Experiment 1). LH surge was calculated as the first sample in which concentration of LH exceeded the baseline (mean of first 2 samples) by 2 standard deviations.

Figure 3. The proportion of dry (DRY) and lactating (LACT) ewes treated with (GS) or without (CON) a gonadotropin mixture following a 5-day progesterone pre-treatment during anestrus showing a LH surge (Experiment 2). LH surge was calculated as the first sample in which concentration of LH exceeded the baseline (mean of first 2 samples) by 2 standard deviations.

Figure 4. Estrous response in dry (DRY) and lactating (LACT) ewes treated with (GS) or without (CON) a gonadotropin mixture following a 5-day progesterone pre-treatment during anestrus (Experiment 2).

Figure 5. Conception rate in dry (DRY) and lactating (LACT) ewes treated with (GS) or without (CON) a gonadotropin mixture following a 5-day progesterone pre-treatment during anestrus. Conception rate was calculated as the proportion of ewes diagnosed to be pregnant of the ewes observed in estrus following introduction of ram (Experiment 2).

Figure 6. Pregnancy rates in dry (DRY) and lactating (LACT) ewes treated with (GS) or without (CON) a gonadotropin mixture following a 5-day progesterone pre-treatment during anestrus. Pregnancy rate was evaluated as the proportion of ewes diagnosed to be pregnant of the ewes exposed via transrectal ultrasonography 30days after ram introduction (Experiment 2).

Figure 7. Effect of gonadotropin stimulation (GS) and level of feed supplementation (SUPP) on the proportion of ewes showing a LH surge following ram introduction during anestrus (Experiment 3).

(a)

(b)

(c)

(d)

(e)

Figure 8. (a, b, c) Pattern of LH secretion in ewes #6674, #6402 and #6433 with detectable LH surge, (d, e) Pattern of LH secretion in ewes # 6882 and #6941 without detectable LH surge. The time of bleed (h) represents the 15 different time periods when blood was collected in experiment 2 and 3 ; -2 to 48 hours after ram introduction.

Figure 9. (a , b). The pattern of LH secretion in ewes # 6513 and #6432 detected in estrus, respectively and was pregnant but without a detectable LH surge. The time of bleed (h) are the various point in time (h) at which blood was collected from the ewes as stated in Figure 8 above .

Figure 10. Effect of gonadotropin stimulation (GS) and level of feed supplementation (SUPP) on the estrous response in lactating ewes during anestrus (Experiment 3).

Figure 11. Effect of gonadotropin stimulation (GS) and level of feed supplementation (SUPP) on pregnancy rate in lactating ewes during anestrus (Experiment 3). Pregnancy rate was evaluated as the proportion of ewes diagnosed to be pregnant via transrectal ultrasonography 30days after ram introduction of the ewes exposed.

DISCUSSION

Increasing the frequency of lambing requires that ewes are rebred early postpartum regardless of the season. Fall-lambing ewes can be successfully rebred early in the postpartum period (Poindron et al., 1980; Wright et al., 1989; Ungerfeld and Sanchez-Davila, 2012) when the photoperiod is still stimulatory to reproduction. However, early rebreeding of lactating ewes during seasonal anestrus has been less successful (Cognie et al., 1975; Martemucci and D'Alessandro, 2010). During seasonal anestrus, LH pulsatility (Karsch et al., 1980), amplitude of LH pulses, and pituitary LH levels are lower than during the breeding season (Clarke and Cummins, 1985). Further, in lactating ewes, the inhibitory effect of suckling and lactation might further inhibit the secretion of gonadotropins. Lactation suppresses LH secretion as a result of a profound suppression of GnRH release from the hypothalamus and a decrease in pituitary responsiveness to GnRH stimulation in the presence of a large suckling stimulus in rats (Fox and Smith, 1984). Suckling also inhibits the secretion of LH in ewes (Stellflug et al., 2001). Weaning can eliminate the suppressive effect of lactation on reproduction and facilitate rebreeding of ewes in the early postpartum period (Pevsner et al., 2010) by removal of the suckling effect and reducing the nutrient demand on the ewes. However, weaning has been known to affect the growth and survival of lambs (Knights et al., 2012; Simpson-Rastle et al., 2012; Ferda, 2014).

Successful breeding of ewes during the non-breeding season is dependent on the ability of rams to induce increase secretion of gonadotropins culminating in an LH surge and ovulation, the “ram-effect” (Knights et al., 2002). Further, fertile mating can be induced by progesterone pre-treatment with or without gonadotropin stimulation at the time of progesterone withdrawal and introduction of rams (Jabbar et al., 1994; Knights et al., 2001ab; D’Souza et al., 2014, Knights et al., 2015). This raises the possibility that the poor fertility observed in lactating ewes during

seasonal anestrous might be due to the inability of rams to induce an LH surge and that the reported positive effects of exogenous gonadotropins might be due to an enhancement of the proportion of ewes ovulating. In experiments 1 and 2, neither the proportion of ewes with a detectable LH surge nor the peak and timing of the LH surge following ram introduction were affected by lactation status. Lactation status did not affect the proportion of ewes displaying estrus, however, both the pregnancy rate and conception rate were higher in dry ewes. Resumption of reproductive activity occurred sooner in weaned ewes as compared to lactating ewes (Mallampati et al.1971; Restall, 1971) and low fertility was also reported in lactating (Hulet and Foote, 1967) and early postpartum ewes (Restall et al., 1978). Assuming a similar proportion of dry and lactating ewes ovulated, the current finding suggests that the environment necessary for fertilization and or maintenance of the early embryo might be compromised in lactating ewes bred during seasonal anestrous.

Gonadotropin stimulation increased or tended to increase the proportion of ewes with a detectable LH surge following introduction of rams independent of lactation status or level of nutritional supplementation. The mean peak LH concentration and the time to peak concentration in ewes with detectable LH surge in this study were not affected by any of the treatments or their interaction in this study. The time to peak concentration of LH surge in this study is similar to what was reported in ewes introduced to rams during late anestrus (Chanvallon et al., 2011) and during the mid-breeding season (Ungerfeld et al.,2005). The mean peak LH concentration in this study is comparable to the finding of Knights et al. (2002) in progesterone pretreated, ram-induced ewes during anestrous. As noted earlier, both pituitary content and gonadotropin secretion is suppressed during anestrous which might explain why the proportion of ewes with a detectable LH surge following ram introduction was lower in ewes not receiving, than those

receiving, GS. These findings suggest that a major benefit of using exogenous gonadotropins in estrus induction protocols might be to increase the proportion of ewes ovulating.

Gonadotropin stimulation increased the estrous response and the magnitude of the effect tended to be greater in lactating ewes (Experiment 2), but was independent of the level of supplementation (Experiment 3). The pregnancy rate in lactating ewes was also increased by GS (Experiment 3). Interestingly GS did not affect conception rate, which indicates that the increases in estrus response, as measured by evidence of mating, accounts for the higher pregnancy rate observed in gonadotropin supplemented females. Estrogen production or the responsiveness to estrogen might be compromised in lactating ewes during anestrus (Shevah et al., 1974) necessitating additional gonadotropin support beyond that induced by ram introduction.

Lactation has a high metabolic demand and can result in significant weight loss (Mbayahaga et al., 1998), and lower body condition scores which in turn reduce the secretion of gonadotropins (Haresign, 1981), delays and reduces the amplitude of the LH surge (Smart et al., 1994), which prevents ovulation (McNeilly, 1997) and negatively impacts fertility (Fletcher, 1973; Hunter and Van Aarde, 1973; McNeilly, 2001). Higher live weights due to an improved plane of nutrition can increase ovulation rate, fertility and embryo survival (Smith, 1991, Scaramuzzi et al., 2006; Ferguson et al., 2011, Oldham et al., 2011). Mennasol et al. (2012) reported that feed supplementation (high plane of nutrition) can compensate for the weight loss during lactation and also bring about a resumption of ovarian activity. There is also a consistent reduction in the duration of seasonal anestrus in ewes maintained at higher body condition score (≥ 2.7 ; Forcada et al., 1992). In the current study, increasing the plane of nutrition of lactating ewes early postpartum did not increase the proportion of females with a detectable LH surge which may be

due to the neuroendocrine effect of suckling stimulus. However animals on the high plane of nutrition had higher pregnancy rates, and tended to have a higher estrous response. Together these findings suggest that increasing the plane of nutrition might not be necessary to increase the proportion of females ovulating but might be acting to increase the expression of estrus and early embryonic survival.

Across experiments almost 50% of lactating Katahdin ewes exhibited an LH surge following ram introduction during anestrus without gonadotropin stimulation. Despite this, the estrous response and pregnancy rate were low which suggests that the combined negative effect of lactation and anestrus season is still evident in Katahdin sheep.

Previous studies have not fully evaluated the potential synergistic effect of gonadotropin and nutritional supplementation on rebreeding lactating ewes early postpartum. In the current study the interaction between plane of nutrition and gonadotropin stimulation on pregnancy rates in lactating ewes was not significant. However, the 63% first service pregnancy rate achieved with the use of a high plane of nutritional supplementation and gonadotropin stimulation was comparable to that achieved in dry ewes during anestrus (Tritschler et al., 1991; Knights et al., 2001a; Dogan and Nur, 2006; Knights et al., 2015) and during the breeding season (Ungerfeld and Sanchez-Davila, 2012). In conclusion, during anestrus, early postpartum fertility is reduced by lactation in Katahdin ewes, but fertility in lactating ewes can be increased to that of dry ewes with nutritional supplementation and by providing exogenous gonadotropin as part of the EIP.

IMPLICATIONS

Nutritional supplementation with exogenous gonadotropin effectively improved fertility rate in lactating ewes in their early post-partum period and can be used to successfully breed lactating ewes during anestrous season.

The combination of both treatments may therefore be appropriate for use in accelerated lambing program. Rebreeding lactating ewes in spring (non-breeding season) would be useful to avoid early weaning of lambs which is reportedly deleterious to the lambs.

On annual production basis, there is an increase in the cost of production associated with out-of-season breeding. However, this can be offset by;

- i. Increasing the proportion of ewes lambing
- ii. Higher market value (selling prices) of lambs, as a result of declining lamb sales during the winter and spring (Singh-Knights et al., 2004).

Therefore, increasing frequency of lambing by breeding lactating ewes out-of-season can increase the productivity and profitability of sheep operation and so contribute to revitalizing the sheep industry in the US.

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