Effect of biostimulation on the duration of postpartum anestrus in hair sheep: A review

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ABSTRACT
Objective: To describe the effect of biostimulation, understood as the male effect and suckling management, on the reestablishment of ovarian activity during the postpartum anestrus in hair sheep.
Design/methodology/approach: A comprehensive review and analysis of scientific literature were conducted, focusing primarily on the biostimulatory effects of the male and the influence of suckling on reproductive activity during the postpartum anestrus in ewes.
Results: Suckling during the postpartum period induces a marked inhibition of reproductive activity. The sudden introduction of the male into pens containing lactating females elicits a positive stimulus for the reactivation of ovarian activity during postpartum anestrus. The abrupt exposure of ewes to the male following parturition, coupled with the limitation of mother-lamb contact during the postpartum period, significantly reduces the interval between parturition and first ovulation in hair sheep.
Study limitations/implications: There is limited research on the physiological effect of the male during postpartum anestrus and its association with the resumption of ovarian function in ewes, as well as its potential interactions with the inhibitory effect of suckling on reproductive activity.
Findings/conclusions: The positive stimulus reflected by an increase in the pulsatile secretion of luteinizing hormone (LH) exerted by the male during postpartum anestrus is more effective, can be prolonged, and may induce ovulation when suckling is managed. Specifically, restricting contact between the ewe and the lamb for a defined period helps mitigate the negative effect, preventing the suppression of LH pulsatility. According to current literature, controlled suckling is among the most effective strategies to shorten the parturition-to-first-ovulation interval in hair sheep.

Keywords: Male effect, suckling, postpartum anestrus, ewes.

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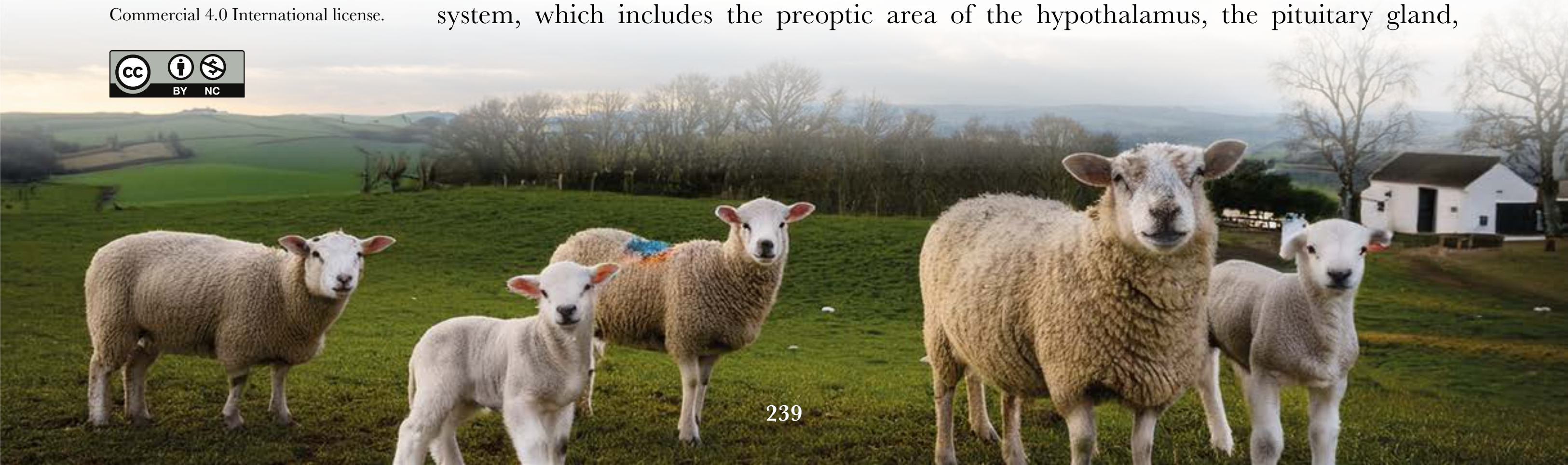
INTRODUCTION

Reproductive activity is a complex physiological process regulated by a series of neuroendocrine mechanisms modulated by both endogenous and exogenous factors. These factors interact dynamically to either stimulate or inhibit the pulsatile secretion of gonadotropin-releasing hormone (GnRH), the central axis of the reproductive system, which includes the preoptic area of the hypothalamus, the pituitary gland,



and the gonads (Malpaux *et al.*, 1996). Among the main factors that negatively affect reproductive efficiency in ruminants is the prolongation of the interval between parturition and the first ovulation, a condition with significant economic implications for livestock production systems. The primary cause of this postpartum inhibition is the suckling stimulus, determined by the frequency and intensity of nursing —that is, the number of events and the duration of each suckling episode exerted by the offspring on the mammary gland (Pérez-Hernández *et al.*, 2002; Camacho-Ronquillo *et al.*, 2008). Numerous scientific studies have addressed strategies to reduce the lambing interval in sheep, focusing mainly on lactation management, including practices such as early weaning, temporary weaning, restricted suckling, and controlled suckling. Additionally, interventions based on socio-sexual stimuli, photoperiod modulation, and nutritional management improvements have also been evaluated. In small ruminants inhabiting temperate and subtropical regions, reproductive seasonality constitutes a biological limitation that affects the availability and production of food and livestock derivatives throughout the year (Ramírez-Ramírez *et al.*, 2021). To mitigate the effects of this seasonality, exogenous reproductive manipulation strategies have been developed to schedule parturitions outside the natural mating season, ensuring continuous production or targeting specific periods. One such strategy is out-of-season mating, achieved by stimulating male reproductive activity to trigger, through the so-called "male effect," ovarian reactivation in anestrous females (Martin *et al.*, 2004; Hawken & Martin, 2012). In this context, the aim of this literature review is to analyze the role of biostimulation particularly the male effect and suckling management in modulating the duration of postpartum anestrus. This strategy represents a sustainable alternative for reproductive management, as it reduces dependence on exogenous hormonal treatments and contributes to improving reproductive efficiency in sheep production systems.

Reproductive season

Reproductive activity in small ruminants is finely regulated by the interaction between physiological processes and neuroendocrine mechanisms integrated within the hypothalamic-pituitary-gonadal (HPG) axis. In this context, neurotransmitters act as essential chemical messengers secreted by the central nervous system (CNS), facilitating synaptic communication between neurons (AL-Jaryan *et al.*, 2023). Within the reproductive domain, their role is crucial as they modulate the secretion and release of gonadotropic hormones such as luteinizing hormone (LH) and follicle-stimulating hormone (FSH), both of which are fundamental to follicular development and ovulation. Alterations in neurotransmitter secretion patterns can significantly impact the functionality of the reproductive axis, thereby affecting the efficiency of reproductive processes (Hull *et al.*, 1999). During the reproductive season, ewes exhibit an estrous cycle averaging 17 days in length, with a range between 15 and 20 days. Estrus, or the phase of sexual receptivity, lasts for a short period averaging between 24 and 36 hours, although it may vary from 16 to 59 hours. Ovulation occurs spontaneously within this window. At both uterine and endocrine levels, functional modifications are observed, associated with fluctuations in basal concentrations of key hormones such as progesterone (P4), estradiol (E2), and gonadotropin-releasing hormone (GnRH), the latter of which regulates the secretion of LH and FSH, promoting follicular growth and ovulation (Goodman & Karsch, 1980). Hormonally, P4 is the predominant hormone of the estrous cycle, remaining elevated for approximately 11 of the 17 days. Estradiol, in contrast, is secreted mainly over a period of three to four days and is chiefly responsible for the expression of estrous behavior (Goodman & Karsch, 1980; Karsch *et al.*, 1989). A sustained rise in E2 induces a positive feedback effect on the hypothalamic axis, generating a preovulatory GnRH surge, which in turn increases the frequency of LH secretion an essential event for ovulation to occur. Subsequently, the luteal phase is established, marked by a progressive increase in P4 levels, while the concentration and secretion frequency of GnRH and FSH rise, and LH levels gradually decline (AL-Jaryan *et al.*, 2023). However, during the annual reproductive cycle of ewes, periods of ovarian inactivity known as seasonal anestrus occur. This phenomenon is primarily influenced by environmental factors such as photoperiodicity especially extended daylight and the suckling stimulus. Both factors enhance CNS sensitivity to the inhibitory effects of estradiol through negative feedback, resulting in a diminished frequency and amplitude of GnRH and LH secretion, thereby preventing ovulation (Arroyo & Camacho-Escobar, 2009).

Seasonal anestrus

In ewes, seasonal anestrus is defined by the absence of estrous cycles, estrous behavior, and ovulatory events. This period occurs during the long days of the year, when the duration of melatonin secretion a key hormone in photoperiod regulation decreases significantly (Thiéry *et al.*, 2002). In sheep located in temperate and subtropical regions of the Northern Hemisphere, the breeding season takes place during the short days, spanning from early autumn to late winter. Conversely, seasonal anestrus extends from late winter through late summer, coinciding with long-day periods. The duration of postpartum anestrus may vary depending on the timing of parturition and the length of the lactation period, both of which are critical modulators of the resumption of ovarian activity (Delgadillo *et al.*, 2020). During seasonal anestrus, sensitivity of the retrochiasmatic lateral region of the hypothalamus to the inhibitory effects of estradiol (E2) is heightened, thereby intensifying the negative feedback exerted by this hormone (Gallegos-Sánchez *et al.*, 1997). As a result, the frequency of GnRH pulses and consequently LH secretion is reduced, preventing follicular maturation and blocking ovulation (Arroyo & Camacho-Escobar, 2009). This basal state of the hypothalamic-pituitary-gonadal axis represents a physiological adaptation to environmental conditions, ensuring that reproduction is synchronized with periods of optimal resource availability.

Postpartum anestrus

During the postpartum period, females undergo a series of physiological and physical changes that hinder the immediate restoration of fertility. One of the primary contributing factors to this phenomenon is the suckling effect, which activates mechanoreceptors located in the nipple. This stimulation triggers neural signals to the hypothalamus that promote the release of endogenous opioid peptides (EOPs), primarily beta-endorphin (Morales *et al.*, 2001). These peptides exert an inhibitory effect on the secretion of gonadotropin-releasing hormone (GnRH). At the neuroendocrine level, neurons of the kisspeptin/neurokinin B/dynorphin (KNDy) complex play a central role in regulating the pulsatile secretion of GnRH in mammals (Lehman *et al.*, 2010; Maeda *et al.*, 2010; Uenoyama *et al.*, 2014, 2021). GnRH is essential for the production of the pituitary gonadotropins LH and FSH; thus, a reduction in its pulsatile release leads to decreased LH secretion, directly impairing the resumption of ovarian activity (Godfrey *et al.*, 1998). Several studies have documented the reproductive responses of ewes during the postpartum period under different management approaches. For example, Wei *et al.* (2016), in a study on anestrous Lanzhou ewes, evaluated various hormonal protocols for estrus induction. They found that treatment with fluorogestone and equine chorionic gonadotropin (eCG) resulted in a higher conception rate (85.72%) compared to the protocol based on cloprostenol and eCG (57.14%). Additionally, Ungerfeld *et al.* (2020) demonstrated that biostimulation through male exposure effectively induced estrus, ovulation, and even pregnancy, despite ongoing lactation in the ewes. These findings suggest that postpartum reproductive management can be approached either through the use of exogenous hormonal treatments or via sexual biostimulation strategies, with the latter representing a more sustainable alternative that involves reduced pharmacological intervention.

Management of postpartum anestrus

In hair sheep inhabiting tropical regions, it has been observed that suckling inhibits the pulsatile secretion of GnRH during the postpartum period. However, when suckling is controlled and the presence of the male is permitted, inter-lambing intervals tend to be significantly shortened (Morales-Terán et al., 2011). In this context, the presence of the lamb and the frequency and intensity of suckling constitute the most significant stimulus in suppressing the resumption of ovarian activity following parturition. Conversely, partial or total separation of the offspring promotes the return to estrus and the occurrence of ovulation in a shorter timeframe (Arroyo & Camacho-Escobar, 2009; Arroyo *et al.*, 2011; Morales-Terán *et al.*, 2011; Castillo-Maldonado *et al.*, 2013). During lactation, suckling imposes a high energetic demand on the mother, leading to a reduction in the frequency of GnRH and LH pulses. This neuroendocrine alteration contributes to ovulation inhibition and prolongation of postpartum anestrus. To counteract this effect and facilitate the early restoration of ovarian activity, various management strategies involving mother-lamb contact have been implemented, such as early weaning, temporary weaning, and controlled suckling. These practices allow modulation of mammary stimulation and, consequently, reduce the inhibitory effect on the hypothalamic-pituitary-gonadal axis, promoting a more efficient reproductive recovery in postpartum females.

Suckling management strategies to reduce the parturition-to-first-ovulation interval

Suckling is one of the main factors inhibiting the reestablishment of ovarian activity during the postpartum period in ewes. Its effect on the central nervous system sensitizes the preoptic area of the hypothalamus to the negative feedback exerted by estradiol (E2), thereby reducing the frequency of GnRH and LH pulses and consequently blocking the reactivation of the reproductive axis (Morales-Terán *et al.*, 2004; Freitas De Melo & Ungerfeld, 2016). During the first two weeks postpartum, ewes allow lambs to suckle with high frequency but low intensity. As the postpartum period progresses, this pattern reverses: frequency decreases while suckling intensity increases, maintaining the inhibitory stimulus on the hypothalamic-pituitary-gonadal axis. To counteract this effect, various management strategies have been developed to modulate mother-lamb contact with the aim of inducing earlier ovulation and shortening the duration of postpartum anestrus.

Early weaning

Early weaning is a commonly used practice in hair sheep, particularly in tropical regions, to induce ovulation in lactating females. This strategy involves the abrupt separation of lambs between 20 and 30 days postpartum, triggering the reactivation of the reproductive axis and promoting ovulation within three to five days after weaning onset (Castillo-Maldonado *et al.*, 2013). Multiple studies have shown that early weaning does not negatively affect the physiological condition of ewes and, on the contrary, enables an earlier resumption of reproductive activity compared to those subjected to late weaning. This benefit is attributed to a longer period for the female to regain optimal body condition (between 2.5 and 3.0 on a 1 to 5 scale) before the next mating, significantly improving conception rates (Kinghts *et al.*, 2012). However, this strategy has been associated with severe stress in lambs, increasing the risk of disease and necessitating more intensive management by the producer. Therefore, its implementation is primarily recommended in specialized dairy production systems where lambs can be fed high-quality milk replacers.

Temporary weaning

Temporary weaning consists of separating lambs from their mothers for a short period during lactation to induce the return to estrus. Castillo-Maldonado (2012) evaluated this strategy in hair sheep by implementing lamb separation on day 43 postpartum for a duration of 48 hours. The study showed that most females subjected to this practice exhibited estrus and significantly reduced the parturition-to-first-ovulation interval. However, adverse effects were reported in lambs, including behavioral stress and, in some cases, maternal rejection upon reintroduction. These issues may compromise animal welfare and productivity efficiency if appropriate complementary measures are not applied.

Controlled suckling

Controlled suckling is a technique developed by the Colegio de Postgraduados with the aim of reducing the duration of postpartum anestrus in hair sheep. This strategy involves gradually reducing the lamb's dependence on the mother by limiting contact between them starting from the seventh day postpartum. The protocol includes two daily contact sessions one in the morning and one in the afternoon each lasting 30 minutes. This restricted suckling management promotes the reactivation of ovarian activity by diminishing the inhibitory stimulus that suckling exerts on the neuroendocrine axis. Simultaneously, alternative high-quality feed sources (creep feeding) are provided to ensure adequate lamb growth. Implementing controlled suckling enables an earlier return to estrus, enhances reproductive efficiency in ewes, and supports a more uniform weight distribution among lambs within the group. Authors such as Camacho-Ronquillo *et al.* (2008), Herrera Corredor *et al.* (2008), Morales-Terán *et al.* (2011), and Castillo-Maldonado *et al.* (2013) have reported significant reductions in the parturition-to-first-insemination interval by limiting lamb access to the mother to just 30 minutes twice daily. This practice is recommended starting from the first postpartum week, once the lambs have consumed colostrum and developed sufficient immunological strength.

Management of socio-sexual stimuli during postpartum anestrus

Sexual biostimulation is a phenomenon whereby males or females induce changes in the reproductive physiology and performance of other individuals through various stimuli, such as pheromones, genital stimulation, or other external signals (Fiol & Ungerfeld, 2012; Landeta-Hernández *et al.*, 2023). Sexually active males emit pheromones capable of stimulating the pulsatile secretion of GnRH and LH in anestrous females a phenomenon known as the "male effect" which induces the reinitiation of ovarian activity and potential ovulation (Scaramuzzi & Martin, 2008; Arellano-Lezama *et al.*, 2013). As a result of this stimulus, some females in the flock may enter estrus, ovulate, or even become pregnant. Sexual biostimulation is a valuable tool for inducing estrus, particularly due to its low cost, which facilitates its integration into flock reproductive management. The sudden introduction of a ram into pens with females increases the pulsatile secretion of GnRH and LH due to heightened activity of neurons in the A12 nucleus and the locus coeruleus complex (Fabre-Nys *et al.*, 2016). This increase in GnRH and LH pulse frequency persists for at least 12 hours, although pulse amplitude declines after one to two hours. Therefore, both sudden introduction and replacement of the male can induce ovulation (Ungerfeld *et al.*, 2004; Arellano-Lezama *et al.*, 2013). In some ewes, the first estrus appears with the second ovulation, occurring between 17 and 20 days after ram introduction. In others, a short luteal phase of 4 to 5 days is followed by a second ovulation without external estrus signs, after which a normal-duration luteal phase occurs. From this point, a third ovulation associated with overt estrus is typically observed (Ungerfeld *et al.*, 2004; Ungerfeld, 2007; Ungerfeld & Núñez, 2011). Ultrasound and laparoscopy observations have confirmed the occurrence of ovulations evidenced by corpora 4 and 6 days following ram introduction. Short luteal phases caused by anovulatory follicles, or normal luteal phases with luteinized follicles, have also been documented (Ungerfeld *et al.*, 2004; Pellicer-Rubio *et al.*, 2013). The rise in LH pulses coincides with an increase in the number of large follicles (>5 mm), as these pulses are essential for the development of the preovulatory follicle (Luo *et al.*, 2011; Seekallu *et al.*, 2009). Several studies have reported an increase in LH secretion frequency in response to ram introduction. The signals that trigger the resumption of reproductive activity are associated with the concentration of androgens produced by the male, which may be influenced by factors such as age and reproductive season (Arellano-Lezama *et al.*, 2013). The "female effect," or the influence of the female on the male, induces an increase in testosterone secretion in rams, which directly affects their sexual behavior and mating success. Exposure of males to estrous females enhances their ability to induce ovulation in anestrous females (Ungerfeld *et al.*, 2004). Meanwhile, the "female-female effect" involves stimulating ovarian activity through the introduction of a female in estrus into a group of anestrous ewes. This practice reactivates ovarian function in the other females and may potentiate the response to the male effect, increasing the number of ewes that ovulate during either seasonal or postpartum anestrus, thereby improving flock reproductive efficiency (AL-Jaryan *et al.*, 2023).

What do the results of biostimulation during postpartum anestrus suggest?

To evaluate the efficacy of sexual biostimulation strategies (male effect) and suckling management in the reestablishment of postpartum ovarian activity in hair sheep, Arrieta *et al.* (2024; unpublished data) conducted an experimental study. The design included four treatments distributed as follows: 14 females with continuous suckling (CS), 14 females with continuous suckling plus male effect (CS+ME), 14 females with controlled suckling (Cs), and 14 females with controlled suckling plus male effect (Cs+ME).

The results showed that the interaction between the male effect and controlled suckling significantly reduced the interval between parturition and the first manifestation of estrus ($p<0.05$), compared to the other treatments (Table 1).

Moreover, as shown in Table 1, not all females exhibited estrus, with the absence of estrus occurring exclusively in the continuous suckling groups. This finding suggests that estrus inhibition may be associated with the suppression of LH pulsatile secretion induced by the suckling stimulus. On the other hand, regarding pregnancy rate and type of lambing, no significant differences were observed between treatments ($p>0.05$; Table 2), indicating that the applied strategies did not affect these reproductive parameters.

Regarding prolificacy and fertility, there is insufficient evidence to suggest that the presence or absence of the male prior to mating is a determining factor for the number of offspring born (Table 3).

The occurrence of a higher number of multiple births in some treatments does not indicate that the type of lambing is determined by sexual biostimulation, as no significant differences were found between treatments ($p>0.05$). Therefore, it is suggested that in lactating hair sheep, the male effect primarily contributes to reducing the interval between parturition and the first manifestation of estrus. These results are consistent with those reported by other authors, such as Morales-Terán *et al.* (2011), who observed

Table 1. Percentage of Hair Sheep Exhibiting Estrus and Average Time to Estrus Onset by Treatment.

Treatments	n	Ewes in estrus (%)	Time of onset of estrus (hrs)
CS	14	12 (85.7)	$51.50\pm2.99_{a}$
CS+ME	14	12 (85.7)	$48.86\pm2.99_{a}$
Cs	14	14 (100)	$43.50\pm2.12_{a}$
Cs+ME	14	14 (100)	$35.50\pm2.04_{b}$

Means with different superscripts are significantly different ($p<0.05$).
CS=continuous suckling; CS+ME=continuous suckling plus male effect; Cs=controlled suckling; Cs+ME=controlled suckling plus male effect.

Table 2. Pregnancy percentage by treatment in hair sheep.

Treatment	n	Pregnant females	Percentage
CS	12	9	75.0
CS+ME	12	10	83.3
Cs	14	12	85.7
Cs+ME	14	12	85.7

Means with different superscripts are significantly different ($p<0.05$).
CS=continuous suckling; CS+ME=continuous suckling plus male effect; Cs=controlled suckling; Cs+ME=controlled suckling plus male effect.

Table 3. Prolificacy and fertility by treatment in hair sheep.

Treatment	n	Prolificacy	Fertility
CS	12	1.88	1.14
CS+ME	12	2.0	1.43
Cs	14	1.67	1.43
Cs+ME	14	1.83	1.57

Means with different superscripts are significantly different ($p<0.05$).
CS=continuous suckling; CS+ME=continuous suckling plus male effect; Cs=controlled suckling; Cs+ME= controlled suckling plus male effect.

that the combination of continuous suckling with the male effect (CS+ME) induces the reestablishment of ovarian activity before day 60 postpartum the time at which weaning is typically performed in hair sheep.

CONCLUSIONS

The relevance of this review lies in the description and analysis of the various strategies used for managing lactation during the postpartum period in hair sheep. It highlights that the duration of the interval between parturition and the first ovulation is a key factor in flock reproductive efficiency, and identifies critical areas of research that should be addressed in the future. Furthermore, it reveals room for improvement in sheep production systems, particularly in tropical and subtropical environments. However, a deeper understanding is still needed regarding the physiological mechanisms occurring at the hypothalamic and ovarian levels in response to sexual biostimulation, in order to optimize its application. The selection and implementation of appropriate management strategies that effectively induce the reestablishment of postpartum ovarian activity and reduce the inter-lambing interval represent a key tool for enhancing productivity and profitability in sheep production systems.

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