AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Suckling, male effect and kisspeptin in the reproductive management of ewes in postpartum anestrus

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ABSTRACT

Objective: To describe the management strategies of controlled suckling and the male effect to reduce postpartum anestrus, and to show the participation of kisspeptin to regulate the effect of both factors.

Design/Methodology/Approach: A review of scientific publications was conducted, in order to show the importance of suckling and the male effect as strategies to reduce postpartum anestrus in the ewe, as well as the relation of kisspeptin with both factors.

Results: Seasonal anestrus can be avoided with the use of breeds adapted to the local environment, such as Pelibuey. Postpartum anestrus occurs mainly as a result of suckling, since the latter inhibits the pulsating secretion of the gonadotropin-releasing hormone (GnRH) and the luteinizing hormone (LH). The exact path of this inhibition is unknown, although it seems that endogenous opioid peptides and kisspeptin are intermediaries. Controlled suckling and the male effect are management strategies that improve the reproductive behavior of postpartum ewes. Kisspeptin regulates the influence of the male effect through the secretion of GnRH/LH.

Study Limitations/Implications: To understand the impacts of suckling and the male effect on the duration of postpartum anestrus, as well as the participation of kisspeptin in the regulation of both effects, will allow designing management strategies to improve the reproductive efficiency of the ewes.

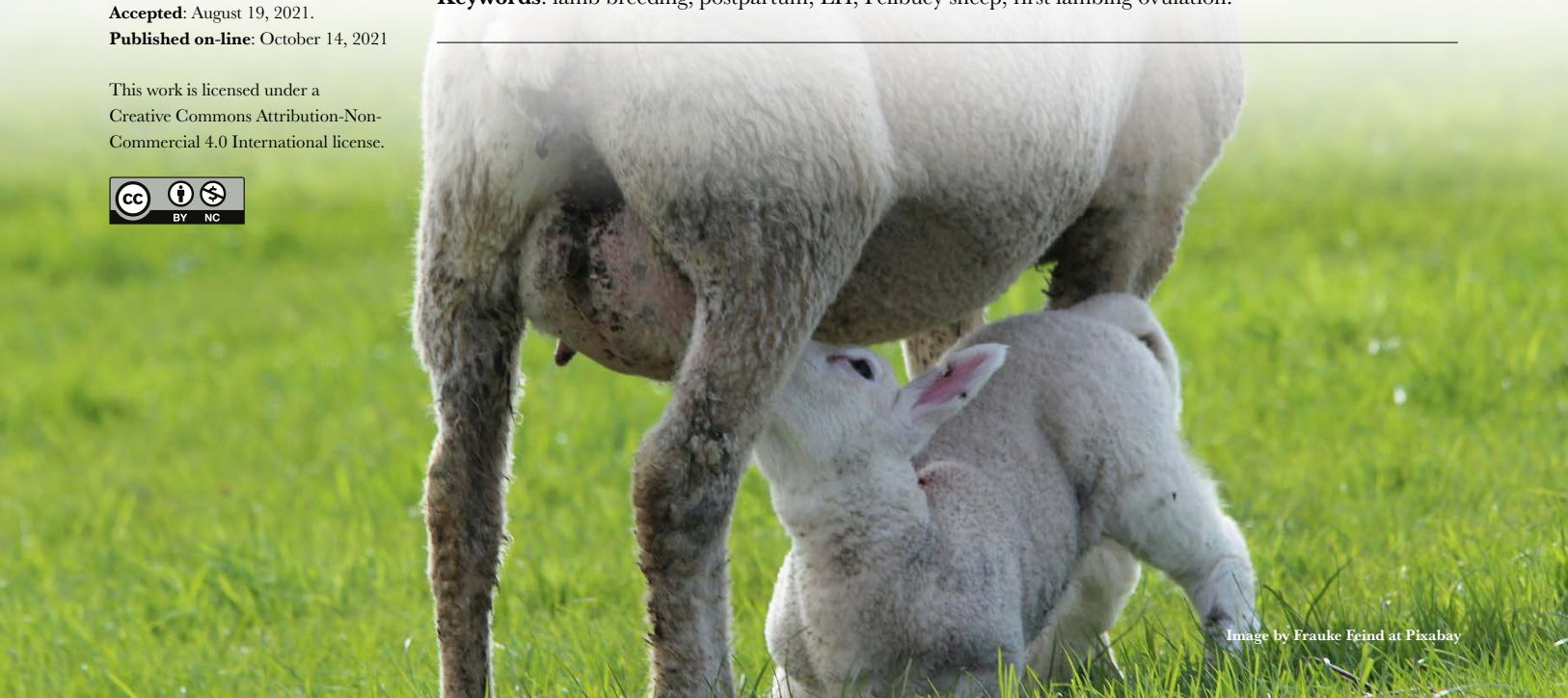
Findings/Conclusions: Controlled suckling and the male effect reduce postpartum anestrus and improve the reproductive behavior of the ewes; advancing knowledge of the kisspeptin effect could improve the effectiveness of both techniques.

Keywords: lamb breeding, postpartum, LH, Pelibuey sheep, first lambing ovulation.

Citation: Hernández-Hernández, J. M., Gallegos-Sánchez, J., Becerril-Pérez, C. M., Pro-Martínez, A., Pérez-Hernández, P. (2021). Suckling, male effect and kisspeptin in the reproductive management of ewes in postpartum anestrus. *Agro Productividad*. https://doi.org/10.32854/agrop.v14i8.2104

Editor in Chief: Dr. Jorge Cadena Iñiguez

Received: April 15, 2021.
Accepted: August 19, 2021.
Published on-line: October 14, 2021



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INTRODUCTION

Sheep reproduction is key for the profitability of the flocks because the lamb is the main product, which is why the reproductive management needs to be the most efficient possible. In an intensive production system there are two births per year, and the number of lambs born depends on the breed.

The season of the year (photoperiod) is one of the factors that inhibit the reproduction of the ewe (Clarke *et al.*, 1984), because this species evolved and adapted to the climate conditions, to reproduce in the days with less light hours in the year (fall-winter) and give birth during the spring and the summer, when the climate conditions and availability of food are the best.

The difference of light and darkness hours is lower the closer to the Equator, which is why in Mexico the hair breeds introduced adapted and are less susceptible to changes in the photoperiod. Thus, studies about seasonality performed in Mexico with the Pelibuey breed show that it is capable of reproducing throughout the year, while wool-producing ewes enter anestrus in spring (Arroyo *et al.*, 2007). Therefore, using hair breeds in cross-breeding with wool breeds is a strategy to decrease the effect of seasonality; and to improve the daily weight gain of the lambs and the quality of the carcass, Dorper or meat-producing breeds can be used.

When seasonality is not the problem, the ewe has a reproductive cycle approximately every 17 days; however, when lambing takes place, suckling by the lamb takes on importance to inhibit the reproductive activity. The filial relationship established between the mother and the lamb plus the suction of the mammary gland (suckling) begins during the first minutes after the birth, while the mother "cleans" the lamb of the placenta residues and stimulates it to consume colostrum (Viker *et al.*, 1993). Suckling inhibits the pulsating secretion of the gonadotropin-releasing hormone (GnRH)/luteinizing hormone (LH); that is, it inhibits the capacity of the ewe to enter estrus and to ovulate (Camacho *et al.*, 2008); thus, lamb weaning restarts the estrus and the ewe's ovulation (Salloum and Claus, 2005).

The effect of suckling begins with the suction of the mammary gland, the sight and detection of the lamb's aroma (Griffith and Williams, 1996). This stimulus reaches the hypothalamus via the spinal cord, where it inhibits the pulsating secretion of GnRH (Yamada *et al.*, 2007) and as consequence of the pulsating secretion of LH, which is essential for the final maturation of the follicles and the ovulation (Mandiki *et al.*, 1990). In recent years the discovery of kisspeptin (Kp) has helped to explain several of the unknowns in the reproductive physiology of the ewe and it seems to also be related to suckling (Yamada *et al.*, 2007).

The effect of suckling in postpartum anestrus in the ewe can be reduced through controlled suckling and the male effect, and Kp seems to have a relation in both effects (Fabre-Nys *et al.*, 2015). Therefore, the objective of this literature review is to describe the management strategies of controlled suckling and the male effect in order to reduce postpartum anestrus, and to show the relationship there is between suckling, the male effect and the Kp during this period.

Reduction of the postpartum anestrus with controlled suckling

Controlled suckling is a reproductive management strategy that reduces the negative effect of suckling on sheep reproduction after lambing, and it is conducted as follows: 1) Immediately after the birth, making sure that the lamb is consuming colostrum and suckling continually from the mother during the first week of life; 2) Starting on day 7 postpartum, separating the mother from the lamb in pens to avoid direct contact, mutual viewing and calling; 3) The lambs have free-access water in the pen, concentrated food according to age, and shade; 4) Since day 7 of age and until weaning, uniting the mothers for 30 min with their lambs to suckle twice a day (morning and afternoon). Starting on day 35 postpartum, introducing a male to induce heat and mounting for females in estrus, or performing the synchronization of estruses and artificially inseminating the ewes.

Controlled suckling, twice per day for 30 min, improves the postpartum reproductive behavior of the ewes. With this modality of suckling, the ovulation rate increases from 70 to 88.8% and the interval from birth to first ovulation is reduced from 60.5 to 52.6 days in comparison to ewes with continuous suckling (Morales-Terán *et al.*, 2004); the ovulation rate increases, although the days until first ovulation were not different (51.1 *vs.* 56.7; Morales-Terán *et al.*, 2011); and the reproductive behavior of ewes synchronized with progesterone improves (Camacho *et al.*, 2008). This happens because controlled suckling reduces the suction stimulus, visual and olfactory contact of the lambs with the ewes, which stimulates the pulsating secretion of GnRH/LH gradually and provokes the ovulation and manifestation of estrus (Pérez-Hernández *et al.*, 2009).

Controlled suckling does not affect the weight gain of the lambs compared to continuous suckling when creep-feeding and basic cares of the lambs are offered (Morales-Terán *et al.*, 2011). In addition, this management of ewes and lambs decreases the levels of stress that happen at the moment of weaning in mothers and lambs observed in continuous suckling, and the weight gain from birth to 41 days of age of the lambs is similar in controlled suckling and continuous suckling (Castillo-Maldonado *et al.*, 2013), when their diet depends most on the milk consumed from their mothers (Pérez-Hernández *et al.*, 2009).

Male effect

The separation and reintroduction of rams to ewes in seasonal anestrus stimulates their reproductive activity (Hawken *et al.*, 2007), and this is known as the "male effect". The management consists in the following: 1) The females and the males must be separated before lambing to avoid visual, auditory contact and the aroma or pheromones; 2) Starting on day 7 postpartum and until weaning or mating, males selected previously with high libido are introduced to the group of females; 3) To avoid copulation, the male is protected with an apron and introduced into the pens with ewes in anestrus during 30 min, twice per day (morning and afternoon); 4) To avoid familiarization, maintain novelty and the potency of the stimulus, it is recommended to rotate males every day.

Exposure of ewes to the male in postpartum anestrus decreases the interval from birth to first ovulation to 27.7 days and manages for 100% of the ewes to ovulate in the first 60 days postpartum compared to 53.6 d and 83.3%, respectively, in ewes with continuous suckling (Cruz-Espinoza, 2011, unpublished data), and improves the percentage of gestating ewes

(90%) in comparison to those without exposure to the male (43%; Hernández-Hernández, 2018; unpublished data).

There are factors that influence the response of ewes to the male effect, such as the prior isolation of females and males, the breed and the previous experience of ewes with the male. Initially, it was believed that a period of separation of males and females previous to the introduction of the males was necessary for this technique to be effective (Martin *et al.*, 1986); however, new studies show contradictory results (Delgadillo *et al.*, 2009). Hawken *et al.* (2009) found that the continuous presence of the male does not increase the pulsating secretion of LH (0.26±0.04 pulses in 6 h); however, introducing new males to the group of ewes (without the need for prior isolation) increases the secretion of LH (0.87±0.06 pulses in 6 h). In turn, in ewes in seasonal anestrus that did not present ovulation after 65 d of contact with males, 90% ovulated after 4 d of contact with new males (Pearce and Oldham, 1988).

In sheep breeds with marked seasonality, they only respond to the male effect in the last days of the anestrus period, and the less seasonal ones can still respond in the middle of the anestrus season (Martin *et al.*, 1986). In the Merino breed, the male effect is always effective; however, in the Suffolk breed it functions at the end of the reproductive season and at the beginning of it (Martin *et al.*, 2004). On the other hand, the response to the male effect among ewes with and without previous experience with males is different, so the experience could be affecting the meaning that ewes assign to the aroma of the males (Gelez and Fabre-Nys, 2004). The main olfactory system projects towards areas responsible for the cognitive analysis of the stimuli that the animal perceives or from past experience, before reaching the area that controls the secretion of LH and it is possible that this is how the experience of the male effect has an impact (Cohen-Tannoudji *et al.*, 1989). Therefore, it is important to consider the factors previously mentioned at the time of introducing the male effect in the flocks.

The male effect happens through the pheromones produced by the stud, so impregnating the sebaceous glands with wax or the exposure of ewes in seasonal anestrus to wool from the studs induces ovulation, but this does not happen when urine is used (Knight and Lynch, 1980). The substances that exert the male effect are secreted on the skin and impregnated on the wool, at least in sheep (Knight *et al.*, 1983). Although mixed fatty acid compounds have been identified, the pheromones or exact substances responsible for the male effect have not yet been isolated (Rosa and Bryant, 2002) and it has been proposed that such a substance could be derived from bacterial fermentation from skin exudates that are impregnated on the wool (Hawken and Martin, 2012). The pheromones stimulate the pulsating secretion of GnRH/LH and with this, the final maturation of the follicles that will potentially be ovulatory.

Kisspeptin and its relationship with the male effect and suckling

Kp is derived from the Kiss1 gene and gives origin to a hormone of 54 amino acids (kisspeptin 54 or metastine) which can be divided enzymatically to form peptides of 10, 13, 14 amino acids (Kotani *et al.*, 2001). The last 10 amino acids of Kp contain the biological power (Caraty *et al.*, 2012). In ewes, the existence of large populations of neuronal Kp

bodies in the preoptic area (POA) and the arcuate nucleus (ARC; Smith *et al.*, 2007) of the hypothalamus has been proven (Figure 1).

Relaciones neuroendócrinas de la kisspeptina (Kp), efecto macho, amamantamiento y fotoperiodo en la secreción de GnRH y LH de ovejas. A15: núcleo dopaminérgico; ARC: núcleo arcuato; D_2: receptor de dopamina; Dyn: dinorfina; EM: eminencia media; E_2: estradiol; ERα: receptor alfa de E_2; Kiss1r: receptor de Kp; KOR: receptor de Dyn; NKB: neuroquinina; NK3R: receptor de NKB; P_4: progesterona; POA: área preóptica; POE: péptidos opioides endógenos.

There are a large number of neuronal projections of Kp towards the internal and external region of the middle eminence, and in apparent contact with small blood capillaries (Franceschini *et al.*, 2006), where projections of the GnRH neurons end in the portal hypothalamus hypophysis system, and they secrete their content (Figure 1). In addition to this, it has been shown that the presence of the Kp receptor (Kiss1r) in GnRH neurons (Messager *et al.*, 2005). Also, in ewes, 93% of Kp neurons in the ARC express the alfa estradiol receptor (ERα), while 50% of the Kp neurons express it in the POA (Franceschini *et al.*, 2006).

Kp regulates the secretion of GnRH and LH in sheep and other species like humans, bovines, monkeys and many other. In ewes, exogenous Kp stimulates the secretion of

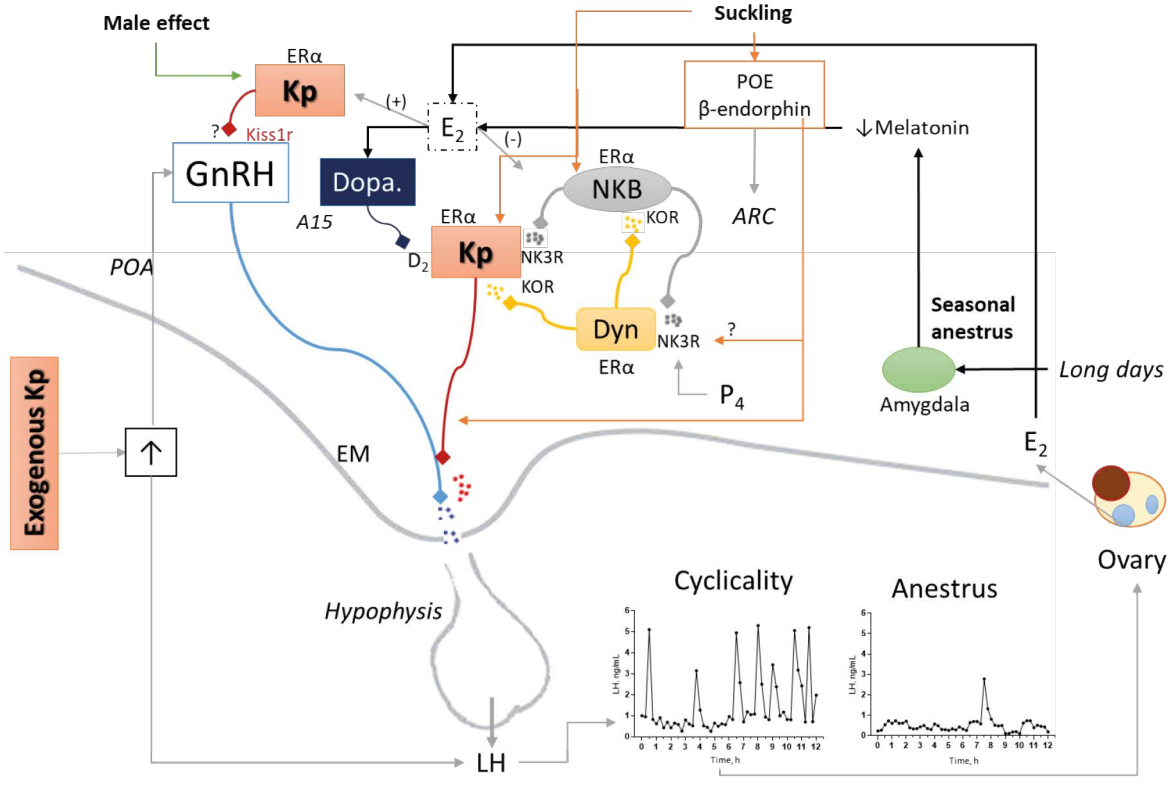

Figure 1. Neuroendocrine relationships of kisspeptin (Kp), male effect, suckling and photoperiod in the secretion of GnRH and LH of sheep. A15: dopaminergic nucleus; ARC: arcuate nucleus; D_2: dopamine receptor; Dyn: dynorphin; EM: median eminence; E_2: estradiol; ERα: E_2 receptor alpha; Kiss1r: Kp receptor; KOR: Dyn receptor; NKB: neurokinin; NK3R: NKB receptor; P_4: progesterone; POA: preoptic area; POE: endogenous opioid peptides.

GnRH, LH, FSH and estradiol (E_2) during the seasonal anestrus, the reproductive season (Caraty *et al.*, 2007) and the ewe's puberty (Redmond *et al.*, 2011). It also induces the pre-ovulatory peak of LH in ewes during seasonal anestrus (Sébert *et al.*, 2010). Just as Kp participates in these reproductive processes, its participation in the regulation of postpartum anestrus cannot be dismissed. In rats, suckling inhibits the expression of Kiss1 and the application of exogenous Kp increases the pulsating secretion and concentration of LH (Yamada *et al.*, 2007). The application of Kp in ewes in postpartum anestrus increases the pulsating secretion of LH (Hernández-Hernández, 2018; unpublished data).

Kp regulates the action of the pheromones of the male effect on the secretion of GnRH/LH (Figure 1). The specific form by which the stimulus of the male effect reaches the GnRH neurons is still unknown, although there is the possibility of Kp being involved, because its secretion is activated at the moment of the male effect (Fabre-Nys *et al.*, 2015). In goats there are neuronal projections from the amygdala to the ARC; in sheep a residue derived from Fos genetic activation has been detected in the nucleus which indicates neuronal activity due to the male effect (Jouhanneau *et al.*, 2013). The ewes subjected to the male effect increase ARNm of Kiss1 in the ARC, while the administration of an antagonist (P-271) to Kp completely blocks the response to the male effect (Bond *et al.*, 2013).

CONCLUSIONS

Suckling prolongs the duration of postpartum anestrus of ewes by inhibiting the pulsating secretion of GnRH/LH necessary for final follicle maturation and ovulation. Kp participates in the regulation of the secretion of GnRH/LH in different reproductive stages and possibly also participates in postpartum anestrus. Controlled suckling and the male effect are management strategies that the producer can implement to improve the reproductive behavior of the ewes in the postpartum period and seasonal anestrus. Kp also regulates the influence of the male effect on the secretion of GnRH/LH; however, the mechanism is still not clear. The exact mechanism of the regulation of suckling on the secretion of GnRH/LH and how the Kp influences in it is also unknown.

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