



## INFLUENCE OF CONTROLLED SUCKLING AND THE MALE EFFECT ON THE RESUMPTION OF POSTPARTUM OVARIAN ACTIVITY IN PELIBUEY SHEEP

### [INFLUENCIA DEL AMAMANTAMIENTO CONTROLADO Y DEL EFECTO MACHO SOBRE EL RESTABLECIMIENTO DE LA ACTIVIDAD OVÁRICA POSPARTO EN LA OVEJA PELIBUEY]

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#### SUMMARY

The effect of suckling and its interaction with the male effect on duration of postpartum anestrus in Pelibuey ewes and on live weight of the ewes and their lambs was determined. Seven days after lambing 56 adult ewes and their lambs were randomly assigned to the treatments: T1) Continuous suckling (CS; n=14); T2) Controlled suckling (RS; n=14); T3) Continuous suckling with ram (CSR; n=14); and T4) Controlled suckling with ram (RSR; n=14). A ram fitted with an apron was introduced with the ewes from day 7 to 60 postpartum for 30 min twice daily. Live weight of ewes and lambs was recorded weekly from lambing until weaning. Ovulation percentage in the first 60 days postpartum was greater in RSR (100 %;  $P \leq 0.05$ ) than in RS, CSR and CS (57.1, 64.8 and 35.7 %, respectively). Controlled suckling shortened ( $P \leq 0.05$ ) the interval lambing-first ovulation (ILFO) in RSR (42.07±2.8 days) compared with RS, CSR and CS (51.07±3.24, 50.50±3.26 and 56.71±1.73 days, respectively). No differences were found in live weight of ewes and lambs ( $P > 0.05$ ). The interaction controlled suckling-male effect increased the percentage of ovulation before day 60 postpartum and shortened the ILFO, with no effect on the live weight of ewes and lambs.

**Keywords:** Postpartum anestrus; suckling; male effect; Pelibuey sheep.

#### RESUMEN

Se determinó el efecto del amamantamiento y del efecto macho sobre la duración del anestro posparto en ovejas Pelibuey y sobre el peso corporal de éstas y sus crías. Siete días después del parto 56 ovejas adultas y sus crías fueron asignadas al azar a los tratamientos: T1) Amamantamiento continuo (CS; n=14); T2) Amamantamiento controlado (RS; n=14); T3) Amamantamiento continuo con carnero (CSR; n=14); y T4) Amamantamiento controlado con carnero (RSR; n=14). Un carnero con mandil fue introducido con las ovejas del día 7 al 60 posparto por 30 min dos veces al día. El peso corporal de ovejas y corderos se registró semanalmente del parto al destete. El porcentaje de ovulación en los primeros 60 días posparto fue mayor en RSR (100 %;  $P \leq 0.05$ ) que en RS, CSR y CS (57.1, 64.8 y 35.7 %, respectivamente). El control del amamantamiento redujo ( $P \leq 0.05$ ) el intervalo parto-primera ovulación (ILFO) en RSR (42.07±2.8 días) con respecto a RS, CSR y CS (51.07±3.24, 50.50±3.26 y 56.71±1.73 días, respectivamente). No hubo diferencias ( $P > 0.05$ ) en peso corporal de ovejas y corderos. La interacción amamantamiento controlado-efecto macho aumentó el porcentaje de ovulación antes del día 60 posparto y redujo el ILFO, sin afectar el peso corporal de ovejas y corderos.

**Palabras clave:** anestro posparto, amamantamiento, efecto macho, oveja Pelibuey.

## INTRODUCTION

After parturition, the endocrine mechanisms that control ovulation in the ewe are interrupted by several factors, such as suckling, which prolongs the duration of postpartum anestrus. Suckling prolongs the interval lambing-first ovulation (ILFO; Moss *et al.*, 1980; González-Reyna *et al.*, 1991) due to the inhibition of the frequency of pulsatile LH secretion and therefore, of the occurrence of the first LH release after lambing (Mandiki *et al.*, 1990). Morales-Terán *et al.* (2004) observed a direct relationship between suckling period and ILFO in Pelibuey ewes.

Another main factor is the interaction of the female with the male (male effect), since exposure of ewes to rams provokes a rapid increase in the pulsatile LH frequency, followed by a preovulatory LH peak that culminates in ovulation, during the seasonal anestrus (Martin *et al.*, 1986). It is also known that the variation in the response to the male effect in sheep and goats is due to the interaction genotype-latitude, as it determines the proportion of females that can ovulate in response to the male presence (Delgadillo *et al.*, 2009). For this reason, the male effect has a great potential for estrus synchronization in sheep and goat breeds that show seasonal anestrus, and during the transition to the reproductive season (Delgadillo *et al.*, 2009). However, in the Pelibuey ewe the effect of the ram on the induction of ovulation during the postpartum anestrus period is not known. In other latitudes and breeds, it has been observed that the interval lambing-conception is reduced when rams are introduced into the pens of the ewes immediately after lambing during autumn (Wright *et al.*, 1989) and spring (Ungerfeld *et al.*, 2001).

This study evaluated the effect of controlled suckling in combination with the male effect on resumption of postpartum ovarian activity in Pelibuey ewes, and the changes in live weight of the ewes during the postpartum period and in live weight of their lambs.

## MATERIALS AND METHODS

### Location

The study was conducted at the Laboratorio de Reproducción de Ovinos y Caprinos (LaROCa), owned by Colegio de Postgraduados Campus Montecillo, in the State of Mexico, Mexico (Lat. 19° 29' N, Long. 98° 53' W, at 2240 m altitude). The climate is sub-humid temperate with summer rains, mean annual temperature of 15.2 °C and mean annual rainfall of 636.5 mm.

### Experimental animals and management

A total of 56 adult anestrus Pelibuey ewes and their lambs (single and twin lambs) were used. The ewes lambed during the early spring with an average weight of 42.3±1.64 kg and body condition score of 3.5 (1 to 5 scale; Russel *et al.*, 1969). The ewes were fed a hay diet (2 kg ewe<sup>-1</sup> d<sup>-1</sup>) and received 500 g ewe<sup>-1</sup> d<sup>-1</sup> of a commercial concentrate (15.2 % CP and 2.5 Mcal ME kg<sup>-1</sup>); the food was provided to groups of ewes in concrete feeders. The lambs fed on their mothers milk and at day 7 of age they started receiving creep food (20 % CP) *ad libitum*.

### Treatments

Seven days after parturition, the ewes and their lambs were randomly assigned to one of four treatments using a completely random design: T1) Continuous suckling (CS; n=14); T2) Controlled suckling (RS; n=14); T3) Continuous suckling with the ram presence (CSR; n=14); and T4) Controlled suckling with the ram presence (RSR; n=14). The ewes in CS remained 24 h a day with their lambs until weaning, whereas the RS ewes suckled their lambs during two periods of 30 min each daily (07:30 h and 15:00 h), and the rest of the time the ewes were kept separated from their lambs. The ewes were weighed weekly from lambing until day 60 postpartum, when weaning was carried out; the lambs were weighed weekly from birth until three weeks after weaning. For the CSR and RSR treatments, six adult, non-castrated and sexually experienced Pelibuey rams were used; the rams were trained on semen collection using an artificial vagina, and macro/micro seminal tests were performed on them. For these treatment groups, a ram fitted with an apron was introduced into the pens of the ewes during two periods of 30 min a day (07:30 h and 15:00 h) from day 7 postpartum until weaning (day 60). The rotation of rams was made every second day to avoid the females to get used to one particular male. All the ewes were kept out of contact with the male from pregnancy to day 7 postpartum, and starting from that time, depending on the treatment assigned.

### Determination of the resumption of ovarian activity

On days 7 to 60 postpartum blood samples were collected from the ewes via jugular venipuncture twice a week, between 7:00 and 8:30 a.m. (Pérez-Hernández *et al.*, 2002a), to measure the serum progesterone concentrations to determine the time of resumption of ovarian activity. Immediately after collection, blood samples were centrifuged at 3500 rpm for 20 min to separate the serum, which was stored at -20 °C until

analyzed. Serum progesterone was determined by solid-phase radioimmunoassay (Srikandakumar *et al.*, 1986). An ewe was considered as having resumed ovarian activity when two consecutive serum samples had 0.5 ng mL<sup>-1</sup> of progesterone, or a single sample had > 1 ng mL<sup>-1</sup> of progesterone (Thorburn *et al.*, 1969). The assay had a sensitivity of 0.3 ng mL<sup>-1</sup> and the intra- and inter-assay variation coefficients were 3.6 and 14.1 % respectively.

### Statistical analysis

The ILFO was analyzed by the statistical method for lifetime data (ovulation times) through survival curves (Parmar and Machin, 1995), with the Kaplan-Meier Estimator. To determine the differences in the number of ewes that ovulated before day 60 postpartum and

days to first ovulation, the t-Student and the Bonferroni tests were respectively used. The changes in live weight of ewes and lambs were analyzed by repeated measures in time using the MIXED procedure of the SAS (2004).

## RESULTS AND DISCUSSION

### Percentage of ovulation and first ovulation postpartum

Percentage of ewes that ovulated was greater ( $P \leq 0.05$ ) in RSR ewes, and was similar ( $P > 0.05$ ) among RS and CSR ewes. All the RSR ewes ovulated before day 45 postpartum and their ILFO was shorter ( $P \leq 0.05$ ) (Table 1).

**Table 1.** Postpartum reproductive performance of Pelibuey ewes managed under continuous or controlled suckling, non-exposed or exposed to the male effect, during the first 60 days postpartum.

Treatment	N	Ewes that ovulated %	Days to first ovulation
Continuous suckling without exposure to the male effect	14	35.7 <sup>a</sup>	56.7±1.7 <sup>a</sup>
Controlled suckling without exposure to the male effect	14	57.1 <sup>b</sup>	51.1±3.2 <sup>a</sup>
Continuous suckling with exposure to the male effect	14	64.8 <sup>b</sup>	50.5±3.2 <sup>a</sup>
Controlled suckling with exposure to the male effect	14	100 <sup>c</sup>	42.1±2.8 <sup>b</sup>

<sup>a,b,c</sup>Means with different superscript within a column are different ( $P \leq 0.05$ )

These results indicate that suckling inhibited the resumption of postpartum ovarian activity in Pelibuey ewes, which is in agreement with the reports by other authors (Morales-Terán *et al.*, 2004; Pérez *et al.*, 2008). Suckling inhibits LH pulsatile secretion during the early postpartum period (Williams *et al.*, 1987). The hypophyseal and plasma LH concentrations in females suckling their offspring gradually increases as the postpartum period progresses, until reaching levels similar to those present in cyclic females (Nett *et al.*, 1988). Therefore, it is suggested that control of suckling to short periods during the day (one or two periods of 30 min each), together with exposure of the ewes to the male starting in the early days after lambing, increases the GnRH/LH secretion, which leads to ovulation before day 45 postpartum. This management strategy of ewes, lambs and rams is an alternative to reduce the postpartum anestrus in Pelibuey sheep, without using drugs to induce estrus .

In this study, the high percentage of RSR ewes ovulating during the first 60 days postpartum in

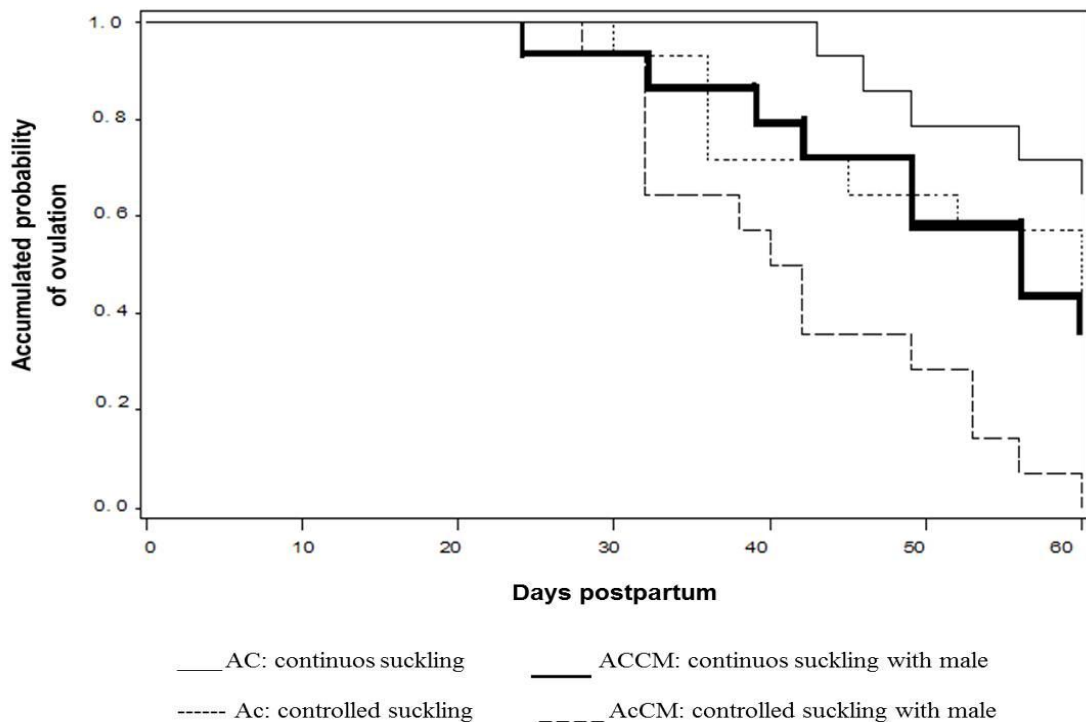
response to the male exposure starting on day 7 after lambing (Table 1), as well as the proportion of RS ewes that ovulated (Table 1), indicate that control of suckling and male exposure during the early postpartum period stimulate the Pelibuey ewes to have an earlier resumption of ovarian activity after lambing, and that the interaction suckling-male effect, besides reducing the ILFO, also increases the percentage of ewes that ovulate; this has been observed in dual-purpose cattle when suckling has been delayed and cows have been exposed to bulls (Pérez-Hernández *et al.*, 2002b; Pérez-Hernández and Gallegos-Sánchez, 2010), and in Brown Swiss cows in which the interval calving-conception and the intercalving interval have been shortened (Izaguirre-Flores *et al.*, 2007). Lindsay and Signoret (1980) observed that in postpartum wool ewes exposed or not to the male effect, a greater percentage of ewes ovulated when exposed to the male, and this response was greater as the postpartum period progressed, which is in accordance with the results obtained in the present study, where the inhibitory effect caused by suckling decreased as the

postpartum period progressed, and is also in agreement with the report by Álvarez and Zarco (2001), that the male effect depends on the time that passes from parturition and on the proportion of spontaneous cyclic females.

The absence of ovulation and cyclicity in postpartum females is due to failure in the follicular development, caused by the inadequate LH secretion due to the inhibitory effect that the estradiol exerts on its release, very similar to what occurs in females during the seasonal anestrus (Karsch *et al.*, 1980). Thus, it is suggested that the stimulus exerted by exposing the females to the male during the postpartum period increases the LH secretion due to the decrease of the

inhibitory effect of estradiol on LH secretion (Martin *et al.*, 1983). Therefore, the females that ovulate in response to the introduction of males do this as a result of the increase in the frequency of LH secretion by interfering with the estradiol negative feedback on the hypothalamus (Martin *et al.*, 1986; Álvarez and Zarco, 2001). Thus, the male stimulus induces the resumption of the ovarian activity in ewes during the postpartum period.

The differences in the probability of ovulation obtained in this study through the survival curves, showed that most of the RSR ewes ovulated earlier than the ewes of the other treatment groups (Figure 1).

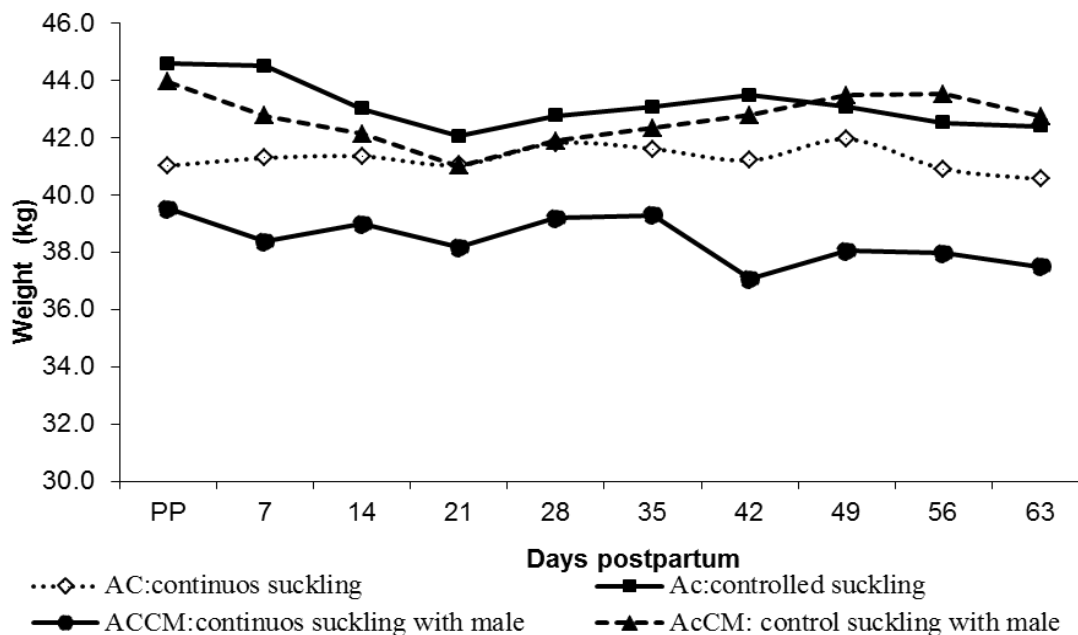


**Figure 1.** Survival curve for the resumption of ovarian activity in postpartum Pelibuey ewes managed under continuous or controlled suckling, either exposed or non-exposed to the male presence.

**Live weight changes in the ewes**

The suckling regime plus the presence or absence of the male had no effect ( $P > 0.05$ ) on live weight of the ewes (Figure 2). Live weight was affected by the postpartum period and by the interaction treatment x postpartum period ( $P < 0.0001$ ); the ewes with controlled suckling, either exposed or non-exposed to

the male, had a tendency to recover their live weight sooner (Figure 2) due to the control of suckling, since their lambs only suckled 30 min twice a day. Pavón *et al.* (1987) showed that the lactation peak occurs in the third week postpartum, and it is the time when the ewe mobilizes and uses its energy, protein and mineral body reserves (Pond *et al.*, 1995), leading to weight loss.



**Figure 2.** Live weight of Pelibuey ewes managed under continuous or controlled suckling, either exposed or non-exposed to the male presence.

Although suckling and the male presence had no effect on the live weight of the ewes in this study, a slight sooner recovery was observed in the ewes under controlled suckling either exposed or non-exposed to the male presence, which is similar to the previous report by Morales-Terán *et al.* (2004) in Pelibuey ewes suckling for 30 min twice daily.

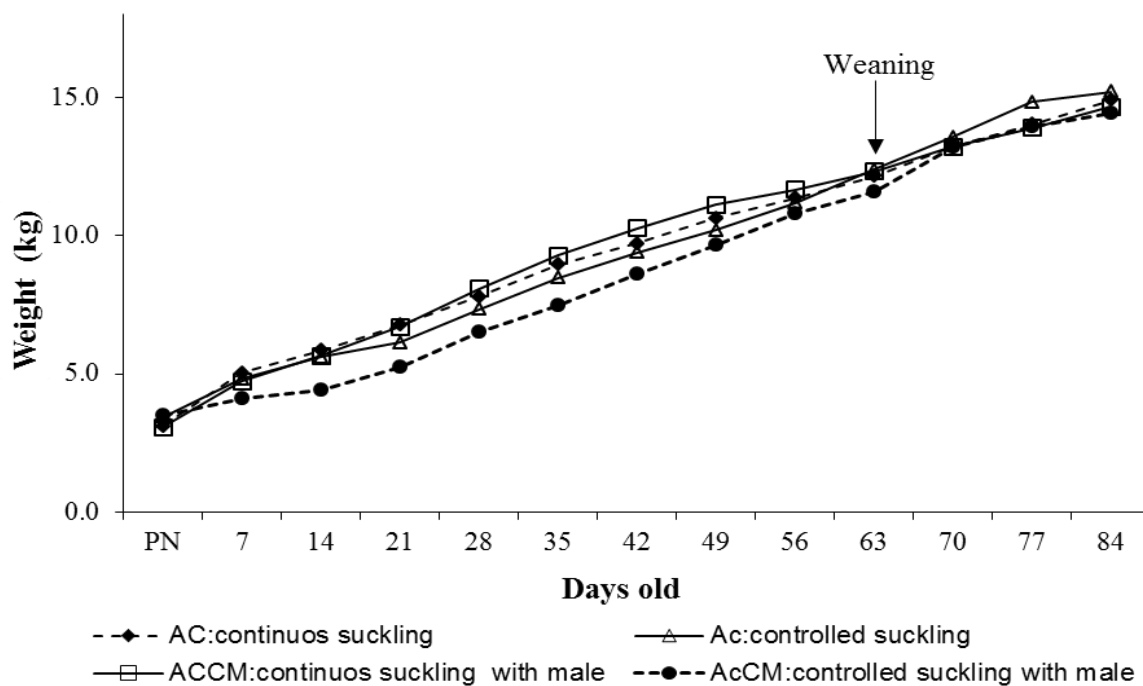
#### Live weight changes in the lambs

The period and the interaction period x treatment affected ( $P < 0.0001$ ) the change in the live weight of the lambs. The weight at birth and up to 7 days of age was similar ( $P > 0.0001$ ) among treatments; however, from day 14 until day 49, the lambs under continuous suckling belonging to the CS and CSR groups, and under controlled suckling of the RS group had a higher weight ( $P < 0.0001$ ) than the lambs under controlled suckling of the RSR group; after day 49 and until the end of the study live weight was similar ( $P > 0.0001$ ) among treatments (Figure 3). This can be attributed to the fact that the lambs under continuous suckling stayed the whole time with their mothers and therefore, consumed more milk (Rondón *et al.*, 1994), which is similar to the report by Morales-Terán *et al.* (2004). These results together suggest that two

suckling periods of 30 min each daily are enough for the lamb to consume sufficient amounts of milk from its mother, which along with the early intake of solid food, probably cause the lambs to have a similar development to those under continuous suckling, as has been observed in dual-purpose calves when suckling has been limited to short periods during the day (Pérez-Hernández *et al.*, 2002 a,b; Pérez-Hernández *et al.*, 2006; Izaguirre-Flores *et al.*, 2007). Additionally, it was observed that this type of management of ewes and lambs prevented the presentation of problems at weaning, since the lambs under controlled suckling adapted themselves to consuming solid food at an earlier age.

#### CONCLUSION

The interaction of controlled suckling with the male effect starting at day 7 after lambing increased the percentage of Pelibuey ewes that resumed ovarian activity earlier than day 60 postpartum and reduced the ILFO, without affecting the live weight of the ewes and their lambs, so exposure of Pelibuey ewes to the male effect along with control of suckling reduced the postpartum anestrus in these females.



**Figure 3.** Live weight (kg) of Pelibuey lambs under continuous and controlled suckling, with their mothers being either exposed or non-exposed to the male presence.

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### REFERENCES

- Álvarez, R. L., Zarco, Q. L. A. 2001. Los fenómenos de bioestimulación sexual en ovejas y cabras. *Veterinaria México*. 32: 117-129.
- Delgadillo, J. A., Gelez, G., Ungerfeld, R., Hawken, P. A. R., Martin, G. B. 2009. The “male effect” in sheep and goats. Revisiting the dogmas. *Behavioral Brain Research*. 200: 304-314.
- González-Reyna, A., Valencia, Z. M., Foote, W. C., Murphy, B. D. 1991. Hair sheep in México: reproduction in the Pelibuey sheep. *Animal Breeding Abstracts*. 59: 509-524.
- Izaguirre-Flores, F., Martínez-Tinajero, J. J., Sánchez-Orozco, L., Ramón-Castro, M. A., Pérez-Hernández, P., Martínez-Priego, G. 2007. Influencia del amamantamiento y presencia del toro en el comportamiento productivo y reproductivo de vacas Pardo Suizo en el trópico húmedo. *Revista Científica FCV-LUZ*. 6: 614-620.
- Karsch, F. J., Goodman, R. L., Legan, S. L. 1980. Feedback basis of seasonal breeding: test of an hypothesis. *Journal of Reproduction and Fertility*. 58: 521-535.
- Lindsay, D. R., Signoret, J. P. 1980. Influence of behavior on reproduction. *Proceedings of the 9<sup>th</sup> International Congress of Animal Reproduction and Artificial Insemination*. Madrid, Spain. pp. 83-92.
- Mandiki, S. N. M., Fossion, M., Paquay, R. 1990. Effects of suckling mode on endocrine control of reproductive activity resumption in Texel ewes lambing in July or November. *Theriogenology*. 33: 397-413.

- Martin, G. B., Oldham, C. M., Cognie, Y., Pearce, D. T. 1986. The physiological responses of anovulatory ewes to the introduction of rams. A review. *Livestock Production Science*. 15: 219-247.
- Martin, G. B., Scaramuzzi, R. J., Lindsay, D. R. 1983. Effect of introduction of rams during anoestrous season on the pulsatile secretion of LH in ovariectomized ewes. *Journal of Reproduction and Fertility*. 67: 47-55.
- Morales-Terán, G., Pro, M. A., Figueroa, S. B., Sánchez del R., C., Gallegos-Sánchez, J. 2004. Amamantamiento continuo o restringido y su relación con la duración del anestro postparto en ovejas Pelibuey. *Agrociencia*. 38: 165-171.
- Moss, G. E., Adams, T. E., Niswender, G. D., Nett, T. M. 1980. Effects of parturition and suckling on concentrations of pituitary responsiveness to GnRH in ewes. *Journal of Animal Science*. 50: 496-502.
- Nett, T. M., Cermak, D., Broden, T., Manns, J., Niswender, G. D. 1988. Pituitary receptors for GnRH and estradiol, and pituitary content of gonadotropins in beef cows. II. Changes during the postpartum period. *Domestic Animal Endocrinology*. 5: 81-89.
- Parmar, M. K. B., Machin, D. 1995. *Survival analysis: A practical approach*. John Wiley and Sons Ltd. Chichester, England.
- Pavón, M., Fuentes, J., Lima, T., Albuernes, T. R., Efremov, A., Perón, N. 1987. Estudio de la producción de leche de la oveja Pelibuey, Pelibuey x Suffolk y Pelibuey x Corriedale y el crecimiento del nacimiento al destete de sus corderos. *Revista Cubana de Reproducción Animal*. 13: 39-53.
- Pérez-Hernández, P., Gallegos-Sánchez, J. 2010. Efecto macho en la reproducción de las hembras bovinas. Madrid-Bury, N. (Ed.). Cuadernos Científicos Girarz 8. Ediciones Astro Data S.A. Maracaibo, Venezuela. Pp. 125-136.
- Pérez-Hernández, P., Becerril-Pérez, C. M., Lamothe-Zavaleta, C., Torres-Hernández, G., López-Ortiz, S., Gallegos-Sánchez, J. 2006. Efecto del amamantamiento retrasado en la actividad posparto de las vacas y los becerros de doble propósito. *Interciencia*. 31: 748-752.
- Pérez-Hernández, P., Garcia-Winder, M., Gallegos-Sánchez, J. 2002a. Postpartum anoestrus is reduced by increasing the within-day milking to suckling interval in dual purpose cows. *Animal Reproduction Science*. 73: 159-168.
- Pérez-Hernández, P., Garcia-Winder, M., Gallegos-Sánchez, J. 2002b. Bull exposure and an increased within-day milking to suckling interval reduced postpartum anoestrus in dual purpose cows. *Animal Reproduction Science*. 74: 111-119.
- Pérez, H. P., Hernández, V. V. M., Figueroa, S. B., Torres, H. G., Rivera, D. P., Gallegos-Sánchez, J. 2008. Efecto del tipo de amamantamiento en la actividad ovárica postparto de ovejas Pelibuey y tasas de crecimiento de corderos en los primeros 90 días de edad. *Revista Científica FCV-LUZ*. 39: 343-349.
- Pond, W. G., Church, D. C., Pond, R. R. 1995. *Basic animal nutrition and feeding*. 4ta. Ed. John Wiley and Sons. USA.
- Rondón, Z., Yépez, G., Navarro, N., Combellas, J., Arvelo, C. 1994. Resultados preliminares de la evaluación del potencial de producción de leche en ovejas West African sometidas a ordeño. Sociedad Española de Ovinotecnia y Caprinotecnia. (Ed). *Producción Ovina y Caprina*. Serie Estudios N° 14. España. pp. 427-432.
- Russel, J. F., Doney, J. M., Gunn, R. G. 1969. Subjective assessment of body fat in live sheep. *The Journal of Agricultural Science*. 72: 451-454.
- SAS. 2004. *Statistic visual*. Version 8.1. Institute Inc. Campus Drive. Cary, North Carolina. USA.
- Srikandakumar, A., Ingraham, R. H., Ellsworth, M., Archbald, L. F., Liao, A., Godke, R. A. 1986. Comparison of a solid-phase, no-extraction radioimmunoassay for progesterone with an extraction assay for monitoring luteal function in the mare, bitch and cow. *Theriogenology*. 26: 779-793.

- Thorburn, G. D., Bassett, J. M., Smith, I. D. 1969. Progesterone concentrations in the peripheral plasma of sheep during the oestrous cycle. *Journal of Endocrinology*. 45: 459-469.
- Ungerfeld, R., Silva, L., Laca, M., Carvajal, B., Rubianes, E. 2001. Fertility of estrus induced with the “ram effect” in lactating and dry Corriedale ewes during the non-breeding season. 35<sup>th</sup> Congress of the International Society of Applied Ethology. Davis, USA. August 4-8.
- Williams, G. L., Kosiorowski, M., Osborn, R. G., Kirsch, J. D., Slanger, W. D. 1987. The postweaning rise of tonic luteinizing hormone secretion in anestrous cows is not prevented by chronic milking or the physical presence of the calf. *Biology of Reproduction*. 36: 1079-1084.
- Wright, P. J., Geytenbeeck, P. E., Clarke, I. J., Hoskinson, R. M. 1989. The efficacy of ram introduction, GnRH administration, and immunization against androstenedione and oestrone for the induction of oestrus and ovulation in anoestrus post-partum ewes. *Animal Reproduction Science*. 21: 237-247.

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