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INFLUENCE OF RESTRICTED FEMALE-LAMB CONTACT IN LENGTH OF POSTPARTUM ANESTROUS IN PELIBUEY SHEEP

[INFLUENCIA DEL CONTACTO RESTRINGIDO HEMBRA-CORDERO EN LA DURACIÓN DEL ANESTRO POSPARTO EN OVEJAS PELIBUEY]

J. Arroyo*, M. A. Camacho-Escobar, N.Y. Ávila-Serrano and J. A. Hoffman

*Universidad del Mar, Campus Puerto Escondido. Km 3.5 Carr. Puerto Escondido–Sola de Vega. Puerto Escondido 71980, Oaxaca, México. E-mail: *arroyo@zicatela.umar.mx,*
**Corresponding Author*

SUMMARY

The study was carried out to evaluate the effect of restricting the ewe-lamb contact on the length of postpartum anestrous. We used 28 adult Pelibuey sheep and their young. One day after birth, females were randomly assigned to one of two testing groups. Restricted Suckling to 17 hours per day (RS, n = 17 sheep, n = 21 lambs), separation of ewes from lambs for 7 h / day (8:00 to 15:00 h). Unrestricted Suckling (US, n = 11 sheep, n = 13 lambs), with contact and interaction between the ewes and lambs 24 h/day. Blood samples were collected from sheep, birth to 105 days postpartum and progesterone concentrations determined by radioimmunoassay. The ewes and lambs were weighed every 15 days and mortality was recorded during the experiment. The intervals between calving to first ovulation in females, were analyzed using an ANOVA, the weaning weight and daily gain in lambs were evaluated with an analysis of covariance, and weight changes in females with multivariate analysis for repeated measurements over a determined period of time. The proportion of females ovulating at 105 d postpartum and lamb mortality were evaluated using x^2 test. The postpartum anestrous in RS (83.2 $\pm$ 5.5 days) was higher (P<0.05) than in US (58.8 $\pm$ 5.5 days), 100% of US females ovulated within 105 days postpartum, in contrast (P<0.05), only 64.7% in RS ovulated in the same period. US lambs gained more weight (172 $\pm$ 0.6 g / d, P <0.05) and had a higher weaning weight (13.2 $\pm$ 0.5 kg, P <0.05) compared with RS (143 $\pm$ 0.6 g / d and 11.4 ± 0.4 kg, respectively). The sheep did not lose weight during lactation and mortality in lambs was similar (P> 0.05) between groups. The restriction of the ewe-lamb contact for 7 h/day in Pelibuey prolongs the duration of postpartum anestrous, and in the offspring, it reduced the weaning weight and daily weight gain, without affecting mortality. We suggest that this type of management causes psychosocial stress in sheep and possibly inhibits the secretion of LH and delays postpartum ovulation.

Keywords: Hair sheep; tropic; postpartum anestrous; lactation; suckling.

RESUMEN

El estudio se realizó con el propósito de evaluar el efecto de la restricción del contacto hembra-cordero en la duración del anestro posparto. Se utilizaron 28 ovejas Pelibuey adultas con sus crías. Un día después del parto, las hembras se asignaron aleatoriamente a dos condiciones de manejo. Amamantamiento restringido a 17 h/día (RS; n=17 ovejas; n=21 corderos), separación oveja-cría por 7 h/día (8:00 a 15:00 h). Amamantamiento continuo (US; n=11 ovejas; n=13 corderos), contacto e interacción oveja-cordero 24 h/día. Se colectaron muestras sanguíneas en las ovejas, del parto a los 105 días posparto y se determinó la concentración de progesterona por radioinmunoanálisis. Las ovejas y los corderos se pesaron cada 15 días y se registró la mortalidad durante el experimento. El intervalo parto - primera ovulación en las hembras, se analizó con un ANOVA; el peso al destete y la ganancia diaria de peso en los corderos se evaluaron con un Análisis de Covarianza y los cambios de peso en las hembras con un análisis multivariado para mediciones repetidas en el tiempo. La proporción de hembras ovulando a los 105 d posparto y la mortalidad de los corderos se evaluaron con una prueba de x^2. El anestro posparto en RS (83.2 $\pm$ 5.5 días) fue mayor (P<0.05) que en US (58.8 $\pm$ 5.5 días); 100 % de las hembras US ovularon dentro de los 105 días posparto; en contraste (P<0.05), sólo 64.7 % en RS ovularon en el mismo periodo. Los corderos US ganaron más peso (172 $\pm$ 0.6 g/d; P<0.05) y tuvieron un peso al destete mayor (13.2 $\pm$ 0.5 kg; P<0.05) en comparación con RS (143 $\pm$ 0.6 g/d y 11.4 $\pm$ 0.4 kg, respectivamente). Las ovejas no perdieron peso durante la lactancia y la mortalidad en los corderos fue similar (P>0.05) entre grupos. La restricción del contacto hembra-cordero por 7 h/día en ovejas Pelibuey prolonga la duración del anestro posparto y en las crías, reduce el peso al destete y la ganancia diaria de peso, sin afectar la mortalidad. Se sugiere que este manejo causa estrés psicosocial en las ovejas y probablemente, inhibe la secreción pulsátil de LH y retrasa la ovulación posparto.

Palabras clave: Ovinos de pelo; trópico; anestro posparto; lactancia; amamantamiento.

INTRODUCTION

Anovulation in the female during the postpartum period is an adaptive strategy that promotes the survival of the offspring (Nowak *et al.,* 2000). In mammals, lactation suppresses ovarian activity. In sheep and deer, postpartum anestrous appears to be short and may coincide with the onset of seasonal anestrous (McNeilly, 2001, García *et al.,* 2002, Arroyo *et al.,* 2009). Some evidence suggests that the restriction of female contact with their young during the postpartum period in sheep, reduces the interval between calving, first estrus and ovulation, without affecting the development of the lamb (Schirar *et al.,* 1989; Mandiki *et al.,* 1989; Morales-Terán *et al.,* 2004, Pérez-Hernández *et al.,* 2009). Other authors (Arroyo-Ledezma *et al.,* 2000) found that restricted suckling does not hasten the first postpartum ovulation and has no effect on lamb production parameters. Therefore, the purpose of this study was to establish the effect of restricted contact and interaction between females and offspring during the postpartum anestrous in Pelibuey sheep.

MATERIALS AND METHODS

Geographic location

The study was conducted at the Experimental Unit of the Universidad del Mar, Campus Puerto Escondido, Oaxaca, Mexico (15° 52' north latitude, at sea level altitude), warm humid climate (AWo AWl) with rains in summer and an annual mean temperature of 28 ° C. The study was carried out between October and February.

Animals.

We used 28 adult Pelibuey female multiparous sheep who had 3 to 4 previous calvings, with an average weight of 30.2 $\pm$ 4.8 kg at the beginning of the experiment, and 34 lambs born to the experimental females. Males, previously employed for insemination of females, were of the same breed. The management system consisted of grazing for 7 h/day after which the animals were penned. One day after birth (October-November), ewes and lambs were randomly assigned into two postpartum groups: 1) Restricted suckling with ewe-lamb contact 17 hours per day (RS, n = 17 sheep, n = 21 lambs), and lambs were isolated for 7 h/day, from 8:00 to 15:00 h. in other pens. 2) Unrestricted suckling (US, n = 11 sheep, n = 13 lambs), with ewe-lamb contact 24 h/day.

Food

Females with and without lambs, grazed in pastures with African Star Grass *(Cynodon plectostachyus),* Bermuda Grass *(Cynodon dactylon),* Guacima *(Guazuma ulmifolia)* and Cocuite *(Gliricidia sepium).* In addition to grazing, the sheep were fed a commercial concentrate (16% PC; 300g/animal/day), dehydrated alfalfa, mineral salts and water *ad libitum*. Housed lambs were fed dehydrated alfalfa *(Medicago sativa),* a commercial concentrate (16% crude protein) and water *ad libitum.*

Sample Collection and Processing

Weekly venous blood samples were collected from females in both groups, from the jugular vein, using heparinized vacutainer tubes (BD Vacutainer ®, Becton Dickinson, USA), beginning one day after calving and up to 105 days postpartum. In the first hour after collection, samples were centrifuged at 3000 rpm, plasma was separated and stored at -20^{o}C until analysis. We determined the concentration of progesterone in the Laboratory of Endocrinology, Faculty of Veterinary Medicine, UNAM, by solid phase radioimmunoassay (Pulido *et al.,* 1991). The sensitivity of the test and coefficients of variation intra and inter assay were 0.02 ng/ml, 2% and 6%, respectively. It was felt that female ovulatory activity was restored when the concentration of P_4 was $\geq$ 1 ng/ml in at least two consecutive samples (Arroyo-Ledezma *et al.,* 2000, Cerna *et al.,* 2000, Arroyo *et al.* 2007).

Although not the main objective of the study, the productive performance of ewes and lambs were evaluated, and were therefore weighed every two weeks, from day 1 to 105 postpartum and the number of deceased lambs were recorded during the experimental period.

Statistical Analysis

The interval from calving to first ovulation (ICO) was compared between groups with a variance analysis, PROC GLM (SAS, version 6.0). Weight changes in females during the experiment were tested with multivariate analysis for repeated measurements over a determined period of time (SAS, version 6.0). The average daily weight gain (DWG) and weaning weight (WW) in the lambs were compared between groups with a covariance analysis (SAS, version 6.0) and was used as covariate birth weight. The mortality rate in lambs was compared between groups with a chi-square test.

RESULTS AND DISCUSSION

Restricted ewe-lamb contact for 7 h/day did not shorten the duration of postpartum anestrous in Pelibuey sheep in the tropic (Table 1). Conversely, this management prolonged the time to onset of ovulatory activity (Table 1). This observation is contrary to the results obtained by other authors (Morales-Terán *et al.,*

2004, Pérez-Hernández *et al.,* 2009), who reported a decrease in the ICO in restricted suckling ewes (female-lamb contact one or two periods 30 min/day) in a temperate climate. The ICO in US (58.8 ± 5.5 days, mean ± SE), in the present study, is close to the values reported by other authors in sheep nursing continuously [Arroyo-Ledezma *et al.* (2000), 54.3 ± 9.4 days; Morales-Terán *et al.* (2004), 60.5 ± 2.7 days; Pérez-Hernández *et al.* (2009), 52.6 ± 2.0 days]. A relevant observation is that, in this study, 100% of US ewes ovulated to 58.8 ± 5.5 days postpartum, a situation different from the results of Arroyo-Ledezma *et al.* (2000), Morales-Terán *et al.* (2004) and Pérez-Hernández *et al.* (2009), who, at 90 days postpartum, reported a low percentage of ovulation in ewes exposed to unrestricted suckling (16.6%, 70% and 18.8%, respectively). During the postpartum period, the presence of nursing lambs delays ovulation (Novak *et al.,* 2000; Rhodes *et al.,* 2003; Pérez-Hernández *et al.,* 2002; Millesi *et al.,* 2008). In this process, endogenous opioid peptides (EOPs), mainly synthesized in the hypothalamic A12 nucleus, acting in the preoptic area, in the ventromedial nucleus and median eminence (Malven, 1995, Arroyo *et al.,* 2009), reduce the pulsatile secretion of GnRH/LH and block ovulation. Apparently, in sheep, EOPs inhibit pulsatile LH secretion in the postpartum period, regardless of the presence of the lamb (Gregg *et al.,* 1986; Malven and Hudgens, 1987; Newton *et al.,* 1988).

Table 1. Reestablishment of postpartum ovulatory activity in Pelibuey sheep under two lactation programs.

Treatment	n	Ewes ovulating in the first 105 days postpartum		First postpartum ovulation (Days)
		%	No.	
RS	17	64.7[a]	11	83.2 ± 5.5[a]
US	11	100[b]	11	58.8 ± 5.5[b]

[ab] Different literals in the same column indicate difference (P <0.05). (mean ± S.E.)

RS: Restricted suckling to 17 hours per day (ewe-lamb separation for 7 hours per day during lactation).

US: Continuous or unrestricted suckling (ewe-lamb contact and interaction for 24 hours per day).

In this study, contrary to expectations, restricted ewe-lamb contact for 7 h/day, prolonged the ICO. We suggest that an inhibitory mechanism, which did not involve EOPs, delayed ovulation in sheep RS. In 64.7% of these females, the duration of postpartum anestrous lasted 23.2 days after weaning, which occurred at 60 days postpartum. 35.5% of the sheep from this group did not ovulate in the first 105 days postpartum. So why did sheep separated from their lambs show a longer period of postpartum anestrous? This observation is difficult to explain, but it has been reported that in sheep, psychosocial stressors are generated by factors such as social isolation, restriction of movement, vision, and also exposure to signals caused by predators (odor or canine vocalizations) (Tilbrook *et al.,* 2000, Breen *et al.,* 2007; Wagenmaker *et al.,* 2009). These stressful events activate the hypothalamic-pituitary-adrenal; neurons located in the paraventricular nucleus, synthesize and release corticotropin-releasing factor and arginine vasopressin. These neuropeptides, secreted into the hypothalamic-pituitary portal system, are carried to the pituitary, where they stimulate the corticotroph and synthesize a series of peptides derived from pro-opiomelanocortin, including adrenocorticotropic hormone (ACTH), and the beta-endorphin and the stimulating hormone of the alpha-melanocyte. Specifically, ACTH acts on the cortex of the adrenal glands to stimulate the synthesis and secretion of glucocorticoids (Tilbrook *et al.,* 2000). Then, psychosocial stress increases blood levels of glucocorticoids and that inhibits the pulsatile secretion of GnRH/LH in the hypothalamus and pituitary, respectively (Breen and Karsch, 2004, Breen *et al.,* 2007; Wagenmaker *et al.,* 2009) which delays the pre-ovulatory surge of LH and therefore ovulation. It is suggested that in the present study, daily separation of the ewes from lambs, produced a type of chronic psychosocial stress, previously unreported, that behaviorally, is characterized by frequent vocalizations of females, reluctance to move away from the lambs with repeated visits to the confinement area of lambs during the hours of separation. This type of stress could generate the observed delay in ovulation in ewes RS, which can extend beyond weaning, which is also a stressful event. If this hypothesis is correct, then why do the sheep under restricted lactating programs for one or two periods of 30 min/day, showed no delay in the reestablishment of postpartum ovarian activity and conversely, reducing the interval between calving and first postpartum ovulation (Morales-Terán *et al.,* 2004; Pérez-Hernández *et al.,* 2009)? A possible explanation could be associated with the conduct developed by the female during calving and lactation periods in which the priority is caring for the young. Then, the inhibition of reproductive activity during the postpartum period promotes the welfare of lambs, and thus avoiding distractions that may compromise the survival of the newborn (Novak et al. 2000). While the female is in constant contact with the lamb, the bond between them is maintained and reproduction is not a priority for the female. When the ewe-lamb contact is restricted to short periods (30 - 60 min/day), the sheep, being apart from the newborn, is exposed to various distractions. As a result, the mother-lamb bond weakens and loses priority for the mother, even when still nursing. Under these conditions, it is suggested that the neuroendocrine mechanism that inhibits the pulsatile secretion of GnRH/LH, with the participation

of the EOPs and estradiol (basal concentration), concludes and leads to the restoration of ovulatory activity. If this is correct, then separating the ewe from the lamb 7 h/day, is not enough time to interrupt that relationship bond, the temporary separation only creates stress in the female, which could delay the first postpartum ovulation, through the mechanisms described above.

Body weight of females differed (P<0.05) between groups (34.6 $\pm$ 1.0 and 29.5 $\pm$ 1.3 kg in US and RS, respectively), but this difference is due to the effect of time (P <0.01) and no treatment, because there is no interaction between time and treatment (P> 0.05). There was no weight loss in ewes during the postpartum period (data not shown), despite the negative energy balance which was probably due to the expenditure of energy and nutrients required for milk production, previously reported in various lactating animals (Canfield and Butler, 1991; Xu *et al*., 2009).

Reproductive seasonality may be another factor that prolongs the reestablishment of ovarian activity postpartum (McNeilly, 2001; García *et al.,* 2002, Arroyo *et al.,* 2009). But deliveries were in October and November, weaning in January and February at which time the hair sheep in Mexico, are in full reproductive activity (Arroyo *et al.,* 2007). Therefore, it is not feasible to say in the present study, that the neural mechanisms regulating postpartum and seasonal anestrous are sequentially activated, nor that the first postpartum ovulation is delayed.

The WW and DWG were higher (P <0.05) in lambs US, compared with RS (Table 2). There was no difference (P>0.05) between groups in mortality during lactation (Table 2).

Body weight at weaning, reported in this study for both groups is in the range reported in hair lambs (Arroyo-Ledezma *et al.,* 2000; Gonzalez *et al.,* 2002; Macedo and Arredondo, 2008; Pérez-Hernández *et al.,* 2009), ranging from 7.8 to 21.7 kg, and this variability is due to factors such as age at weaning, sex and mode of suckling. With respect to this variable, it was reported that weaning weight was greater when the lambs were in constant contact with the ewes during lactation (Arroyo-Ledezma *et al.,* 2000; Morales-Terán *et al.,* 2004; Perez -Hernández *et al.,* 2009), which also occurred in this study. With regard to DWG, the values recorded in this report (Table 2) are in the range established for young hair lambs (88 to 226 g/day; Arroyo-Ledezma *et al.,* 2000; Gonzalez *et al.,* 2002; Macedo and Arredondo, 2008; Pérez-Hernández *et al.,* 2009). Apparently, this variable is not altered negatively by restricting suckling (Arroyo-Ledezma *et al.,* 2000; Morales-Terán *et al.,* 2004; Pérez-Hernández *et al.,* 2009), but in this study, the temporary restriction of ewe-lamb contact and interaction during lactation decreased (P <0.05) DWG. Probably, the lower consumption of milk and the separation anxiety lambs experienced, and hierarchical competition negatively affected the production indicator. Other factors that affect the DWG and WW in lambs are the type of birth, breed and sex of the offspring (Gonzalez *et al.,* 2002; Macedo and Arredondo, 2008). A low percentage of RS and US sheep had twin births in this study (11% and 9%, respectively), the proportion of male and female lambs in both groups was homogeneous, about 50% for each sex. Therefore, it is unlikely that the observed differences of WW and DWG between groups, were associated with these factors. It is necessary to carry out specific experiments in order to verify the above. Probably US lambs gained more weight because they consumed a greater quantity of milk while being allowed continuous interaction with the ewe. It can be inferred that ewes learned to select higher quality forage, since the adult sheep, by experience, selected grass with the best palatability and nutritional quality (Aldezabal *et al.,* 2002). The percentage of mortality (Table 2) in the lambs was similar (P>0.05) between groups and within the range established for Pelibuey in tropical conditions (5.2-11.2%) (González-Reyna *et al.,* 1991); therefore, restricted ewe-lamb contact during lactation has no effect on lamb mortality. It has been established (Macedo *et al*., 2010) that in extensive sheep production systems there is a greater (P <0.05) prevalence of mortality (30.9%) in lambs before weaning, compared to intensive systems (4.6%). The leading causes of death in both cases are the starvation-exposure syndrome, trauma, asphyxia, respiratory and enteric infections, dystocia, and attack by predators. Grazing can be a risk factor for lambs due to different hazards to which they are exposed while accompanying the females in the pasture. In this study, confining or grazing lambs did not affect mortality.

Table 2. Productive performance of Pelibuey lambs with two nursing programs during the period of lactation.

Treatment	n	Weaning Weight (kg; LS means ± S.E.)	Daily Weight Gain (g; LS means ± S.E.)	Mortality (%)
RS	21	11.4 ± 0.4[a]	143 ± 0.6[a]	4.7[a]
US	13	13.2 ± 0.5[b]	172 ± 0.6[b]	7.6[a]

[ab] Literals in the same column indicate difference (P <0.05).
RS: Restricted suckling to 17 hours per day (separated ewe-lamb for 7 h per day during lactation).
US: Continuous or unrestricted suckling (ewe-lamb contact and interaction for 24 hours per day).

CONCLUSION

We conclude that in the tropics, restricted ewe-lamb contact for 7 h/day, prolonged the duration of postpartum anestrous in Pelibuey sheep and retarded the growth of the lambs, without affecting mortality. It is suggested that this model of ewe-lamb contact generates chronic psychosocial stress in females, which may reduce pulsatile LH secretion and delay ovulation, but this has yet to be confirmed.

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