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Short communication

Effect of the length of the suckling period and dietary energy intake in lactation on the duration of postpartum anestrus in Creole goats

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ABSTRACT

The objective of this study was to determine the effect of the length of the suckling period and the dietary energy level on the duration of postpartum anestrus in Creole goats. Twenty-eight multiparous pregnant goats (bodyweight BW 39.9 ± 6.4 kg; mean ± SEM) each bearing a single fetus were used in a 2 suckling period (30 vs. 60 d) × 2 levels of energy intake (low (L) vs. high (H)) factorial design. Kids were separated from their dams 3 d after birth and then suckled twice per day until weaned at 30 or 60 d postpartum. Dams weaned at 30 d postpartum were hand milked for the next 30 d. Goats were individually fed chopped alfalfa hay + balanced concentrates (45:55 DM basis) at 3% of BW in the last month of pregnancy and at the same level to the L goats in the postpartum period when the H goats were fed at 4% of BW. Energy balance was estimated by difference between energy intake and calculated energy requirements. Duration of postpartum anestrus was estimated by determining the occurrence of ovulation from changes in blood progesterone concentrations. There were no significant lengths of suckling x energy intake level interactions. Goats on all treatments lost weight until 45 d postpartum with more weight loss on the low than the high energy intake level (L 4.06 ± 0.43 vs. H 1.99 ± 0.64 kg; $P < 0.05$). The daily 4% FCM production was similar for all treatments (overall mean 1.10 ± 0.01 kg). The difference between the postpartum anestrus period between low (129 d) and high (128 d) dietary energy levels was not significant but no goat ovulated while in negative energy balance. The effect of suckling on the resumption of ovarian activity was highly significant ($P < 0.001$) with goats weaned 30 d postpartum resuming ovulation 102 d after parturition compared to 155 d for those weaned 60 d postpartum. This study illustrates the importance of the bond between the doe and its kid in the prolongation of the anestrus period in goats.

1. Introduction

The reproductive activity of goats has a seasonal pattern which is related to the annual variations of photoperiod, mainly in goats of high and middle latitudes (Fatet et al., 2011). However, many authors have shown that in sheep and goats the effect of photoperiod on sexual activity can be modulated or completely overridden by factors such as maternal nutrition and kids nursing (or suckling) (Delgadillo et al., 1998; Scaramuzzi et al., 2006).

It is well known that nutrition is an important factor affecting reproductive function and influencing the onset of ovarian cyclicity in postpartum ewes and goats (Fatet et al., 2011). Several studies have shown that reproductive performance is related to body weight changes, indicating that body weight increase shortens the duration of postpartum anestrus. These changes can be obtained through supplementation with high energy and/or high protein diets (Meza-Herrera and Tena-Sempere, 2012). Another way to assess the effect of nutrition on reproduction is through energy balance. When the animal's total energy requirements are more than the total energy intake, animals are in "negative energy balance". The effect of negative energy balance on reproduction is characterized by anovulation and anestrus (Scaramuzzi et al., 2006).

The presence of the offspring and suckling are other factors that influence ovarian activity. It has been shown that suckling prolongs postpartum anestrus in cows (Stagg et al., 1998; Sinclair et al., 2002; Montiel and Ahuja, 2005), ewes (Schirar et al., 1990) and goats (Takayama et al., 2010). However, other studies have shown that

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ovulation may occur during the suckling period (Delgadillo et al., 1998).

To develop management methods that enable producers to shorten the period of postpartum anestrus, breed animals all year round and stabilize production, it is necessary to have a deeper knowledge of the factors that control the annual reproductive cycle of goats and their interactions. However, in spite of all the background information, there are no studies on the effect of dietary energy level and its interaction with the effect of natural suckling on the duration of postpartum anestrus in Creole goats. Accordingly, the objectives of this study were: 1) to determine the effect of suckling length and dietary energy level on postpartum anestrus; and 2) to establish which factor is the main regulator of the resumption of postpartum ovarian activity in Creole goats. We hypothesize that a high energy intake and a reduction of the suckling period will decrease the duration of postpartum anestrus. And also that between both factors, dietary energy will be the main factor regulating the resumption of ovarian activity, so goats with higher energy intake will ovulate first regardless of the weaning time of their kids.

2. Material and methods

2.1. Animals, management and treatments

All experimental procedures and animal care practices were in agreement with the Guide for Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010). Experimental trials were conducted in the facilities of the Argentinean Institute of Arid Land Research located in the Scientific Technological Center CONICET Mendoza, Argentina.

In September 2012, approximately 30 days before parturition, a herd of adult female Creole goats from the farming establishment "El Empeño" (Malargüe, Mendoza, 35° 57′ 57″ S, 68° 46′ 15.4″ W) was examined by trans-abdominal ultrasonography. Twenty-eight multiparous adult goats between the third and sixth birth, carrying a single pregnancy (one fetus) in the last month of gestation were selected. During the last month of pregnancy (adaptation period) all goats were individually fed with a daily ration composed of chopped alfalfa hay and balanced pelleted feed (45% and 55%, respectively on a dry matter basis) at a rate of 3% of body weight (BW). All animals were treated for internal and external parasites with ivermectin (0.2 mg kg^{-1} BW). Clean drinking water and mineral licks were always available *ad libitum* (Paez Lama et al., 2014).

At the beginning of parturition, goats were assigned to four experimental groups (n = 7) balanced for body weight (BW 39.9 ± 6.4 kg; mean ± SEM) and body condition score (BCS 1.82 ± 0.22; mean ± SEM, scale 1–5). All goats gave birth between 1st and 31st of October 2012. The experiment had a 2 × 2 factorial design with two levels of dietary energy level (high and low, H and L groups respectively) and two lengths of suckling period (60 and 30 days long groups). All goat kids were separated from their dams at 3 days of age. Thereafter, goat kids were allowed to suckle milk from their respective dams for about 30 min twice daily at 0730 and 1930 h. In the long suckling groups (H60 and L60) kids suckled milk until the end of dam lactation period (at 60 postpartum days). In the short suckling groups (H30 and L30) kids were weaned at 30 postpartum days and then the dams were hand milked (at 0730 and 1930 h) until 60 postpartum days. Short suckling groups were hand milked to prevent that the weaning of kids decreased the energy requirements of goats and produce an overlap between the effect of suckling length and energy balance. Immediately after weaning, kids were moved to a farm located 19 km away, to avoid any kind of interaction with the mother. A daily ration composed of chopped alfalfa hay (40% on DM basis) and balanced pelleted diet (60% on DM basis) was individually offered to dams at a rate of 3% and 4% of BW (at 2 postpartum days) in the groups of low (L30 and L60 groups) and high energy intake (H30 and H60 groups), respectively. Alfalfa hay and pelleted diet were offered twice a day (at 0800 and 2000 h) in separate containers. Refusals were collected and weighed before offering rations. The rations were calculated in order to allow high energy groups leave the negative energy balance before weaning the kids (while low energy after the weaning), based on the nutritional requirements according to CSIRO (2007) and information obtained from previous trials on goats of the same breed (Paez et al., 2001). The experimental diet was offered steadily from the beginning of parturition (October 1, 2012) until the end of the trial when all goats ovulated (April 1, 2013).

Table 1
Chemical composition (on DM basis) of milk produced and of ration offered to goats during trials.

Composition (%)	Milk[a]	Balanced pelleted	Alfalfa hay	Ration[b]
Dry matter	13.8	92.5	92.9	92.7
Crude or milk protein	4.2	14.0	15.1	14.4
NDF		21.6	42.7	30.0
ADF		9.3	31.6	18.2
Ether extract or milk fat	5.1	3.2	1.9	2.7
Ash	0.8	6.2	11.2	8.2
ME MJ/kg[c]	3.5	10.0	9.0	9.6

[a] Composition expressed per kg of fresh milk. Average values for the whole assay and groups.
[b] Ration composed by 40% alfalfa hay and 60% balanced pelleted on a DM basis.
[c] Estimated as: MJ/kg = 1.4694 + (0.4025 x milk fat%) (Nsahlai et al., 2004).

2.2. Sampling and analyses

Every week, the intake, body weight and milk production were individually measured during three consecutive days and then averaged as a weekly value. During the suckling period, milk production was determined through milk consumption of kids (difference between the weight of kids before and after suckling, digital hanging scale with accuracy 10 g). During the milking period the milk production was determined by weighing the milk container. Every week individual milk samples (50 ml) were taken. During suckling period a composite sample was formed from subsamples taken at the beginning, middle and before finishing suckling. While during milking period the sample was taken from the container at the end of milking. Blood samples were taken by jugular venipuncture twice a week from day 3 to day 170 postpartum. The samples were placed into tubes containing anticoagulant (EDTA) and then centrifuged at 3000 rpm for 5 min. The plasma was stored at −20 °C until analysis.

The chemical composition of goat milk was determined in the Laboratory of Food Quality, EEA INTA Salta Argentina, using a precalibrated automatic analyzer (Lactostar, Funke Gerber); and alfalfa hay and balanced pelleted diet were analyzed in the Laboratory of Nutrition and Forage Quality Assessment, EEA INTA Balcarce Argentina (Table 1). The production of 4% fat-corrected milk (FCM), was calculated according to the following formula: 4% FCM (kg d^{-1}) = 0.4 × kg milk yield + 15 × kg fat yield (Haenlein, 2007). The blood progesterone concentration was determined using solid-phase radioimmunoassay with a commercial kit (Progesterone Coat-A-Count RIA kit, Siemens, USA). To validate the use of this kit for goat plasma, some representative plasma samples were extracted twice with ether, evaporated to dryness and reconstituted in one volume of PBS (Phosphate-buffered saline), pH 7.4. Different volumes of unextracted and extracted plasma from the same samples were run in parallel to determine linearity of the assay and interference of plasma proteins in the assay. We found similar values for extracted and unextracted samples and that serial

Table 2
Intervals (days) between parturition to zero energy balance (EB); zero energy balance to ovulation; parturition to ovulation and weaning to ovulation according to dietary energy level and lengths of suckling period in Creole goats.

Intervals	Groups					E		S		Effect[a]		
	H60	L60	H30	L30	S.E.M.	H	L	30	60	E	S	E × S
Parturition-zero EB, d[b]	44.3[b]	60.0[a]	41.5[b]	60.0[a]	3.1	42.9[a]	60.0[b]	50.7	52.2	*	NS	NS
Zero EB-ovulation, d	109.7[a]	96.5[a]	60.2[b]	42.0[b]	4.9	84.9	69.2	51.1[a]	103.1[b]	NS	*	NS
Parturition-ovulation, d	154.0[a]	156.5[a]	101.7[b]	102.0[b]	4.8	127.8	129.2	101.8[a]	155.3[b]	NS	***	NS
Weaning-ovulation, d	94.0[a]	96.5[a]	71.7[b]	72.0[b]	3.0	82.8	84.2	71.8[a]	95.3[b]	NS	***	NS

*$P \leq 0.05$, ***$P \leq 0.001$, NS not significant.
[a] The effects were length of suckling period (S, 30 or 60 days), energy level in diet (E, high and low energy) and their interaction (E × S).
[b] Zero EB, zero energy balance equal to time when energy intake is equal to energy requirement.

dilutions gave a linear response parallel to the standard curve. Thus, we used unextracted plasma for the determinations. Intrassay coefficient of variation at 40% binding was <10% and all the samples were run in a single assay.

2.3. Calculations

It was considered that ovulation occurred when blood progesterone concentration remained above $1\,\text{ng}\,\text{ml}^{-1}$ in at least three consecutive samples (Rivera et al., 2003). The following intervals were calculated: days from parturition to zero energy balance, equal to time when energy requirements are equal to energy intake (Parturition—zero EB); days from zero energy balance to ovulation (Zero EB—ovulation); days from parturition to first ovulation (Parturition—ovulation); and days from weaning to ovulation (Weaning—ovulation).

The energy balance was calculated every fortnight by the difference between metabolizable energy intake (MEI) and metabolizable energy requirements (MER). The MER were calculated adding the metabolizable maintenance (MEM) and milk production (MEL) energy requirements. All equations for calculating the energy balance are described in CSIRO (2007).

2.4. Statistical analysis

All data were statistically analyzed using the GLM procedure of Infostat statistical package (InfoStat, 2012 version).

The data of body weight were analyzed with a repeated measures design according to the following model:

$$Y_{ijk} = \mu + IBW + E_i + S_j + T_k + A_l + \epsilon_{ijkl}$$

where Y_{ijk} is dependent variable, μ is overall mean, IBW is bodyweight at 2 postpartum days as a covariable, E_i is fixed effect of energy level in the diet i, where i = high and low energy, equal to ration offer at 3 and 4% of BW, S_j = fixed effect of suckling length j, where j = 30 and 60 days long, T_k is fixed effect of time, A_l is random kid effect, and ϵ_{ijkl} is experimental error. IBW as covariable was significant and therefore was included in the model. The interaction was not significant and therefore was excluded from the model.

The data of energy intake and energy balance were analyzed with a repeated measures design according to the following model:

$$Y_{ijk} = \mu + E_i + S_j + T_k + A_l + \epsilon_{ijkl}$$

where Y_{ijk} is dependent variable, μ is overall mean, E_i is fixed effect of energy level in the diet i, where i = high and low energy, equal to ration offer at 3 and 4% of BW, S_j = fixed effect of suckling length j, where j = 30 and 60 days long, T_k is fixed effect of time, A_l is random kid effect, and ϵ_{ijkl} is experimental error. The interactions were not significant and therefore were excluded from the model.

The data of 4% FCM production and the intervals from: Parturition—zero EB, Zero EB—ovulation, Parturition—ovulation and Weaning—ovulation were analyzed according to the following linear model:

$$Y_{ijk} = \mu + E_i + S_j + \epsilon_{ijk}$$

where Y_{ijk} is dependent variable, μ is overall mean, E_i is fixed effect of energy level in the diet i, where i = high and low energy, equal to ration offer at 3 and 4% of BW, S_j = fixed effect of suckling length j, where j = 30 and 60 days long, and ϵ_{ijk} is experimental error. The interaction was not significant and therefore was excluded from the model.

The covariance structure for each variable analyzed was chosen by comparing several models with different covariance structures. The covariance structure yielding the lowest AIC value (Akaike's Information Criterion) was selected (Di Rienzo et al., 2011). When significant effect of energy or suckling length was found, differences between means were determined by Fisher's LSD test. For all variables analyzed, significance was stated at $P < 0.05$.

The Pearson correlation between the interval from Parturition—zero EB and Parturition—ovulation was calculated.

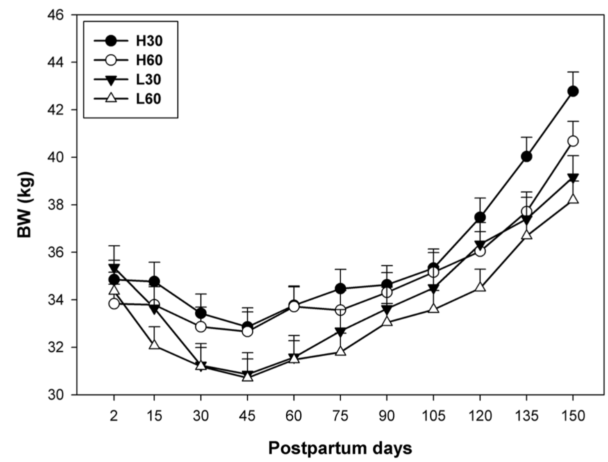

Fig. 1. Body weight (mean ± S.E., values adjusted with BW at 2 postpartum days as covariable) of Creole goats according to energy intake and lengths of suckling period. H30 (High energy and short suckling), L30 (Low energy and short suckling), H60 (High energy and long suckling) and L60 (Low energy and long suckling).

3. Results and discussion

As can be seen in Fig. 1, goats of all groups lost body weight from parturition until 45 postpartum days, but high energy groups showed less weight loss ($P < 0.05$) than low energy groups. Goats from L30, L60, H30 and H60 groups decreased on average 3.85, 4.27, 1.87 and 2.11 kg from 2 postpartum days until 45 postpartum days which represent 10.9, 11.7, 5.3 and 6.0% of their body weight, respectively. This is in agreement with many authors who argue that goats are able to mobilize significant amounts of body fat during early lactation in order to maintain milk production according to their genetic potential, being able to lose between 3–6 kg

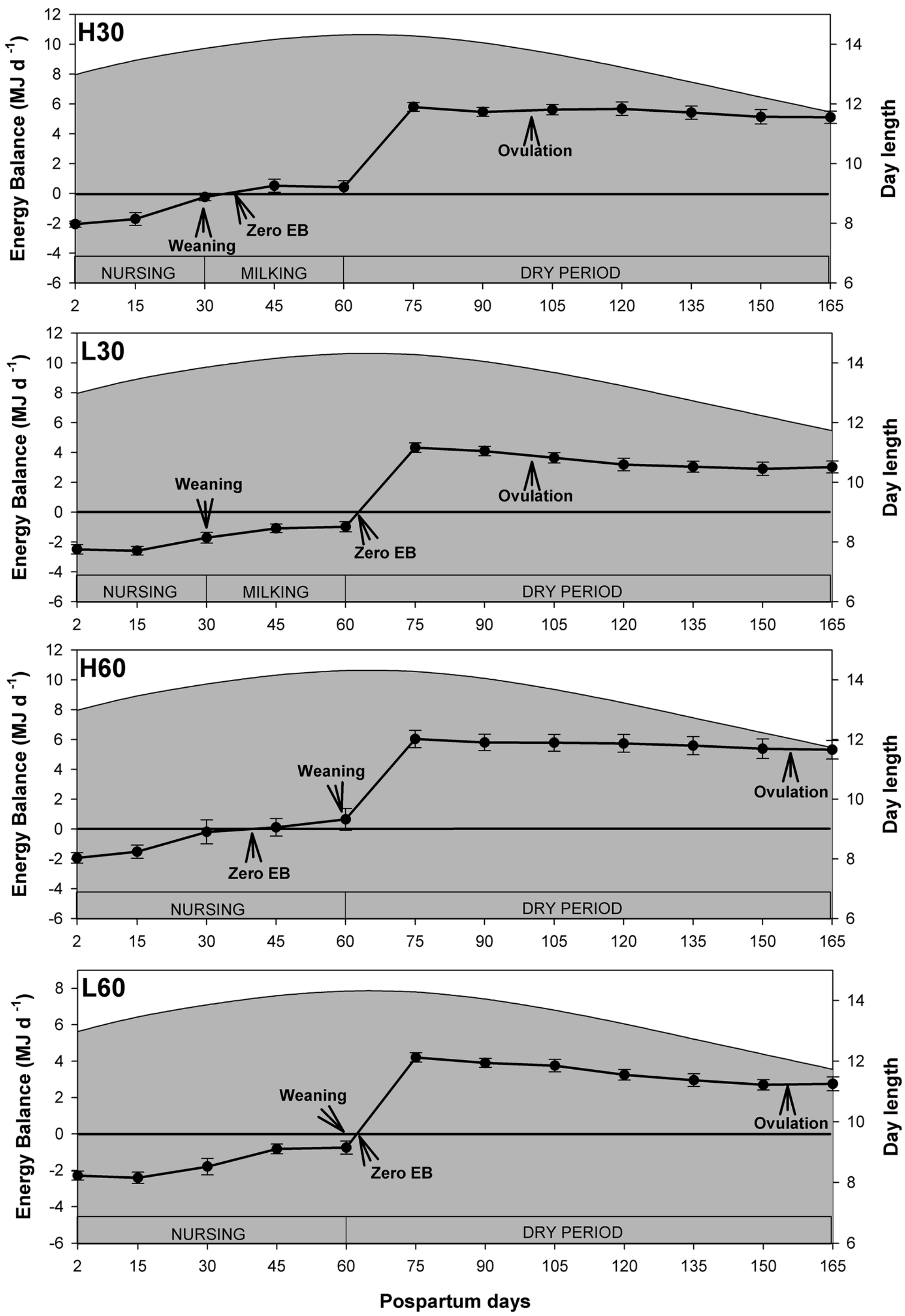

Fig. 2. Energy balance (mean ± S.E.) of Creole goats according to energy intake and lengths of suckling period. H30 (High energy and short suckling), L30 (Low energy and short suckling), H60 (High energy and long suckling) and L60 (Low energy and long suckling). The gray shadow represents the daylight hours. Zero EB (zero energy balance).

of their body weight (Ngwa et al., 2009; Dønnem et al., 2011). Between 90 and 120 postpartum days, no differences were found in body weight between groups ($P > 0.05$), while at 150 postpartum days body weight of high energy groups was significantly higher ($P < 0.01$) than that of low energy groups.

In this study, no effect ($P > 0.05$) of energy level in diet on milk production was found. The 4% FCM production of goats from L30, L60, H30 and H60 groups was on average 1.09, 1.07, 1.14 and 1.10 kg d^{-1}, respectively. These milk productions require on average 6.03, 5.87, 6.31 and 6.07 MJ ME d^{-1} representing 50.9, 53.0, 51.3 and 52.9% of total metabolizable energy requirements for L30, L60, H30 and H60 groups, respectively. For the first and second month of lactation, no effect of suckling length on 4% FCM production was observed ($P > 0.05$). So between 30 and 60 postpartum days, milk production of goats suckling their kids (L60 and H60) was not different from goats hand milked (L30 and H30). This coincides with results of Peris et al. (1997) and Keskin and BiÇer (2002) who found no difference in milk production between natural rearing and mechanical milking systems.

The effect of suckling length on the resumption of ovarian activity was highly significant ($P < 0.001$); goats from short suckling groups (H30 and L30) ovulated on average 72 days after removing their kids, while goats from long suckling groups (H60 and L60) ovulated on average 95 days after weaning their kids. Many authors have found that the duration of suckling influences the length of postpartum anestrus in goats (Delgadillo et al., 1998; Takayama et al., 2010), sheep (Schirar et al., 1990; Ascari et al., 2013) and cattle (Montiel and Ahuja, 2005). At parturition, goats develop their maternal behavior and establish a selective union with their own kids mainly through olfactory recognition. In the first hours after parturition visual and auditory recognition of the kids is also developed (Poindron et al., 2007). The presence of the young strongly inhibits the resumption of postpartum sexual activity, and sensory stimulation caused by suckling is a very important component of this process (Ascari et al., 2013). During early lactation prolactin concentration is increased in goats that nurse their kids as well as in those that are machining milked (Hernandez et al., 2007). Prolactin has an inhibitory effect on GnRH, so high levels of prolactin reduce the concentration of follicle stimulating hormone (FSH) and decrease the frequency of pulses of luteinizing hormone (LH), inhibiting ovulation (Ascari et al., 2013). Thus, lactation and kid suckling delays the resumption of postpartum ovarian activity. However, in our study goats separated from their kids at 30 postpartum days ovulated earlier than goats separated from their kids at 60 postpartum days, although goats weaned at 30 postpartum days were hand milked until 60 postpartum days. This could be because the stimulus produced by kid suckling produces a higher prolactin response than the stimulus caused by manual milking. Accordingly, Hernandez et al. (2007) found that goats allowed nursing their kid for 5 h showed higher concentrations of prolactin than mothers totally separated from them and only milked once daily. These results are also consistent with that found in cattle, where the suckling effect and maternal bond were more important than the level of postpartum nutrition in regulating the resumption of postpartum ovarian activity (Stagg et al., 1998; Sinclair et al., 2002).

As was expected, the average daily energy intake of high energy groups (0.83 MJ kg BW$^{0.75}$) was higher ($P < 0.001$) than low energy groups (0.69 MJ kg BW$^{0.75}$). Therefore, the energy balance of high energy groups was higher than low energy groups at 15 postpartum days ($P < 0.01$) and between 30 and 150 postpartum days ($P < 0.001$). There was no effect of suckling length ($P > 0.05$) on energy balance. The intervals from Parturition—zero EB were shorter ($P < 0.05$) in high energy groups. In addition, high energy groups left the negative energy balance before the end of lactation, while low energy groups were only able to reach the positive energy balance at weaning time (Fig. 2). While, differences in the intervals Zero EB—ovulation, Parturition—ovulation and Weaning—ovulation between groups of high and low energy were not significant, suggesting that the energy intake had no effect on the duration of postpartum anestrus in Creole goats (Table 2). Many authors have reported that nutrition is one of the major factors that affect reproduction (Zarazaga et al., 2005; Mellado et al., 2006) and determine the resumption of ovarian activity after parturition. The weight loss produced by a decrease in the quantity and or quality of diet prolongs the duration of postpartum anestrus in cows (Canfield and Butler, 1991) and sheep (Mbayahaga et al., 1998). Although, low energy groups lost more body weight than high energy groups no differences were found in the interval from parturition – ovulation between these groups.

Scaramuzzi et al. (2006), argue that one way of linking nutrition and reproduction is through the energy balance. In cows, it has been shown that the energy balance is directly related to the duration of postpartum anestrus (Montiel and Ahuja, 2005). In agreement with this Canfield and Butler (1991) found that in dairy cows there is a high correlation ($r = 0.85$) between the energy nadir (most negative value of the energy balance) and ovulation. However, in this study, the correlation between intervals from Parturition—zero EB and Parturition—ovulation was very low and not significant ($r = 0.16$, $P > 0.05$). Furthermore, no differences were found in the interval from zero EB—ovulation between high and low energy groups (Table 2). Although there was apparently no significant effect of energy balance or nutrition on the duration of postpartum anestrus, it is very important to point out that no goat ovulated during its interval of negative energy balance. In agreement, Meza-Herrera and Tena-Sempere (2012) argue that the energy balance is one of the most important internal signals for an animal to determine whether or not to trigger the onset or resumption of the reproductive function. In this study, the lack of statistical difference in the duration of postpartum anestrus between groups of high and low energy might be due to that when the positive energy balance was achieved, the resumption of ovarian activity became controlled by the effect of suckling length, masking the effect of the energy balance. However, under these study conditions this could not be verified.

4. Conclusions

The dietary energy level had no effect on the duration of postpartum anestrus. The length of the suckling period was the main factor regulating the resumption of postpartum ovarian activity in Creole goats. This information can contribute to develop management methods that would allow shorten the period of postpartum anestrus, breed animals all year round and stabilize production, improving the productivity of goat farming systems.

Conflict of interest

The authors declare that there are no conflicts of interest that could inappropriately influence the impartiality of the present work.

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