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Postpartum ovarian activity of Alpine goats consuming different energy levels

Actividad ovárica postparto de cabras Alpinas consumiendo diferentes niveles de energía

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ABSTRACT

34 Alpine goats were used with the objective of evaluating the effect of energy density in the diet, on the return to postpartum ovarian activity. Three experimental groups were formed: E80: 80% of the postpartum energy requirements, E100: 100% of the requirements and E120: 120% of the requirements. The diet was provided 3 weeks before and up to 60 days after delivery. Female weight was determined every five days and the ovaries were scanned by ultrasonography twice a week to assess follicular growth. The day the females ovulated was determined by radioimmunoassay, based on the progesterone concentration. The goats in E120, observed higher weights than in E80 and E100 ($P \leq 0.05$). In E100 the first follicle presented earlier (27.09 ± 08.5 days; $P \leq 0.05$) and presented the highest percentage of ovulation (54.54%). There were no differences in the calving-ovulation interval between E100 and E120 ($P < 0.05$). The level of energy consumption in the diet stimulates postpartum ovarian activity; however, a low percentage of animals ovulate, which may indicate that there are other factors that can condition the complete recovery of the ovary, such as suckling.

Keywords: Postpartum, energy, ovarian follicles, ovulatory activity.

RESUMEN

Se utilizaron 34 cabras Alpinas con el objetivo de evaluar el efecto de la densidad de energía en la dieta, en el retorno a la actividad ovárica postparto. Se conformaron tres grupos: E80: 80% de los requerimientos de energía en postparto, E100:100% de los requerimientos y E120: 120% de los requerimientos. La dieta se proporcionó 3 semanas antes y hasta 60 días después del parto. Se determinó el peso de las hembras cada cinco días y se escanearon los ovarios por ultrasonografía dos veces por semana, para evaluar el crecimiento folicular. Por medio de Radioinmunoanálisis se determinó el día en que las hembras ovularon, con base en la concentración de progesterona. Las cabras en E120, observaron pesos más altos que en E80 y E100 ($P \leq 0.05$). En E100 el primer folículo se presentó más temprano (27.09±08.5 días; $P \leq 0.05$) y presentó el mayor porcentaje de ovulación (54.54%). No hubo diferencias en el intervalo parto-ovulación entre E100 y E120 ($P<0.05$). El nivel de consumo de energía en la dieta estimula la actividad ovárica postparto; sin embargo, un bajo porcentaje de animales ovulan lo que puede indicar que hay otros factores que pueden condicionar la recuperación completa del ovario como el amamantamiento.

Palabras Clave: Postparto, energía, folículos ováricos, actividad ovulatoria.

INTRODUCTION

In the goat, as well as in the rest of the ruminant animals, the last third of gestation is one of the most critical periods of the production cycle. Between the final three weeks of gestation and the initial three weeks of lactation, the metabolic demand for fetal growth and milk production increases, and these physiological events coincide with a decrease in dry matter consumption; which can cause a negative energy balance (NEB). Thus, when energy is scarce, the physiological mechanisms that contribute to its metabolism will favor those processes that ensure the viability of the individual, over those processes that promote growth, longevity and reproduction, delaying the appearance of estrous cycles after delivery (Van Knegsel *et al*., 2005).

During the first week postpartum, waves of follicular growth have been observed in cattle, regardless of the degree of NEB in response to the elevation of Follicle Stimulating Hormone (FSH), which decreases in the second week postpartum. Conversely, estrogen levels decrease in the first postpartum week and begin to slowly increase after the first week, which coincides with the development of the dominant follicle (DF; Beam and Buttler, 1997; Emerick *et al*., 2010). Canfield and Butler (1990) point out that the pulsatility of LH is suppressed until the nadir value of NEB is reached; time in which the LH pulse rate begins to increase stimulating ovulation. DF ovulation occurs only when an LH pulse occurs every 40-60 minutes, to stimulate peak estradiol production, positive feedback, and a pre-ovulatory surge in LH and FSH. These pulsatile episodes are detected around 10-20 days postpartum in milking cows; while in lactating females a delay is observed in the presentation of pulsatile LH secretion (Crowe *et al*., 1998).

On the other hand, the causes of postpartum anestrus in small ruminants is multifactorial, coupled with the nutritional conditions of the handling and the photoperiod; breastfeeding is another potential factor that can delay postpartum ovulatory cycles, since it inhibits the secretion of Gonadotropin Releasing Hormone (GnRH; Morales-Terán *et al*., 2004) that will trigger the hormonal events that lead to ovulation; In this regard, breastfeeding control strategies have been implemented aimed at advancing ovarian cyclicity in the postpartum period.

Based on the above, the objective of the present investigation was to determine whether or not the different levels of energy consumption favor the restart of postpartum ovarian activity in goats that give birth in the reproductive season and are breastfeeding.

MATERIAL AND METHODS

Location

The study was carried out at the facilities of the Caprine Unit of the Faculty of Agronomy and Veterinary Medicine of the Autonomous University of San Luis Potosí, located in the Municipality of Soledad de Graciano Sánchez, San Luis Potosí, Mexico. It is located at 22º11 'North Latitude and 100º56' West Longitude, at an altitude of 1850 meters above sea level. The climate is dry temperate, with a southwestern fringe of semi-dry temperate climate. The average annual temperature is 17.1 °C, the warm temperature includes the months of March to October and the cold period from November to February. Its pluvial precipitation is 362 mm (García, 2004).

Animals and treatments

In order for the parturitions to occur in the month of November and thus eliminate effects confused by photoperiod, in the month of July, multiparous French Alpine breed goats were synchronized, weighing 45±4.3 kg and body condition of 3 on a scale of 1 a 5 (Ghosh *et al*., 2019). Intravaginal devices (CIDR-Pfizer®; 0.3 g natural progesterone) were used which remained for 9 days, 24 hours before removing the devices, 300 IU of eCG (Folligon®-Intervet) were applied intramuscularly. Twelve hours after removing the device, estrus was detected, using males provided with an apron to prevent copulation. Once the female was detected in estrus, a single service was given by means of natural breeding, with an Alpine stallion with a body condition of 3. The pregnancy diagnosis was made 30 days after service by real-time ultrasonography (Sonovet PICO, Universal Medical Systems Inc) and 3.5 MHz convex transducer.

Feeding management

Three weeks before parturition, the treatments were randomly assigned to the females, to form the following experimental groups: E80 (diet that covers 80% of the energy of the stage, NRC, 2007), E100 (diet with 100% of the requirements) and E120 (diet with 120% of requirements; table 1).

Table 1. Energy contribution of the diet and Dry Matter

Treatment	Diet energy (Mcal/Kg DM)	Dry mass (Kg DM/days)
E80	1.48	1.60
E100	1.85	2.00
E120	2.22	2.22

E80: Food with low energy density; E100: Power supply with adequate energy density; E120: Power supply with high energy density. DM: Dry matter

The goats were subjected to an adaptation period to the new diet for 7 days, where their food (A) was gradually replaced by the experimental diets (D), in proportions 85% A: 15% D, 70% A: 30% D, until reaching 100% D. The diet was provided twice a day, for 2 months after delivery (weaning).

The diet was formulated based on chopped alfalfa, oat hay, grain and corn silage, and mineral salts. The goats had free access to water.

Calving care and handling of young

Females and calves were weighed 24 hours after parturition, to avoid that handling interfered with the recognition of the calf and the intake of colostrum. The females were weighed every 5 days, after parturition and until weaning. The pups stayed all the time with the females and after 8 days they received a supplement with 20% CP, in a special pen where the female did not have access. Goats with simple delivery were used, which was determined at the time the treatment was assigned.

Postpartum follicular development

Fifteen days after delivery, twice a week until weaning, an ovarian scan was performed and the day on which the first follicle ≥ 6 mm in diameter appeared on the ovary surface was recorded (González-Bulnes *et al*., 2002, by ultrasonography (Sonovet PICO, Universal Medical Systems Inc), using a 7.5 MHz linear transrectal transducer, coupled to a rigid manipulator, after emptying the rectum, with the goat in a standing position.

Reestablishment of postpartum ovarian activity (Delivery-ovulation interval)

From the 15th day postpartum, blood samples were collected from the jugular vein twice a week; using sterile 0.8 × 38 mm needles (Becton Dickinson and Company, Franklin Lakes, USA) and sterile 10 mL Vacutainer collection tubes (Corvac Sherwood Medical, Saint Louis, Mo, USA). The samples were centrifuged for 15 min at 1500 rpm to separate the blood serum, which was stored in 1.5 mL polypropylene microtubes (MCT-150C, AxygenMR Scientific, INC., Union City California, USA), at −20 °C for subsequent serial analysis at the end of the collection period.

The reestablishment of postpartum ovarian activity was evaluated through progesterone concentrations, which were determined by radioimmunoassay (RIA) in solid phase, with assay sensitivity of 0.1 ng mL^{-1} and with an intra-assay coefficient of variation of 4.1%. To determine if the goat restored its ovarian activity (ovulation), the criterion of Srikandakumar *et al*. (1986) who point out that when two consecutive samples present 0.5 ng mL $^{-1}$, or more than 1 ng mL^{-1} of progesterone in a single sample, then there is a functional corpus luteum in the ovary and therefore the female has ovulated. According to the laboratory analysis, the day on which increases in progesterone concentration were observed, according to the criteria established above, was recorded.

Statistical analysis

For the variable live weight change, an analysis of repeated measures over time was used with the MIXED procedure of SAS (2002). To determine differences in the number of goats that ovulated before 60 d postpartum, the chi-square homogeneity test will be used. The variables, days on which the first follicle with the largest diameter appeared and the day of ovulation, were analyzed using a completely randomized experimental design and Tukey's tests ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Change of live weight in the postpartum period

There were no differences in the weight of the females at parturition (P> 0.05). From the 15th day postpartum, goats in E120 presented the highest weights (Table 2) and those in group E80, the lowest. This behavior was maintained until day 30 postpartum; However, from day 35 postpartum, goats E80 and E100 began to lose weight, presenting differences (P≤0.05) with respect to goats at E120; what was observed until the end of the evaluation period. On day 65, after delivery and when weaning had already been carried out, a slight tendency to increase weight is observed in the three groups; however, the difference in weight continues to be maintained between treatments E120 vs E100 and E80 (Table 2).

Table 2. Live weight of French Alpine goats consuming different energy levels in the postpartum period

		Postpartum days												
Treatments	n	Delivery weight	15	20	25	30	35	40	45	50	55	60	65	SE
E80	11	57.2a	43.3c	40.1c	39.6c	38.5c	38.7b	38.8b	38.8b	38.7b	38.8b	39.2b	40.7b	5.2
E100	11	58.4a	43.9b	42.8b	42.0b	40.0b	39.6b	39.7b	38.7b	38.4b	38.6b	39.4b	41.5b	5.1
E120	12	57.5a	45.5a	45.2a	44.9a	43.7a	43.8a	44.2a	44.5a	44.5[a]	44.7a	45.2a	48.7a	3.8

Treat: Treatment, n = Number of goats per treatment.
E80: Food with low energy density; E100: Power supply with adequate energy density; E120: Power supply with high energy density.
a, b, c Means with different literals in a row are different (P≤0.05), SE = Standard error.

Lactation is one of the factors that contribute to the greatest loss of weight and body condition in ruminants after parturition. Pérez-Hernández *et al*. (2009) point out that when the young remain in contact with the mother all day, the females lose more weight, and since milk production is stimulated, removing fat tissue reserves, which prolongs the postpartum anestrus. There is controversy among researchers about the role of weight and body condition in postpartum reproduction, Lwelyin *et al*. (1992), point out that ovarian activity restarts even before changes in weight and body condition occur in African goats. Similarly, Mbayahaga *et al*. (1998), found no correlation in the time to which the first postpartum estrus occurs and weight loss in female goats. However, more recent studies do report a delay in the reestablishment of ovarian cyclicity in ewes, due to the loss of weight due to lactation (Godfrey and Dodson, 2003; Robinson *et al*., 2002). Nascimento *et al*. (2014) in goats weaned at 15 days in the postpartum period, did not find a strong correlation between weight and postpartum ovarian activity, for which they concluded that there are other factors such as the number of calving, milk production, age of the mother and involution uterine; which have a joint effect on the return to postpartum estrous activity.

In this study, the goats in E120 maintained their weight in the postpartum period, so it can be attributed to the fact that the energy consumption, higher than their needs for the physiological stage, has been directed to the accumulation of reserves in adipose tissue. The weight differences that existed in the E80 and E100 groups, with respect to E120, coincide with the time in which the goats reach their lactation peak, so the metabolic demand for energy is greater and the energy restriction in the case of E80 and E100 levels may not be enough to cover it and maintain the weight.

Day the first major follicle appears on the surface of the ovary

In goats, as well as in other ruminants, energy participation plays an even more important role, since the metabolic processes that occur between the antepartum and early postpartum period affect the restart of ovarian activity, as well as reproductive efficiency. During this period the body prioritizes functions such as basal metabolism and lactation over reproductive processes; thus, a negative energy balance inhibits the restart of ovarian activity in the postpartum period (Canfield and Butler, 1990).

In all three groups, ovarian activity resumed after the second week postpartum. Goats in group E100 presented a follicle with a larger diameter before 30 days postpartum (P≤0.05; Table 3). In the E80 and E120 groups, on average they were observed days later, although these were more dispersed in time, compared to E100. There was no difference in these two groups (P> 0.05).

Table 3. Day after parturition in which the first follicle reaches the largest size in goats that consumed different energy levels

Energy consumption	N	Presence of the largest diameter follicle (day postpartum)
E80	11	54.33±17.19[a]
E100	11	27.09±08.5b
E120	12	41.27±19.72[a]

a, b Means with different literal in the same with column are different (P≤0.05)

The high frequency of pulsatile LH secretion is decisive for the final maturation of the follicles, in cattle, the secretion of normal LH patterns occurs 10 to 20 days postpartum, Savio *et al*. (1990) indicate an interval for the detection of the first dominant follicle by means of ultrasonography of 11.6 ± 8.9 days, observing the first ovulation in dairy cows with normal puerperium and not affected by cystic ovarian diseases around 15 and 21 days postpartum (Opsomer *et al*., 1996); while in lactating cows it tends to be delayed. In the case of sheep, the first postpartum estrus occurs between 25 and 60 days (Morales-Terán *et al,* 2004).

In sheep and goats, the study of follicular dynamics during the postpartum period is more limited; however Al-Gubory and Martinet (1986) point out that in wool ewes, the number of preantral follicles (2-4 mm) in each ovary increases on day 5 postpartum and this correlates with an increase in FSH secretion after birth. Similarly, Rubianes and Ungerfeld (1993) observed on day 1 postpartum follicles with a diameter of 1–2 mm and on day 5, follicles 2–4 mm, and it is until after day 17 postpartum, when uterine involution is has completed, which are observed on the surface of the ovary follicles larger than 4 mm. Bartlewski *et al*. (2000) by means of ultrasonography observed follicles greater than 4 mm up to day 21 postpartum. Zongo *et al*. (2015) point out that in Sahelian goats, ovarian activity begins in the first week postpartum, characterized by 1 to 3 medium-sized follicles in both ovaries. Thus, one of the physiological events that mark the beginning of postpartum ovarian activity in small ruminants is the development of follicles at a diameter where they have the potential to ovulate (≥6mm; González-Bulnes *et al*., 2002).

The results of the study agree with those reported by these researchers. Although smaller diameter follicles were observed in each ovarian scan, larger follicles were observed after the third week postpartum. It should be noted that in goats at E80 and E120, a wide range of days was observed in the appearance of this follicle, contrary to E100, where goats presented this follicle in a more grouped form. Although the growth of the follicle is energy dependent, it can be seen that as long as the goat meets its energy demands 100% it is enough to promote the hormonal mechanisms that trigger ovulation after giving birth. Likewise, the growth of the follicle until reaching an ovulation diameter is consistent with the time in which the uterine involution has already ended. In this regard, Greyling and van Nierker (1991) point out that caruncular structures return to their normal size at 26.3 days postpartum; while at approximately 28 days the uterine involution has been completed, in goats. Salmazo *et al*. (2008) point out that insulin-like growth factors I and II (IGF-I and IGF-II) and lutenizing hormone (LH) participate in the relationship between energy balance and reproduction. When there is an energy deficit, the low insulin concentration in the blood reduces the secretion of gonadotropin-releasing hormone (GnRH), and as a consequence, gonadotropins decrease, as well as the proliferation of the granulosa cells of the follicle and the activity of the aromatase enzyme (Scaramuzzi *et al*, 2006), therefore postpartum ovarian activity is delayed.

Postpartum ovulatory activity

Based on the progesterone blood analysis, it is confirmed that the percentage of goats that ovulated was higher ($P \leq 0.05$) in the goats that met their energy needs according to their requirements for the postpartum period (E100); while females at E80, only one goat ovulated (table 4). According to the criteria of Srikandakumar *et al*. (1986), a female restored her ovarian activity when the progesterone concentration in two consecutive samples is equal to or greater than 0.5 ng mL^{-1}, or when the progesterone concentration is greater than 1 ng mL^{-1} in a single sample. Therefore, following this criterion, the goats in the E100 group ovulated in a higher proportion (54.54%) compared to the E80 and E120 groups.

Table 4. Ovulatory response and calving-first ovulation interval of goats that consumed different energy levels in the postpartum period

Energy consumption	n	Goats that ovulated (%)	Delivery Interval - First ovulation (days)
E80	11	9.09 (1/11)c	43.00
E100	11	54.54 (6/11) a	37.33±5.32a
E120	12	33.33 (4/12)b	34.00±5.71a

a, b Means with different literal in the same with column are different (P≤0.05)

There were no differences (P> 0.05) in the calving-first ovulation interval in goats at E100 and E120 (table 4). Only one goat ovulated at E80 and ovulation occurred until day 43 postpartum. Until the end of the experimental period, no more ovulatory response was observed.

Delgadillo *et al.* (1998) point out that the length of the postpartum anestrum in goats located in subtropical regions is strongly influenced by the time of year in which the parturitions occur, being the longest anovulatory period when parturition occurs in January, compared to the months of May and October. The delivery of these females occurred in the month of November, the full reproductive season for the region. The birth scheduling was done at this time to avoid a confused effect by photoperiod, so it was expected that the higher energy consumption would accelerate the recovery of ovarian activity. The observed response indicates that although energy consumption can promote follicular development, reaching ovulation diameters, as observed in the three groups (Table 2), there are other factors that prevent their ovulation.

The delay in the onset of ovulation after delivery has multifactorial causes, in addition to deficiencies in energy consumption. It has been reported that breastfeeding is a factor that can inhibit GnRH secretion, delaying the onset of ovulation (Morales-Terán *et al*., 2004; Castillo- Maldonado *et al*., 2013). Inhibition of GnRH secretion at the mid-eminence level due to breastfeeding is exerted via endogenous opioids (enkephalins and β-endorphin; Gordon *et al.*, 1997), by acting directly on GnRH-producing neurons (Leshin *et al.*, 1991).

The low ovulatory response observed in goats can be attributed more to the negative effect of suckling on gonadotropin secretion, regardless of energy consumption, as all goats remained with their young during the experimental period.

In sheep, it has been determined that restricting suckling to 30 minutes, 2 times a day, advances the parturition-ovulation interval (Pérez Hernández *et al*. 2009; Arroyo *et al*., 2009; Morales-Terán, 2004; in cattle breastfeeding restriction has also shown positive effects on the reestablishment of postpartum ovarian activity (Zárate-Martínez *et al*., 2010); however, this management strategy for this production system may not be viable, since for the conditions of the study region, the main production objective is the sale of kids at weaning, for which the offspring must remain until this time with the mother so that they can reach the weights required for the market.

The results of this experiment, in the females that ovulated, do not coincide with the results in ewes that remained all the time with their young, where the calving-first ovulation interval occurs at 60.5 ± 2.7 days (Morales-Terán; 2004) and 52.6 ± 2.0 days, and in these females it appeared a few days after 30 days postpartum (table 4). In this sense, these results may indicate that the energy level stimulates follicular growth and ovulation; However, the inhibitory effect of suckling can be even stronger, preventing females, even if they have developed follicles with ovulatory potential in the early postpartum period, from ovulating, even eliminating the effect of the photoperiod.

CONCLUSIONS

Energy consumption promoted a return to postpartum ovarian activity in goats; follicular growth resumed in the first postpartum weeks in animals that met their energy requirements and in those that received an extra supply of energy in the diet. During this stage of high metabolic demand, females maintained their body weight; However, even having the positive effect of the photoperiod, given that the parturitions occurred in the reproductive season, not all the animals ovulated, which indicates that there is another factor that may be inhibiting ovulatory activity, such as suckling the young.

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