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ORIGINAL ARTICLE

A comparison of behavioral and biochemical changes associated with pain between primiparous and multiparous goats around parturition.

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ABSTRACT. This study aimed to examine the physiological, biochemical, and behavioral responses of primiparous and multiparous goats during peripartum, as well as their relationship with the discomfort caused by parturition. Eleven primiparous and 10 multiparous dairy goats maintained under intensive conditions were used in this study. Goat behavior was monitored immediately before and during expulsion. The behavior of the mother and kid was recorded during the first 2 h postpartum. In the three stages of parturition, the intensity of maternal vocalizations, changes in facial Grimace scale, and blood samples to determine the cortisol, estradiol, and protein concentrations were determined. The weight of the kids and litter size were also considered. Primiparous goats took longer to expel the kid than did multiparous goats ($P = 0.04$). A negative correlation was found between cortisol concentration and maternal motivation score at 12 and 8 days prepartum ($P \leq 0.06$), as well as at 24 and 48 h postpartum ($P \leq 0.05$). Cortisol concentrations at 4 h postpartum were negatively correlated with the latency of the first lick to the kid ($P = 0.01$). The latency to suckle was shorter in twin births than in single births kids ($P = 0.02$). Single birth kids weighed more than twin births kids ($P = 0.004$). The duration of the first grooming of the kid was shorter in the heavier than in the lighter kids ($P = 0.028$). The intensity of the vocalizations emitted by the mother in the pre-birth period was greater in the births of heavy offsprings than in those of light offsprings ($P = 0.032$). The expulsion phase was affected only in primiparous goats. Regardless of parity, cortisol and estradiol concentrations were elevated during parturition; the facial Grimace score and the intensity of vocalizations were greater in the expulsive and postpartum phases.

Keywords: *Capra aegagrus hircus L.*, birth, pain, maternal behavior, facial expression, hormones.

INTRODUCTION

The study of maternal behavior and the mother-offspring relationship in goats is scattered; however, its importance lies in understanding in depth the factors associated with this event, which may be related to the mortality rate of the kids. Preweaning mortality in goats is a significant economic concern, with rates ranging from 11.5% to 29% across studies (Dessie & Tilahun, 2021; Ershaduzzaman *et al.*, 2007; Snyman, 2010). Birth weight is a crucial factor affecting kid survival, with lower birth weights associated with higher mortality rates (Chauhan *et al.*, 2019; Yitagesu & Alemnew, 2022). Other factors that influence mortality include breed, sex, litter size, parturition season, and dam characteristics (Ebozoje & Ngere, 1995; Ukanwoko *et al.*, 2012). Male kids, twins, and those born in unfavorable seasons also tend to have higher mortality rates (Snyman, 2010; Yitagesu & Alemnew, 2022). Among these factors, those related to the characteristics of the mother that stand out, are maternal experience and care for the offspring. Therefore, it is necessary to delve deeper into the events that occur around parturition, which could affect offspring survival.

On the other hand, it has been widely demonstrated in sheep that changes in the concentrations of some steroid hormones, such as progesterone and estradiol, play an important role in triggering maternal behavior (Kendrick & Keverne, 1991; Lévy, 2022; Poindron & Le Neindre, 1980); however, such regulation in goats remains unknown. Meanwhile in sheep and goats, it has been shown that proprioceptive factors such as cervicovaginal stimulation caused by the expulsion of the fetus facilitates the establishment of the selective bond (Kendrick *et al.*, 1991; Keverne *et al.*, 1983; Lévy, 2022; Poindron *et al.*, 1988). In goats, when labor is blocked with early epidural anesthesia, maternal motivation is also inhibited, whereas if such blockage occurs after the goat has had contractions (late epidural), the female can express

maternal behaviors (Poindron *et al*., 2007). In conjunction with changes in the concentrations of the hormones mentioned above, labor, particularly the process of expelling the fetus, is a determining factor for the display of maternal behavior. However, as it is an event that causes discomfort and possibly pain, it could have negative consequences for animals that experience it for the first time.

In the case of human, pain is defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage" (IASP, 1979). This definition has received criticism, mainly because it "emphasizes verbal self-report at the expense of nonverbal behaviors that may provide vital information, especially in non-human animals and humans with impaired cognition or language skills" (Raja *et al*., 2020). The definition of pain was updated in 2020, and after a two-years review process by experts in the field, the International Association for the Study of Pain (IASP) modified it as "An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage". The following note is made in this definition: "Verbal description is only one of several behaviors to express pain; inability to communicate does not negate the possibility that a human or a non-human animal experiences pain" (Raja *et al*., 2020).

Alternatively, Zimmerman (1986) proposed a definition for the study of pain in animals, where pain is described as "an aversive sensory experience caused by an actual or potential injury that elicits protective motor and autonomic responses, leads to learned avoidance, and may modify species-specific behavior, including social behavior".

Pain during parturition, although a natural response, can vary in intensity depending on each individual and associated factor. This event can also cause different responses among animals, and, as parturition is an unpleasant event, it can compromise the well-being of the animal afflicted by it (Weary *et al*., 2006). Nevertheless, studies on pain in goats, especially during kidding, and their relationship with behavioral and physiological factors are still scarce.

When parturition is prolonged due to difficulties and becomes dystocia, the pain and discomfort in the female may be greater. Dystocia occurs when labor becomes prolonged, and can be due to insufficient cervical dilatation, uterine torsion, malpresentation, very large fetuses in young mothers, malformations of the fetus, among other factors (Balasopoulou *et al*., 2022; Rahim & Arthur, 1982). Therefore, dystocia can result in maternal exhaustion, lack of interest in the newborn, injuries and even the death of the dam (El-Hamamy & Arulkumaran, 2005). Difficult parturition in goats and kids' mortality have been sparsely studied. A study of goats in Iraq conducted by Majeed and Taha (1989) reported that the most frequent dystocia was caused by malpresentation of the fetus, followed by failure of the cervix to dilate. In the same study, dystocia was reported to be more frequent when kids were male, with a mortality rate of up to 60%. The authors also reported that the frequency of dystocic births was higher in young primiparous goats.

Dystocia is one of the factors studied in goats and other species that causes problems in mother-kid relationship in the postpartum period. According to Redfearn *et al.* (2023), sheep that presented dystocia at parturition showed less maternal interest, whereas their avoidance behaviors towards the newborn increased. In several animal species, discomfort during pregnancy and parturition has been related to changes in the female body associated with these stages, such as adaptation to pain (Driesse *et al*., 2012; Ness & Gebhart, 1990) and changes in the organs involved (Shnol H. *et al*., 2014). Experiencing pain during parturition is an indication of an important process for the individual, as well as an intense physiological response that suggests the search for a safe area for its development (Russell *et al*., 2001). This adaptation will ultimately benefit both the parturient mother and her offspring, and, in the case of goats, the protective isolation possibly induced by the pain of kidding will allow the female to deliver her offspring in a place isolated from disturbances and allow her to form an exclusive bond (Romeyer & Poindron, 1992; Romeyer *et al*., 1994). In addition, the perception of pain is a crucial alert system for monitoring the physical integrity of the body (Bateson, 1991; Monteiro *et al*., 2019).

In veterinary medicine, various studies have evaluated the expression of pain at parturition from different perspectives, such as ethology in sows (Ison *et al*., 2016), and endocrine changes and adrenal responses in cattle (Barrier *et al*., 2012; Mohammad & Abdel-Rahman, 2013; Vannucchi *et al*., 2015) and goats (Hydbring *et al*., 1999; Olsson *et al*., 2004). Animals show behavioral and physiological changes in response to certain stimuli. When they experience pain, they evince several non-verbal signals, such as changes in posture, gestures, and colors (Bateson, 1991; Bellegarde *et al*., 2017). Facial expressions are visual signals that can be recognized and used to evaluate their valence. Mammals can change their facial expression in response to different stimuli, which can be used to assess emotional states, including pain (Diogo *et al*., 2009). Facial expression has been defined as a non-verbal language tool based on gestures and reactions linked to positive or negative emotions, thoughts and particular states of mind (Gudiña, 2023). Thus, pain can be identified through facial movements such as grimace. Grimace scales were first developed a decade ago for use in rodents and currently exist for 10 different mammalian species (Mogil *et al*., 2020).

The analysis of facial expression is based on the changes and alterations that an individual's face can produce under various situations, which is why they are called "facial pain scales", "grimace scales" or "GRIMACE scale". The evaluation of facial expression is the assessment of changes in the face or muscle groups known as "units of action" to a stimulus which are considered involuntary to the experience of pain in the animal (Sotocinal *et al*., 2011). However, very little information is available regarding the application of these scales to evaluate pain in goats. In a recent study conducted by Weeder *et al*. (2023) a facial grimace scale was developed using facial expressions in goats with induced transient lameness, so that it could be assessed with certainty that they

were in the nociceptive stage. In that study, four facial action units were considered: ear position, nostril shape and dilation, orbital tightening, and cheek tightening. The scale used in the present study was adapted from a scale used in sheep, which includes one more unit of action, the lip and jaw profile McLennan *et al.* (2016). Therefore, our work contributes to the understanding of the expression of pain in goats through measurements such as facial grimaces.

To make an objective and comprehensive assessment of pain in animals, it is also important to consider the measurement of biological markers such as some hormones and metabolites. Thus, it is known that variations that occur in the hypothalamus-pituitary-adrenal axis in response to painful stimuli are mostly evaluated by measuring glucocorticoid production. Some studies have shown that under certain circumstances, variations in the concentrations of these hormones could also be associated with negative experiences such as pain (Sapolsky *et al.*, 2000; Sneddon *et al.*, 2014). In a study in goats, where the type of parturition and cortisol concentrations were evaluated, it was found that the highest concentrations in peripartum and during parturition were in goats with pathological deliveries and dystocia (Probo *et al.*, 2011).

The study of factors associated with parturition and mother in goats could contribute to increasing the knowledge of the causes of mortality in kids, particularly those that occur in the first days after parturition. Likewise, events such as complicated and painful parturitions should be easily detected by the producer or manager and treated to guarantee the well-being of the mothers, and consequently, of the newborn. This would help improve the productivity of the herd and the economic situation of goat producers.

The hypothesis of this study is that the process that occurs around parturition in goats causes pain, which in turn affects behavioral, physiological, and biochemical responses, which vary depending on maternal experience. In this regard, the aim of this study was to examine the physiological, biochemical, and behavioral responses of primiparous and multiparous goats during peripartum, as well as the relationship with the discomfort caused by parturition.

MATERIALS AND METHODS

For our measurements, based on the two parturition stages "cervical dilation", and "fetal expulsion and expulsion of membrane" (Purohit, 2010), three phases were stablished: 1) Prepartum phase: equivalent to the cervical dilation phase, where discomfort is observed in the female; there are some pre-parturition signs and contraction, and the amniotic sac appears; 2) Parturition phase: equivalent to fetal expulsion, where the limbs appear and the kid is expelled, and the first postpartum behaviors also begin; and 3) Postpartum phase: equivalent to the membrane expulsion, when greater mother–kid interaction is observed, the first nursing takes place, and the expulsion of the placenta begins.

Subjects and place of study

The study was carried out in the goat production unit of the Facultad de Estudios Superiores Cuautitlán, of Universidad Nacional Autónoma de México, located at 19°31′35'' N and 99°11′42'' W at an altitude of 2256 m.a.s.l., which has approximately 80 adult and young females and 10 bucks. This flock has a reproductive seasonality beginning in July and ending in October. Our study covered mating in August, which corresponded to the sexual season, and the parturitions occurred from January 16 to February 1 of the following year. We selected twenty-one (10 multiparous and 11 primiparous) adult dairy-type female goat crosses of the French Alpine and Toggenburg breeds. A simple stratified probabilistic sampling was carried out on a population of 80 animals; 40 were chosen to meet the following criteria: adult females with more than one previous parturition, and adult females with the possibility of becoming pregnant for the first time. The exclusion criteria were: adult females with more than one parturition; with the possibility of becoming pregnant but presenting a chronic disease that affected their reproduction; or animals that were too old (> 6 years).

The animals were housed in two 12 × 12 m pens and were fed twice a day according to the nutritional needs indicated by the US National Research Counsil (NRC, 2007), with a diet composed of corn silage, oat hay, alfalfa hay and a balanced commercial concentrate, which provided 12% protein and 2.6 Mcal/kg of metabolizable energy. The animals had ad libitum access to water.

To obtain a group of pregnant females in a short period of time, estrus was synchronized by applying intravaginal sponges containing flurogestone acetate (20 mg, Chronogest® CR, Intervet) for 12 days. One day prior to removing the sponge, 1 ml (IM) of Lutalyse® containing 5 mg of Dinoprost Tromethamine-PGF2α (Zoetis CR) was applied. On day 12, upon removal of the sponge, 200 to 400 IU of Novormon 5000®, which contains equine chorionic gonadotropin (Zoetis CR), was administered intramuscularly to each goat. The females were immediately exposed to sexually active male goats, which were provided with a harness with a marker crayon. Approximately 60 days after the introduction of the males, pregnancy diagnosis was made using real-time ultrasound equipment. After mating, 30 pregnant females were identified, of which only 21 described in the groups could be measured.

Experimental process

Experimental groups

From mating to gestation, the goats remained in pens where primiparous and multiparous females were mixed. For this experiment, the groups were named as follows:

• Nulliparous or primiparous group (n = 11) (before and after parturition, respectively): At the beginning of mating, they had an average body weight of 38 ± 3 kg, average body condition score of 2.24 ± 0.45 and were 2 years of age.

• Multiparous group (n = 10): females who had had at least one previous parturition. At the beginning of mating, they

had an average body weight of 50 ± 2 kg, an average body condition score of 2.5 ± 0.25, and were 3 to 5 years of age.

The numbers and descriptions of the goats that gave birth were as follows: multiparous goats (n = 10) gave birth (six single births and four twin births) to six males and eight females, and primiparous goats (n = 11) gave birth (seven single and four twin births) to seven males and eight females.

Body weight and body condition score in goats.

To rule out possible effects of malnutrition, in addition to ensuring that the diet was adequate, the body weight and body condition score (BCS) of the mothers were measured. The goats were weighed on days 12 and 7 prepartum and approximately two hours postpartum, and BCS was measured approximately two hours postpartum according to the scale described by Santucci *et al*. (Santucci *et al*., 1991), which consists of using a scale of 0 to 5 by palpating two anatomical regions, the sternum and lumbar vertebrae. Healthy goats should have a BCS of 2.5 to 4.0; a BCS between 1.0 and 2.0 indicates a management or health problem, whereas a BCS of 4.5 or 5 is almost never observed in goats under normal management conditions. The kids were weighed approximately two hours postpartum, using a portable digital scale brand Trip2 trip® of Chinese origin with a capacity of 40 kg and a precision of 50 grams.

Behavioral observations in the peripartum

The measurements were made using a focal observation method with continuous recording, using indirect observation with the help of video cameras. The records indicated when a goat went into labor, regardless of the time of day. The parturitions of these goats were distributed between 05:00 to 21:00 h

Once the female began labor, the goats were observed and video filmed with portable video cameras (SONY®) placed on a tripod outside the pen, with an observer ensuring that the framing of the subjects would allow for later analysis in the laboratory. This filming allowed the quantification of behaviors before, during, and after parturition, as well as recording the intensity of the sounds emitted by the mother during this process. Filming ended once the mother had delivered the placenta completely. Later, in the laboratory, the videos were edited and analyzed on a desktop computer using the Observer Video Pro® program (Noldus, The Netherlands) to obtain the latencies, frequencies, and durations of the behaviors of the goats and their kids. An expert in maternal behavior in goats and in the management of the program carried out the analysis of all videos.

Behavioral observation around and immediately after parturition

This process was performed in a manner similar to that described by Terrazas *et al*. (2009). Once the first parturition began, continuous supervision of the female herd began, covering a period from 05:00 to 21:00 h every day. These observations were made with the support of trained observers located 3–5 m from the animals. When parturition began, the goats were placed (when possible) in a 2 × 2 m individual pen. The behaviors of the goats and their offspring were recorded on a pre-formatted sheet and filmed with the help of portable video cameras, continuously recording for approximately 2 h after the expulsion of the first offspring.

The recorded behaviors in goats were: duration of the second stage of labor (from the moment the amniotic sac appeared until the extremities appeared); time to first parturition (from the moment the extremities appeared until the first kid was expelled); parturition position of the first kid born (lying down or standing); movements of the rump (when at the time of expulsion the goat in a lying position stretches its rump); movements of the limbs (when the goat gives birth in a lying position, it stretches its hind or front limbs); look at the flanks (at the beginning of labor, when the goat turned its head towards the belly area or the back of its body); time to first lick of the kid (the time when the kid was first groomed after expulsion); duration of the first licking; number of vocalizations (any type of bleat, high or low, that the female emitted during this period) until the first suckling; birth assistance of each of the kids born (when the goat had a parturition with dystocia and it was necessary to perform obstetric management to deliver the kid (Coll-Roman *et al*., 2023; El-Hamamy & Arulkumaran, 2005; Rahim & Arthur, 1982). Obstetric management was always performed by two people with more than ten years of experience in goat parturition.

The recorded behaviors of the kids were latency from expulsion to first attempt to stand; latency from expulsion to first stand; latency from expulsion to first udder search; latency from expulsion to first suckling.

A different person from the one filming recorded the intensity of the vocalizations emitted by the female using a decibel meter (Steren HER-402, China). Only vocalizations considered to be high bleats (when the animal emitted sounds with its mouth open) were recorded. Low bleats (vocalizations emitted with the mouth closed) were not considered, because they are normally associated with maternal calls emitted by goats immediately before giving birth and while caring for their young (Briefer & McElligott, 2011; Lickliter, 1985). Data were obtained from at least 60 vocalizations in total, and this was performed in three stages: during the period that included the exposure of the amniotic sac and the limbs, during the phase of expulsion of the offspring, and minutes after the expulsion. The device was always placed no less than 30 cm near the animal's mouth to avoid disturbing the animals.

Finally, at the end of filming, approximately two hours after parturition, and in the same pen where the goats were weighed and had their temperature measured, the maternal motivation score was evaluated, according to the description of Everett-Hincks *et al*. (2004), and was categorized into three values: Score 1 = little to no interest: when the mother shows no interest and does not follow her kid; she walks away or does something else and does not make low bleats. Score 2 = medium interest: when the mother does not approach her kid but follows it with her eyes and makes low bleats. Score 3 =

very interested: when the mother follows her kid, approaches it, sniffs it, licks it and continues to vocalize.

Determination of the Facial Grimace Score

With the help of the video filming carried out peripartum, three video fractions were taken with a duration of 3 min each. These features correspond to three stages of the process: during labor, at parturition, and postpartum. The analysis of these video filming sections was carried out by a person trained to recognize the scale points and determine the grimace value, from which 10 photos were taken at random, the grimace scale measurement was performed, and then an average was taken. Using the scale used in sheep and the procedure described by McLennan *et al*. (2016) five facial points were analyzed: a) the orbital area (eye contour), b) the cheeks (masseter muscles), c) the position of the ears, d) the profile of the lips and jaw, and e) the shape of the nose and filter. Each facial point was assigned a value of 0 (change not present or "normal position"), 1 (partially present) or 2 (present). Each animal obtained a score for each of the five facial points analyzed. These were added together to determine the final grimace value, with a 0 to10 score.

Hormonal and biochemical determinations

Blood samples were collected approximately on days 12 and 8 prepartum, during parturition, and at 4, 8, 24 and 48 h postpartum. The samples were collected by jugular puncture into two tubes, one with EDTA and the other without anticoagulant. Once collected, the samples were refrigerated and subjected to centrifugation for 20 min at 1500 rpm. Serum and plasma were collected and stored at -20°C until determination in the laboratory. Cortisol and estrogens were determined in the plasma, while the proteins (total, albumin, and globulin) were determined in the serum. Cortisol and estradiol determinations were performed in the Reproduction Laboratory of the Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México using the ELISA technique, with a commercial kit (DRG Diagnostics©, Marburg, Germany), with a dynamic range of 2.5 - 800 ng / mL; the analytical sensitivity of the assay was 2,5 ng ml (6.9 nmol/L). The intra-assay coefficient of variation was 9.99 %. The globulin concentration in the serum (G, g/dL) was obtained as the difference between the total protein and albumin levels. Proteins were determined using commercial reagents (Total protein and Albumin AA) from the Wiener Lab brand (Argentina) and colorimetry.

Statistical analysis

A repeated measurement analysis of variance was performed on the physiological and productive variables (cortisol, estradiol, protein and weight) using the GLIMMIX procedure of SAS® software (SAS on demand for academics). The model included the fixed effect of parity, litter size, time, and the interaction between them. The difference between animals within each parity was considered a random effect. An autoregressive covariance structure of order 1 was used.

The behavior, grimace score and body condition score were analyzed with the nonparametric Kruskal–Wallis and Mann–Whitney U tests for comparisons between groups and litter size, as well as the Friedman and Wilcoxon Signed-Rank Test for comparisons within groups. The proportions of goats that needed birth assistance or not were analyzed with the help of a Fisher test considering a random distribution. The possible existence of a relation between variables from behavior and concentrations of cortisol was determined using the Pearson correlation procedures. A weight categorization into two types was made in the born kids: light (less than 3.0 kg) and heavy (more than 3.0 kg). All variables were compared between these two categories with the Student´s t-test and the Mann-Whitney U test. The data were analyzed using a statistics software (SYSTAT® 13.0). The results are expressed as mean ± SEM, and as median and ranges. Data were considered significant at an alpha level ≤ 0.05 and a trend when $0.05 < P \leq 0.10$.

RESULTS

Behavioral observations during peripartum

Regarding the behavior of the mother, the time to first parturition of the offspring was greater in primiparous females than in multiparous females ($P = 0.04$). There was a tendency for primiparous females to take a longer time in the second stage of labor than multiparous females ($P = 0.08$). No significant difference was observed in the movements of the limbs ($P = 0.81$), in the movements of the rump ($P = 0.38$) or in the frequency of looking at the flanks ($P = 0.69$) between the groups of primiparous and multiparous mothers (Table 1).

The latency to licking the kid ($P = 0.40$) and the duration of the first lick ($P = 0.93$) did not differ between the groups, nor were there differences in the number of vocalizations that the mothers emitted until the first nurse between primiparous and multiparous mothers ($P = 0.308$). The maternal motivation score did not show a significant difference between the groups ($P = 0.29$) (Table 1). None of the behaviors recorded in the mother during the peripartum period were affected by litter size ($P > 0.05$).

Table 2 shows that there were no differences between the primiparous and multiparous groups ($P > 0.05$) regarding the intensity of the vocalizations emitted by the females in the three observation periods (prepartum, during parturition and postpartum). When comparisons were made within a group, the primiparous goats tended to emit vocalizations with greater intensity in the postpartum period than in the prepartum period ($P = 0.068$) (Table 2). The intensity of vocalizations emitted by mothers at any stage was not affected by litter size ($P > 0.05$).

Regarding the proportion of goats that took a given position to give birth to the first kid, we found that 8/11 primiparous and 5/10 multiparous goats delivered while recumbent and this did not differ between them ($P = 0.40$). All other goats delivered from a standing position (3/11 vs. 5/10, respectively, $P = 0.48$).

Table 1.
Frequency or duration of the behaviors (median and minimum–maximum ranges) recorded in the peripartum period in primiparous and multiparous goats (Mann–Whitney U test).

Behaviors	Primiparous (n=11)	Multiparous (n=10)	*P* - value
Number of limb movements	5.5 (1-11)	5 (2-21)	0.80
Number of rump movements	5 (2-18)	7 (7-7)	0.38
Number of looks to the flanks	5 (1-8)	8.5 (1-16)	0.68
Duration of second stage of labor (sec)	200 (22-532)	40.5 (13-172)	0.089
Time to first birth or parturition (sec)	467 (254-1496)	235.5 (26-294)	0.04
Kid lick latency (sec)	4 (2-5)	4 (3-6)	0.40
First lick duration (sec)	86.5 (13-229)	93 (13-240)	0.93
Number of vocalizations until the first suckling	92 (57-144)	67.5 (45-135)	0.30
Maternal motivation score	2.5 (1-3)	3 (1-3)	0.29

Table 2.
Intensity of vocalizations (median and minimum–maximum ranges) recorded during prepartum, parturition and postpartum periods in primiparous and multiparous goats (Friedman and Wilcoxon Signed-Rank Test).

Stage	Primiparous (n = 11) (dB)	Multiparous (n = 10) (dB)	*P* - value
Prepartum	63.5 (55.9-76.1)	61.2 (53.3-68.6)	0.30
Parturition	60.3 (52.7-81.8)	60 (53.1-73.3)	0.83
Postpartum	55.5 (54.6-63.1)	61.1 (58.5-63.8)	0.34

Concerning the need of birth assistance for each of the kids born, primiparous females tended to require more assistance during parturition than multiparous females (10/15 vs. 3/14) ($P = 0.052$). The rest of the kids born in each group did not require assistance and this did not differ between parities (5/10 vs. 11/14) ($P = 0.13$).

When identifying the sex of the kids during the assistance to parturition, no differences were found between males and females (six males and eight females) ($P = 0.59$). Also, litter size did not affect the number of kids requiring help to be born (six single and eight double) ($P = 0.59$). Finally, in the categorization by weight, no difference was found between the heavy and light kids that required assistance to born (seven light and seven heavy) ($P = 1.00$).

Behavior of the kid after parturition

The time from expulsion to first trying to stand did not differ between those offspring born from primiparous and multiparous mothers (Table 3). Likewise, the time from expulsion to first udder search ($P = 0.30$) and the time from expulsion to first suckling ($P = 0.25$) did not show a significant difference between the kids born to the two groups (Table 3). The latency to stand tended to be lower in twin-birth kids than in single-birth kids [124 (29-1388) vs 953 (195-1790) sec ($P = 0.079$)]. Therefore, the latency of the first suck was lower in kids born from double birth than in those from single birth [59 (28-628) vs 1183.5 (194-2662) sec ($P = 0.025$)]. The other behaviors evaluated in the kids were not affected by the type of delivery ($P > 0.05$).

Facial Grimace Score

In the facial grimace score assessment to primiparous and multiparous goats, no significant differences were observed either during prepartum or at parturition ($P > 0.05$), while in the postpartum measurement, multiparous goats tended to show higher scores on the pain scale than primiparous goats ($P = 0.096$) (Table 4).

When compared within each group throughout the three observation periods, it was found that the grimace scale scores tended to be higher at parturition compared to prepartum (primiparous, $P = 0.066$ and multiparous, $P = 0.068$), while the score was significantly higher at parturition than postpartum (primiparous, $P = 0.01$ and multiparous, $P = 0.02$). The grimace scale score was not affected at any stage by the litter size ($P > 0.05$).

Table 3.
Latency of the behaviors (median and minimum–maximum ranges) recorded during the first 2 h postpartum in the kids born to primiparous and multiparous goats (Mann–Whitney U test).

Behaviors	Primiparous (n=11)	Multiparous (n=10)	*P* - value
Time from expulsion to first trying to stand (sec)	139 (60-648)	176-5 (29-567)	1.0
Time from expulsion to first stand (sec)	1043.5 (89-1790)	629 (29-983)	0.18
Time from expulsion to first udder search (sec)	1288 (55-1878)	806 (28-1154)	0.30
Time from expulsion to first suckling (sec)	1213 (59-2662)	805 (28-1154)	0.25

Table 4.
Facial grimace score (median and minimum–maximum ranges) of primiparous and multiparous goats during prepartum, at parturition and postpartum.

Stage	Primiparous (n=11)	Multiparous (n=10)	*P* - value
Prepartum	3.5 (1-3) B	4.5 (3-7) B	0.44
Parturition	7.5 (5-9) a	8 (5-9) a	0.27
Postpartum	3 (1-4) b	3 (2-4) b	0.096
Time from expulsion to first suckling (sec)	1213 (59-2662)	805 (28-1154)	0.25

a, b indicates differences between parturition and postpartum within groups: $P < 0.05$, Friedman and Wilcoxon Signed-Rank Test. B indicates tendency to differ between prepartum vs parturition: $P = 0.06$.

Body weight and body condition score of goats

The multiparous goats weighed more than the primiparous ones ($P = 0.01$). Likewise, a significant time effect was observed ($P < 0.001$) in both groups, as well as an increase in weight from day 12 to 7 prior to parturition, and a weight loss from day 7 prior to parturition until parturition (Figure 1). No effect of the parity x time interaction was found ($P = 0.55$). Regarding the BCS at parturition, there was no significant difference between the primiparous and multiparous goats [(2.0 (2-3) vs. 2.5 (1.5-3.0), respectively) ($P = 0.45$)]. Neither the weight at the different stages nor the body condition at parturition were affected by the litter size ($P > 0.05$).

Body weight of kids

Weight at birth of kids was not different between those born to primiparous versus multiparous goats (2.94 ± 0.19 vs 2.86 ± 0.18 kg, respectively) ($P = 0.75$). However, litter size affected birth weight of kids, with single kids weighing more than twin kids (3.35 ± 0.20 vs 2.59 ± 0.14 kg, respectively) ($P = 0.004$). Finally, male kids tended to weigh more than female kids (3.17 ± 0.21 vs 2.66 ± 0.14 kg, respectively) ($P = 0.06$).

Hormonal and Biochemical Determinations

There was no parity effect on either cortisol ($P = 0.42$) or estradiol ($P = 0.3$) plasmatic concentrations. There was also no interaction between parity and time for cortisol ($P = 0.74$)

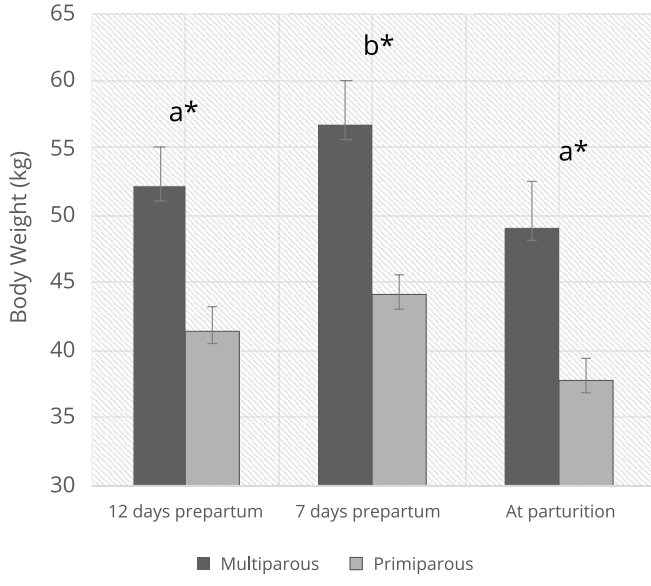

Figure 1.
Body weight (mean ± SEM) of primiparous and multiparous goats on days 12 and 7 prepartum, and at parturition. Different letters indicate significant differences between gestation days ($P < 0.001$). * Multiparous goats weighed significantly more than primiparous goats (Multivariate Repeated Measures Analysis, $P = 0.010$).

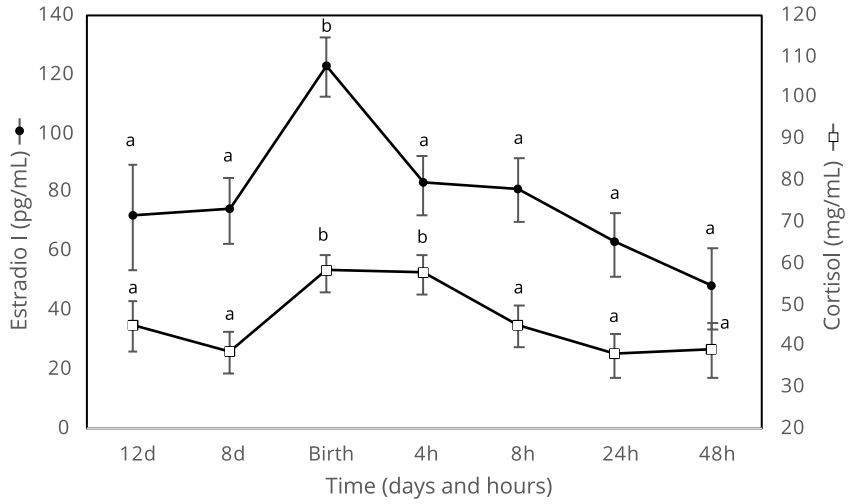

Figure 2.
Plasmatic concentrations (mean ± SEM) of estradiol and cortisol in goats during pregnancy and postpartum. Data are pooled from the two groups. Multiparous n = 8, primiparous n = 8.

and estradiol (P = 0.49). Both cortisol and estradiol concentrations varied over time (P < 0.01). Cortisol concentrations increased from 12 d prepartum to parturition, when it reached its highest value, and then decreased at 24 h, when it reached basal values and remained stable (P = 0.01) (Figure 2). Estradiol concentration increased from 12 d prepartum to parturition, when it reached its highest value, and then rapidly decreased at 4 h, when it reached basal values (P = 0.009) (Figure 2). Cortisol concentrations at parturition tended to be higher in goats from twin birth than in those from single birth, (63.2 ± 3.8 vs 51.15 ± 5.6 ng/mL, respectively) (P = 0.095)

A negative correlation was found between prepartum cortisol concentrations and maternal motivation score (12 d: r = -0.86, P = 0.001; 8 d: r = -0.71, P = 0.006). A negative correlation was found between postpartum cortisol concentrations and maternal motivation score (24 h: r = -0.55, P = 0.04; 48 h: r = -0.65, P = 0.050). Finally, cortisol at 4 h postpartum was negatively correlated with the latency of the first lick to the kid (r = -0.75, P = 0.01).

The concentrations detected of total proteins (P = 0.64), albumin (P = 0.29) and globulin (P = 0.32) were not affected neither by parity, nor by the interaction between parity x time ($P \leq 0.69$). For total protein (P = 0.17) and globulin (P = 0.99) concentrations, time had no effect, while it did affect albumin concentration (P = 0.004), which increased from parturition until reaching the highest value at 8 h (P = 0.007). Afterwards, albumin decreased from 8 h to 24 h (P = 0.01), when it remained stable (Figure 3). Protein concentrations evaluated at different stages were not affected by litter size (P > 0.05).

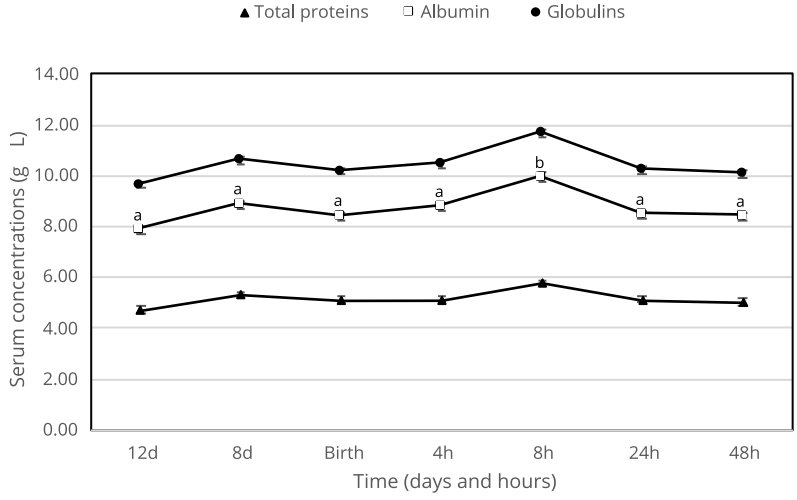

Figure 3.
Serum concentrations (mean ± SEM) of total protein, albumin and globulin, in goats during pregnancy and postpartum. Data are pooled from the two groups. Multiparous n = 8, primiparous n = 8.

Effect of litter weight on the different variables

Eleven kids were categorized as heavy (more than 3.0 kg) and 10 kids as light (less than 3.0 kg). In that regard, the duration of the first lick towards the kid was shorter in the group of heavy kids than in the group of light kids [15 (13-140) vs 143 (43-240) sec, respectively, (P = 0.028)]. The intensity of the goats' vocalizations emitted prepartum was greater in the group of heavy kids than in the light kids group [63.5 (57.8-76.1) vs 57.9 (53.3-65-4) dB, respectively, (P = 0.032)].

Neither the Grimace scale at different stages nor the concentrations of cortisol, estradiol and proteins were affected by the weight of the kids at birth (P > 0.05).

DISCUSSION

The hypothesis that the process that occurs around parturition in goats causes pain, which in turn affects behavioral, physiological and biochemical responses, which vary depending on maternal experience, could be partially confirmed by some of our results.

We found that primiparous goats took longer to expel their offspring and tend to require more assistance at birth than multiparous goats. These results are associated with the duration of expulsion of the kid and are related to those obtained in female water buffalo where females that presented dystocia at parturition had also a longer duration of parturition (Mohammad & Abdel-Rahman, 2013). In addition, these results are consistent with those reported by González-Stagnaro and Madrid-Bury (2004), where they found that primiparous Creole goats had a longer duration of their contraction and expulsion phases than multiparous ones. In some species, prolonged newborn expulsion times may be associated with parturition problems, such as signs of dystocia. In a retrospective study evaluating the information of 198 periparturient goats, it was found that 47% had at least one reproductive problem. Of the females studied in the mentioned study, a positive interaction was found between small breeds and the percentage of attendance at parturition (Coll-Roman *et al*., 2023).

In a study of goats that had been treated for obstetric problems, it was found that 51 of the 89 animals studied had dystocia. The causes of dystocia described by the authors were classified as those attributed to the fetus or those attributed to the mother. The most frequent were dystocia attributed to the fetus, of which flexion of the body and head were the main causes of these dystocia. While from those derived from the mother, the most frequent were those related to a small pelvis, uterine inertia and ringwomb (Rahim & Arthur, 1982) which is more frequent in females giving birth for the first time, as was the case in our study. This also coincides with what was found in the present study where it was observed that primiparous goats tend to emit more insensitive vocalizations in the pre-birth period. This could indicate that there is already a greater difficulty in the progress of labor. The latter coincides with what was reported in another study in primiparous goats of the Murciano-Granadina breed, where it was found that grunting sounds are emitted more frequently by females close to giving birth, particularly from one hour before delivery, and that their emission increases considerably 15 minutes before expulsion (Otal *et al*., 2010).

Studies carried out in sheep also report that, of the causes of dystocia associated with the mother, the most frequent diagnosis was insufficient cervical dilation (Balasopoulou *et al*., 2022). In fact, other studies carried out in both sheep and goats report lack of dilation as a main cause of dystocia (Sobiraj, 1994). On the other hand, in a study conducted in Dorset sheep, it was determined that the incidence of dystocia was significantly higher in two-year-old sheep than in any other later age group (George, 1976).

Regarding behaviors that could be indicative of pain in the mother, no difference was found between primiparous and multiparous goats. Although there are various studies in goats where other behaviors performed by the female during parturition are reported, such as looking at her flanks and stretching her limbs in the lateral recumbent position (Ismail, 2017; Purohit, 2010; Ramirez *et al*., 1995), there are almost no reports where the frequency of presentation of these behaviors has been studied and even fewer making comparisons between primiparous and multiparous females. However, in a recent study where an automated system for detecting parturition in goats was developed, the behavior of stretching the legs when they feel pain during kidding was included in the device to calculate the labor pain index (Kim *et al*., 2024).

Other signs of pain that could be evaluated in the mothers in this study was the Grimace pain scale, where it was found that the values obtained were similar in both groups during the prepartum and parturition periods, regardless of parity. However, the grimace scores were higher for both primiparous and multiparous animals in the expulsion phase. It is also important to clarify that the values obtained in this postpartum stage were lower (around 3) than those obtained in the expulsion stage, where it was observed that both primiparous and multiparous goats had scores close to 9, indicative of severe pain, which could coincide with the high concentrations of cortisol identified during parturition and up to 4 h after. In a study of heifers and goats, the relationship between hormone concentrations that are indicators of stress during parturition and that could reflect discomfort and pain was investigated. In both species, cortisol concentration was found to be highest when the first calf was born (Hybring *et al*., 1999). However, as discussed later in our study it seems that elevated cortisol might not have such effects but might be more related to litter size.

The grimace scores obtained in the present study are similar to those reported in studies carried out in sheep that were exposed to painful procedures or suffered from disease (Guesgen *et al*., 2014; McLennan *et al*., 2016). There are few studies where facial or behavioral scales have been used to evaluate pain in goats. In a study where the visual analogue scale (VAS) was applied to evaluate pain in goats during an electroejaculation procedure, it was found that the values of said scale were higher in the untreated males than in the

sedated or anesthetized ones (Abril-Sanchez *et al*., 2018). Meanwhile, in a recent study in goats where lameness induction was performed and the grimace facial scale was validated, it was found that goats exhibited higher grimace scores 24 h after lameness induction (Weeder *et al*., 2023). Due to the limitation of our study with a small sample size, it is recommended to expand the research to evaluate pain by using facial pain scales in goats.

In the case of the offspring, the behaviors evaluated are in accordance with what was previously reported for the offspring of females kept in stables that have gone through the gestation period with a minimum of stressors (Baxter *et al*., 2016) or whose mothers were fed with energy supplements at the end of pregnancy (Ramírez-Vera *et al*., 2012). In fact, the behaviors of the offspring, such as the latencies of standing, looking for the udder and suckling, were lower than those reported in the literature, even in the group of primiparous females. This indicates that the vitality of the kids in the present study, in both primiparous and multiparous groups, is appropriate to ensure early feeding and adequate affiliation with their mothers.

It was also found that the latency to stand up tended to be shorter in those with twin births than in those with single births, and therefore the latency to suckle for the first time was shorter in the kids in twin births. In different studies on goats, it has been reported that the mortality rate is higher in multiple-birth offspring than in single-birth offspring, and these differences are also associated with the body weight of the offspring at birth (Chauhan *et al*., 2019; Yitagesu & Alemnew, 2022). In fact, in our work we also found that single-birth goats weighed more than those with twin births. However, related to behavior, our study could coincide with another work on primiparous goats where it was observed that the behavior of the mothers towards the offspring varies depending on the size of the litter; the latency of the first sniff and the first lick was lower when the birth was double than when single (Otal *et al*., 2010). This also coincides with what was found in the present study where, when the kids were categorized by weight between heavy and light, the duration of the first cleaning episode carried out by the mother was shorter in the kids weighing more than 3 kg than in the lighter ones. Therefore, it is necessary to elucidate the factors that induce mortality when goats come from multiple births.

No effects were found on cortisol and estradiol concentrations due to parity. Although primiparous goats tended to require more help during delivery, this did not lead to higher cortisol concentrations; therefore, it could not be attributed to a stressful effect derived from the difficulty of delivery, but rather to a metabolic event, since in the present study, a tendency to higher cortisol concentrations was also found at delivery in goats that had twin births compared to those with single births. This could be associated with the nutritional demands that affect the mother's metabolism, since it has been reported that goats that are well fed in the second half of gestation have higher cortisol concentrations than goats that only received 70% of their nutritional requirements (Terrazas et al., 2012).

However, for both steroids, an increase was observed 12 d prior parturition, with the highest concentrations found at parturition. These results coincide with previous studies in pregnant goats fed with 70% of their nutritional requirements and well-fed goats (Terrazas *et al*., 2012).

In the case of cortisol, the study of Terrazas *et al*. (2012) showed a sudden increase of this hormone during parturition in both groups, however the concentrations were higher in well-fed goats compared to the malnourished ones in the period from 41 to 10 d prior parturition. As previously mentioned, the highest concentrations of cortisol were at the time of labor, which coincides with the high scores on the facial grimace scale obtained in the present study, confirming that the goats at this stage experienced severe pain. This is not consistent with what was reported by Weeder *et al*. (2023), where lameness was induced in goats, since cortisol concentrations did not vary due to lameness although the animals already had elevated concentrations 24 h prior the induction of treatment. However, in that study, the authors report that goats exhibited more facial grimacing at 24 h post-lameness induction.

In goats, fluctuation in cortisol concentrations is associated with changes in reproductive functions, which is also associated with the photoperiod. The labor process is activated by the increase in cortisol; at this point, there is a greater adrenal response in the fetus towards the adrenocorticotropic hormone. The initial increase of cortisol concentration in plasma at parturition has been related to the intraplacental exchange between the mother and the offspring. The resulting cortisol enters the maternal circulation inducing labor by activating the production of PF2α. The elevation of cortisol concentration at parturition is partially the result of the pain and stress that accompany this process; the mother goes through the increasing need for glucocorticoids to accelerate breast growth and induce lactation (Probo *et al*., 2011).

The similarities in the behavior of the plasma cortisol and estradiol curves independently of the maternal experience indicate a similar adrenal regulation in female goats. In goats with eutocic parturitions, this cortisol increase occurs during peripartum (Hydbring *et al*., 1999). On the other hand, a significant reduction occurs 24 h after the expulsion of the kid. In dystocic parturition, the peak of cortisol is reached exactly during expulsion and the decrease begins after 36 h postpartum. Dystocia during parturition requires greater muscular work; it also stimulates the onset of inflammatory and stress processes in the mother, so that an increase in plasma values of this hormone is expected and highlights the level of stress associated with birth (Probo *et al*., 2011).

In this study, the pattern of the plasma cortisol curve is similar in the two groups during peripartum, indicating a similar regulation of labor regardless of experience. Likewise, physiological stress requires the mobilization of energy sources and glucocorticoids that have a central role in the regulation of the metabolism through the hypothalamic–pituitary–adrenal pathway (Oyola & Handa, 2017). This is reflected

in a sudden increase in glucose concentrations, particularly at parturition (Terrazas *et al*., 2012).

A negative correlation was also found between cortisol concentrations and maternal motivation at 12 and 8 d prepartum, as well as at 24 and 48 h postpartum. This indicates that high concentrations of this biomarker reflect poor maternal motivation score. These results could be comparable to those reported by Nunes *et al*., 2001 in male marmots that show that there is a relationship between paternal behavior, hormones and paternal experience, as they found that cortisol concentrations were negatively correlated with brood-bearing rates in all males. These results coincide with previous findings in malnourished goats, which when provided with only 70% of their energy and protein requirements in the second half of gestation, the effects were more detrimental on the behavior of the mother than of the kid. Furthermore, it has a higher impact on the establishment of maternal non-olfactory recognition than on maternal care at parturition or the establishment of maternal selectivity, than goats with 100% of their nutritional requirements (Terrazas *et al*., 2009). A later study also showed that malnourished goats had high concentrations of cortisol in that period of gestation than well-fed goats (Terrazas *et al*., 2012).

Contrary to what was found with maternal interest, it seems that in goats high maternal cortisol concentration 4 h postpartum were negatively related to their vitality, since the offspring took less time to take the nipple for the first time. This result could be related to the size of the litter, although in our study we did not find differences in cortisol concentrations between single and twin births. It was observed that the offspring from twin births tended to get up faster and therefore, reached the nipple sooner than those from single births. This result is similar to that reported in another study in goats showing that when mothers suffered more stress during pregnancy and therefore had higher cortisol concentrations, their offspring had better cognitive abilities (Vas *et al*., 2019). The differences found in the correlation of cortisol with the maternal motivation index and the behavior of the offspring require further research, since very little is known about the endocrine control of this behavior in goats.

During peripartum, placental synthesis of progesterone changes to estrogens causing an increase in the estradiol/progesterone ratio (Ford *et al*., 1998; Probo *et al*., 2011). In the present study, no effects of maternal experience on estradiol concentrations were observed, indicating that this hormone has a normal progression independent of parity, contrary to what had been suggested in sheep (Dwyer *et al*., 2003). Our results coincide with those recently reported in goats in which, despite there being differences in the behavior of mothers with and without experience, estradiol concentrations are not different (Cano-Suarez *et al*., 2024). However, a significant variation was found over time, which coincides with previous studies in which it was determined, in well-fed and malnourished goats, that estradiol concentrations begin to rise 12 d prior parturition (Terrazas *et al*., 2012). In this study, these values decreased significantly from parturition up to 24 h afterwards. The significant differences related to the sampling time in the present study suggest that this variation may be normal in goat species. According to what was reported by Currie *et al.* (1988) and Capezzuto *et al.* (2008), in goats, there is no difference in the estradiol pattern at peripartum in relation to the type of parturition (eutocic or dystocic). Regardless of this factor, the antepartum increase in estradiol is gradual and reaches its maximum value at parturition followed by a rapid and significant decrease on the first day after expulsion (Olsson *et al*., 2004; Perveen *et al*., 2019).

In the case of serum proteins, plasma albumin concentrations are elevated in pregnant goats compared to non-pregnant females of a similar age. Our results are similar to those obtained in male goats from which semen was extracted by electroejaculation and it was found that albumin was also elevated in those animals that were not anesthetized or sedated after treatment (Abril-Sanchez *et al*., 2018). Albumin, as some other proteins, are considered biomarkers present in blood serum that can be used as disease indicators; produced due to infections, inflammations or traumas (Murata *et al*., 2004).

However, the general behavior observed for this protein is similar in the two groups (Doweiko & Nompleggi, 1991; Mazzaferro *et al*., 2002). Considering the parity of the goats, the patterns obtained from the protein, albumin and globulin curves were similar between groups, suggesting similar control during this period. The profile of the albumin concentration has been related to the mechanisms of response to heat stress where the values are negatively affected (Inbaraj *et al*., 2018; Perveen *et al*., 2019). For its part in our case, the state of stress that suggests parturition did not significantly or observably affect the concentration of these metabolites in the graphic representation in our study groups.

It is concluded that primiparous goats have longer time spans to give birth and require more help at birth than multiparous goats. Also, primiparous goats tend to emit more insensitive vocalizations in the prepartum than in the postpartum period. The grimace scale scores in the goats of both parities tended to be greater during the expulsion phase than before or after this stage. In the kids, it was found that the latency to stand up tended to be shorter in those from twin births than in those from single births, and therefore the latency to suckle for the first time was shorter in the kids from twin births. The kids from single births weighed more than those from twin births at birth. The duration of the first grooming of the kid was shorter in the heavier than in the lighter offsprings. The intensity of the vocalizations emitted by the mother in the pre-birth period was greater in the births of heavy offspring than in those of light offspring. Cortisol and estrogens in goats from both groups increased significantly from day 12 until parturition. Cortisol tended to be higher at parturition in goats from twin birth than in goats from single birth. A negative correlation was found between cortisol concentrations in the prepartum and postpartum periods, with the maternal motivation scale. Likewise, cortisol at 4 h postpartum was negatively correlated with the latency of the first suckling of the goats.

Conflict of interest

All authors declare that there is no conflict of interest

Author contributions

A.M.T.G., J.P.D., V.M.D.S., R.S.G., K.U.E. contributed to design and conduct of the study. A.M.T.G., K.U.E., R.S.G., V.M.D.S., P.C.C.S., J.J.R.E., O.S.F. executed the experiment and collect the data. S.R analyses the blood samples. A.M.T.G., K.U.E., J.P.D. analyzed the data. All authors interpreted the data, critically revised the manuscript for important intellectual content and approved the final version.

Ethical statement

This protocol was approved by the Subcommittee on the Use and Care of Animals with the reference number SICUAE.MC-2019 / 2-16.

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