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PARTURITION CHARACTERISTICS AND UTERINE INVOLUTION IN NATIVE SHEEP FROM BRAZILIAN PANTANAL

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ABSTRACT

The aim of this study was to describe aspects of the parturition and uterine involution in native sheep from Brazilian Pantanal. Fifty-seven pluriparous ewes were used in this study. At parturition, the placenta expulsion (h), weight (g), total diameter of cotyledons, sex and weight of newborn, and single or twin parturition were evaluated. The uterine involution was evaluated by leucocytes profile, desquamation cells (cervix-uterine smears) and transrectal ultrasonography at 1, 7, 14, 21 and 28 days post-partum. The weight of the placenta and total of cotyledons were higher (P<0.05) in female lambs born from single and twin parturition. Placental expulsion time and cotyledons diameter were not different (P>0.05) between sex and parturition classification. The total of neutrophils in twin parturitions were higher in days 1, 7 and 14 postpartum (78.5±9.5; 58.5±9.6 and 31.9±9.8, respectively). Macrophages were different (P<0.05) at 1 and 14 days postpartum in ewes with twin parturition (12.5±1.6 and 6.7±1.6, respectively). The regression analysis model revealed a quadratic curve estimation (P<0.001) to the diameter, area and uterine volume considering all postpartum period. In conclusion, the parturition characteristics are similar to other breeds. Leukocytary profile and the transrectal ultrasonography exam suggest that the uterine involution in native sheep of Brazilian Pantanal varies among 14 and 21 days postpartum.

KEYWORDS: leukocytes; ovine; parturitium; postpartum period; ultrasonography.

CARACTERÍSTICAS DO PARTO E INVOLUÇÃO UTERINA EM OVELHAS NATIVAS DO PANTANAL BRASILEIRO

RESUMO

O objetivo deste estudo foi descrever aspectos do parto e da involução uterina em ovelhas nativas do Pantanal. Foram estudadas 57 fêmeas pluríparas com cio sincronizado. Ao parto, avaliou-se sua classificação (simples ou gemelar), o tempo de expulsão da placenta (h), peso da placenta (g), total de diâmetro dos cotilédones, além do sexo e peso do cordeiro. A involução uterina foi avaliada pelo perfil leucocitário e de células de descamação (esfregaços cérvico-uterinos) e pela ultrassonografia uterina transretal nos dias 1, 7, 14, 21 e 28 dias pós-parto. O peso da placenta foi superior (P<0,05) nas fêmeas nascidas tanto dos partos simples quanto nos gemelares seguido pelo total de cotilédones. O tempo de expulsão da placenta e diâmetro dos cotilédones não diferiu (P<0,05) entre o sexo e parto. Os períodos 1, 7 e 14 dias pós-parto apresentaram valores superiores (P<0,05) para neutrófilos nos partos gemelares (78,5±9,5; 58,5±9,6 e 31,9±9,8, respectivamente). Macrófagos diferiram (P<0,05) nos períodos 1 e 14 para fêmeas com partos gemelares (12,5±1,6 e 6,7±1,6, respectivamente). A análise da regressão revelou ajuste quadrático (P<0,001) para o diâmetro, área e volume uterinos considerando todo o período pós-parto. As características observadas ao parto nas ovelhas nativas são semelhantes a ovelhas de

outras raças. O perfil leucocitário e análise ultrassonográfica sugerem que a involução uterina nas ovelhas nativas do Pantanal varia entre 14 e 21 dias após o parto.

PALAVRAS-CHAVE: leucócitos; ovinos; parto; período pós-parto; ultrassonografia.

INTRODUÇÃO

Sheep is estimated to have been introduced into Pantanal region along with the European-origin bovines by the Portuguese and Spanish, which came from the Plata Basin and Paraguay, almost three centuries ago. Bovines come originally from temperate climate, but after acclimating to the tropical characteristics of the local environment conditions, they formed a creole type called *Tucura* or *Pantaneiro* (CORREA FILHO, 1926 cited by ROSA et al., 2007). Similarly to bovines, sheep was also gradually acclimated to little management care, and were kept almost exclusively on native pastures of the extensive sandy plains with few subdivisions, allowing the use of selective grazing and use of watering places (GARCIA, 1986). Pantanal native sheep make up a crossbred genetic group, of undefined breed, but with European predominance and fully adapted to regional conditions (McMANUS et al., 2005; SANTOS et al., 2010). Several aspects of the physiopathology of reproduction are not yet known and represent challenges to increase the reproductive rates.

In sheep intensive production, the postpartum period may be considered critical for the continuity of the females' reproductive function. During this period, the uterus returns to regular size, position and tone required for a new pregnancy, in response to the interaction of the hypothalamic-pituitary-gonadal axis. Immediately after delivery, the uterine content is readily expelled due to endometrial contractile activity because of high levels of prostaglandin (E_2 and $F_{2\alpha}$) and estradiol 17_{β} (THORBURN et al., 1991; BARTLEWSKI et al., 2000). This period, known as puerperium, ranges from 23 to 35 days the ewe but can also reach up to 10 weeks with significant decrease in conception rates (Van WYK et al. 1972; KIRAKOFE, 1980).

During the first postpartum week, the vasculature that irrigates the placentones degenerates, resulting in the formation of uterine lochia, a dense brown exudate produced by autolysis of endometrial erythrocytes associated with the dissolution of the fetal and maternal blades of the placentones and expulsion of the remaining cotyledons (Van WYK et al. 1972). In the second week, necrotic plaques are released in caruncular involution, and the lochia becomes less dense and more transparent. Reepithelialization of the caruncular region occurs during the third or fourth postpartum week, along with the decrease in uterine size and length (O'SHEA & WRIGHT, 1984).

Although the effects of uterine infections on the postpartum period in sheep is known (TZORA et al., 2002), the white blood cell count, as well as other physiopathological indicators have been little explored during uterine involution in healthy females, especially in Pantanal-native ewes. The physiological changes observed in the uterine environment are, in part, interacted with leukocyte population and muscle tone in response to luteal activity (GOTTSHAL & HANSEN, 1992; HAYDER & ALI, 2008). Thus, the evaluation of uterine function indicators, including cellular immune profile and postpartum ultrasound examination, adds some subsidies to clinical diagnosis and prognosis of fertility potential of females, especially in intensive production systems. This study aimed to establish some parameters of clinical importance for labor and uterine involution in native sheep from Brazilian Pantanal.

MATERIAL AND METHODS

We selected 57 females from farms in Pantanal, Mato Grosso do Sul State. The ewes were two years old, pluriparous, with an average weight of 39.5 ± 5.20 kg and body condition score ranging from 2.5 to 3 (RUSSEL et al., 1969). The ewes were kept at the Sheep Production Technology Center (Universidade Anhanguera, Fazenda Três Barras, Campo Grande, MS). All females were considered suitable after gynecological examination (HAIBEL, 1990; SHARKEY et al., 2001). In order to concentrate lambing period, all females were submitted to oestrus synchronization by applying intravaginal progesterone pessaries (0.33 g progesterone, Eazi-Breed CIDR) for 06 days, with application of 200 to 300 IU of eCG *(equine chorion gonadotropin)* on the fifth day and use of directed mounts (Pantanal-native males with proven fertility) upon presentation of estrus (DIAS et al., 2001).

Near parturition, the ewes were kept in a protected place with water and pasture *ad libitum*. Upon observation of the first clinical signs of lambing, the ewe was separated from the group, weighed and kept under constant observation. After delivery, we recorded the time (hours) of elimination, the placental weight, sex and weight of the lamb, as well as the type of delivery (single or twin). Regarding the placenta, we recorded the total cotyledon and the average diameter of ten structures randomly observed.

We performed consecutive postpartum analyzes on days 1, 7, 14, 21, and 28. In each of these periods, females were submitted to vaginal gynecological examination and transrectal ultrasound. We collected cervical-uterine samples *(swabs)* and immediately spread them on slides and fixed them with methanol. Subsequently, the slides were stained by Panoptic solution (cyclohexadiene 0.1%, azobenze sulphonate 0.1% and phenothiazine 0.1%). We performed the reading in 15 random fields (bright field microscopy, 400x), considering the total number of neutrophils, macrophages, lymphocytes, eosinophils and cell desquamation. The transrectal ultrasonography (Pie Medical with 8.0 MHz linear transducer) was used to measure the diameter (cm), area (cm^2) and volume (cm^3) of the uterus.

The values obtained after delivery (time of elimination of the placenta, lamb weight, and total cotyledon and its diameter) were submitted to analysis of variance, linear model with random main effect and interactive between sex and type of delivery. Leukocytes and desquamation were compared among postpartum periods (1 to 28 days) by analysis of variance, linear model for measures repeated according to the type of delivery. The simple linear regression model was used to estimate the variation of the uterine diameter, area and volume according to the postpartum period.

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RESULTS AND DISCUSSION

Although the native sheep of Pantanal are considered mongrel, they exhibit similar phenotypic characteristics; resistance, hardiness and functionality adapted to the conditions of the region, and offer interesting genetic variability regarding germplasm conservation (MARIANTE et al., 2011). This fact has generated an increased interest in learning more about the productive conditions of the livestock and the potential for adapting to the herds sustainable management techniques (SANTOS et al., 2010).

The results of the variables observed at delivery are described in Table 1. Overall, these findings are similar to those reported for other sheep breeds, although they are unique for native sheep from Pantanal. However, these results are probably higher than those verified in extensive production systems of this same region, since the animals received sanitary and nutritional management adequate to the reproductive category, besides being managed in a technological production center. The rate of twin births is an example of this aspect. It increased sharply, allegedly by a nutritional effect or by the use of oestrus synchronization and concentrate lambing (NAQVI et al., 2001; MORI et al., 2006). Of the total births observed, 38 (66.6%) were single and 19 (33.3%) twin pregnancies, resulting in 76 lambs, 39 (51.3%) males and 37 (48.7%) females.

The average time of placenta elimination was more than three hours for most of the ewes, regardless of the type of birth and sex of the newborn. This result contrasts with the findings by ARTHUR (1979), who reported a period of one to at most three hours in both single and twin births. This difference may be related to the genetic origin of the animals, i.e., the effect of the different breeds that compose the native sheep (McMANUS et al. 2010). In addition, the weight and size of the female, estrous synchronization, the paternal genetic, and the order of delivery can interfere with this result (KEISLER, 2007). On the other hand, the time of placenta elimination presented no correlation with the other variables studied at birth (Table 2), corroborating studies showing that this relationship is confirmed only in cases of severe uterine infection with placental retention (TZORA et al., 2002; HINDSON & WINTER, 2007). In this study, however, any female retained the placenta or presented postpartum uterine infection.

Table 1. Adjusted mean (± SDM) for variables observed at birth in sheep native to Pantanal of Mato Grosso do Sul, submitted to estrus synchronization and lambing concentration

Variables	Simple (N = 38)		Twin (N = 19)	
	Male (N = 20)	Female (N = 18)	Male (N = 19)	Female (N = 19)
Time of placenta expulsion (h)	3.3 ±0.18[a]	3.4±0.19[a]	3.3±0.19[a]	2.9 ±0.20[a]
Placental weight (g)	407.1±54.24[a]	471.4±56.42[b]	502.4±56.00[b]	547.5±62.21[c]
Total cotyledons	74.4 ±4.0[a]	65.3 ±4.6[b]	91.5 ±4.2[c]	86.2 ±5.0[c]
Cotyledon diameter (cm)	17.2 ±1.5[a]	17.7 ±1.7[a]	21.1 ±1.6[a]	21.7 ±1.9[a]
Lamb Weight (Kg)	3.7±0.13[a]	3.7±0.13[a]	2.6±0.13[b]	2.5±0.15[b]

± standard error of the mean, [a, b, c,] indicates a significant effect ($p < 0.05$) of birth and sex of lambs.

Table 2. Correlation analysis (Pearson's model) for the variables obtained after delivery for sheep native to Pantanal of Mato Grosso do Sul

Variables	Placental weight (g)	Total cotyledons	Cotyledon diameter (cm)	Lamb Weight (Kg)
Time of placenta expulsion (h)	-0.13	-0.16	0.02	0.08
Placental weight (g)	_	-0.06	0.56**	-0.01
Total cotyledons	_	_	-0.25*	-0.04
Diameter of cotyledons	_	_	_	0.21*

* $P < 0.05$, ** $p < 0.01$

The observed correlation between placental weight and cotyledons diameter suggests increased vascular area, possibly originated from the group of ewes with twin pregnancies who had heavier placentas. In this group, total cotyledons were higher compared to the group of ewes with single pregnancy. These aspects revealed that ovine placental vasculature adapts to the type of gestation and it may vary within females of the same breed (PENNINGAA & LONGOB, 1998; SHOENAU et al., 2005).

The means and deviations of leukocytes and desquamation cells observed up to 28 days postpartum are shown in Figure 1. After placenta expulsion, the uterus continues the process of contraction due to the effect of estrogen and prostaglandin $F2_{\alpha}$ on the myometrium. However, the momentary opening of the cervix favors the entry of bacteria leading to an immediate cellular immune response (SHELDON, 2004). Besides infection naturally developed, the endometrium starts a regenerative process, along with the elimination of large amount of mucosal uterine exudate (O'SHEA & WRIGHT, 1984). Blood leukocytes showed that from the first to seventh postpartum days, females with twin pregnancy had a more marked response, characterized by a significant increase of neutrophils and macrophages, in contrast to the lymphocyte profile. This response appears to be related to the larger uterine area, but it suggests that it is physiological according to the changes of the intrauterine environment, being directly associated with phagocytosis of placental structures remaining from postpartum (NASAR et al., 2002). Ewes with simple birth reduced the leukocyte percentage earlier, assuming a faster regenerative process that benefits uterine involution. The cell desquamation profile was contrary to leukocyte profile, with higher averages on the 28th day postpartum. This finding is possibly due to endometrial proliferative activity in this period, in accordance with the reorganization of the endometrial and endocrine structure of the reproductive tract. According to O'SHEA & WRIGHT (1984), on the 23rd day postpartum, there are still some degenerative plaques of placental tissue along the uterine caruncles accompanied by

epithelial cells. GRAY et al. (2003) emphasized this aspect by verifying proliferation of endometrial layer until the 28th day postpartum.

Ultrasound analysis resulted in quadratic adjustments for the uterine diameter, area and volume (Figure 2). However, measurements were less sensitive (correlation coefficients below 50%) for ewes that had twins. This is partly due to the high variation, naturally expected, of uterine size, directly related to the amount of exudate especially until the seventh day postpartum. In subsequent periods, at the 14th and 21st days, there was a substantial reduction in the measures, and on the 28th day the values were considered normal for lactating females (HAUSER & BOSTED, 2002). These findings confirm other studies, although there are methodological, environmental, management and breed differences among experiments. In equatorial climate KIRAKOFE (1980) determined the uterine involution on the 21st day postpartum, contrasting with findings by HAYDER & ALI (2008), who observed a reduction in uterine diameter only on the 28th and 35th days postpartum in subtropical conditions. Therefore, sonographic findings together with the leukocyte profile observed in this study suggest that uterine involution in sheep native to Pantanal occurs between the 14th and 21st days.

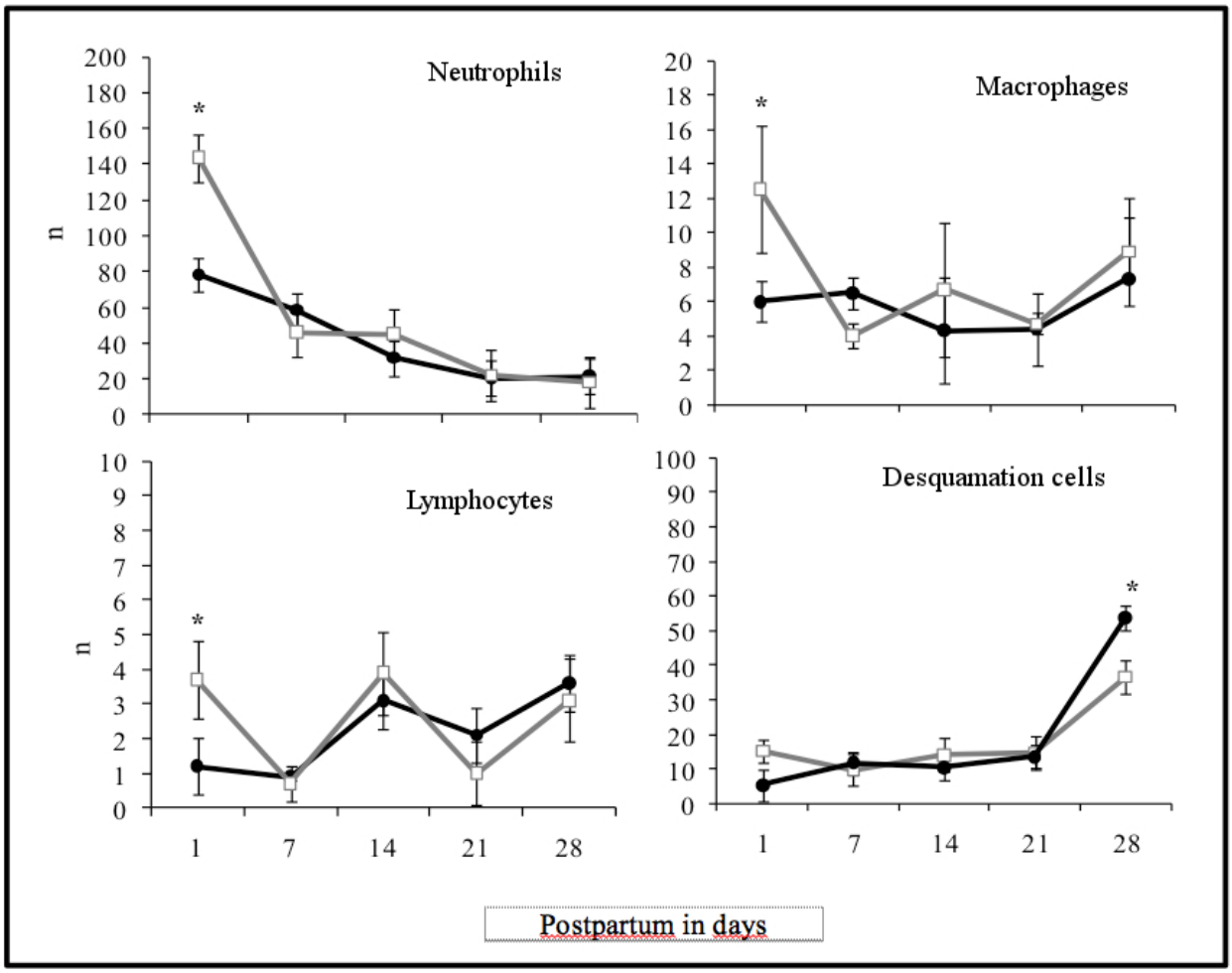

Figure 1. Adjusted mean (± SDM) for leukocytes and desquamation cells observed at simple (●) and twin birth (□) according to the postpartum period in sheep native to Pantanal.

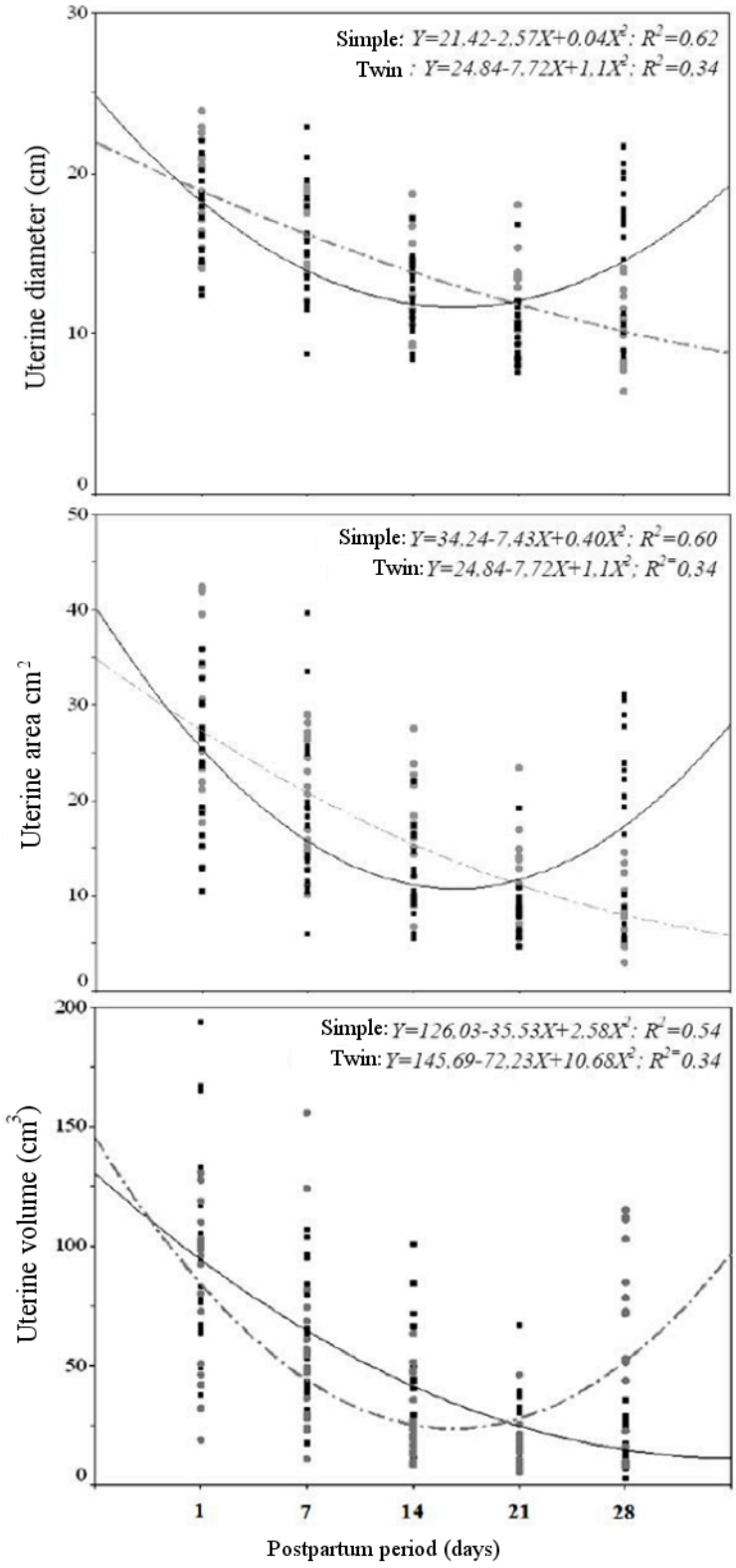

Figure 2. Regression analysis* of the ultrasonographic measures estimated during postpartum of sheep native to Pantanal of Mato Grosso do Sul, with simple (—·—) and twin (——) pregnancy.
* All models are significant (P<0.001).

In conclusion, the parameters observed at birth agree with those reported by other studies and, in general, improve the understanding the physiology of reproduction in ewes from Pantanal. The association of the cervical and uterine leukocyte profile with the transrectal ultrasound brings important elements for the prognosis of uterine function during postpartum. The sheep native to the Pantanal have satisfactory uterine regeneration, allowing its use in intensive breeding programs in technical production systems.

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