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Short communication

Some aspects of the puerperium after singleton and twin parturitions in Santa Inês ewes submitted to energy restriction during pregnancy



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ABSTRACT

The present work aimed to characterize the uterine involution after singleton and twin parturitions in Santa Inês ewes submitted to two nutritional diets. Sixteen pregnant pluri-parous Santa Inês ewes, with age ranging from 3 to 5 years, were used. The animals were randomized into four experimental groups of four animals each. The experiment was a 2 × 2 factorial with number of lambs (singleton versus twin pregnancy) and diet (maintenance diet versus maintenance diet with 15% energy deficit). To determinate the number of fetuses, all ewes were submitted to an ultrasonographic evaluation at days 30, and checked at 45 and 60 days of pregnancy. Genital tract involution was followed by vaginal smear cytology and transrectal evaluation of the uterus by ultrasonography. Changes in cells from the vaginal wall were not useful to characterize the involution process of the genital tract in ewes, however leukocyte cells were. Ultrasonographic observations pointed to a longer period for reduction and stabilization of uterine size after twin parturition, and there was no alteration of the uterine involution process due to the level of nutritional restriction.

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1. Introduction

The interval between successive parturitions is limited by the involution process and the return to cyclic ovarian activity (Jainudeen et al., 2004). There are conflicting reports regarding uterine involution in ewes, which may reflect differences in breeds and management. In serial

investigations using ultrasonography, 97% of the uterine involution in German crossbred ewes occurred approximately day 17 postpartum (Hauser and Bostedt, 2002); in this study, uterine size decreased by 50% of its size, 5 days postpartum. In crossbred wool-type ewes, macroscopic uterine involution was completed approximately 24 days post-partum, although recovery of the uterine epithelial cell layer did not occur before 45 days (van Wyk et al., 1972; Call et al., 1976). Moreover, reports indicates substantial variation in the period needed for the full recovery of the uterus of ewes after parturition (van Wyk et al., 1972; Call et al., 1976; McEntee, 1990; Hauser and Bostedt, 2002; Hayder and Ali, 2008; Nasciutti et al., 2011).

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Nutritional conditions, before and after conception, play an important role in the reproductive efficiency of ewes (Viñoles, 2003). Nutrition, mainly energy status, is essential for the reproductive efficiency of ruminants, affecting age at puberty, duration of postpartum anestrus, embryonic survival rate (Diskin et al., 2003; Rhind, 2004), size of new-born lambs (Robinson, 1990; Susin, 1996), expression of maternal instincts, milk production and service period (Susin, 1996). Nevertheless, there is apparently no data regarding the effect of sub-nutrition on uterine involution in ewes. The Santa Inês breed is not seasonal in the equatorial and tropical regions (Rodrigues, 2001), and is submitted, under natural conditions, to great annual variations in the quality and quantity of available nutrients. Considering the little information available on this breed and the great variation observed among studies, the objective of this study was to characterize uterine involution in Santa Inês ewes receiving maintenance diet or maintenance diet with a 15% energy deficit during single or twin pregnancy of.

2. Materials and methods

The experiment was conducted at 19°55'15" South latitude and 43°56'16" West longitude, during the months of July to September corresponding to the end of the winter (dry) period and beginning of spring. The experiment had a 2 × 2 factorial design (singleton or twin pregnancy versus two diets).

Single and twin pregnancies were identified through ultrasonography by day 30 and rechecked after 45 and 60 days of pregnancy, out of a group of fifty ewes (3–5 years old), mated following estrous synchronization (10 days of intravaginal sponge with 60 mg of medroxyprogesterone plus 200 IU of eCG¹ plus 250 mg² cloprostenol injected the day of progesterone withdrawal). Two twin bearing groups (four ewes per group/G1 and G3) and two singleton bearing groups (four ewes per group/G2 and G4) were formed. After 70 days of gestation, G1 and G2 were submitted to a diet based on the nutritional recommendations of the National Research Council – Nutrient Requirements of Sheep (NRC, 1985), and G3 and G4 received a diet with 15% less energy than that recommended by the NRC for twin and single pregnancies, respectively (all together four different diets).

All animals were kept in metabolic pens until close to parturition, and were then transferred to individual cages. The body condition score at parturition was between 3 and 4, on a scale of 1–5 according to Russel et al. (1969). Starting one week after birth, lambs were allowed access to their mothers from 04:00 PM to 07:00 AM. The rest of the day, they were group-housed close to their mothers, they were fed a diet based on the nutritional recommendations (NRC, 1985) and water was given ad libitum.

Gynecological evaluations were performed 5 days before the expected parturition date, as well as postpartum, at 3 day-intervals from lambing to day 16, and once weekly from day 21 to 56. Transrectal ultrasonography of the genital tract was performed with ewes in the standing position. An Aloka®SSD-500 ultrasound scanner with a 5 MHz linear array transducer was used. Uterine depth was measured and corresponded to the greatest distance between the dorsal and ventral aspects of the uterus (Hauser and Bostedt, 2002). Volume and echogenicity of the intra luminal uterine secretion was recorded based on classification scores. The localization of the uterus was classified as abdominal, pelvic-abdominal or pelvic. Additionally, only after parturition, vascularity of the vaginal wall, the status of the external cervix, the presence or absence of vaginal edema and lacerations, as well as the type of intravaginal fluids were investigated by means of a vaginal speculum.

Colpocytology of the vaginal wall and fornix was collected through a swab (Ceconete, Cecon®, São Paulo, Brazil). Swabs were gently rolled

on two glass microscope slides, which were immediately fixed in alcohol (10 s), air dried and stained with the fast Panótico method (Laborclin®, São Paulo, Brazil). Cells were classified as: superficial anucleated, superficial nucleated, intermediary, parabasal, basal, metaestrus and FOAM (Schutte, 1967a,b). Slides were examined under a 400× bright field optical microscope (Olympus BX41®, Tokyo, Japan). One hundred cells were evaluated on each of the two slides, for each sample. The presence of leucocytes was classified as one, two or three crosses. One cross indicated an average of ≤5 leucocytes per field, two crosses 6–15, and three crosses indicated ≥16 per field (40×).

The Mann-Whitney non parametric test was used to evaluate the association between type of parturition and type of diet, as well as other non parametric variables. The Chi-Square test was used to assess the association between type of parturition and type of diet and other categorical non-parametric variables. The relation between postpartum period and quantitative (parametric) variables was evaluated using Pearson' and Spearman and Newman Keuls's tests (SNK). The vaginal length and the cellular type frequency were evaluated by SNK tests. The non-parametric Kruskal–Wallis test was used to verify the association between postpartum day and a categorical variable.

Differences were considered significant when $P < 0.05$. Analyses were carried out using the statistical programs SAEG and ESSP and the software Excel.

3. Results

All parturitions were normal and occurred in about a one-week period. Lambs born from ewes submitted to nutritional restriction were lighter than lambs from ewes without restriction, both in the singleton and twin parturition groups. On average, the weight of lambs from twin parturitions corresponded to 12% of the weight of the mother, against 9% in singleton parturitions. There was no difference in the uterine regression between diet groups and no interaction between types of gestation and diets was observed. Table 1 presents the uterine regression and R^2 coefficients. Different uterine reduction rates were only observed between singleton (–0.051 cm/day) and twin (–0.030 cm/day) parturitions (Tables 2 and 3).

The ultrasound evaluation demonstrated that mean uterine depth decreased in all ewes from day 1 post-partum until day 50. The sharpest drop was observed from day 1 to day 16, after parturition, corresponding to more than 50% of total regression. The mean values of uterine depth as a function of type of diet (A) and type of parturition (B) are shown in Fig. 1. Temporal regression of the uterine depth was not different in ewes receiving different types of diet, whereas uterine regression was slower after twin parturition ($P = 0.001$). Uterine depth regression stabilized on average on day 35 and 49 postpartum, for single and twin parturitions, respectively.

The position of the uterus on day 1 postpartum was abdominal in 45% and pelvic-abdominal in 55% of the ewes, respectively. On day 4, uterine position was intra-abdominal, pelvic-abdominal or intra-pelvic in 15, 25 and 60% of the cases, respectively. On day 7, 95% of the females had the uterus located in the pelvis, while 5% still had it in a pelvic-abdominal position.

Discrete lacerations were reported in 40% of vulvas, in 95% of vaginas and in 23% of cervixes. Gynecological examination on day 28 showed recovery of all ewes, without any intervention.

Hyperemia of vaginal vestibules, vagina and cervix was observed in up to 95% of the females soon after parturition,

¹ Novormon® – Intervet

² Veteglan® – Calier

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