CLINICAL OBSERVATIONS OF POSTPARTUM UTERINE INVOLUTION IN CROSSBRED DAIRY COWS

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ABSTRACT

The puerperium is the period between birth and complete uterine involution, which is around 40 days postpartum (dpp) in cattle. This study evaluated the dynamics of uterine involution in 19 crossbred dairy cows, who had given one or more births. The evaluation was done by observing the vital characteristics, body score, ultrasonographic and gynecological examinations, and the odor and characteristics of vaginal secretions. These evaluations were done during eight different moments of puerperium: day 0, 3, 7, 10, 14, 21, 28, and 43. The results from this study demonstrated that there are specific differences relative to uterine involution in crossbred dairy cows at 21 and 28 dpp. Additionally, these results suggest that the diagnosis and treatment of clinical endometritis normally done by evaluating the characteristics and the odor of vaginal secretions should be revised, since spontaneous recuperation of endometritis occurred at 43 dpp.

Keywords: Puerperium, vaginal secretions, crossbred dairy cows, gynecological evaluation, transrectal ultrasonography.

INTRODUCTION

Fertility after parturition of dairy cows is considered as the principal economic factor of milk producing farms. Alteration or extension of this phase results in progressive economic lost (PATEL et al., 2006). The sustainability of dairy herds depends primarily on the parturition program and the benefits associated with the physiological consequences (BELLO; PURSLEY, 2006).

The puerperium is defined as the period between gestation and complete uterine involution, being approximately 40 days postpartum in cows. Four events occur concomitantly after birth and expulsion of the placenta: uterine involution, endometrial regeneration, the return of the ovarian cycle, and elimination of bacterial contamination (SHELDON, 2004; SHELDON et al., 2008).

The uterus is sterile during gestation, whereas during or soon after birth, when relaxation of the vulva and cervical dilation occur, microorganisms from the atmosphere, skin, and feces are known to contaminate the uterine lumen (SHELDON, 2004; SHELDON; DOBSON, 2004). Bacterial contamination of cows during postpartum is a dynamic situation, with alterations of bacterial populations and spontaneous recontaminations during the first weeks of postpartum (GRIFFIN; HARTGAN; NUNN, 1974). However, a few weeks thereafter, there is significant reduction of uterine infection and most animals might recuperate from these infections (GRUNERT et al., 2005). Elliot et al. (1968) have demonstrated that the proportion of animals positively diagnosed with uterine bacterial infection is reduced from 93% at 15 days postpartum (dpp) to 9% by 46 to 60 dpp.

Although most cows would have eliminated bacteria during the first five weeks, a small percentage remains infected. Additionally, dystocia, placental retention, and metabolic diseases might exert negative influence (FONSECA et al., 1983).

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The innate immune system is mainly responsible for the elimination of uterine bacterial contamination during postpartum and consist of anatomic, physiological, inflammatory, and phagocytic barriers. The vulva, vaginal vestibule, and cervix are anatomic barriers that prevent the ascension of bacteria to the genital tract. Physiologic barriers include the copious amounts of mucus secreted by the vagina and cervix during estrus. The principal phagocytic barrier is composed of neutrophils that migrate to the uterine lumen in response to invading bacteria, and inflammatory barriers include the nonspecific defense molecules such as lactoferrin and acute phase proteins (GRIFFIN; HARTGAN; NUNN, 1974; ZERBE et al., 2000; SHELDON et al., 2002). However, it is known that the functional capacity of neutrophils is reduced during postpartum, although the exact mechanism still has not been clarified (BAGGIOLINI, 1998). Therefore, this uncertainty justifies the implementation of an early system of diagnosis and treatment of uterine infections.

One alternative that can facilitate the diagnosis of uterine diseases under field conditions is the evaluation of the possible accumulation of purulent vaginal secretion. Further, the severity of these alterations can be verified by evaluating the physical characteristics and the odor of the vaginal secretion (SHELDON; NOAKES, 1998; LEBLANC et al., 2002; WILLIAMS et al., 2005). Additionally, it was verified that purulent vaginal secretions with fetid odor were related to the growth of pathogenic but not opportunist bacteria (WILLIAMS et al., 2005). However, to diagnose uterine infections of cows during puerperium, knowledge of the dynamics of uterine involution and its normal characteristics is of paramount importance. Consequently, the objective of this study was to evaluate the dynamics of uterine involution in dairy cows that demonstrated clear vaginal mucus aspects at 43 days postpartum.

MATERIALS AND METHODS

Animals, geographical location, diet, and herd management. The experiment was done at the Gloria Experimental Farm of the Federal University of Uberlândia, Minas Gerais, Brazil. Nineteen crossbred dairy cows, having two or more calves, with parturitions occurring between July and September, were used during this study. All evaluations were done during the dry period of the region, which extended between the months of April and September, where the average photoperiod was 12 hours/day. All animals were confined during the experiment and received a ration that contained 24% of crude protein; water was administered *ad libitum*. These animals are annually vaccinated against rabies, clostridial diseases, leptospirosis, foot and mouth disease, and paratyphoid. Additionally, all females are vaccinated against brucellosis between three and eight months of age, and are annually evaluated for tuberculosis, leptospirosis, and brucellosis. For reproductive management, the involuntary waiting period was considered as 43 days, pregnancy diagnosis at 28 days after artificial insemination (AI), and pregnancy confirmation at 45 days.

Clinical evaluation. A complete clinical evaluation of the cows was done on the date of parturition, and then on days 3, 7, 10, 14, 21, 28, and 43 postpartum (FEITOSA, 2008). Only females without clinical alterations were included in this study. The following parameters were evaluated: body score (FERREIRA, 1990), classified *as* cachectic (1), thin (2), good (3), fat (4), and obese (5); and vital parameters: rectal temperature (*T°C*), cardiac frequency (CF), respiratory frequency (RF), ruminal frequency (RumF), and color of mucus membranes.

Gynecological evaluation. This consisted of a combination of rectal palpation, ultrasonographic evaluation, and a physical evaluation of the vagina and vulva. All gynecological examinations and samplings were performed by the same vet. During the rectal palpation, the anatomical location (abdominal or pelvic

cavity) and the degree of uterine contraction (relaxed, medium, and Medison Co. Ltd, Seoul, Korea) examination determined the diameters of gravid and non-gravid uterine horn. The physical evaluation was done with a vaginal speculum, to evaluate the vagina, for alterations of color, edema, and lacerations, and the external cervical ostium, where the form (conical, rosette, and loose) and all of the above-mentioned parameters were observed.

Collection and evaluation of vaginal secretion. This was done as previously described (WILLIAMS et al., 2005); briefly, the vulvar region was cleaned and then a gloved hand was introduced to collect the vaginal secretion. The secretion was then classified based on its color and the proportion of its content: absent, clear mucus, bloody-mucus, chocolate-colored mucus, purulent mucus, and purulent. Additionally, the mucus was evaluated relative to its odor: odorless, fetid, very fetid, and extremely fetid.

Statistical analyses. This was done by evaluating the averages, standard deviations, and percentages of the descriptive statistics by using the program Minitab Release 15 (Minitab Inc, Pennsylvania, USA). To evaluate the puerperal influences, the variables were submitted to the Kolmogorov-Smirnov (K.S.) Test so that the data can be classified as having a normal distribution or not. The variables with normal distributions (temperature, heart rate, ruminal frequency, respiratory rate, and the diameter of the uterus) were submitted to the variance analysis (ANOVA). In this case, the differences of the averages were analyzed by the Tukey-Kramer multiple comparison Test; in both instances, the level of significance was considered as 5% ($p \leq 0.05$).

Further, those variables that did not demonstrate a normal distribution (the elevated) were evaluated. The ultrasonographic (Eureka AS-600®, the following: the uterine neck, immediately after the cervix, neck of the color of vaginal, cervical, and vulvar mucus membranes; shape of the cervix; vaginal, cervical, and vulvar lesions; uterine anatomic location; and the odor of vaginal secretion) were analyzed by the Kruskal-Wallis test (non-parametric ANOVA). While the differences of these averages were analyzed by the Dunn multiple comparison test. Additionally, to evaluate the differences in the diameter between the gravid and non-gravid uterus, the obtained variables were constantly submitted to the non-paired T-test, where a significance level of 5% ($p \leq 0.05$) was used (VIEIRA, 2003).

RESULTS AND DISCUSSION

Only non-treated cows, without any alteration during the clinical and gynecological evaluations, and whose vaginal secretions at 43 dpp demonstrated clear and odorless mucus were included into this study. Initially, 23 cows were evaluated, but two were excluded due to acute puerperal metritis (*n*=2) and two with mucopurulent (*n*=1) and sanguinopurulent (*n*=1) vaginal secretion.

There was no significant variation in the temperature and the cardiac rate during the puerperal period (Table 1). The temperature varied between 37.3° ± 3.0 and 38.8° ± 0.6, while the cardiac frequency oscillated between 74.2 ± 13.1 (beats/minute; bpm) and 79.7 ± 11.8 bpm; these values being within the reference limits (FEITOSA, 2008). However, there was significant difference ($p \leq 0.5$) when the respiratory rate was evaluated, the variation being between 29.7 ± 11.8 bpm and 44.4 ± 16.7 bpm. Nevertheless, these values were also within reference limits (FEITOSA,2008).

Table 1. The influence of puerperium on vital parameters and body condition of crossbred dairy cows

Postpartum days	Body score	Heart rate (beats/min)	Respiratory rate (bests/min)	Temperature (°C)	Ruminal movements (mov/3min)
0	2.9 ± 0.5 [a]	78.1 ± 14.1 [a]	44.4 ±16.7 [a]	38.7 ± 0.8 [a]	1.8 ±1.4 [a]
3	2.7 ± 0.4 [a]	79.7 ± 11.8 [a]	34.8 ± 8.9 [ab]	38.8 ± 0.6 [a]	4.9 ±1.2 [b]
7	2.6 ± 0.4 [a]	75.8 ± 10.1 [a]	31.0 ± 6.6 [b]	38.6 ± 0.6 [a]	4.4 ±1.3 [b]
10	2.7 ± 0.5 [a]	79.2± 10.7 [a]	36.8 ± 9.4 [ab]	38.7 ± 0.6[a]	4.4 ±1.4 [b]
14	2.6 ± 0.5 [a]	77.0 ± 8.8 [a]	32.2 ± 5.9 [b]	38.1 ± 0.7 [a]	4.0 ±1.3 [ab]
21	2.7 ± 0.5 [a]	74.2 ± 13.1 [a]	29.7 ±11.8 [b]	38.0 ± 1.6 [a]	4.8 ±1.4 [b]
28	2.7 ± 0.5 [a]	74.8 ± 10.1 [a]	33.3 ± 6.2 [ab]	37.8 ± 2.6 [a]	4.6 ±1.0 [b]
43	2.7 ± 0.6 [a]	78.2 ± 11.4 [a]	36.4 ± 7.4 [ab]	37.3 ± 3.0 [a]	3.8 ±0.9 [ab]

Note: different small letters in the same column indicate statistical difference (p≤0.05).

Significant differences (p≤0.5) were observed when the ruminal frequencies were evaluated during this experiment, having variations between 1.8 ± 1.4 and 4.9 ± 1.2 ruminal movement/each 3 minutes (Table 1). Two to three ruminal movements during a period of two minutes are considered as normal (DIRKSEN; GRÜNDER; STÖBER, 1993). During this study, 21% (4/19) of the cows evaluated demonstrated ruminal atony, 68% (13/19) had ruminal hypotonia, while only 11% (2/19) had normal ruminal movements at parturition. In all ruminants, there is physiological reduction in ruminal contractility that can be increased by a few factors. These include reduced dietary ingestion, which begin effective from the third to second week before birth and is accentuated at birth, and body score, which determines a reduced level of dietary ingestion in fat cows (DIRKSEN; GRÜNDER; STÖBER, 1993; SANTOS, 2006). Reduced ruminal motility at parturition can have negative influence on the pH, microflora, and fermentation of the rumen, which associated with the increase in energy requirements for milk production (negative energy balance), might predispose the affected animal to metabolic disturbances, such as ketosis and abomasal displacement (RADOSTITIS et al., 2002; SANTOS, 2006). It must be remembered that only healthy cows were used during this study, but these could have had subclinical conditions, which would probably have not been diagnosed under the current evaluation method.

There was no significant variation of the body score during the puerperal period (Table 1), since all cows had a good body score (FERREIRA, 1990). Dairy cows will normally lose weight during postpartum (DUFFIELD, 2000). However, these animals probably maintained a good body score due to the good availability of diet, median milk production (21.6 kg/cow), and the absence of an ascending milk production curve that is prominent during the initial stage of lactation.

Significant statistical differences (p≤0.5) were observed when the diameters of the gravid and non-gravid uterus of the cows evaluated were compared; this being constant up to day 14 postpartum (Table 2). Different results were described (OKANO; TOMIZUKA, 1987), where an unrecognizable difference was related only after the fourth week postpartum. Nevertheless, similar results to those of this study, relative to the diameter of uterine involution, were described in Holstein-Friesian cows during the physiological puerperal period (MATEUS et al., 2002), and it was suggested that the evaluation of the uterine neck is an efficient diagnostic method for alterations related to uterine involution. Uterine involution involves physical shrinkage, necrosis and sloughing of caruncles, and the regeneration of the endometrium and result in significant reduction in the size of the uterus during the first three months of postpartum. It is only after this moment that endometrial regeneration begins, and is completed between the third and eight week postpartum (SHELDON et al., 2008).

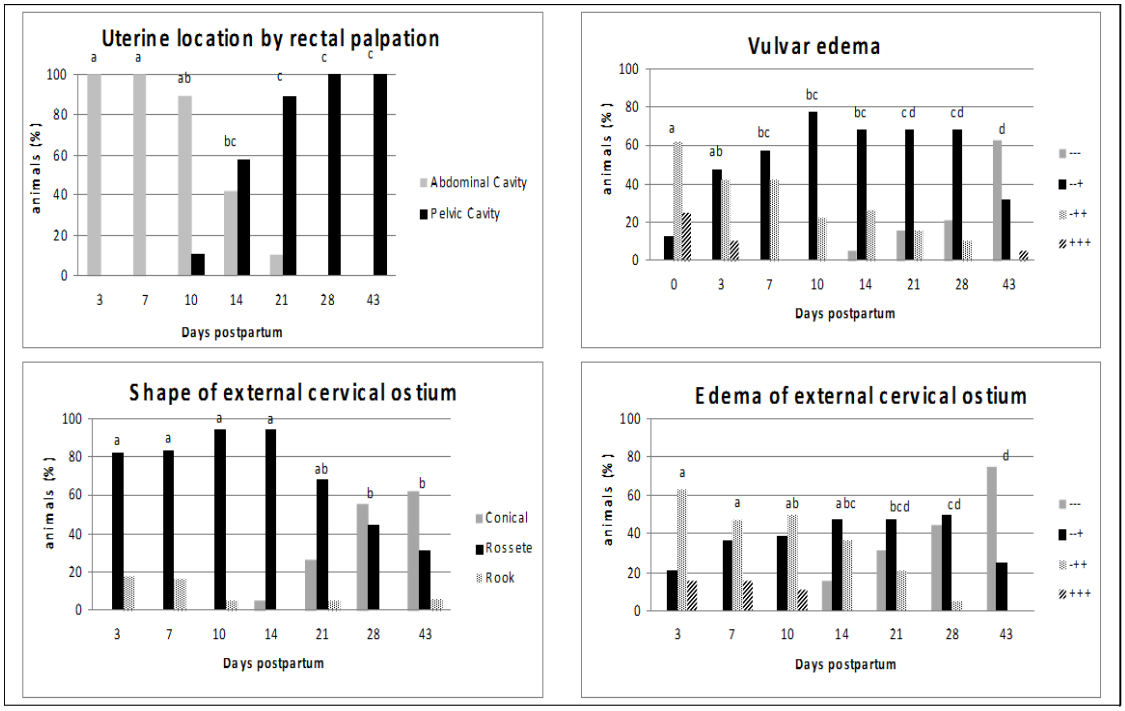
Further, the lochia, the vaginal discharge that occurs after birth, is a combination of eliminated caruncles, fetal fluids, destroyed cell layers of the superficial endometrium, and blood from the ruptured umbilical vessels (PRESTES; LANDIM-ALVARENGA, 2006).

Table 2. The relationship between uterine involution and the diameter of the uterus of cows during puerperium

Anatomic location/ physiological state	Diameter (mm) during days of postpartum					
	7	10	14	21	28	43
Neck	44.3 ± 7.8^{a}	40.2 ± 7.6^{ab}	35.7 ± 11.7 ^{b}	26.7 ± 5.4 ^{c}	25.1 ± 9.1^{c}	19.7 ± 2.9 ^{c}
Non-gravid uterine horn	-	-	17.3 ± 3.2 ^{abA}	18.1 ± 2.2 ^{aA}	16.9 ± 4.0^{abA}	14.7 ± 2.4 ^{bA}
Gravid uterine horn	-	-	23.8 ±6.9 ^{aB}	19.1 ± 2.6 ^{bA}	18.2 ± 4.5 ^{bA}	15.6 ± 2.9 ^{bA}

Note: different small letters in the same line indicate statistical difference (p≤0.05); large different letters between the gravid and non-gravid uterine horns indicate statistical difference (p≤0.05).

During this study it was observed that effective uterine involution occurred from the second week postpartum; the uterus of 58% (11/19) of the cows evaluated by rectal palpation was located within the abdominal cavity at 14 dpp, while at 28 dpp the uterus of all cows was within the pelvic cavity (Figure 1). Similar results were obtained in dairy Gir cattle, where it was demonstrated that the uterus remained within the abdominal cavity in 95% of animals during the first week postpartum, in 91.8% of animals during the second week, with only 17.2% at 42 dpp (GONZÁLEZ SÁNCHEZ; BIANCHINI SOBRINHO; GONÇALVES, 1999). Factors that can physiologically affect the duration of uterine involution include: the number of births, where the duration of uterine involution increases proportionately with the number of births (MORROW; ROBERTS; MCENTEE, 1969; EDUVIE, 1985; VASCONCELOS; SILVA; REIS, 1993; GONZÁLEZ SÁNCHEZ; BIANCHINI SOBRINHO; GONÇALVES, 1999), and the influence of hormones (MORROW; ROBERTS; MCENTEE, 1969).



Observe: small letters in different columns indicate statistical differences (p≤0.05)

Figure 1. Uterine location, vulvar edema, and shape and edema of the external cervical ostium during puerperium of crossbred dairy cows.

During this study, mucus-hemorrhagic vaginal secretions (Figure 2) occurred in a large percentage of cows during dpp 3 (31.3%), 7 (18.8%), 10 (50%), and 14 (23.5), while the intensity of chocolate-colored mucus secretions oscillated during the same period (dpp 3 – 6.3%; dpp 7 – 37.5%; dpp 10 – 25%; and dpp 14 23.5%). However, the frequency of these vaginal secretions was reduced at 21 dpp, and by 28 dpp there was no manifestation of these secretions in any cow. These vaginal secretions can be associated with the blood-colored lochia which is normally observed effective from 3 dpp, and between 7 – 14 dpp the lochia is admixed with an increasing volume of blood due to hemorrhage of the caruncles during the separation process (PRESTES; LANDIM-ALVARENGA, 2006). Additionally, the lochia disappears completely at around 30 dpp when the maternal caruncles are fully reestablished.

Purulent vaginal secretions (Figure 2) were observed initially at 3 dpp (18.8%) and continued until 28 dpp (23.5%). Similar results were described in other studies (ELLIOT et al., 1968; GRIFFIN; HARTGAN; NUNN, 1974; SHELDON et al., 2002) and suggest that there is a bacterial uterine contamination that is eliminated during the puerperal period, in which the neutrophils are the principal phagocytes.

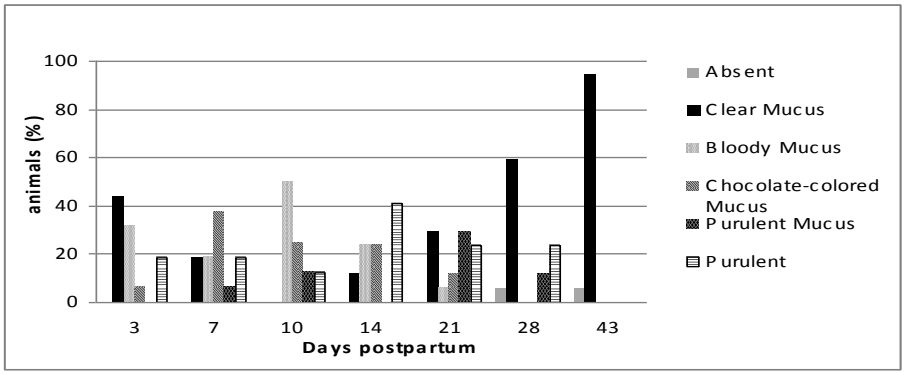


Figure 2. The characteristics of the vaginal secretions of crossbred dairy cows during puerperium.

The combined finding of mucus-purulent (29.4%) and purulent vaginal (23.5%) secretions at 21 dpp associated with fetid (21%) and very fetid (5.3%) odor of vaginal secretions (Figure 3) in cows at 21 and 28 dpp are highly suggestive of manifestations of clinical endometritis (LESLIE, 1983; SHELDON et al., 2002; WILLIAMS et al., 2005). However, at 43 dpp and without therapy, the vaginal mucus secretions of all cows was odorless (100%), with most of these (94.1%) demonstrated clear mucus secretions, while few cows (5.9%) did not had any mucus vaginal secretions (Figures 2-3). These findings suggest that these animals recuperated spontaneously from the previous infection. Clinically, endometritis has been classified based on the degree of severity, and animals with discrete infections demonstrated spontaneous recuperation without antibiotic therapy (MATEUS et al., 2002). The results from this study and previous investigations (HOEDEMAKER, 1998; MATEUS et al., 2002) have demonstrated spontaneous recovery in cows with discrete puerperal endometritis occurring up to 43 dpp, and therefore question the validity of antibiotic therapy during this phase.

Vulvar, vaginal vestibular, and cervical ostium lacerations were more pronounced at the time of birth, and occurred in 80% of all animals (Figure 4). Three critical areas have been identified during the passage of the fetus: the vulva, the hymen ring, and cervix; this in association with the vagina, vaginal vestibule, and the sacroischial ligaments constitutes the soft fetal passage (PRESTES; LANDIM-ALVARENGA, 2006). Additionally, the findings from this

study demonstrated that there was rapid regeneration of vaginal mucus membranes and the external cervical ostium, characterized by the distinct pink color of these areas at 10 dpp (Figure 4).

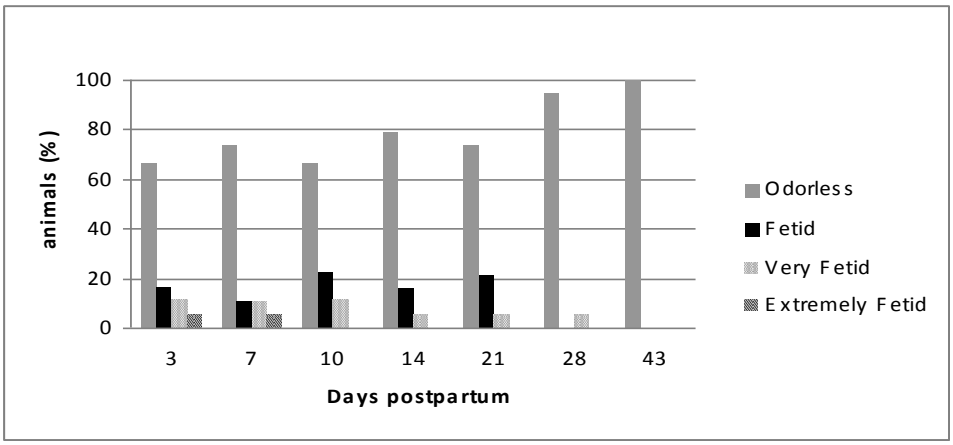


Figure 3. The manifestations of the odor of vaginal secretions in crossbred dairy cows during puerperium.

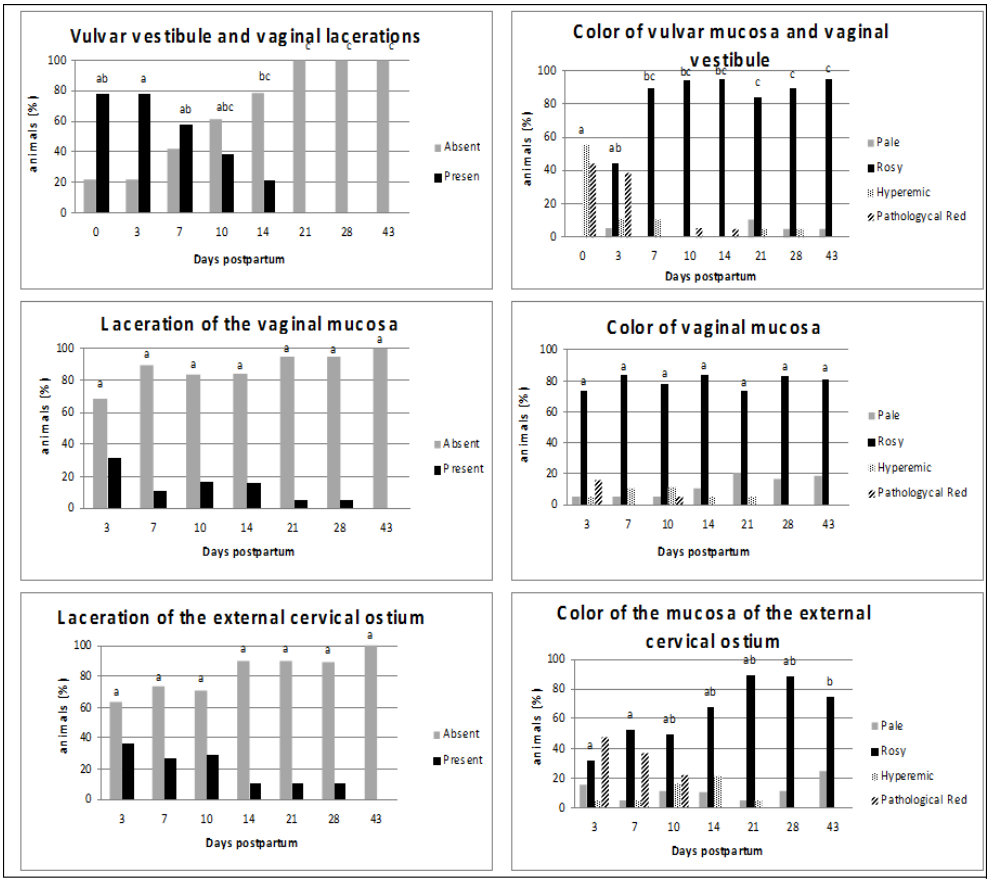


Figure 4. Comparative analyses of the lesions and color of the vulva, vaginal vestibule, vagina, and external cervical ostium of dairy cows during puerperium.

Normally, tissue repair and regeneration of damaged vaginal structures occurs during the puerperal period. However, bacterial infections is known to occur in 85% of cows at 15 dpp (GRIFFIN; HARTGAN; NUNN, 1974), and the severity of infection might predispose different degrees of lacerations to the

uterine layers, with subsequent bacteriemia and/or toxemia. Further, the adsorption of bacterial toxins might induce abomasal hypotony, which can predispose the affected animal to abomasal dislocation (SHELDON et al., 2008).

During postpartum, the vulvar edema is more pronounced and associated with concomitant edema of the vaginal and external cervical ostium, all membranes are more hyperemic; these processes are mediated by estrogen and relaxin (PRESTES; LANDIM-ALVARENGA, 2006). This seems to have occurred during this study, since the edema at the vulva and external cervical ostium were gradually reduced as puerperium progresses, with marked reduction in edema at these locations effective from 7 dpp (Figure 4). Thereafter, there was progressive marked reduction in edema at these structures, and this might be related to the slow recuperation of the cervix, whose involution normally begins 12 hours postpartum and continue towards the end at 43 dpp (PRESTES; LANDIM-ALVARENGA, 2006).

CONCLUSION

The specific characteristics related to uterine involution of crossbred dairy cows must be considered during the clinical and gynecological evaluations. While the diagnosis and treatment of clinical endometritis, by evaluating the characteristic of odor and vaginal secretions during 21 – 28 dpp must be revised, since this study has demonstrated spontaneous recuperation in crossbred dairy cows at 43 dpp, who had previously demonstrated mucus-purulent and purulent vaginal secretions.

Avaliação clínica da involução uterina de vacas mestiças leiteiras

RESUMO

Puerpério é o período compreendido entre o parto e o momento em que ocorre a completa involução uterina em torno de 40 dias pós-parto (dpp). Com o objetivo de avaliar a dinâmica de involução uterina em vacas leiteiras foram examinadas 19 vacas, mestiças, com dois ou mais partos em oito momentos no puerpério: dia do parto, dia 3, 7, 10, 14, 21, 28 e 43 dpp, onde se verificou os parâmetros vitais, escore de condição corporal, exames ginecológico e ultrassonográfico, características e odor de secreção vaginal. Concluiu-se que existem características particulares referentes à involução uterina nas vacas mestiças leiteiras e que o diagnóstico e tratamento da endometrite clínica, através da avaliação aos 21 e 28 dpp das características e odor de secreção vaginal devem ser revistos, pois se verificou cura espontânea desta infecção aos 43 dpp.
Palavras-chave: puerpério, secreção vaginal, raça mestiça, exame ginecológico, ultrassonografia transretal.

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