Subclinical endometritis in beef cattle in early and late postpartum: Cytology, bacteriology, haptoglobin and test strip efficiency to evaluate the evolution of the disease

Test strip efficiency and use of markers of inflammation to evaluate positivness to subclinical endometritis in early and late postpartum of beef cattle.

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Contents

Detrimental effect of fertility are caused from uterine inflammation mainly, subclinical endometritis (SEM). As demonstrate in previous work Piedmontese cattle Unexpectedly are affected by a higher rate of infertility and SEM. The Objective of the study is to assess the presence of SEM at 30 and 60 days postpartum evaluating the correlation between uterine cytology and microbiology, analyzing the SEM using test strip and haptoglobin. 50 healthy cows were enrolled and sampled at 30 and 60 days postpartum, cytology and bacteriology, test strip and haptoglobin were measured. ROC curve sets the optimal cut-off at 6.5% at 30 days and 2.55 at 60 days for a Partum to Conception (PC) of 120 days. Presence of a suckling calf and parity not significantly affect the PC. The cytological positivity was negatively correlated with fertility, 46 days at 30 days but not at 60 days. A positive bacteriological test was not correlated with an increase in the PC at either 30 or 60 days postpartum. Test strips were useful between 30 and 60 days pp in cows with a PC beyond 120 days. ROC curve for protein at 30 days postpartum sets a cut-off of 2 for PC. No difference in serum haptoglobin between negative or positive cytology/bacteriology in postpartum.

Introduction

Good reproductive performance is one of the parameters that influences the production efficiency of dairy and beef cows and that is necessary to maintain for the economical sustainability of farms. The multifactorial failure of reproductive efficiency can reduce profits: inadequate nutrition, poor reproductive management, or a negative energy balance during the postpartum interval could have a detrimental influence on the immune system, thus allowing the invasion of pathogens. Uterine pathologies that affect cows during the postpartum interval, are considered among the most important causes of reproductive inefficiency and are widely described in the literature for both dairy (Gilbert et al. 2005; Leblanc et al. 2008, Bichalo et al 2016) and beef cows (Santos et al. 2009; Ricci et al. 2015). The Piemontese cow, is a white, double-muscled breed, due to a mutation of the myostatin gene (Hanset et al. 1982), spread worldwide for his superior meat quality and low levels of cholesterol (Shanin and Berg 1985; Hanset et al. 1982). In knownd that double-muscled breeds, are affected by a higher rate of dystocia problems and subsequent low fertility. Very little is known about the reproductive performance, nutritional needs, breeding and farming management of this breed: Only 60% of farms apply artificial insemination and an even smaller percentage collect and process reproductive data (ANABORAPI 2014). Inflammation of the genital tract is a common condition in dairy and beef cows but not all the cows affected by uterine contamination in Post partum will develop uterine diseases. Only when bacterial growth exceeds the competence of the immune system, clinical uterine disease appears (Sigh et al. 2008, Galvao et al. 2010). Uterine pathologies have been demonstrated to have a strong detrimental effect on reproductive performance (Leblanc 2008; Sheldon et al. 2009; Williams et al. 2013; De Boer et al. 2015, Toni et al. 2015, Bicalho et al 2016). Subclinical

endomtetritis **(SEM)** is an inflammatory condition of the uterus in the absence of clinical signs that can reach 25.9% between 40 and 60 days after parturition (Cheong et al. 2011) and is characterized by an infiltration of neutrophils (PMN) in the endometrium resulting in a significant reduction in reproductive performance (Kasimanickam et al. 2004; Sheldon et al. 2006; Foldi et al. 2006 and Sheldon et al. 2009). Uterine cytology is considered the most reliable and accurate diagnostic technique to evaluate the presence of SEM: it is easy to perform, specific and inexpensive (Gilbert et al. 2005) but need a laboratory support. Samples can be obtained by using the uterine lavage technique or the cytobrush (Barlund et al. 2008; Gilbert et al. 2005; Kasimanickam et al. 2004 and Thomè et al. 2016) and Pascottini et al. (2015) have described a new rapid and inexpensive diagnostic technique by using a cytotape. An increased proportion of PMN is prognostic of subsequent impaired reproductive performance (Kasimanickam et al. 2004; Gilbert et al. 2005 and Barlund et al. 2008, Galvão et. al 2010). The threshold value for the proportion of PMN needed to define SEM is still controversial, and a range of 4–18% has been proposed in literature (Kasimanickam et al. 2004; Gilbert et al. 2005; Barlund et al. 2008). Because of its asymptomatic condition, it is difficult to formulate an early diagnosis of SEM before the negative effects on reproductive performance are expressed. Very few studies are related to SEM in beef cows (Santos et al. 2009, Ricci et al. 2015) showing different results. The acute phase proteins are a group of plasma proteins released into the bloodstream in response to inflammation or stress. One of the most important of these proteins is haptoglobin (Hp), which is often described in the literature as a diagnostic tool (Chan et al. 210). Some authors (Santos et al. 2006 and Cheong et al. 2012) presented a field test helpful for the diagnosis of SEM in dairy cows, using Multisix 10 SG test strip

directly in the uterine fluid and reported good results. Given the limited data in the literature on SEM in beef cows and the importance of this breed, the following work aims to: confirm the presence of SEM in Piemontese cattle and the progression of the disease from 30 to 60 days postpartum, analyze effects of SEM on reproductive performance, evaluate the correlation between the cytology and microbiology of the uterine contents and serum levels of acute phase inflammatory proteins and analyze the diagnostic methods for SEM based on the use of colorimetric tests and test strip test as a field method for SEM evaluation.

Materials and methods

Fifthy healty postpartum Piedmontese cows were enrolled in this study (8 primiparous and 42 multiparous) from three different farms of similar size (approximately 70 breeding cows) and with similar nutritional protocols. All farms were officially free of infectious diseases (tuberculosis, brucellosis), and animals were regularly vaccinated for BVD and IBR. The cows were housed in free stalls with appropriate accessibility to water and food. All cows were visited at 30 and 60 days postpartum by the same veterinarian.

All animals with a history of dystocia, retained placenta, uterine pathologies or metabolic or locomotory afflictions from parturition to 60 days pospartum were excluded from the study, as well as cows with an increased body temperature, even if the increase was only temporary. Cows with foul-smelling drainage between 30 to 60 days postpartum or with purulent or abnormal vaginal discharge (mucus level 1, 2 or 3) (Sheldon et al. 2005) were also excluded from the study. Additional exclusion criteria included any detectable abnormality of the reproductive tract such as ovarian or uterine adhesions and all animals with a BCS <2.5. Immediately following the

visit, the enrolled cows were subjected to blood withdrawal and to the uterine cytology protocol.

Blood sampling

At 30 and 60 days postpartum **(pp),** a blood samples was taken. The withdrawal was performed by venipuncture from the medial caudal blood vessel using an 8 ml evacuated serum collection tube and a 20 G needle (Vacutainer, Venoject, Terumo®, Leueven, Belgium); the samples were immediately placed on ice and transported to the laboratroy within 4 hours. The blood was centrifuged at 2000 rpm for 10 minutes and the plasma was harvested and stored at -20°C in 1 ml SafeLock Tubes (Eppendorf, Hamburg, Germany).

Cytologic sampling

Each cow was sampled twice by cytological examination of endometrial samples at 30 and 60 days postpartum (dpp). Endometrial cells for diagnosis of SEM were obtained by an infusion of 20 ml of sterile saline solution into the uterus with a plastic infusion catheter (53.5 cm, Bovivet uterine catheter, Kruuse, Langeskov, Denmark) followed by uterine massage and aspiration of approximately 10 or more ml of fluid into a 50 ml syringe (Terumo, Rome, Italy). The recovered fluid was transferred to two sterile plastic tubes and placed on ice in a portable cooler. One tube was immediately transported to the I.Z.S. laboratories (Torino, Italy) for bacteriological analysis and the other tube was processed within 4 h after collection as described by Santos et al. 2009. Briefly, the sample was vortexed and centrifuged onto a glass slide in a cytocentrifuge (Shandon Cytospin, Tharmac GmbH, Waldsolms, Germany). The slides were air-dried and stained with a Hemacolor rapid staining kit (Merck KGaA, Darmstadt, Germany), and each slide was examined

using 400x magnification. Two different examiners separately counted a minimum of 200 cells (endometrial cells, PMNs and squamous cells) in 10 fields on the slide.

Bacteriological analysis

Bacterial characterization and an antibiogram were performed for each cow as described previously in Ricci et al. 2015. Briefly, selected blood, chocolate and Gassner agar media were used to isolate aerobes, anaerobes and total microbial growth. Gassner media was also used for Enterobacteriaceae species, and Baird-Parker media was used for *Staphylococcus* spp. and *Streptococcus* spp., PPLO agar media was used for *Mycoplasma* spp., and Sabouraud dextrose chloramphenicol agar was used for the isolation of yeasts and molds. Finally, a brain heart infusion (BHI) broth media was used for the final microbial growth following direct inoculation. All plates were incubated at 37°C for 24-48 h. Phenotypic analysis of bacterial colonies with API bacterial identification columns was used to identify the different bacterial colonies. For slow-growing bacteria species (>24-48 h), genotypic identification using PCR for the rDNA 16S rRNA gene sequence was used; the resulting gene sequences were compared with an online database to identify matching species (Benedetto et al.. 2007).

Test strip evaluation

Immediately after uterine lavage, an aliquot of each uterine flushing media (30 and 60 days postpartum) was tested for leukocyte esterease (LE), pH and protein concentration (Prot) using dry chemistry Combur-Test® 9 strips (Roche, Grenzach, Germany) as described by Cheong et al. (2012).

Following manifacturer's instructions, **LE**, which reflects the quantity of leukocytes present in the sample, was classified as either **negative**, the absence of leukocytes in the sample; **1+**, a slight presence of leukocytes in the sample (10-25 Leuc/uL); **2+**, a

moderate number of leukocytes in the sample (75 Leuc/uL); or **3+**, a large number of leucocytes in the sample (500 Leuc/uL). Similarly, the values of proteins were classified as **Neg,** an absence of protein in the sample; **1+**, a slight presence of protein in the sample (up to 0.3 g/L); 2+, a moderate level of protein in the sample (from 0.4 g/l to 1 g/L); or 3+, a high level of protein in the sample (from 1.1 g/L to 5 g/L). The **pH** was classified using values from 5 to 9.

Acute phase protein analysis

Serum samples were analyzed using a colorimetric test for the quantitative measurement of haptoglobin (Hp). The test uses a procedure that measures haptoglobin–haemoglobin complexing by estimating the differences in peroxidase activity (CPPA), with results expressed as optical density (Cooke et al. 2013).

Statistical analysis

The Shapiro-Wilk test was used to evaluate the normality of the data. Correlations between continuous variables were analyzed using the non-parametric Wilcoxon test. PMN proportions, interaction effects, cut-off points and PC correlations were analyzed using a Kruskal-Wallis test with a Bonferroni correction for post-hoc comparisons.

Variables such as the PMN proportion, Hp concentration, and LE, pH and Prot values in the uterine lavage samples were evaluated for variations (Var) between day 30 and 60 pp. Categories were therefore created depending on the trend between 30 and 60 dpp (increasing INC; decreasing, DEC; unchanged, MED). Logistic regression (GLM) was used to analyze category trends, class scores, lactation and the number of inseminations.

To assess the possible effects of SEM on reproductive performance, the PC was evaluated with the cut-off set at 120 days after parturition as the satisfactory limit for

the Piedmontese cattle breed; Cox's proportional hazard ratio was used to analyze survival. A receiver operating characteristic (ROC) curve model was used to determine the optimal cut-off point for evaluating SEM at 30 and 60 dpp and the cut-off values for LE, pH and Prot at 130 dpp. P values <0.05 were considered significant, and trends were considered present at P values between 0.06 and 0.08. Statistical analysis was performed using the software R (Ver 2.15.2).

Results

The ROC curve for the proportion of PMN at 30 days postpartum shows that the optimal cut-off occurs at 6.5% (If 54%, Sp 85.7%, AUC 0.64, P <0.05) for a PC of 120 days (Graph 1), whereas the proportion of PMN at 60 days after birth shows the optimal cut-off at 2.5% (If 54%; Sp 66%; AUC 0.56; P <0.05) (Graph 2) for a PC over 120 days. These cut-offs allow the discrimination of animals with SEM and an increasing PC from healthy animals. Sono uguali all'altro lavoro??

The presence of a suckling calf did not significantly affect the degree of variation in the PC (P> 0.05) or SEM at 30 or 60 days postpartum compared with cows from which the calf was removed (P > 0.05). There was an interesting relation between the presence of a delivery parlor and a decrease in the PC interval compared with cows that do not calve in a calving parlor (104 dpp vs. 141 dpp, P = 0.036). (Graph 3)

Parity did not appear to have an effect on the PC interval or on SEM (P> 0.05). Based on Cox's proportional hazard ratio, 46% (23/50) of the animals have a PC > 120 days, whereas 52% (26/50) of the animals have a PC <120 days.

The cytological presence of SEM was correlated with fertility. At 30 days after parturition, animals positive for SEM showed an increase in PC of 46 days (93 days vs. 139 days, P< 0.013), whereas at 60 days the PC did not differ significantly between the groups (109 days vs 119 days, P> 0.05) (graph 4a, 4b).

At day 30 postpartum, 20% of cows (10/50) had sterile cultures, 50% (25/50) showed a single bacteria species, 22% (11/50) showed two bacteria species, 8% (4/50) showed three bacteria species, and only one animal showed five bacteria species. At 60 days postpartum, 33% of cows (16/50) had sterile cultures, 48% (24/50) showed a single bacteria species, 12% (6/50) showed two bacteria species, and 4% (2/50) showed three bacteria species. Based on the classification of bacterial species available in the literature (Williams et al. 2005), the recognized pathogens isolated included *T. pyogenes* in 7 samples at 30 days postpartum (all animals possessed a positive cytology, but it did not extend the PC) and 1 sample at 60 days (one animal with positive cytology and increased PC) and *E.coli* in 3 samples at 30 days postpartum (two animals with positive cytology and extended PC) and in 2 samples at 60 days (one animal with positive cytology and an increase in PC). Potential pathogens isolated included *Bacillus licheniformis* (in 1 sample at 30 days postpartum) and *Enterococcus faecalis* (in 2 samples at 30 days postpartum and in 1 sample at 60 days postpartum); contaminants/opportunists included *Proteus* (in 1 sample at 60 days postpartum) (Table 1). A positive bacteriological test was not correlated with an increase in the PC interval at either 30 or 60 days postpartum ($P > 0.05$); 42% (21/50) of animals with positive bacteriological results at 30 days showed a PC of more than 120 day pp with an average PC of 171 days. However, a correlation was found between positive cytology or positive bacteriology at 30 days postpartum and an increase in PC. Cows positive for either cytology or bacteriology at 30 days postpartum had a 6-fold (OR = 6.3) greater risk for increased PC compared with animals that tested negative. A similar finding was not found for either cytology or bacteriology at 60 days postpartum.

Sixty-four percent (32/50) of the animals showed a positive cytology for SEM at 30 days postpartum, and 44% (22/50) were positive at 60 days postpartum. Among cows, 28% (14/50) showed a positive cytology at either 30 or 60 days postpartum with an average PC of 123 days, 18% (9/50) showed a negative cytology at either 30 or 60 days postpartum with an average PC of 97 days, 38% (19/50) tested positive only for cytology at 30 days with an average PC of 149 days, and 17.5% (8/45) showed a negative cytology at 30 days and a positive cytology at 60 days with an average PC of 89 days (Graph5).

A positive SEM was associated with a PC greater than 120 days at 30 days postpartum ($P < 0.004$; OR = 4.6) but not at 60 days postpartum. Variations in the proportion of PMN from 30 to 60 days were not, correlated with an increase in the PC interval beyond 120 days ($P = 0.23$), although the animals with a positive cytology at either 30 or 60 days postpartum showed a PC 27 days longer compared with healthy subjects. Cows exhibiting a decrease in the proportion of PMN from day 30 to day 60 postpartum represented 64.4% (29/45) of the animals and showed an average PC of 124 days. Cows that experienced an increase in the proportion of PMN from day 30 to day 60 postpartum represented 28.8% (13/45) of the animals and had an average PC of 118 days. Cows that maintained a constant proportion of PMN (from day 30 to day 60 postpartum represented 6.6% (3/45) of the animals and showed an average PC of 123 days (Graph 5).

Test strip performance

Changes in LE and pH between 30 and 60 days postpartum for all categories (INC, DEC, MED) showed significant differences ($P > 0.05$) in the PC beyond 120 days. The ROC curve for the protein values at 30 days postpartum (Prot30) indicated that a cut-off of 2 was correlated with an increase in the PC above 120 days (Se 100%; Sp

12.5%; AUC 0, 54; P <0.05). Ninety-three percent (42/45) of animals had values of Prot30 ≥ 2 with an average PC of 127 days; 7% (3/45) of the animals had values of Prot30 <2 with an average PC of 67 days. The statistical analysis also showed that along with a change in the proportion of PMNs from 30 to 60 days postpartum, there was a change in protein from 30 to 60 days postpartum (P = 0.08) that did not show a significant relation to the increase in PC among the various groups (INC, DEC, MED).

Acute phase inflammatory protein (haptoglobin)

Hp concentration was measured for each blood sample. No difference was found between aninals with negative or positive cytology or bacteriology at either 30 or 60 days (P>0.05). Seventeen cows (38%) showed an acute inflammation response at 30 days postpartum and 10 (22.2%) showed an acute response at 60 days. Variations from 30 to 60 days was calculated for each individual included in the study. Seventy-seven percent (33/43) of the animals showed a decrease in Hp from 30 to 60 days postpartum, and in these animals the average PC was 132 days; 21% (9/43) showed an increase in Hp from 30 to 60 days postpartum, and in these animals, the average PC was 106 days; and finally, 2% (1/43) of the animals showed no change in Hp between 30 and 60 days postpartum, and in these animals, the PC was 72 days. The statistical analysis did not show any significant correlation between haptoglobin and other parameters (P> 0.05) (Graph 6a,b).

Discussion

The results obtained in the present study support the thesis that SEM is present in cows of the Piemontese beef breed (Ricci et al. 2015).

The presence of a suckling calf is often considered a stressful condition for the cow that is correlated with acyclicity or decreased reproductive performance, as reported

in literature (Yavas and Walton 2000; Montiel et al. 2005). In the present study, however, this factor did not significantly influence the interval from calving to conception, or the presence of SEM at 30 or 60 days postpartum compared with cows without calves. Due to differences in housing (tie or free stalls), the presence of the suckling calf and the intensive farming conditions of Piedmontese cows is different from other studies on beef cows (Sawa A and Bogucki 2011). The presence or absence of a delivery parlor room on the farm appears to influence the incidence of SEM and subsequent reproductive performance. The greater stress of parturition in Piedmontese in comparison with milking cows requires precise and proper management of the cow during delivery and during the initial postpartum period. Parity does not appear to have any effect on SEM or positive bacteriology tests. According to the literature (Kauffman et al. 2009), cows with more than 2.5 IA/pregnancy are more likely to have an increased PC interval with a positive cytology. Sampling at both 30 and 60 days postpartum allowed the analysis of trends in different variables over time. The two cut-off values utilized in this study for the diagnosis of SEM were obtained from the ROC curve at either 30 or 60 days postpartum and appear to be quite different from those proposed in the literature for milking cows (Kasimanickam et al. 2004; Gilbert et al. 2005; Barlund et al. 2008) (Galvão et al. 2009a; Dubuc et al. 2010; Madoz et al. 2014).. To date, only two studies (Santos et al. 2009, Ricci et al.. 2015) have considered SEM in beef cattle. In the former study, 137 beef cows were sampled from 2 to 87 days postpartum, and the researchers concluded that beef cows had the ability to clear uterine inflammation after the resumption of ovarian cyclicity thus producing no effects on reproductive performance; in contrast, the latter study were 97 Piedmontese cows were analyzed between 28 and 68 days postpartum stated a 31% incidence of SEM in Piedmontese

cattle and an increase of 40 days in PC**.** In this study, the cut-off values were chosen based on the results of the ROC curves and highlighted the detrimental effects of SEM on reproduction. Choosing the proper cut-off value appears to be important for the correct diagnosis of SEM when working with different breeds. The lack of scientific information on uterine infections in the Piedmontese cattle breed has made difficult to perform an accurate analysis of SEM. The results of this study support the hypothesis that a greater proportion of animals were positive for SEM at 30 days postpartum than at 60 days postpartum. with a PC>120 days at 30 days postpartum but not at 60 days postpartum.

This finding suggests that uterine inflammation at 30 days postpartum is more crucial for determining reproductive performance in both Cit30+/Cit60- and Cit30+/Cit60+ cathegories, than for SEM diagnosed at 60 days postpartum.

These results lead to the hypothesis that late SEM has a lesser impact on reproductive performance if it affects previously healthy animals. This could be attributed to an increased ability to resolve the inflammatory process at 60 days after birth due to higher competence or efficacy of the immune system.

the presence of SEM at 30 days postpartum has a greater effect on the ability of the uterus to reach optimal conditions for the recovery of fertility within a short time. The positive SEM values reported by Santos et al.. (2009) and Ricci et al. (2015) are higher than those reported in the literature (Kasimanickam et al. 2004; Sheldon et al. 2006; Dubuc et al. 2010; Barlund et al. 2008; Madoz et al. 2013; Ribeiro et al. 2013) on dairy cows and for different pospartum periods. The highly positive bacteriological results at 30 and, in particular, 60 days postpartum were unexpected; in fact, the literature indicates that most cows have completed uterine involution and re-epithelialization of the endometrium before the end of puerperium (Griffin et al.

1974; Sheldon, 2004a and 2004b) and that bacteria can be isolated from the uterine lumen from 2 weeks after parturition (Hussein et al.. 1990; Sheldon et al.. 2002) until 5 weeks postpartum (Leblanc et al.. 2002, Bichalo et al 2016). Of the recognized pathogenic bacteria reported in the literature, some bacterial species were isolated (Williams et al. 2005, Bichalo et al 2016): *T. pyogenes* at 30 days pp with no increase in PC and at 60 days pp with an effect on PC; *E. coli* was also found at 30 and 60 days postpartum. As reported in other studies, the most relevant bacteria species isolated in this study were: *Aerococcus, Bacillus, Corynebacterium, Enterococcus, Escherichia, Histophylus, Staphylococcus and Streptococcus* (Noakes et al. 1991; Sens et al. 2013). Bacteriological isolation is thus not directly correlated with an increase in the PC interval at either 30 or 60 days postpartum, but it is interesting that a large number of animals positive for bacteria at 30 days showed an average PC of 171 days; greater risk is shown for animals positive for either cytology and bacteriology at 30 days than for animals that test negative. The results of the bacteriological analysis in this study are confirmed by a recent study (Barański et al. 2012) in which SEM appears to be related to the ability of the endometrium to recover after parturition, thus supporting the hypothesis of a greater impact of bacteria at 30 days rather than 60 days postpartum.

A significative correlation was also found between positive cytology and positive bacteriology at 30 days after parturition; animals with a positive cytology had an average of up to 145 PC and were 6 times more likely to show a positive bacteriological result and an increased PC compared with animals with a negative cytology. These data are consistent with the literature (Kasimanickam et al. 2004; Gilbert et al. 2005; Barlund et al. 2008; Galvão et al. 2009b; Cheong et al. 2011). This study attempted to demonstrate the effectiveness of the test strip as a diagnostic

tool usable in the field by obtaining an initial selection of animals with pathologies; those pathologies were subsequently confirmed by laboratory examination. These results are in contrast to the literature. The two studies to which we have referred, both on dairy cows (Couto et al. 2012; Cheong et al. 2012), reported a good correlation between the values of leukocyte esterase (LE ≥ 3+), pH (pH ≥ 7) and positive cytology and a subsequent decrease in reproductive performance. In the present study, we found no significant change in LE or pH between 30 and 60 days postpartum, and none of the three categories of performance (INC, DEC, MED) showed significant differences (P> 0.05) in PC over 120 days. In contrast, although the literature does not show any association between protein and reproductive performance, the ROC curve at 30 days postpartum indicated an association of this variable with increased PC beyond 120 days, with a cut-off of 2. The sensitivity of 100%, even with a low specificity, little better in Sp at 60 days, allows the ability to accept, from a clinical point of view and under field conditions, the risk of obtaining a high number of false positives because it drastically reduces the likelihood of not diagnosing any positive animal. We assume that a larger number of animals will allow a higher accuracy in the detection of positives and negatives using this method. The statistical analysis has also shown a tendency for proteins and the proportion of PMN to change between 30 and 60 days postpartum. To obtain greater specificity and greater precision with regard to this trend, it would be appropriate to repeat testing on a larger sample of animals. The literature suggests that acute phase proteins, particularly haptoglobin, can be used as parameters to diagnose uterine inflammatory conditions in cattle (Ceciliani et al. 2012; Alsemgeest et al. 2014). In the present study, we did not find any correlations between Hp and the other parameters considered, which is consistent with the results of another recent study

(Yasui et al. 2014), in which the concentration of serum Hp was not associated with the presence of SEM or with decreasing reproductive performance. At 30 days, 38% of the animals exhibited an acute inflammatory condition, whereas 22% exhibited this condition at 60 days. For each individual included in the study was also calculated the variation in the Hp parameter from 30 to 60 days.

A substantial number of animals (77%) showed a decrease in Hp between 30 and 60 days postpartum and an increase in PC, whereas 21% of the animals manifested an increase in Hp between 30 and 60 days postpartum with an average PC of 106 days; only 2% of the animals showed no change in Hp values between 30 and 60 days postpartum, and in those animals, the PC was 72 days. Although there was no statistical correlation, it can be hypothesized that the higher concentration of Hp at 30 days could be detrimental to reproductive performance and could delay PC.

Conclusions

This study shows that uterine diseases in subclinical forms are detrimental in Piemontese cattle, with a greater incidence in early than in late postpartum. Cytology analysis and bacteriology at at two different times between 30 and 60 days postpartum, allows to evaluate the progession of the disease, and to detect the negative impact on the fertility, which results in an increase in the PC interval. The test strip results have demonstrated utility at 30 days postpartum for screeening for cows that are at risk of developing an increased PC. Further studies are needed to confirm the value of this test, which could become highly useful in theriogenological practice.

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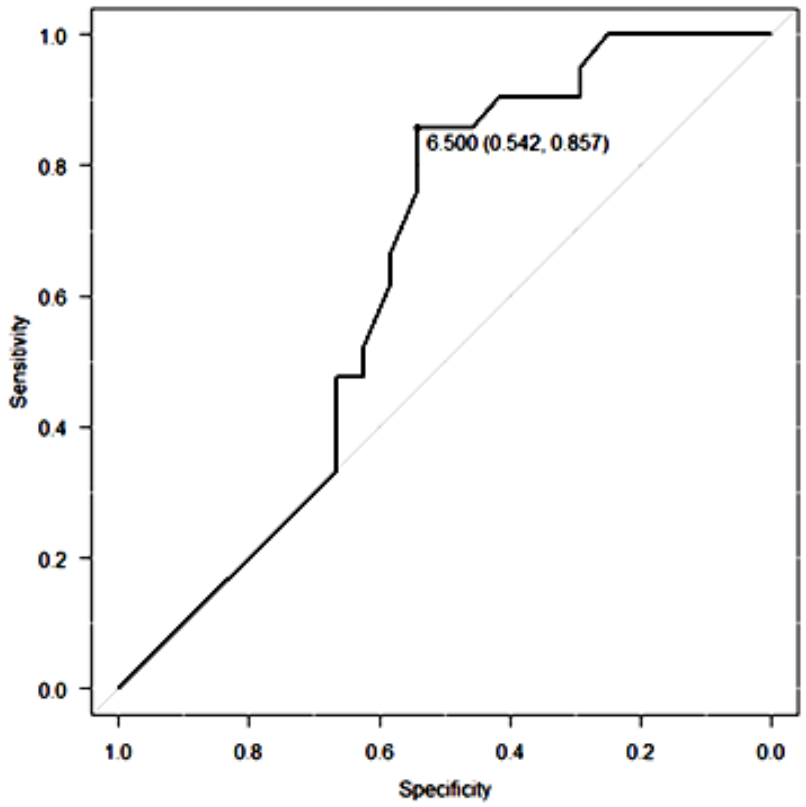
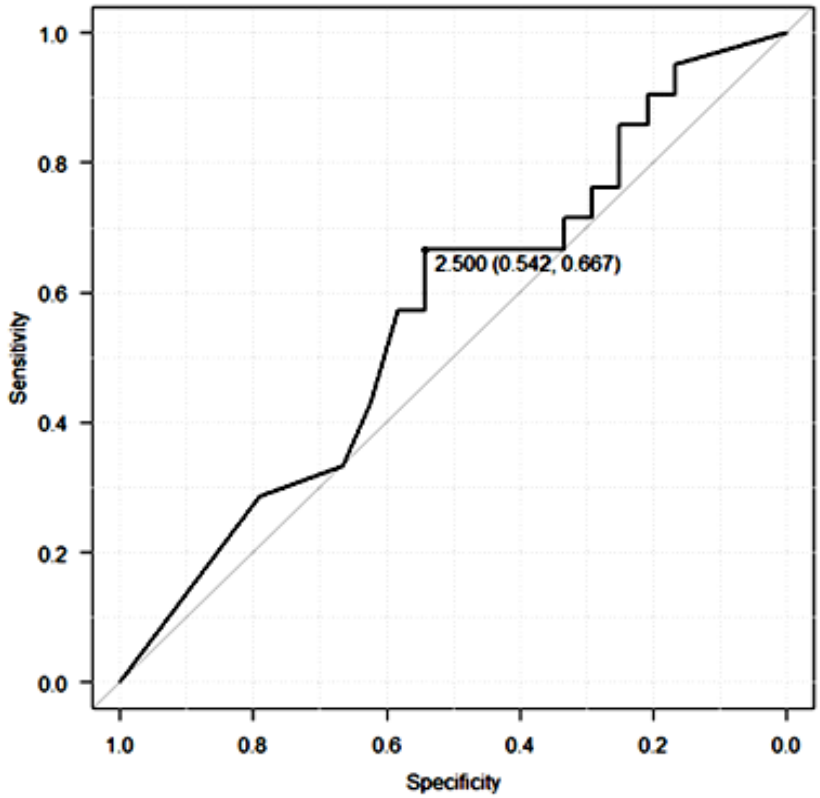
Microbiology at 30 days pp.	Microbiology at 60 days pp.
Trueperella pyogenes (7)	Aerococcus spp. (3)
Serratia marcescens (5)	**Escherichia coli (3)**
Sphingomonas paucimobilis (5)	Pantoea spp. (3)
Escherichia coli (3)	Staphylococcus spp. (3)
Facklamia hominis (3)	Alloicoccus otitis (2)
Pantoea spp. (3)	Bacillus spp. (2)
Aerococcus viridans (2)	Corynebacterium spp. (2)
Bacillus spp. (2)	Staphylococcus epidermidis (2)
Brevibacterium non reactive (2)	Staphylococcus sciuri (2)
Corynebacterium spp. (2)	Staphylococcus warneri (2)
Enterococcus faecalis (2)	Achromobacter (1)
Streptococcus uberis (2)	Acinetobacter lwoffi (1)
Bacillus licheniformis (1)	Acinetobacter spp. (1)
Bacillus pumilus (1)	Aerococcus viridians (1)
Corynebacterium accolens (1)	Alcaligenes faecalis (1)
Corynebacterium cystitidis (1)	Cellulomonas/Microbacterium (1)
Corynebacterium mastitidis (1)	Corynebacterium glucuronolyticum (1)
Corynebacterium renale (1)	Corynebacterium minitussimum (1)
Dermacoccus spp. (1)	Corynebacterium pseudotuberculosis (1)
Dietzia maris (1)	Enterococcus faecalis (1)
Dolosigranulum pigrum (1)	Enterococcus hirae (1)
Enterococcus spp. (1)	Mycobacterium mucogenicum (1)
Escherichia vulneris (1)	Pasteurella spp. (1)
Globicatella sanguinis (1)	Proteus (1)
Granulicatella adiacens (1)	Staphylococcus equorum (1)
Helcococcus kunzii (1)	Streptococcus pluranimalium (1)

Histophylus somni (1)	**Trueperella pyogenes (1)**
Kocuria rosea (1)	
Lactococcus lactis (1)	
Leifsonia aquatica (1)	
Pasteurella canis (1)	
Rhodococcus equi (1)	
Staphylococcus auricolaris (1)	
Staphylococcus spp. (1)	
Streptococcus spp. (1)	

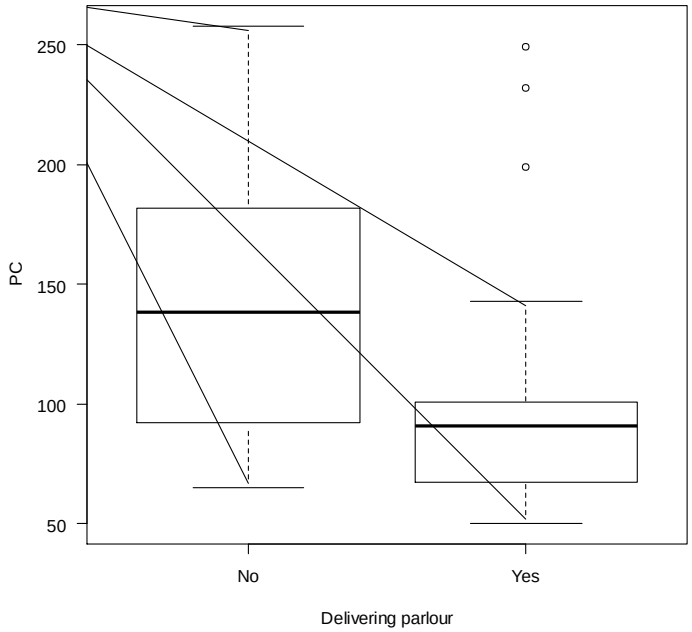
Table 1: Bacteriological findings (frequncy) at 30 days pp. and 60 days pp. The parentheses indicates the number of positive samples for each bacterial species.



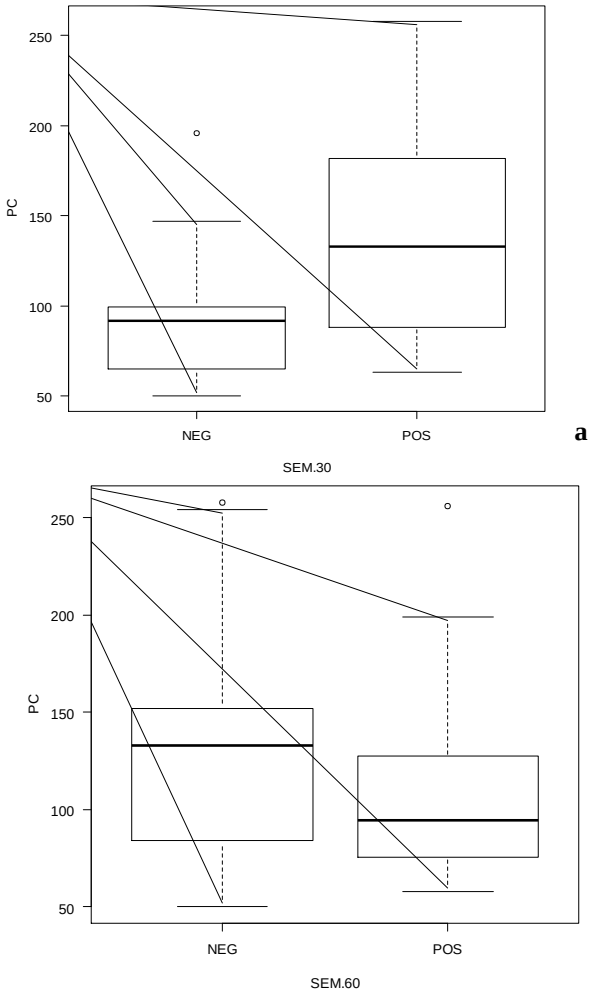
Graph 1: ROC outcome for PMN cut off at 30 days postpartum



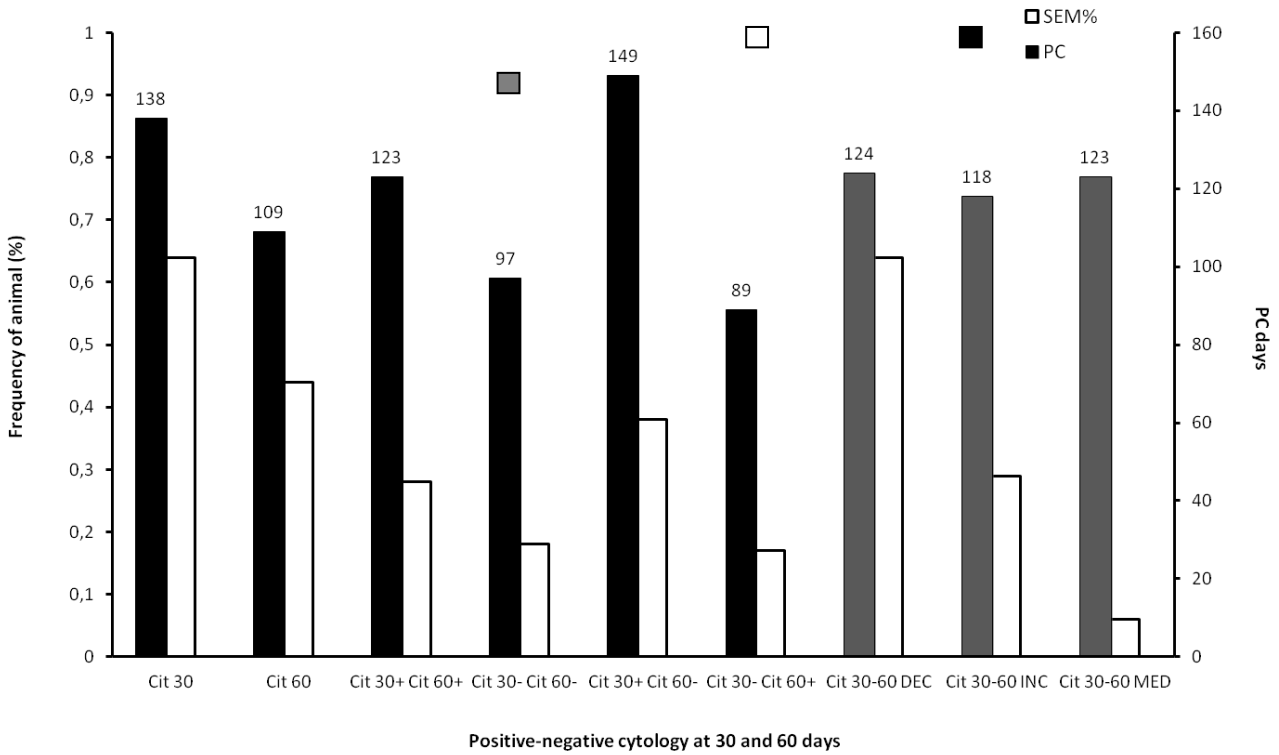
Graph 2: ROC outcome for PMN cut off at 60 days postpartum



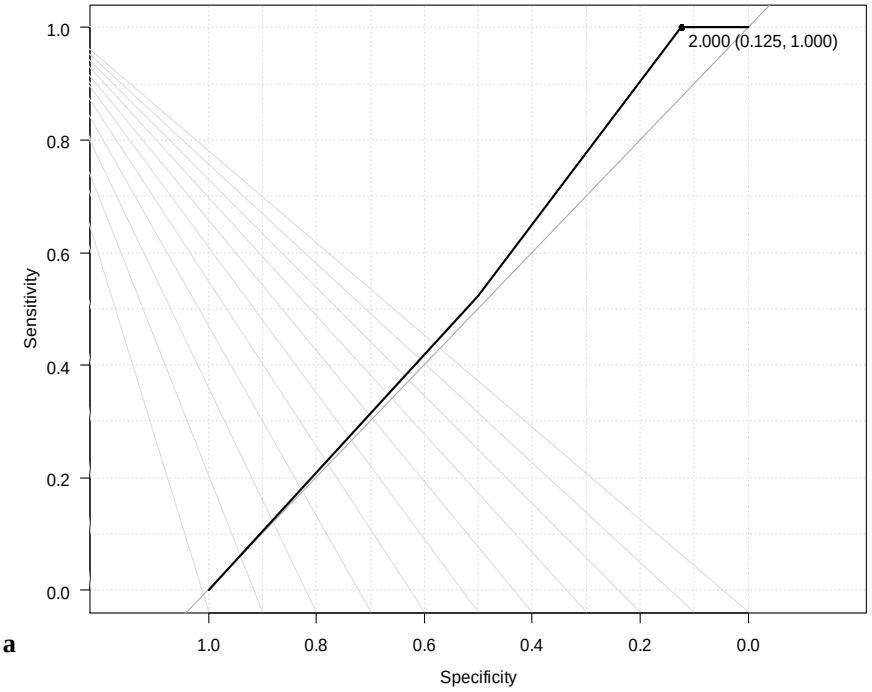
Graph 3: Effect of delivering parlour on partum to conception days, P=0.013



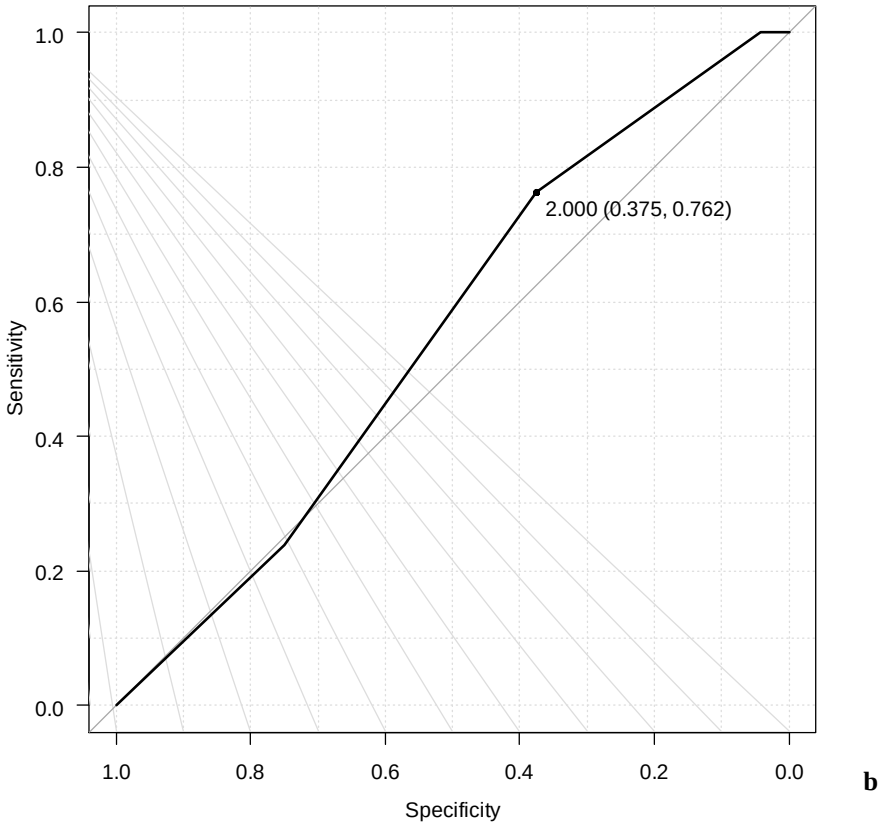
bGraph 4a, 4b: Impact of SEM at 30 (a) and 60 (b) days post partum on partum to conception (PC) days.



Graph 5: Impact of SEM on Partum to conception at 30 and 60 days in different cytological categories and PMN variation from 30 to 60 days. SEM incidence, partum to conception (PC) days for cytology categories , partum to conception days (PC) for trend cathegories.



a



Graph 6a,b : ROC output for protein (strip test) at 30 (a) and 60 (b) days and PC>120 days. a): cut off 2 (Sp12%, Se 100%) b) cut off 2 (Sp 37%, Se 76%).