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

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ABSTRACT

In cows, detrimental effects on fertility are mainly caused by clinical and subclinical endometritis (SEM). As demonstrated in previous work, Piedmontese cattle are affected by a higher rate of infertility and presence of SEM. The objective of this study is to assess the pattern of SEM at 30 and 60 days postpartum by evaluating the correlation between uterine cytology and microbiology, analyzing SEM consequences on reproductive career and verifying the reliability of rising inflammatory proteins - haptoglobin and the test strip test. Seventy healthy cows were enrolled and sampled at 30 and 60 days postpartum; cytology and bacteriology as well as haptoglobin and test strip were evaluated. The ROC curve for cytology set the optimal cut-off at 6.5% at 30 days and 2.5% at 60 days for a Partum-to-Conception (PC) interval of 120 days. The cytological positivity was negatively correlated with fertility, 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. The presence of a calving parlor affect the fertility ($P < 0.05$) but not the presence of parity or suckling calf and parity. The ROC curve for strip test protein at 30 days postpartum set a cut-off of 2% for PC. No difference in serum haptoglobin was observed between negative or positive cytology/bacteriology in postpartum cattle. The test strip results for proteins have demonstrated a utility at 30 days postpartum for screening the cows that are at risk of developing an increased PC > 120 days.

1. Introduction

Good reproductive performance is a parameter that influences the production efficiency of dairy, beef cows, and especially in beef cattle is necessary to maintain the economical sustainability of farms. The multifactorial causes of poor reproductive efficiency include inadequate nutrition, poor reproductive management and a negative energy balance during the postpartum interval, which can 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 [1–3] and beef cows [4,5].

The Piedmontese cow is a white, double-muscled breed, due to a mutation of the myostatin gene [6]. It has spread worldwide because of its superior meat quality and low levels of cholesterol [6,7]. It is known that double-muscled breeds are affected by a higher rate of dystocia problems and subsequent lower fertility. Very little is known about the reproductive performance, nutritional needs, and breeding and farming management of this breed; in fact, only 60% of farms apply artificial insemination and an even smaller percentage collect and process reproductive data [8]. Inflammation of the genital tract is a common condition in dairy and beef cows, but not all of the cows affected by uterine contamination postpartum will develop uterine diseases. Clinical uterine disease appears only when bacterial growth exceeds the competence of the immune system [9,10]. Uterine pathologies have been demonstrated to have a strong detrimental effect on reproductive performance [2,3,11–14]. Subclinical endometritis **(SEM)** is an inflammatory condition of the uterus in the absence of clinical signs that can reach 25.9% of positivity between 40 and 60 days

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after parturition [15] and is characterized by an infiltration of neutrophils (PMN) in the endometrium, resulting in a significant reduction in reproductive performance [11,16–18]. 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 [1] however, laboratory support is needed. Samples can be obtained using the uterine lavage technique or the cytobrush [1,16,19,20]. An increased proportion of PMN in uterine sample is prognostic of subsequent impaired reproductive performance [1,10,16,19]. In addition, Pascottini et al. [21] have described a new, rapid and inexpensive diagnostic technique to evaluate SEM in uterus using a cytotape. 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 the literature [1,16,19]. Because it is an asymptomatic condition, it is difficult to formulate an early diagnosis of SEM before negative effects on reproductive performance occur. Very few studies have investigated SEM in beef cows [4,5] and their results are inconsistent.

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 [22].

Seeking to offer a rapid field diagnosis, some authors [23,24] have presented a field test that could be helpful for the diagnosis of SEM in dairy cows. It involves placing a Multisix 10 SG test strip directly in the uterine fluid, and accurate results have been reported [24]. Although SEM has been studied extensively in the past, most literature reports the use of different techniques to accurately diagnose this pathology and to try to understand what effects it has on fertility in dairy cows; very few studies have shown a correlation between bacterial presence and cytology positivity on fertility [25]. None have compared both cytology and bacteriology in dairy or beef cows, with easier and faster cow-side methods (i.e haptoglobin and test strips), allowing a better diagnosis of affected cows to apply a useful therapy. This study further contributes to the knowledge of SEM focusing on analyzing the evolution of this pathology from early to late postpartum [30–60 days postpartum **(dpp)**], the detrimental effects of bacterial presence on fertility, and on the use different diagnostic methods at increasing days postpartum in beef cows.

The aims of the study are: 1) Evaluate the accuracy of uterine cytology and microbiology to confirm the presence of SEM in Piedmontese cattle at 30 and 60 dpp 2) Analyze the effects of SEM and the precense of bacteria on reproductive performance with a main focus on the evolution of the disease from 30 to 60 days postpartum; 3) Analyze the accuracy of different diagnostic, sierological (haptoglobin concentration) and on filed rapid test (reagent strip test), or a combination of them, to diagnose the cytological SEM at 30 and 60dpp, in beef cows and predict a detrimental effect on PC > 120 days.

2. Materials and methods

Seventy healthy postpartum Piedmontese cows (12 primiparous and 58 multiparous) from three different farms of similar size (approximately 100 breeding cows) with similar nutritional protocols were enrolled in this study. All farms were officially free of infectious diseases (e.g., tuberculosis, brucellosis), and the animals were vaccinated for BVD and IBR. The cows were housed in free stalls with appropriate accessibility to water and food. All cows were visited and scanned at 30 and 60 days postpartum by the same skilled veterinarian. All cows were submitted to AI based on heat detection, at the end of the protocol after 60 days postpartum.

All animals with a history of dystocia, retained placenta, uterine pathologies or metabolic or locomotor afflictions from parturition to 60 days postpartum 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 and 60 days postpartum or with purulent or abnormal vaginal discharge (mucus level 1, 2 or 3) [17] 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.

2.1. Blood sampling

At 30 and 60 dpp blood samples were taken 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 laboratory within 4 h. The blood was centrifuged at 2000 rpm for 10 min and the plasma was harvested and stored at −20 °C in 1-ml SafeLock tubes (Eppendorf, Hamburg, Germany).

2.2. Cytologic sampling

Each cow was sampled twice for cytological examination of the endometrium at 30 and 60 days postpartum. Endometrial cells 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. [4]. 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 400× magnification. Two different examiners separately counted a minimum of 200 cells (i.e., endometrial cells, PMNs and squamous cells) in 10 fields on the slide.

2.3. Bacteriological analysis

Bacterial characterization and an antibiogram were performed for each cow as described previously [5]. Briefly, selected blood, chocolate and Gassner agar media were used to isolate aerobes, anaerobes and total microbial growth, respectively. Gassner media was also used for *Enterobacteriaceae* species, 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 the bacterial colonies with Analytical Profile index **(API)** test for bacterial identification columns was used to identify the different bacterial colonies. For slow-growing bacteria species (>24–48 h), genotypic identification via 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).