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Uterine lavage is efficient to recover endometrial cytology sample and does not interfere with fertility rate after artificial insemination in cows

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ABSTRACT

Productivity rates directly depend on the fertility of a herd, which in turn can be influenced by many factors. Semen deposited in the female reproductive tract is foreign to the body and, in response to this invasion, produces an inflammatory reaction, which is characterized by rapid infusion of polymorphonuclear (PMN) cells. Techniques to obtain an endometrial sample are usually invasive and can mask the true inflammatory response. Ultrasound is a noninvasive technique and can contribute to the diagnosis of postartificial insemination (AI) inflammatory response in cattle. The present study was divided into two experiments. The aim of experiment 1 was to compare two methods of endometrial cytology collection, uterine cytobrush (UC) and uterine lavage (UL), and their effects on uterine hemodynamics that provide information about blood flow. The two methods were evaluated by Doppler ultrasound using the spectral and color modes. For that purpose, 19 Nellore cows were synchronized for timed AI and subjected to UC (n = 9) or UL (n = 10). The techniques were performed 4 hours after AI. The results showed that both techniques allow collection of a good quality sample and with enough PMN cells to perform counting. More PMN cells were obtained by UL than UC. There was no difference in uterine blood flow between the UC and UL groups in any of the periods evaluated (34 hours before and 4, 24, and 48 hours after collection of uterine sample). On the basis of results of experiment 1, the effect of UL on fertility was studied in experiment 2. A total of 128 Nellore cows were synchronized for TAI; 35 cows were subjected to endometrial cytology by UL 4 hours after AI, and 93 were not submitted to any procedure (control). Pregnancy diagnosis was performed by transrectal ultrasound 30 days after AI. Pregnancy rates did not differ between UL (54.29%) and control (56.99%) groups. The results of this study showed that UL allows the collection of more representative cells of the surface of the uterus than UC technique

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and causes no damage to the reproductive tract. Moreover, UL did not affect pregnancy rate when performed 4 hours after AI.

1. Introduction

Productivity rates directly depend on the fertility of a herd, which in turn can be influenced by many factors, such as the conditions of the female reproductive tract, procedures and biotechniques used, and also to all the physical and biochemical events that gametes are subjected to until fertilization occurs. For that reason, the healthiness of the female reproductive tract is extremely important for sperm capacitation and embryonic and fetal development.

From an immunologic point of view, semen deposited in the reproductive tract of the female, either by natural mating or artificial insemination (AI), is foreign to the body, producing an inflammatory reaction in response. According to Troedsson [1] and Robertson [2], this inflammatory reaction is physiological, transient and important to remove the excess of dead spermatozoa and uterine contaminants and is characterized by rapid infusion of leukocytes, mostly polymorphonuclear (PMN) cells [3].

This interaction that occurs between the semen and the uterus, characterizing the postinsemination inflammatory process, is well described in horses [1,2], but little is known in cows because of the difficulty of applying these techniques for evaluation [4,5]. Cytologic specimens can be obtained by uterine biopsy, cotton swabs, cytobrush, and uterine lavage (UL) techniques [4]. To obtain a consistent and reliable result, it is necessary to use a technique that allows collection of representative cells of a large surface of the uterus and causes no damage to the reproductive tract [4,6–9]. However, these techniques are invasive and could induce some inflammatory response after the procedure, thus masking the diagnosis.

Noninvasive techniques, such as ultrasound, when used for the evaluation of the uterine environment can contribute to the diagnosis of post-AI inflammatory response in cattle; B-mode ultrasound is capable of identifying liquid content in the uterine lumen but does not determine its constitution. On the other hand, color Doppler ultrasound has been used to study uterine blood flow in cows at different stages of the estrous cycle and can be used to study the vascular uterine perfusion in endometrial injury [10] in an attempt to assess the inflammatory response of the uterus [11,12]. Although it is expected that uterine inflammatory response after AI causes more subtle changes than in the case of serious injuries, Doppler ultrasound could also possibly verify these changes.

Understanding the interaction that occurs between the uterine environment and the semen deposited in the female reproductive tract as well as the techniques and their influence on fertility can provide information for the development of new biotechniques that improve livestock breeding.

This study was designed to compare the uterine cytobrush (UC) and UL techniques to evaluate which one provides more representative and high-quality samples in cattle. Moreover, this study aimed to evaluate the effects of these techniques on uterine blood flow and to determine the effects of endometrial cytology on fertility, when performed 4 hours after AI.

2. Material and methods

Two experiments were performed. The first experiment used animals belonging to the herd of the University of São Paulo (Campus of Pirassununga, SP, USA), and the second experiment used animals of commercial herds (Fronteira Farm, Cocalinho, MT, Brazil). The study was approved by the Ethics Committee in the use of animals of the School of Veterinary Medicine and Animal Science of the University of São Paulo, protocol number 2083/2010.

2.1. Experiment 1: comparison between cytobrush and UL techniques and their effects on uterine hemodynamics

2.1.1. Animal selection and experimental groups

A total of 50 Nellore cows were evaluated between Days 30 and 50 postpartum with regards to uterine and ovarian scores and body condition. Uterus was classified in scores ranging from 1 to 3 (score 1: uterus with complete involution, without liquid; score 2: uterus showing a small amount of liquid in the lumen, even with incomplete involution, presenting little distention, and score 3: uterus contents liquid in the lumen and may have some kind of infection, has become distended and without involution), according to Oliveira et al. [11]. Ovaries were classified in scores ranging from 1 to 3, according Madureira and Pimentel [13] (Score 1: ovary showed greater than 30 mm diameter with growing follicles with diameter larger than 8.5 mm in the presence of CL or follicles larger than 12 mm in the absence of CL; score 2: ovary to less than 30 mm and follicles between 5 and 8.5 mm, and score 3: ovary with $<$12-mm diameter and follicles up to 5 mm in diameter). Body condition scores were classified from 1 to 9, according to Spitzer [14]. Nineteen cows presenting uterine and ovarian score 1 or 2 and body condition score between 4 and 7 were randomly assigned to one of the two techniques to obtain an endometrial sample: Uterine Cytobrush (UC, $n = 9$) or Uterine Lavage (UL, $n = 10$). The animals were chosen before the hormone treatment.

2.1.2. Timed artificial insemination (TAI)

All cows were submitted to an estrous and ovulation synchronization protocol using a subcutaneous implant containing 3-mg norgestomet (Crestar), followed by 2-mg estradiol benzoate (Estrogin) intramuscular. The implant was removed after 8 days, and the animals received 1 mg of estradiol benzoate, 0.5 mg of sodium cloprostenol (Sincrocio), and 300 IU of equine chorionic gonadotropin (Novormon) intramuscular. Timed artificial insemination in the uterine body was performed 30 hours later by a single inseminator.

A frozen-thawed semen batch was used after being previously evaluated. The parameters of the batch were