ENDOMETRITIS DIAGNOSIS IN REPEAT BREEDER COWS FROM EMBRYO TRANSFER PROGRAMS

DIAGNÓSTICO DE ENDOMETRITE EM VACAS REPETIDORAS DE ESTRO PROVENIENTES DE PROGRAMAS DE TRANSFERÊNCIA DE EMBRIÕES

C. S. OLIVEIRA[1], L. Z. OLIVEIRA[1]*, W. M. GONÇALVES[2], H. MAGALHÃES[2], F. Z. BRANDÃO[3], L. A. G. NOGUEIRA[3]

SUMMARY

Uterine flush technique was used for harvesting biological material and further bacteriological isolation to diagnose endometritis, as well as to determine if subclinical endometritis is causing reproductive failure in cows submitted to embryo transfer. Twenty repeat breeder females from embryo transfer programs, without reproductive pathology symptoms, were selected for the experiment. Samples of uterine flush were plated in aerobic and anaerobic culture systems. Identification of isolated microorganisms was performed with API Index® classification system. The technique presented satisfactory results, allowing recovery of aerobic and anaerobic bacteria recognized as being part of uterine flora in 80% of the animals. Even if the presence of bacteria level III that are not normally associated with endometritis (55%) was superior ($p<0.05$) to pathogenic bacteria level I (20%), bacteria that causes endometritis (level I and II) was isolated in 50% of the animals, and that might represent an important fertility decline factor.

KEY WORDS: Repeat breeders. Cow. Uterine flora. Bacteriological culture. Endometritis.

RESUMO

O presente trabalho teve como objetivo avaliar a técnica de lavagem uterina como método para colheita de material biológico destinado a pesquisa bacteriológica, para o diagnóstico de endometrite bacteriana; e estimar se a mesma representa uma causa significativa de falha reprodutiva em animais submetidos à colheita de embriões. Para tanto, foram selecionadas 20 fêmeas repetidoras de estro oriundas de programas de transferência de embriões, na ausência de sintomatologia clínica de afecções reprodutivas. As amostras dos lavados uterinos foram semeadas em sistemas de cultivo aeróbio e anaeróbio, e o sistema API Index® de classificação bioquímica foi utilizado para a identificação das bactérias. A técnica apresentou-se satisfatória, permitindo a recuperação de bactérias da flora uterina em 80% dos animais. Apesar de a prevalência de gêneros bacterianos grau III não relacionados a endometrites (55%) ter sido superior ($p<0.05$) à presença de bactérias patogênicas grau I (20%), o isolamento de bactérias descritas como causadoras de endometrite (grau I e II) foi possível em 50% dos animais avaliados, o que pode representar um importante fator para o declínio de fertilidade nestes indivíduos.

PALAVRAS-CHAVE: Vacas repetidoras de estro. Flora uterina. Cultivo bacteriológico. Endometrite.

[1]Depto. Medicina Veterinária Preventiva e Reprodução Animal, UNESP. Via de Acesso Prof. Paulo Donato Castellane s/n. ZIP: 14884-900, Jaboticabal/SP.
* Corresponding author: leticiazoccolaro@yahoo.com.br.
[2]PESAGRO RIO – Niterói/RJ.
[3]UFF – Niterói/RJ.

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INTRODUCTION

Repeat breeder cow is defined as the cow that does not become pregnant after three or more services associated with true estrus, in the absence of detectable abnormalities (ZEMJANIS, 1980). In large herds, animal intrinsic factors such as metrites, endometritis, cervicitis and vaginitis contribute to 30% of the causes for repeat estrus. In intensive production systems, estrus monitoring, semen quality, and artificial insemination are well conducted and, therefore, failure to conceive is mainly due to animal intrinsic factors, including uterine infections (KUNZ et al., 2002).

Subclinical endometritis is characterized by the absence of both vaginal discharges and changes to the clinical examination. It is therefore, a disease of difficult diagnosis due to the absence of specific clinical and laboratory findings (ECKERT et al., 2004). Inflammation of the endometrium reduces animal reproductive performance and consequently, increases the number of services per estrus and decreases pregnancy rates (KASIMANICKAM et al., 2004).

Some donors in embryo transfer programs show decrease in fertility rate, failing to present satisfactory results in the production of embryos and pregnancies even in the absence of apparent clinical symptoms. A hypothesis to explain such events is that a subclinical bacterial endometritis develops due to both excessive manipulation of the animal genital tract and administration of exogenous hormones.

This study used the uterine flushing technique to collect samples for bacteriology that was first described by Ball et al. (1988). According to these authors, this technique allows greater recovery of pathogenic bacteria compared to the uterine swab since it increases area coverage of the endometrial sample and bacteria concentration during centrifugation.

Thus, the present study aims at evaluating the microbial flora of repeat breeder cows from embryo transfer programs outside postpartum period using the uterine flush for a bacterial culture.

MATERIAL AND METHODS

Twenty cows (ten Brown Swiss and ten Girolando) that had a high rate of return to estrus were selected for the experiment. On a scale from 1 to 5, these cows were rated between three and four according to their body condition (EDMONSON et al., 1989), and were not in the postpartum period for at least 180 days. These cows were submitted to artificial insemination by a qualified technician at least three times for a 150-day period during estrus. Empty animals were then selected and submitted to general clinical exam and the following specific exams of the genital tract, vaginoscopy and rectal palpation.

The animals with no apparent disease underwent uterine flushing as described by Ball et al. (1988) in order to obtain biological material. Precaution, such as the use of a sleeve, was taken to minimize vaginal contamination, which sets a limitation to the technique.

After low epidural anesthesia, 2 to 4 mL of lidocaine hydrochloride 2% (Person, Eurofarma, Itapevi, Brazil), a Foley catheter (Solidor, São Paulo, Brazil) protected by a sleeve (IMV Technologies, L'Aigle, France) was inserted with the help of a sterile universal mandrel through the vulva. After passing through the first cervical ring, the sleeve was broken, the Foley catheter was then introduced through the cervix and fixed at the entrance of the uterine body. A three way hose (Nutricell, Campinas, Brazil) in closed system was used to introduce 100 to 300 mL of buffered saline solution into the uterine lumen. The uterine horns were massaged and the solution was recovered (minimum 30 mL) and used for bacteriological culture. The chilled material (4-7ºC) was sent to the bacteriology laboratory within 8 hours, where the samples were plated in aerobic and anaerobic culture systems.

Sample processing included centrifugation at 1,400 x *g* per 10 minutes. The precipitate was recovered using a Pasteur pipette. The anaerobic culture used first Tarozzi culture medium and thioglycolate, the sample was plated after boiling for 15 minutes and subsequent rapid cooling in ice bath to remove air. The bottles were sealed with paraffin and kept at 37º C during 48 to 96 hours before they were examined for growth. Samples from the bacterial growth were then divided into Tyga® Agar plates (yeast extract agar and glucose) and subsequently placed in anaerobic jars Aerogen® (Oxoid, Basingstoke, UK) again at 37º C during 48 to 96 hours. Aerobic culture was performed first in glucose broth and simple agar culture media and kept in an oven during 24 to 48 hours at 37º C. Subsequently, colonies from the bacterial growth were transferred to Teague® and Blood agar. Isolated bacterial genera underwent biochemical tests of the classification system API Index® (bioMerieux, Paris, France). Bacteria isolated from the uterine flora were classified according to potential pathogenicity as established by Sheldon et al. (2002): level I, bacteria that cause endometritis often (*Arcanobacterium pyogenes*, *Bacteroides asaccharolytica* and *Bacteroides capilorosus*); level II, bacteria that cause endometritis infrequently (*Bacillus* spp, *Peptostreptococcus assacarolyticus*, *Peptostreptococcus* spp and *Staphylococcus* spp coagulase-positive); and level III, bacteria that are not uterine pathogens (*Clostridium* spp, *Enterobacter* spp, *Klebsiella* spp, *Pseudomonas* spp and *Staphylococcus* spp coagulase-negative).

Statistical analysis of frequency of bacterial pathogenicity level was performed by Fisher exact test, using the program INSTAT (GraphPad Software Inc., San Diego, USA) at 5% significance.

RESULTS AND DISCUSSION

The present study evaluated the uterine flushing technique performed to collect material for bacteriological culture in order to determine the bacteria present in the uterus of repeat breeder cows

from embryo transfer programs and their correlation with endometritis as well.

Uterine flushing technique was useful to collect biological material to study uterine bacterial flora since 80% (16/20) of repeat breeder cows had bacteria isolated from the aerobic and anaerobic cultures. Bacteria and their prevalence in the 20 animals studied were as follows: *Arcanobacterium pyogenes* (10%), *Bacillus spp* (30%), *Bacteroides asaccharolytica* (5%), *Bacteroides capilorosus* (5%), *Clostridium spp* (5%), *Enterobacter spp* (5%), *Klebsiella spp* (5%), *Peptostreptococcus assacarolyticus* (5%), *Peptostreptococcus spp* (5%), *Pseudomonas spp* (5%), *Staphylococcus spp* coagulase-negative (45%) and *Staphylococcus spp* coagulase-positive (5%).

Repeat estrus syndrome may decrease reproductive efficiency of the property and can be triggered by several factors (ECKERT et al., 2004). One hypothesis for the incidence of this syndrome is the colonization of the uterine environment by pathogenic bacteria that causes inflammation of the endometrium without symptoms, thus characterizing subclinical endometritis (KASIMANICKAM et al., 2004).

According to the classification proposed by Sheldon et al. (2002), level I bacteria were found in 20% (4/20) of the cows, with or without other bacteria; level II in 40% (8/20) and level III were present in 55% (11/20) of positive animals. The uterine lumen is a sterile environment, which can be colonized by pathogenic bacteria from the vaginal flora or by environmental pathogens. However, a healthy uterus is able to control these transient infections very effectively, mainly by phagocytic leukocytes acting on uterine bacteria (GILBERT et al., 2004). The failure of uterine defense mechanisms and the presence of highly pathogenic bacteria may determine the persistence of infection and further development into endometritis (DHALIWAL et al., 2001). In this study, 50% (10/20) of cows had bacteria of level I and II, which are related to uterine disorders and seem to suggest endometritis in the absence of apparent symptoms (subclinical endometritis). In contrast, level III bacteria, which are not associated with uterine disorders were observed simultaneously or not with levels I and II. According to statistical analysis, prevalence of levels I and III was significantly different ($p<0.05$). Figure 1 shows the frequency of all three bacterial levels in the studied cows (SHELDON et al., 2002).

The findings reported here suggest that bacterial colonization of the uterine environment of repeat breeder cows, is mainly due to the presence of non pathogenic uterine bacteria. Although the presence of level III bacteria is not considered as evidence of endometritis, they can play a negative role during conception since they change the microenvironment (GILBERT et al., 2004). Thus, the presence of pathogenic bacteria associated with the failure of defense mechanisms contribute to establish endometritis, which by its turn affect negatively cow fertility by changing the uterine microenvironment and/or embryonic infection (DHALIWAL et al., 2001).

From the results, it was concluded that in repeat breeder cows the colonization of the uterine lumen by pathogenic bacteria occurs with 20% frequency, this changes the uterine environment, favors installation of other pathogens and/or negatively impacts survival rates of gametes and embryos, as well as fertility rates.

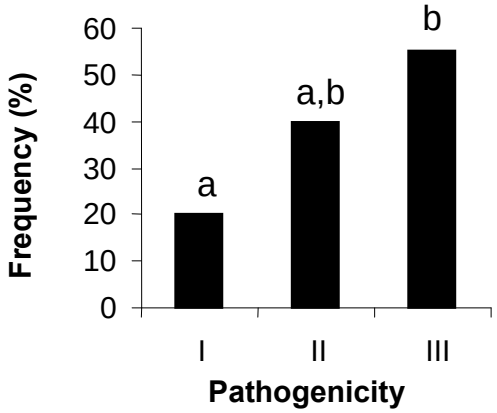

Figure 1 – Frequency of three bacterial groups (classified according to pathogenicity) observed in uterine flushing of 20 repeat breeder cows (different letters on the bars are significantly different according to Fisher at 5%)

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