Small Ruminant Research 113 (2013) 162–166

Contents lists available at SciVerse ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Changes in the vaginal flora of goats following a short-term protocol of oestrus induction and synchronisation with intravaginal sponges as well as their antimicrobial sensitivity

J.K. Oliveira[a], G. Martins[b], L.V. Esteves[a], B. Penna[b], C. Hamond[b], J.F. Fonseca[c], A.L. Rodrigues[a], F.Z. Brandão[a], W. Lilenbaum[b,*]

[a] Faculty of Veterinary Medicine, Universidade Federal Fluminense, CEP 24230-340, Niterói, RJ, Brazil
[b] Laboratory of Veterinary Bacteriology, Universidade Federal Fluminense, CEP 24210-130 Niterói, RJ, Brazil
[c] Embrapa Goats and Sheep, Estrada Sobral/Groaíras, CEP 62010-970, Sobral, CE, Brazil

ARTICLE INFO

Article history:
Received 30 August 2012
Received in revised form 25 January 2013
Accepted 20 February 2013
Available online 9 April 2013

Keywords:
Vaginal flora
Goats
Induction and synchronisation of oestrus
Intravaginal sponges

ABSTRACT

The aim of this study was to characterise the changes in the vaginal flora of goats subjected to the induction and synchronisation of oestrus using intravaginal sponges (IVSs). Mucous samples were collected from the vaginas of 32 Toggenburg goats using sterile swabs. Samples were obtained on the day that the IVSs inserted (D0), the day they were withdrawn (D6), and 24 h (D7), 48 h (D8), and a week (D13) after the sponges were withdrawn. The samples were transferred to the laboratory in transport medium, inoculated on 5% sheep blood agar, and incubated at 37 °C (aerobic culture only). The susceptibility of the cultured bacteria to eight of the most frequently employed antimicrobial agents was determined using the disc diffusion method in Mueller–Hinton agar. Fewer colony-forming units (CFUs) were obtained from the samples that were taken before sponge insertion (D0) compared with those obtained on the other days. After the sponges were inserted, the number of CFUs obtained from the vaginal samples increased, reaching the highest value at the time of sponge withdrawal (D6), when 62.1% (18/29) of the samples yielded $\geq 10^5$ CFU/ml. At this time, more than one type (genus) of bacterial colony was detected. The most prevalent bacterium belonged to the genus *Staphylococcus* sp., except at the time of sponge withdrawal, when the most prevalent bacterium was *Escherichia coli*. The results of this study demonstrated that changes in the prevalent bacterial isolate and the number of CFUs in the vaginal flora of goats subjected to a short-term protocol of oestrus induction and synchronisation using IVS occurred while the IVSs were present and that there was a rapid re-establishment of the normal microbiota after the IVSs were removed.

1. Introduction

Goats are seasonal breeders and are influenced by the geographic latitude and the characteristics of their breed. The Brazilian territories occur in a wide variety of latitudes, which may strongly or weakly influence goat reproduction. In southeastern Brazil, the commercial dairy industries that use specialised dairy breeds must regularly induce or synchronise oestrus to obtain sufficient amounts of milk throughout the year. A combination of progestagens, gonadotropins and prostaglandins is the most commonly used formulation to induce oestrus in goats (Souza et al., 2011). Intravaginal sponges (IVSs) are traditionally utilised in the oestrus synchronisation of small ruminants (Abecia

* Corresponding author at: Department of Microbiology and Parasitology, Hernani Mello Street, 101, Room 309, Niterói, RJ, Brazil. Tel.: +55 21 2629 2435; fax: +55 21 629 2435.
E-mail address: mipwalt@vm.uff.br (W. Lilenbaum).

0921-4488/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.smallrumres.2013.02.011

et al., 2012). The IVSs are impregnated with progestagens, which are effective at lower doses than is natural progesterone (Wildeus, 2000). The protocols vary from short-term (6 days) to long-term (14 days); recent studies have demonstrated no differences among the protocols in their efficacy of synchronisation (Karaca et al., 2010; Vilariño et al., 2011). However, those IVSs are a predisposing factor for vaginal infections because they substantially alter the profile of uterine and vaginal bacteria (Padula and Macmillan, 2006). Vaginitis is a common disease of the reproductive tract of domestic animals that is often caused by opportunistic secondary pathogens (Kustritz, 2006), such as coliforms, mainly *Escherichia coli* (Martins et al., 2009a), which is the bacterium most frequently isolated from the vaginas of ewes (Sargison et al., 2007), goats (Ababneh and Degefa, 2006) and cows (Sheldon et al., 2008).

Therefore, many commercial intravaginal devices manufacturers recommend applying antimicrobial drugs to the sponges prior to insertion. IVSs are known to induce inflammatory responses and the accumulation of vaginal fluids, with the concomitant increase in the bacterial load. The bacterial load significantly decreases after the sponge is withdrawn and reaches normal values by the day of oestrus (Suaréz et al., 2006).

The aim of this study was to characterise the changes in the vaginal flora of goats subjected to the induction and synchronisation of oestrus using IVSs that lacked antimicrobial agents and to determine the antibiotic sensitivity of the vaginal bacteria.

2. Materials and methods

2.1. Location and experimental conditions

This study was conducted during the months of November and December of 2010, which correspond to the anoestrus season of the goats. This study was approved by the Animal Care Committee of Fluminense Federal University, and it was conducted according to the ethical principles of the Sociedade Brasileira de Ciências de Animais de Laboratório.

The goats were kept in an intensive system within suspended pens. The goats were fed corn silage, *Pennisetum purpureum* and *Saccharum officinarum* were available for forage. Additionally, a balanced concentrate supplement was provided according to their milk production (NRC, 2007). Mineralised salt (Salminas Goats®, Nutriplan, Juiz de Fora, MG, Brazil) and drinking water were available *ad libitum*.

2.2. Animals

The samples were obtained from 32 Toggenburg goats that were apparently free of reproductive diseases. Oestrus was induced and synchronised using IVSs impregnated with 60 mg of medroxyprogesterone acetate (Progespon®, Tecnopec, São Paulo, SP, Brazil) lacking antibiotics, which were left in the dams for 6 days. Twenty-four hours before the removal of the sponges, the goats were given an intramuscular injection of equine chorionic gonadotropin (eCG - 200 UI - Novormon 5000®, Tecnopec, SP, Brazil) and 30 μg of d-cloprostenol (Prolise®, ARSA S.R.L., Buenos Aires, Argentina).

2.3. Collection of the vaginal mucous samples

The mucous samples were collected from the posterior vaginal region using a sterile cotton swab. Sampling was conducted on the day of the intravaginal sponge insertion (D0), at sponge withdrawal (D6), 24 h (D7), 48 (D8) hours and a week (D13) after sponge withdrawal. The samples were maintained at 4 °C until the microbiological tests were performed.

2.4. Bacterial culture

The samples were transferred to the laboratory in transport medium (Stuart's medium, Copan, Italy), inoculated on 5% sheep blood agar (Merck, São Paulo, SP, Brasil), and incubated at 37 °C (aerobic culture only). If bacterial growth was apparent after 24 or 48 h of incubation, smears were made, Gram-stained, and examined by microscopy. The samples containing bacteria with morphologies consistent with Gram-negative rods were transferred to EMB Teague agar (Merck, São Paulo, SP, Brasil), whereas those with morphologies suggestive of *Staphylococcus* sp. were transferred to mannitol-salt agar (Merck, São Paulo, SP, Brasil).

2.4.1. Bacterial identification

The bacteria were identified based on their colony characteristics, Gram-staining, pigment production and biochemical reactions, using Triple Sugar Iron (TSI) agar, the citrate, urease, indole, Methyl Red (MR), Voges Proskauer (VP), nitrate and motility tests, the catalase activity test, the tube coagulase test, and assays of their aerobic fermentation of several carbohydrates. The bacteria were classified as described in previous studies (Martins et al., 2009a), according to Bergey's Manual of Determinative Bacteriology (Holt et al., 1994).

2.5. Antibiotic sensitivity test

The susceptibility to a panel of eight of the most frequently used antimicrobial agents was determined using the disc diffusion method with Mueller-Hinton agar (Merck, São Paulo, SP, Brasil), in accordance with the protocols of the Clinical and Laboratory Standards Institute (2008). Briefly, three to five well-isolated colonies of the same morphological type were selected from the agar plate culture and transferred to a tube containing Brain Heart Infusion broth (Merck, São Paulo, SP, Brasil). Standardised inoculum cultures with a turbidity equivalent to a 0.5 McFarland standard were used (corresponding to a concentration of approximately $(1–2) \times 10^6$ CFU/mL). All of the isolates were tested using disks with the following drug concentrations and minimum inhibitory zones: gentamicin – GEN (10 μg, 15 mm), cefalotin – CFL (30 μg, 18 mm), tetracycline – TET (30 μg, 15 mm), ciprofloxacin – CIP (5 μg, 21 mm), florfenicol – FLF (30 μg, 18 mm), and trimethoprim-sulfamethoxazole – SUT (25 μg, 17 mm). In