Theriogenology 85 (2016) 856–861

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Vaginal mucus from ewes treated with progestogen sponges affects quality of ram spermatozoa

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ARTICLE INFO

Article history:
Received 26 March 2015
Received in revised form 26 October 2015
Accepted 26 October 2015

Keywords:
Intravaginal sponge
Estrous synchronization
Sheep
Sperm viability
Vaginitis

ABSTRACT

The use of intravaginal sponges (IS) to synchronize estrous onset in ewes provokes vaginitis, an increase in the vaginal bacterial load, and growth of bacterial species that are not present during spontaneous estrous behavior. The objective of the study was to compare the functional sperm parameters after incubating it with mucus collected from the vagina of ewes during spontaneous estrus or estrous synchronized with IS. Pooled spermatozoa were co-incubated with: (1) vaginal mucus collected from ewes in spontaneous estrus; (2) vaginal mucus collected from ewes in estrus pretreated with progestogen-impregnated IS; (3) synthetic mucus; and (4) medium without mucus as a control group. Sperm samples were evaluated after incubating it for 30 and 90 minutes. The number of colony-forming units (CFUs/mL), pH, and osmolality were greater in the mucus collected from ewes treated with IS than from those untreated ($P = 0.046$; $P < 0.0001$, and $P < 0.0001$, respectively). The percentage of sperm with progressive motility was lower after incubation with vaginal mucus collected from estrous ewes treated with IS than in the other three treatments both, 30 and 90 minutes after incubation ($P = 0.0009$ and $P < 0.0001$, respectively). The sample incubated for 30 minutes with mucus from ewes treated with IS had a lower percentage of sperm with intact plasma membrane than all the other treatments ($P < 0.0001$). The percentage of sperm with functional membrane was significantly lower in the sample incubated for 30 minutes with vaginal mucus from ewes treated with IS than in the other three treatments ($P < 0.0001$). After 90 minutes, the percentage was still lower than that in the sample collected from ewes during their spontaneous estrus ($P = 0.0005$). The lowest percentages of sperm with acrosome damage were observed in sperm incubated with mucus collected from sheep in spontaneous estrus for 30 and 90 minutes ($P < 0.0001$ and $P = 0.008$, respectively). The percentage of apoptotic spermatozoa was greater in samples incubated during 30 minutes with vaginal mucus collected from ewes treated with IS than in the other three groups ($P = 0.0005$). The functionality and the viability of ram sperm is negatively affected by the cervical mucus of ewes pretreated with progestagen-impregnated IS used in estrous synchronization treatments. This may partially explain the decrease in conception rate obtained with treatments with IS.

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http://dx.doi.org/10.1016/j.theriogenology.2015.10.033

1. Introduction

Reproductive biotechnologies can be used to enhance breeding programs in farm animal production [1]. There are several treatments used to synchronize or induce estrus in ewes, but most of them require the application of intravaginal devices containing progesterone or synthetic progestagens during 7 to 14 days before artificial insemination [2]. In most widely used protocols, polyurethane sponges impregnated with progestagens are introduced into the vagina (intravaginal sponges [IS]). However, at IS withdrawal, abnormal hemorrhagic and putrid vaginal discharge is commonly set free [3]. Therefore, progestagen-impregnated IS are a predisposing factor for vaginal infections caused by opportunistic secondary invaders [4]. In ewes, a significant increase in the vaginal flora number and disruption of the bacterial composition has also been observed, as there is an important increase of the presence of Gram negative *Enterobacteriaceae* [4–6]. These bacterial vaginal changes might affect the subsequent fertility, and thus, the reproductive performance. In this sense, Manes et al. [7] reported that the presence of the IS itself is responsible for an important decrease in ewes' conception rates.

Although the number of vaginal bacteria returns to basal values by the day of estrus [3,8], the normal vaginal flora composition is still altered. For example, the presence of opportunistic *Enterobacteriaceae* family was related to vaginitis even 72 hours after IS removal [4,6]. In the same direction, *Escherichia coli* (the most prevalent bacteria after device removal [4,6]), reduces sperm motility through sperm adhesion and agglutination and causes important morphologic changes that alter its function in human spermatozoa [9]. Yániz et al. [10] observed that the contamination of ram semen with enterobacterial species during infection of male genital tract reduces sperm quality parameters. Thus, it maybe expected that the accumulation of bacterial products in the vaginal environment compromises the viability of the spermatozoa, as it also increases the number of apoptotic spermatozoa [11]. Maybe as a consequence of all those factors, the number of sperm breakage increases after the use of progestagen-impregnated IS [12,13], and thus, the cervical sperm reservoir is negatively affected by alterations in the characteristic of the vaginal mucus caused by the vaginitis that often follows the use of IS [14].

Cervical mucus filters out sperm with poor morphology and motility allowing only a small percentage of sperm to go into the uterus [15]. Therefore, spermatozoa are selected according to their ability to progress through natural mucus; as this process is strongly related with the fertilizing capacity of spermatozoa [16], the sperm ability can be evaluated with a cervical mucus penetration test. The penetration of spermatozoa into cervical mucus *in vitro* is a reliable indicator of sperm function [17].

Considering all this information, our hypothesis was that the co-incubation of ram sperm with cervical mucus of ewes treated with progestagen-impregnated IS negatively affects sperm viability and functionality. Thus, the objective was to compare the functional sperm parameters after co-incubating it with mucus collected from the vagina during spontaneous estrus or estrous synchronized with progestagen-impregnated IS.

2. Materials and methods

2.1. Experimental treatments

The experiment was conducted at the Instituto Nacional de Tecnología Agropecuaria Experimental Research Station, in Balcarce, Argentina (37° 45′ S; 53° 18′ W), during the breeding season (March–April; autumn). All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise indicated. All animals used in this study were handled in strict accordance with good animal practice and the conditions approved by the Animal Ethics Committee of Instituto Nacional de Tecnología Agropecuaria.

Semen was collected from 10 fertile mature (5 years old) Texel rams using artificial vagina. Temperature of the water in the lining of the artificial vagina ranged from 40 °C to 44 °C. The ejaculates were evaluated to determine volume, concentration, and sperm wave motion. For sperm wave motion, a sample of sperm, without further dilution, was placed on a microscope slide. The sample was then viewed at 26 °C, and the presence of a swirling motion of the mass of sperm (wave motion) was subjectively assessed on a scale from 0 to 4 according to Cherms [18] and Graham et al. [19]. Samples of sperm displayed strong wave motion (rating of 4) were considered to be of high quality and likely to prove highly fertile, whereas samples not displaying wave motion (rating of 0) probably would have poor fertility, and only ejaculates with wave motion greater than or equal to 4 (0–5 scale) were used for the study. To minimize individual effects, pools were made with semen collected from three rams, including 20×10^8 spermatozoa from each ram. A single ejaculate from each ram/day was used.

Sperm samples were divided in aliquots containing 100×10^6 spermatozoa and the seminal plasma, which were incubated in four experimental treatments in 10-mL sterile tubes at 38 °C in 5% CO_2. Each tube contained 500 μL of: (1) mucus collected from the vagina during its spontaneous estrus; (2) mucus collected from the vagina of an estrous ewe previously treated with progestagen-impregnated IS (for mucus collection in treatments 1 and 2, see Section 2.2); (3) synthetic mucus (for details, see Section 2.5); (4) M199 plus 0.1 mg/mL L-glutamine and 2.2 mg/mL $NaHCO_3$ (M199 modified) without mucus as a control.

Sperm were deposited at the bottom of each tube, under the mucus. After 15 minutes, 500 μL of M199 modified was added to each tube, above the mucus, to give a carrier medium to later facilitate sperm sampling. Then, semen–mucus samples were incubated during 90 minutes, collecting sperm samples at 30 and 90 minutes. At the end of the incubation period, samples were collected from the top of the tube.

2.2. Ewes' management and collection of vaginal mucus samples

One hundred twenty Texel ewes, with a mean body condition score of 3.2 ± 0.7 (scale 1–5), weighing 41.3 ± 2.8 kg (mean ± standard deviation) were injected