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Intravaginal placebo sponges affect negatively the conception rate in sheep

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ABSTRACT

Intravaginal sponges (IS) impregnated with progestagens are commonly used for oestrous synchronization in ewes. However, the use of IS is a predisposing factor for vaginal contamination that can affect negatively fertility. The objective of this work was to determine if the use of IS negatively affects the fertility. The experiment was conducted on a commercial farm (Uruguay), during March-May (mid-breeding season) with 575 mature Australian Merino ewes. Oestrous was recorded daily with vasectomized rams for 5 d in ewes that received a single dose of a PGF2alpha analogue. Two days after oestrous detection, females were randomly allocated to three groups: Group MAP ($n = 200$) received an intravaginal polyurethane sponge impregnated with 60 mg of medroxyprogesterone acetate (MAP) for 13 days. At the same time, sponges without MAP were inserted in 156 ewes (ISG Group), and remained in situ also for 13 days. Other 213 ewes (Group CG) remained as a control of the fertility of the spontaneous oestrus, without treatment. Ewes presenting oestrous were inseminated 12 h later. Conception rates were determined by rectal ultrasound 30 days after oestrus. Conception rate of the CG group was greater than that of MAP and ISG groups (CG: 55.4% vs MAP: 41.5% and ISG: 34.6%; $p = 0.0002$), without differences between the last two. We conclude that the IS is responsible itself for a significant reduction in conception rate in oestrous synchronization treatments in sheep.

1. Introduction

Procedures to synchronize oestrous and ovulation facilitate the use of assisted reproductive technologies. Although there are many techniques available for oestrous synchronization in ewes, the most widely applied protocols include the use of intravaginal sponges (IS) impregnated with progestagens (Abecia et al., 2012). In traditional treatments, progestogens are used for long periods of similar lifespan to a cyclic corpus luteum, regardless of the stage of the cycle or the follicular status of the ovary at the time of treatment (Menchaca and Rubianes, 2004). However, the fertility rate remains variable (Gordon, 1983).

One factor that could be implicated in the failure of fertility of IS synchronized cycles after vaginal insemination is the alteration of the vaginal environment. The use of IS provokes vaginitis with the accumulation of purulent mucous secretion and disruption of the bacterial composition, as there is an important increase in Gram negative *Enterobacteriaceae* (Suárez et al., 2006; Manes et al., 2010; Martins et al., 2009, 2010). At sponge withdrawal, an abnormal

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haemorrhagic and putrid vaginal discharge may be set free (Scudamore, 1988; Suárez et al., 2006). Gatti et al. (2011) proposed that the main cause of the vaginitis is the presence of a foreign body in the vagina. It may not be related to the hormonal treatment, because the increase in bacterial number is similar in ewes treated with IS regardless of its progesterone content. The infiltration of leukocytes in the vaginal epithelium may act against the spermatozoa (Suárez et al., 2006), affecting the reproductive response and the resulting fertility from the oestrous synchronization treatments. Additionally, lipopolysaccharides produced by Gram negative bacteria have been linked to infertility and pregnancy losses in humans (Gorga et al., 2001). The biologically active substances released in the course of an inflammatory response (Fraczek and Kurpisz, 2007) and the action of the Gram negative bacterial components (Yániz et al., 2010) could also have a negative effect on the viability and motility of spermatozoa (Gorga et al., 2001).

Each of these bacterial vaginal changes might affect the reproductive response and the subsequent fertility of an oestrous synchronization treatment. To avoid vaginitis some laboratories recommend the addition of antibiotic to the IS. As this decreases the odour perceived at sponge withdrawal, without affecting the conception rate, Viñoles et al. (2011) claimed that the vaginitis had no effect on sperm survival. However, they did not compare conception rates with an untreated group, so the fertility may have been similarly affected in both groups because the flora present after the local use of antibiotics is completely different to that observed in the vagina of ewes with spontaneous oestrus (Manes et al., 2010).

Considering all this information our hypothesis was that the use of IS itself is at least partially responsible for the lower fertility observed in ewes in oestrous synchronization treatments. As previous reports that assessed vaginal changes produced by the application of sponges did not determine whether those changes affect the fertility of the synchronized oestrus, this study aimed to compare the conception rate obtained after an artificial insemination of oestrous ewes that during a previous normal luteal phase were treated with IS with or without progestagens or that remained without treatment.

2. Materials and methods

2.1. Animals and management

The experiment was conducted on a commercial farm located in Tacuarembó, Uruguay (31°42′ S, 55°49′ W) during the mid-breeding season (March–May; autumn) with 575 Australian Merino ewes. Ewes were all multiparous (4–6 years old), weighed 43.5 ± 3.7 kg; mean ± SD, had a body condition score of 3.4 ± 0.5 (scale 0 = extremely emaciated, 5 = obese; Russel et al., 1969), and remained grazing native pastures throughout all the experimental period.

2.2. Experimental treatments

All ewes were injected intramuscularly with 75 μg of a PGF2alpha analogue (75 μg d-cloprostenol, Celovet-prost, Vetcom, Montevideo, Uruguay). Oestrus was recorded daily by androgenized wethers during 5 days. Wethers received 2 doses of 100 mg of testosterone ciclopentilpropionate (Testosterona, Dispert, Montevideo, Uruguay) 7 days before and on the day of the administration of PGF2alpha to the ewes. Oestrus was checked once a day, and females detected in oestrus were randomly allocated to one of three experimental groups.

Ewes of group MAP (n = 200) received an IS, which remained in situ 13 days, impregnated with 60 mg of medroxyprogesterone acetate (Medroxyprogesterone acetate USP23, Farmabase, Rovereto, Italy) 2 days after oestrus detection. Another 156 ewes (ISG Group) received the same treatment with an IS without medroxyprogesterone. A further 213 ewes remained without treatment to determine the conception rate of the spontaneous oestrus as a control group (group CG). All sponges were previously sterilized with ethylene oxide (Biolene, Buenos Aires, Argentina).

Fifteen days after the first oestrus all ewes were placed with marking androgenized wethers (ratio 1 wether: 11 ewes) for oestrus detection. Marked ewes were identified once a day until 72 h after sponge withdrawal.

2.3. Artificial insemination and pregnancy diagnosis

Ewes detected in oestrus were cervically inseminated 12 h later with fresh semen from 3 adults rams (100 million spermatozoa/ewe). Semen was collected daily using an artificial vagina from rams exposed to ewes in oestrus. Ejaculates were pooled and diluted with UHT skimmed milk to minimize individual effects. Only ejaculates with wave motion ≥4 (0–5 scale) were pooled and used for AI.

Pregnancy was determined by transrectal ultrasonography 30 days after oestrus (Aloka SSD 500 with a 5 MHz transducer, Tokyo, Japan).

2.4. Statistical analysis

Conception rates from each day after IS withdrawal, and final conception rate were compared with Fisher exacts probability test.

3. Results

The interval from device withdrawal to oestrus onset was similar in MAP and ISG group (1.5 ± 0.9 and 1.4 ± 0.9 days, respectively). The conception rate of CG group was significantly greater (p = 0.0002) than that of MAP and ISG groups, without differences between these two (Table 1). No significant differences were observed in the conception rates according to the day after sponge removal between MAP and ISG groups (Table 1).

4. Discussion

The conception rate was negatively affected in ewes treated with IS demonstrating that IS use is responsible for an important decrease in fertility in oestrous synchronization treatments. The sponge as a foreign body induces changes in the normal vaginal environment that favours bacterial growth (Manes et al., 2010) that could have a direct effect on sperm fertilization ability. Although, the number of vaginal bacteria returns to basal values at the time of insemination (Suárez et al., 2006; Gatti et al., 2011) changes in the normal vaginal flora composition such as the presence of opportunistic *Enterobacteriaceae* family were incriminated as a cause of vaginitis in even 72 h after device removal (Manes et al., 2010; Martins et al., 2009). Moreover, Oliveira et al. (2013) found that the normalization of the normal microbiota occurs at least one week after devices removal. The contamination of ram semen with enterobacterial species reduces sperm quality (Yániz et al., 2010). In this sense *Escherichia coli*, the most prevalent bacteria in ewes after device removal (Manes et al., 2010; Martins et al., 2009), reduces sperm motility through sperm adhesion and agglutination and causes important morphological changes that alter its function in human spermatozoa (Schulz et al., 2010). The presence of biologically