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Microbiological and functional evaluation of an alternative device (OB®) for estrous synchronization in ewes

Avaliação microbiológica e funcional de um dispositivo vaginal alternativo (OB®) para sincronização de cio em ovelhas

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ABSTRACT

The use of synthetic progestagens released by vaginal devices is an important tool to overcome the reproductive seasonality in sheep, but cost and/or subsequent vaginitis are limiting factors for their use. To identify economic, simple and innocuous alternative vaginal devices for estrous synchronization/induction protocols in sheep, this study aimed to evaluate the microbiological and functional viability of the human vaginal tampons (OB®) impregnated with medroxyprogesterone acetate (MAP) on reproductive performance of ewes. The study compared them with commercial vaginal inserts (CIDR®) and polyurethane sponges impregnated with MAP. In Experiment 1, the device loss rate, the degree of vaginitis during the device removal, the count and identification of bacterial colonies at the device insertion and removal, and efficiency in estrous synchronization and estrus temporal distribution were evaluated. Pubertal ewes at the beginning of the breeding season were randomly allocated to three experimental groups: CIDR®, PSP (polyurethane sponge) and OB®. No device losses occurred in any group, but the use of OB® caused milder signs of vaginitis than polyurethane sponges, with a similar vaginal bacterial growth and microbiota than the CIDR group. The estrus distribution was more disperse in the CIDR than PSP or OB groups. In Experiment 2, pregnancy rates using CIDR® or OB® devices were compared, with estrus manifestation (85.4% and 89.8%) and pregnancy rates (58.3% and 49.0%) being similar between groups (P>0.05), respectively. In conclusion, the use of human intra-vaginal tampons (OB®) impregnated with MAP was proven highly hygienic, practical and effective as a low-cost alternative for estrous synchronization and AI in sheep.

Key words: *intra-vaginal device, CIDR®, sponge, progestin, vaginitis, ewe.*

RESUMO

O uso de progestágeno sintético liberado por pessários vaginais é uma importante ferramenta para suplantar a sazonalidade reprodutiva em ovelhas. Todavia, seu uso é limitado pelo custo ou pelas subsequentes vaginites. Na busca de uma alternativa simples e de baixo custo para sincronizar estro em ovelhas, este estudo avaliou o tampão vaginal humano (OB®) impregnado com MAP, na performance reprodutiva de ovelhas, comparando com o CIDR® e as esponjas de poliuretano, estas também impregnadas com MAP. No experimento 1 foram avaliados a taxa de perdas; o grau das vaginites no momento da remoção do pessário; a contagem e identificação das colônias bacterianas; bem como a eficiência da sincronização e a distribuição temporal dos cios. As ovelhas foram aleatoriamente distribuídas em um de três grupos experimentais: CIDR, Esponjas e OB, no inicio da estação reprodutiva. Não ocorreram perdas de pessários em qualquer grupo, porém o OB causou menor grau de vaginite em relação às esponjas, com um crescimento bacteriano e microbiota similares ao grupo CIDR. A distribuição dos cios foi mais dispersa no grupo CIDR do que nos grupos Esponja ou OB. No experimento 2, foram comparados o CIDR e OB em relação à manifestação de cio (85,4% e 89,8%) e taxa de prenhez (58,3% e 49,0%), que foram similares (P<0,05). Conclui-se que o pessário OB impregnado com MAP é higiênico, de baixo custo, prático e efetivo como para a sincronização de cios e IA em ovelhas.

Palavras-chave: *pessário intra-vaginal, CIDR®, esponja, progestageno, vaginite, ovelha.*

INTRODUCTION

The reproductive seasonality in sheep usually affects the regularity of meat and dairy supply

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to the industry, which have stimulated researchers to pursue technological alternatives to improve the reproductive efficiency in this species. The administration of exogenous hormones such as the melatonin (LALIOTIS et al., 1998), prostaglandin F2α or its analogues, progesterone or its analogues, or even gonadotrophins, allow follicular stimulation and ovulation synchrony at a reasonable level of fertility (EVANS, 2003). Prostaglandin F2α can be used for estrous synchronization and ovulation throughout the reproductive season (MENCHACA & RUBIANES, 2004). Conversely, during seasonal anestrous, the use of progesterone or synthetic progestagens associated with gonadotropins is required to sufficiently stimulate the follicular development to attain ovulation (GÓMEZ et al., 2006; HASHEMI et al., 2006; BARRETT et al., 2008). However, hormone delivery methods offer distinct advantages and/or limitations through their use. The first way devised to supplement progesterone to females was via oral ingestion by mixing the hormone to the feeding stuff, but the control of the amount ingested by each animal is very difficult. Subcutaneous progesterone implants allow a very precise control of the individual dose but such approach, in addition to being invasive, is not practical. Thus, vaginal devices have been widely accepted as more useful and practical devices for estrous induction and/or synchronization in sheep. Among the variations in vaginal hormone delivery apparatuses, sponges manufactured from high density polyurethane suds, usually impregnated with synthetic progestagens (medroxyprogesterone acetate, MAP; flurogestone acetate, FGA), are the devices of choice in commercial flocks, mainly due to their low cost (AINSWORTH & SHRESTHA, 1983; HASHEMI et al., 2006). For highly valuable animals, a commercial apparatus named CIDR® (Controlled Internal Drug Releasing device, Pfizer Inc., USA) consists of a "Y"-shaped silicon-based device loaded with progesterone for vaginal use (WHEATON et al., 1993). Due to its inherent properties and composition, the rates of device loss, adherence, and vaginitis are much lower with the use of CIDR® than with sponges (SUÁREZ et al., 2006). In addition to lowering the efficiency of the protocol, the local adherence reactions and/or purulent secretion accumulation creates a concern related to the animal welfare. In fact, the use of sponges is only justifiable due to the low cost of the product. Consequently, studies searching for alternative low-cost materials that can safeguard the animal's health, still maintaining or even increasing the reproductive response in sheep estrous synchronization are still a need. A viable replacement option to sponges or CIDR® is the use of vaginal human hygienic tampons (OB® - Johnson & Johnson do Brasil Ind. Com.), made of cellulose polymers and thermoplastic resins as the textile components, and a tampon nucleus in rayon. Such tampons, if applied as vaginal hormone delivery devices, have the advantages of being of low cost, differing in presentation sizes, being disposable and sterile, abiding to standard sanitary requirements for human health and safety. Thus, this study aimed to evaluate the efficiency of the use of human vaginal tampons (OB®) impregnated with medroxyprogesterone acetate (MAP) in sheep estrous distribution, and its effects on inflammatory response and changes in the vaginal microbiota (Experiment 1), and the rates of estrous synchronization and pregnancy after AI in ewes synchronized with CIDR or OB devices (Experiment 2).

METHODS

Procedures of this study were approved by the Animal Ethics Committee of the Santa Catarina State University (UDESC), Brazil. The experiment was conducted on a private commercial farm in the Santa Catarina plateau (lat 27º16'58" S - lon 50º35'04" W) using Texel x Ile de France crossed ewes, with ages ranging from 1.5 to 3.0 years old, and body condition score between 2.5 to 3.0 (1-5). Animals were kept in range conditions under ***Sorghum sudanense*** and ***Pennisetum glaucum*** grass pasture, having access to mineral supplement and water *ad libitum.*

Experiment 1

Sixty healthy females were randomly assigned to one of three experimental groups (n=20) using distinct hormone delivery vaginal devices for estrous synchronization: CIDR group, using CIDR® inserts; PSP group, using polyurethane sponges impregnated with MAP; or OB group, using human intra-vaginal tampons (OB®) also impregnated with MAP. CIDR® implants were of second use, being sterilized by autoclaving. Polyurethane sponges (PSP) were homemade using a 33-density polyurethane foam, cut in cubic shape size 2x2X2cm and trespassed by a cotton string. Sponges were washed, sterilized by autoclaving, and then impregnated with 60mg medroxyprogesterone acetate (MAP) diluted in an acetonealcohol solution (1:3, v/v) with 0.5% gentian violet. Commercial human tampons (OB®) were impregnated with 60mg MAP, following the same procedures as for PSP. To impregnate the OB tampons without damaging the individual wrapping, a syringe attached to a needle was used to inject the MAP solution in the device's grooves. After the impregnating

process, sponges and OB® tampons were let to dry at room temperature for at least 12h to allow evaporation of the acetone-alcohol fraction.

For vaginal device insertion and vaginal sample collections, after cleaning the perineal region, devices of each treatment were inserted into the vaginal canal using a tubular vaginoscope previously disinfected with quaternary ammonia (1:1000). The estrous synchronization protocol consisted of the insertion of the vaginal devices according to each experimental group (CIDR®, PSP, or OB®) on day zero (D0), and removal after ten days (D10), followed by an intramuscular injection of 135mg of sodium D-cloprostenol. Just after the D-cloprostenol administration, a sexually matured vasectomized ram teaser was used to detect the onset of estrus in each group. The incidence of device losses were recorded at the device removal (D10), when the difficulty for device removal from the vaginal canal was additionally evaluated, classifying the degree of adhesion as absent/none (-), minor (+), moderate (++), or intense (+++; totally adhered); moreover, the intensity of vaginitis was also determined by the evaluation of the discharge characteristics (amount, odor, and aspect).

The total bacterial count (colony forming units ml^{-1} - CFU ml^{-1}) and bacterial identification were determined in each group, with individual vaginal sample aseptically collected prior to the device insertion (D0) and immediately after its withdrawal (D10). Collections were carried out using sterile pressed cotton swabs ($1cm^3$), moistened with saline (0.9% NaCl; w/v) solution. Upon collection, samples were placed individually into sterile tubes containing 10 ml of saline solution, which were kept in ice until arrival at the Microbiology Laboratory (CAV/UDESC). For processing, samples were homogenized by mechanical vortexing, diluted in saline (1:10.000) and then seeded in Petri dishes. Culture was carried out in blood agar for 24h in a bacteriological incubator at 37°C. The total bacterial count (CFU mL^{-1}) was done by using a manual bacterial colony count procedure. The bacterial identification was carried out after Gram staining; catalase/oxidase test; triple sugar iron (TSI); sulfite, indol and motility (SIM); citrate test; and urease test. The final identification was performed by optic microscopy under 100-X magnification.

Experiment 2

After a gynecologic examination, 97 healthy pubertal ewes were randomly assigned to two groups: CIDR group (n=48) or OB group (n=49). Synchronization procedures were the same as in first experiment. Estrus detection was performed by the use of a ram teaser. Twelve hours after the onset of estrus, females were artificially inseminated, with females not detected in estrus being inseminated 54h after the device removal. Pregnancy diagnosis was performed by ultrasonography 35 days after breeding.

Data values were analyzed by ANOVA and compared by the Tukey test or χ^2 test, using the Minitab statistical software package (State College, USA), for $P<0.05$.

RESULTS

Experiment 1

At the time of device withdrawal (D10), no device losses were observed in any experiment or treatment group. The total bacteria count obtained from samples collected at D0 was similar between all treatments and increased significantly in all groups at the end of the period (D10). As expected, animals from the PSP group showed a significant increase *(*$P<0.05$*)* in total bacteria count in comparison with the other groups, with counts for the use of OB® being similar to the CIDR group (Figure 1). In the CIDR group, the prevailing bacteria on D0 were the gram – (G-) ***Pasteurella*** sp.*,* ***Klebsiella*** sp. *and* ***Escherichia coli****,* and the *Gram* + (G+) ***Staphylococcus*** sp., whereas in the PSP group, the prevailing bacteria were ***Escherichia coli*** (G-) and ***Staphylococcus*** sp. (G+). In the OB® group, the main bacteria present on D0 were ***Yersinia*** sp. (G-), ***Escherichia coli*** (G-) and ***Staphylococus*** sp. (G+). At the removal of the devices (D10), in addition to the bacteria observed on D0, ***Shigella*** sp. (G-) was also present in the CIDR® group; ***Pasteurella*** sp. (G-), ***Yersinia*** sp. (G-) and ***Streptococcus*** sp. (G+) in the PSP group; and ***Providencia*** sp. and ***Aeromonas*** sp. (G-) in the OB® group.

The degree of local inflammation (vaginitis) was generally minor in the CIDR group, with all animals from this group having no adherence, considered as degree (-), showing neither resistance during the device removal nor significant accumulation of purulent and/or fetid vaginal discharge. Fourteen animals from the PSP group showed moderate device adhesion classified as (++), while severe adhesion (+++) was observed in six animals. Furthermore, in some cases, the sponge was fragmented in pieces making it difficult to remove the entire device from the genital tract. In this group, vaginitis was present in all animals, classified as severe (+++) in 13 and moderate (++) in 7 animals. It was characterized by the accumulation of large amounts of vaginal discharge with repulsive odor, purulent aspect, and containing blood stripes. In the

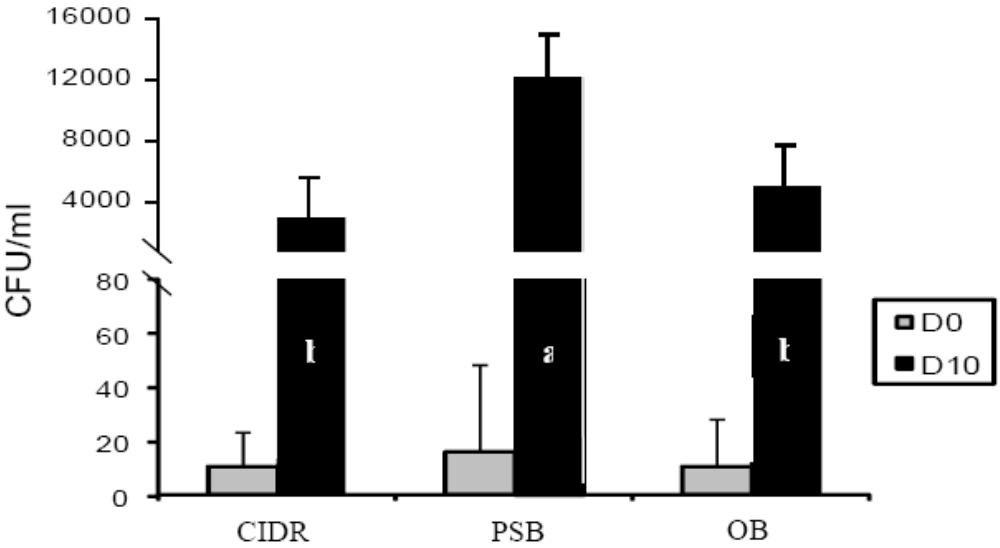

Figure 1 - Colony forming units (CFU ml^{-1}) observed before the insertion (D0) and after the removal (D10) of the vaginal devices used for estrous synchronization in ewes with CIDR®, PSP (polyurethane sponges), and OB® vaginal devices. * P<0.05

OB group, most animals (n=18) had a local vaginal inflammation classified as (+) due to the slight resistance to remove the device, although noticeably lesser than for the sponges. In some cases, the tampon was longitudinally deformed, but without adhesion. No fragmentation or loss of material was observed in the interior of the vagina. In this group, a small discharge accumulation of a whitish serous-milky aspect with no odor was present in the vagina at the time of the device removal.

Six hours after the devices removal, primary signs of estrus were initially observed. The temporal onset of estrous in the CIDR group was more disperse than the others groups (Figure 2). The PSP and OB groups showed a more concentrated distribution of estrus 36h after device removal. However, most animals in all groups showed estrus within a 36h window, from 24 to 60h after the device removal, especially in the PSP and OB groups.

Experiment 2

Estrus manifestation rates were similar between the CIDR and OB groups (85.4% and 89.8%), respectively. Seven females that were not detected in estrus in the CIDR group (14.6%) and 5 in the OB group (10.2%) were subjected to AI 54h after the device removal, resulting in additional 3 and 1 pregnancy, respectively. Overall, pregnancy rates for all bred animals were similar between groups, being 58.3% and 49.0% for the CIDR and OB groups, respectively. When only estrus-detected inseminated ewes were considered, pregnancy rates were also similar, being 61.0% for the CIDR and 52.3% for the OB group (P>0.05).

DISCUSSION

Vaginal devices were first developed in the 1970s, using sponges, impregnated with FGA or MAP (AINSWORTH & SHRESTHA, 1983), which are still in wide use today. Aside from being effective, practical and inexpensive, such devices cause various levels of vaginal irritation, mainly due to its physical abrasiveness, but also due to its features per se, favoring the retention of genital secretions (AL-HAMEDAWI et al., 2003). In the 1980s, the silicon-based CIDR® device was developed (WHEATON et al., 1993), which minimized or eliminated some of the biological effects caused by the use of sponges. However, this device is more expensive, limiting its widespread use in commercial flocks. Human vaginal tampons (OB®) have similar absorption and secretion retention characteristic as sponges, but the former are made with more appropriate and inert material than the latter. The effect of those features were observed in this study, as a significant increase in the number of CFU mL^{-1} was observed at the end of the treatment in the PSP group when compared with the CIDR and the OB groups. The increase in bacteria counts was well expected after the use of sponges (AMIM, 1996;

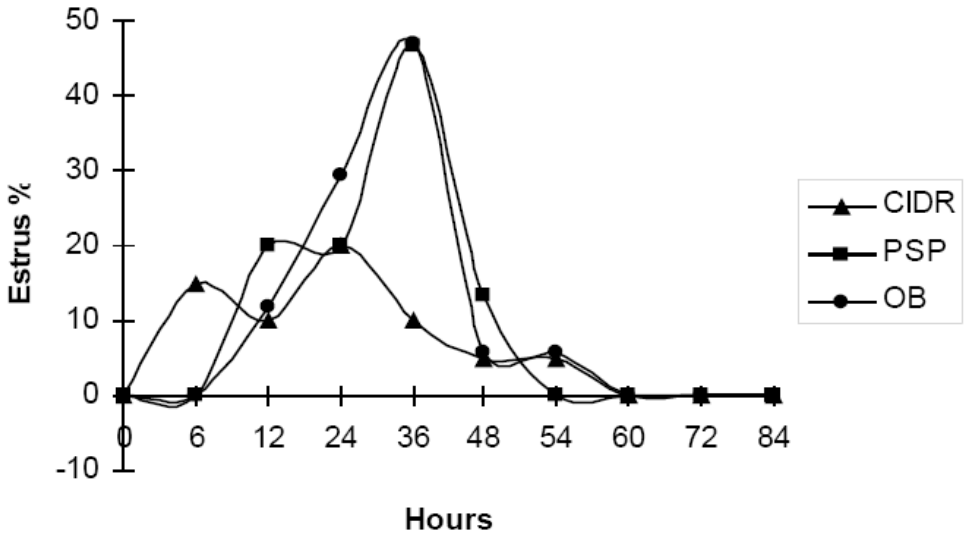

Figure 2 - Temporal distribution of the onset of estrus in ewes synchronized with CIDR®, PSP (polyurethane sponges), and OB® vaginal devices (Time 0=device removal).

MARTINS et al., 2009), even after different periods into the vagina (SUÁREZ et al., 2006). The concomitant use of antibiotics is often suggested to reduce the undesirable effects caused by the sponges, but this practice may induce antibiotic resistance (SUÁREZ et al., 2006; MARTINS et al., 2009). As expected, lower CFU were observed in the CIDR group, with the OB group having a bacteria pattern similar to the use of CIDRs, which indicates advantages in terms of animal welfare, in addition to a reduced propensity for the use of antibiotics along with the devices, as it occurs with sponges.

Most animals in the PSP group presented a local inflammatory reaction classified as (++), attributed to the features described above, with accumulation of secretion with repugnant odor, cloudy aspect and purulent characteristic, associated with blood clots, also observed by MARTINS et al. (2009). The changes in the vaginal canal are attributed to the physical abrasiveness of the device *per se*, in addition to the retention of exsudate and other secretions of the genital tract (AL-HAMEDAWI et al., 2003). The sponge's features also cause a moderate resistance to its removal, aggravating the clinical outcome. The myriad of inflammatory reactions, infectious state, and intensity of the vaginitis favors the absorption of toxins, if present. In this study, although the animals of the three groups had an increase in CFU mL^{-1} on D10, with the presence of ***Streptococcus*** sp. and ***Staphylococcus*** sp., any clinical toxemic manifestation was detected at any given time for any treatment. In a few cases, sponges fragmented at removal, collectively demonstrating that this device is not the most appropriate material to use in such practice, in terms of animal health, despite the surprisingly high pregnancy rates obtained after its use.

From tested groups, a lower local reaction (vaginitis) was observed in the CIDR group, with all animals classified as (-) in the evaluation of the degree of adhesion. In general, no resistance for the removal of the device or any significant accumulations of purulent or fetid secretion were observed in the CIDR group. The silicon-based material minimizes the abrasiveness of the device, reducing the inflammatory response and, unlike sponges and tampons, which retain secretions; the use of CIDRs allows the drainage of any secretion. Distinct to what was observed in the PSP group, animals in the OB group had less intense vaginal reactions, classified as (+), which was associated with a low resistance for removal. In some cases, tampons suffered a longitudinal deformation, nevertheless without any fragmentation or withheld material inside the vagina. The significance of such deformation is yet to be determined. The characteristics of the secretion were similar between the CIDR and the OB groups, with the latter showing a small accumulation of a whitish serous vaginal secretion, without any undesirable odor. Bacteria identified in D0 and D10 did not necessarily match with the vaginal alterations caused by the different devices used in this study. In a recent study, MARTINS et al. (2009) observed a 72.7% prevalence of coliforms, 18.2% ***Klebsiella pneumoniae***,

and the remaining 9.1% were ***Staphylococcus aureus***. All these bacteria were also present in our study. What is physiologically surprising, even by the use of sponges, is the fact that only two days after the device's removal, the vaginal bacterial population returns to levels similar to those observed prior to their insertion (AMIN, 1996; MARTINS et al., 2009). This reduction in the CFU/ml is due to the removal of the irritating agent, also promoted by the increase in local immune response caused by the estrogenic phase during proestrus and estrus.

In the CIDR group, the onset of estrus started earlier, and the temporal distribution of estrus was more disperse than the PSP and OB groups. Such difference in the distribution of estrus in females from different groups was likely to depend more on the hormone used (MAP *vs.* progesterone) than the device *per se*. These results can be related to the low residual effect of the progesterone (GREYLING et al., 1997) in comparison with progestagen (SIMONETTI et al., 2000). The shorter progesterone half life allows a rapid fall in serum levels, triggering an early GnRH discharge. However, our results disagree with those observed by HASHEMI et al. (2006) where they did not observe any differences to the onset of estrus with CIDR (30.1±7.6h) and MAP sponge (29.6 ±5.6h). Although some difference exists in estrus distribution when progesterone and progestagen are compared, fertility rates obtained with both types of hormones are similar for sheep (AINSWORTH & SHRESTHA, 1983; UNGERFELD & RUBIANES, 2002) and goats (MOTLOMELO et al., 2002; ROMANO, 2004).

High rates of estrus manifestations were observed in both the CIDR (85.4%) and OB (89.8%) groups. In the CIDR group, 7 ewes (14.6%) were not detected in estrus, in comparison with 5 (10.2%) in the OB group. This may be a consequence of a more disperse distribution of estrus in the CIDR group. Also, this finding may explain the differences in pregnancy rates observed after the timed AI at 54h after the device removal in ewes without estrus detection in the CIDR group (3 of 4) in comparison with the OB group (1 of 4). However, the overall pregnancy rates observed using CIDR or OB were satisfactory (58.3% or 49.0%, respectively) and did not differ between groups (P>0.05). When only estrus-detected inseminated ewes were considered, pregnancy rates were 61.0% and 52.3% for CIDR and OB groups, respectively, also being statistically similar.

CONCLUSION

The human vaginal tampons (OB) impregnated with MAP was very effective for the synchronization of estrous cycle in sheep. Reproductive responses by the use of tampons were similar to those observed with sponges and CIDR. In addition, tampons were a low-cost alternative to CIDR, as much as sponges are, also resulting in significant improvement in clinical and microbiological profiles after their use in comparison with sponges. In summary, the use of human tampons was proven to be very hygienic and practical, combining the low cost of the sponges, with the lower inflammatory reactions seen with CIDR®, resulting in a similar estrous synchronization response and pregnancy rates than the CIDR device. Nevertheless, new studies need to be carried out to evaluate the possibility of the use of a timed AI protocol without estrus detection using human vaginal tampons (OB) impregnated with MAP for the estrous synchronization in sheep.

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