

ESTRUS SYNCHRONIZATION WITH PROSTAGLANDIN F2 α COMPARED TO PROGESTOGEN TREATMENT ASSOCIATED WITH EQUINE CHORIONIC GONADOTROPIN (eCG) IN SANTA INÊS BREED EWES REARED IN FEDERAL DISTRICT, BRAZIL

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ABSTRACT

The aim of this study was to compare two protocols of estrus synchronization in Santa Inês ewes. Thirty-eight ewes were randomly divided into two groups of estrus synchronization: protocol PGF2 α (two doses of 0.530 mg of PGF2 α , nine days apart) and protocol MAP+eCG (intravaginal sponge impregnated with medroxyprogesterone acetate, for 12 days, and then an injection of 250 IU of eCG). The experiment was in a cross-over design, two estrous cycles apart. On the final day of protocol, a transrectal ultrasound examination was carried out to measure the size of the largest and second largest ovarian follicles and on day 7 of estrous cycle blood was collected to measure serum P4 concentration.

Laparoscopy was carried out on day 11 after the end of protocols to count corpora lutea. Synchronization rate, size of largest and second largest ovarian follicles, interval between the end of the protocol to estrus and ovulation rate did not differ between protocols. Ewes synchronized with MAP+eCG had greater serum P4 concentrations than ewes synchronized with PGF2 α (3.9 and 2.8 ng/mL, respectively, $P < 0.05$). Based on the results, it may be concluded that, although the protocol MAP+eCG was superior in inducing higher serum concentration of P4, the protocol PGF2 α was equivalent regarding estrus synchronization.

KEYWORDS: Follicle size, serum progesterone, sheep, synchronization rate.

RESUMO

SINCRONIZAÇÃO DE ESTRO COM PROSTAGLANDINA F2 α versus PROGESTÁGENO ASSOCIADO À GONADOTROFINA CORIÔNICA EQUINA (eCG) EM OVELHAS SANTA INÊS NO DISTRITO FEDERAL, BRASIL

Este estudo teve por objetivo comparar o uso de um análogo da PGF2 α à associação de progestágeno (MAP) com gonadotrofina coriônica equina (eCG) na sincronização do estro em ovelhas da raça Santa Inês. Foram utilizadas 38 fêmeas ovinas submetidas a dois protocolos de sincronização de estro, com os seguintes protocolos: PGF2 α (duas doses de 0,53 mg de D-cloprostenol com nove dias de intervalo) e protocolo MAP+eCG (pessário intravaginal com 50 mg de acetato de medroxiprogesterona por

doze dias e no momento da remoção do dispositivo aplicação de 250 UI de eCG, IM). Submeteram-se as ovelhas aos diferentes protocolos, com intervalo de dois ciclos estrais. Procedeu-se a uma ultrassonografia transretal no último dia do protocolo, para avaliação do diâmetro do maior e do segundo maior folículo, e à coleta de sangue no dia sete do ciclo estral, para avaliação da concentração sérica de P4. Exame laparoscópico foi realizado no dia 11, após o fim dos protocolos, para contagem de corpos lúteos.

Para os parâmetros taxa de sincronização, diâmetro do maior e do segundo maior folículo, período do final do protocolo ao estro e taxa de ovulação, não se observaram diferenças entre os mesmos. Foi observado que o protocolo MAP+eCG produziu concentrações séricas de P4 maiores do que o protocolo PGF2 α (3,9 e 2,8 ng/

mL, respectivamente, $P < 0,05$). Nas condições do presente estudo, embora o protocolo MAP+eCG tenha apresentado superioridade em relação à concentração sérica de P4, o protocolo PGF2 α foi tão eficiente quanto aquele em sincronizar o estro.

PALAVRAS-CHAVES: Diâmetro folicular, ovino, progesterona sérica, taxa de sincronização.

INTRODUCTION

Sheep industry is a growing activity, pointing to an increase in the number of animals in the Midwest region of Brazil (8,325 in 2000 to 19,000 in 2006), according to data from IBGE (2006). In livestock activity, this species is used for breeding, production and consumption, mainly because the meat of young animals has been offered and crossbreeding provides tastier meat.

Among the breeds found in Brazil, Santa Ines, a woolless, large breed from the Brazilian Northeast, which is easily adapted to any breeding system and pasture, is worth noting. It also presents a good carcass conformation and stands out as being very fertile, prolific, precocious, rustic and resistant to gastrointestinal parasites. This breed has been gaining importance in modern sheep industry, being used both pure and in industrial crossbreeding.

The adaptability of Santa Ines sheep is an important feature in a country like Brazil. Its continental size and its regions, regarding the different characteristics and different climates, can cause changes to the animal's cyclicality. Besides, the reproductive seasonality suffers many influences by photoperiod, nutrition, age, breed and others. It is noteworthy that the choice of breeds suited to local environmental conditions is one of the most important factors for the success of an economically viable farming system. Therefore, it becomes important to study Santa Ines breed in the Federal District region, with the purpose of obtaining a better understanding of these animal's performance in a tropical climate region, with rainy and humid summer and dry and cold winter, during which the grazing is drastically reduced.

Estrus synchronization in sheep is a reproductive biotechnique that allows the concentration of insemination and calving at specific moments of the production systems (EVANS & MAXWELL, 1987).

This technique has been used in small ruminants, through the use of intravaginal sponges impregnated with progestagens, such as fluorogestone acetate (FGA) and medroxyprogesterone acetate (MPA), prostaglandin F2 α (PGF2 α), equine chorionic gonadotropin (eCG), intravaginal progesterone (P4; CIDR), in association or not (DIAS et al., 2001).

The most widely used protocol is the combination of P4 with eCG, which can be used both in the breeding season and beyond, to stimulate ovulation (EVANS & ROBINSON 1980; EVANS & MAXWELL, 1987; RUBIANO ET AL., 1998). Developed protocols recommend maintaining the P4 vaginal device for periods of twelve to fourteen days. These protocols result in high rates of estrus induction in treated animals, but with low fertility. It is due to the time the device remains, which generates an excessive period of follicle growth and hence the aging of the oocyte (VIÑOLAS et al., 2001). Moreover, excessive use of eCG hormones can trigger the production of anti-eCG, which leads to decreased efficiency of the hormonal treatment (BARIL et al., 1996).

As an alternative to estrus synchronization, a protocol based on PGF2 α can be used, which leads to premature regression of the corpus luteum through the interruption of progesterone phase of the estrous cycle; thus, beginning a new cycle (HERRERA et al. 1990). The protocol, which uses two doses of PGF2 α , is an alternative to the producer, to implement estrus synchronization in the property, as a way to improve reproductive efficiency and reduce cost with other managements such as toilets, to concentrate lots with the same requirements in the same period, and to reduce labor costs.

The aim of the present study was to compare the estrus synchronization protocol based on PGF2 α to the MAP protocol associated with eCG.

MATERIAL AND METHODS

The study was conducted at Fazenda Água Limpa, a farm owned by Universidade de Brasília (UNB), in Brasília (DF), located at 15 ° 46 ' South latitude and 47 ° 55' West longitude at an altitude of 1,171 m, with tropical climate, in the period from July to October 2007.

A total of 38 non-pregnant and non-lactating Santa Ines ewes were used. The animals were submitted to examination for general clinical condition, sanitary and reproductive health, with a standard body condition score between 3 and 4 on a scale of 1 to 5 (WHITE & RUSSEL, 1984). The sheep were initially evaluated for estrous cyclicity and estrus onset using a vasectomized male (teaser). The animals were kept in intensive system, receiving water and mineral salt ad libitum and corn silage. Before the beginning of the experiment, the animals were kept together for 45 days, aiming at providing conditions of environmental adaptability and the same nutritional level.

In a first phase, the animals were randomly divided into two groups and submitted to two synchronization protocols (the first day of the protocol was called D0). In PGF2 α protocol, two doses of PGF2 α IM (0.530 mg D-cloprostenol, Ciosin, Schering-Plough, São Paulo) were applied with an interval of nine days (D0 to D9), and in MAP + eCG protocol, vaginal pessaries (BioRep, RS, Brazil, RS; D0) containing 50 mg of MAP were used and inserted into the cranial portion of the vagina via an ewe-specific applicator. The pessaries remained there for twelve days. At the moment of pessaries withdrawal (D12), ewes received 250 IU of eCG IM (Novormon, Schering-Plough, São Paulo). At the end of the protocol, ewes were kept in the presence of a vasectomized male, previously tested, painted with a mixture of oil and powder paint all over its chest for marking the females in estrus. The vasectomized male remained with 38 females until the last manifestation of estrus. The ewes were observed twice a day (7:00 a.m. and 07:00 p.m.) for a period of sixty minutes in each observation, and they were considered in estrus when the ruffians rode the females which remained still and / or when there was ink on the female's rump region.

On D9 of PGF2 α protocol and D12 of MAP + eCG protocol, the ovaries were evaluated by trans-rectal ultrasonography with the aid of the laparoscopy technique. The ewes were prepared for laparoscopy, and they were previously kept fasting for 24 hours and without water for twelve hours. Later, they were placed in a surgical bed in a dorsal recumbency, so that the head was kept down and tilted at an angle of 45 degrees. Trichotomy and abdominal antisepsis were carried out, and 5.0 mL of 2.0% lidocaine were injected where the trocars were inserted. The abdominal cavity was distended with carbon dioxide, and the uterus was visualized with a laparoscope (7.5 mm and 30° Storz), and the ovaries were seized with atraumatic clamps. At the same time, carboximetilcelulose was injected with a 20 mL syringe in the rectum, introducing a bi-frequency linear transducer (6.0 and 8.0 MHz) of the Falco 100 ultrasonic device (Pie Medical, Nutricell, São Paulo). The transducer was connected to a PVC pipe to facilitate its handling in the animals' rectum. The images were obtained at a frequency of 8.0 MHz. When visualized, the ovarian images were frozen, to measure the two largest follicles in the ovaries.

Soon after the procedure, carbon dioxide was withdrawn by abdominal pressure, as well as the transducer and the trocars. Oxytetracycline in aerosol and healing ointment were applied on puncture sites. The sheep were removed from the surgical bed and returned to a paddock supplied with food and water.

Eleven days after the end of the protocol (D20, PGF2 α protocol, and D23, MAP + eCG protocol), the animals underwent the laparoscopic technique, as described above, for corpora lutea evaluation and counting.

Blood samples were collect from all animals for serological determination of the P4 concentration, seven days after the observation of estrus. The collection was carried out by puncturing the jugular vein, in vacuum tubes, which were stored at 5 °C until centrifugation on the day after collection. Soon after, the serum was collected and stored in 2.0 mL conical tubes at -20 °C until the analysis. Serum concentrations of P4 were determined by radioimmune

assay (RIA) by using a commercial kit (Coat-a-count, DPC, Diagnostic Products Co, Los Angeles, CA, USA) and following the manufacturer's recommendations. The intra-assay coefficient of variation was 4.6%, obtained in tests performed in the laboratory of Animal Reproduction, Embrapa Genetic Resources and Biotechnology.

For the second stage, after a period of two estrous cycles, animals were resynchronized in order to alternate treatments.

Tested variables were analyzed for normality and homoscedasticity by Lilliefors and Cochran tests, respectively. The analysis of variables with normal distribution and homoscedasticity (P4 serum concentration) were performed by paired t test. The variables, which did not show normal distribution were analyzed by nonparametric Wilcoxon test. The binomial variables were compared by chi-square test. For the discussion, a significance level of $P \leq 0.05$ was used.

RESULTS AND DISCUSSION

All sheep used in the experiment presented estrus after the use of both synchronization protocols (Table 1). In the study by MENCHACA et al. (2004), estrus behavior was observed within 72 hours after the second dose of PGF2 α in 93.9% and 82.4% of multiparous and nulliparous females, respectively. GODFREY et al. (1997) observed that within three days, 71.4% of females came into estrus after the second application of PGF2 α . RODRIGUES et al. (2004), studying different doses of eCG after the vaginal pessary removal from woolless sheep, observed 100% of estrus in ewes with a dose of 200 IU of eCG. A similar result was reported by AINSWORTH & WOLYNETZ (1982) who verified 96% of estrus in ewes treated with MAP + eCG. Dias et al. (2001) obtained 76.7% of ewes in estrus after the application of 200 IU of eCG. This higher rate of estrus detection in the present study compared to some of the rates

mentioned previously may be due to the introduction of a male at the same day of the end of protocols, in addition to the animals' excellent body condition. According to YILDIZ et al. (2003), the nutritional status of sheep also influences LH secretion. The body condition score measurements reflect the degree and energy storage of the animal and they are related to the reproductive efficiency, rate of brood mortality, and the best results in estrus synchronization programs.

There was no difference ($P > 0.05$) regarding the time period from the end of the protocols to estrus between PGF2 α and MAP+eCG treatments (Table 1). In spite of that, GREYLING & BRINK (1987) claimed that the use of eCG associated with progestogen reduces the interval between implant removal and estrus. Moreover, EVANS & ROBINSON (1980) observed a negative correlation between the dose of eCG used and the time for estrus manifestation after the treatment, with a decrease of 14 hours when using higher doses (0-1600 IU eCG). These same authors, using 200 IU, observed estrus in sheep in counter-season, 48 hours after the end of treatment. In studies by GODFREY et al. (1997) there is a contradiction regarding hours until the onset of estrus after the second PGF2 α dose. In a first study, the authors observed 69.6 hours (GODFREY et al., 1997), similar result to those obtained in this study, while a second paper reported 31.6 hours (GODFREY et al., 1999), using the same protocol. This contrast may be due to the different stage of follicular development of the sheep at the time of treatment (VIÑOLAS & RUBIANO, 1998).

MENCHACA et al. (2004) observed a good synchronization rate, with 80% of sheep with estrus expression between 25 and 48 hours of treatment, with the applications of two doses of PGF2 α analogs within a seven-day interval. Such estrus manifestation occurred earlier than could be observed in this study, within 37 and 72 hours (Figure 1 and Table 2). This difference between experiment results may be due, at least partially, to the twelve-hour interval between the observations of estrus in this study and the non-constant observation.

TABLE 1. Estrus manifestation, the period between the end of the protocol and estrus, diameter of the largest follicle and of the second largest follicle, sheep with CL at the end of the protocol, average follicles ovulated and P4 serum concentration on the seventh day of the estrous cycle in PGF2 α synchronization protocols (two injections of PGF2 α nine days apart, n = 38) and MAP + eCG (intravaginal MAP maintained for twelve days and 250 IU of eCG were applied at the time of implant removal, n = 38) in Santa Ines ewes (percentage or mean \pm SE)

| | PGF2 α | MAP+eCG |
|--|----------------------------|----------------------------|
| Ewes in estrus; % (n/n) | 100,0 (38/38) | 100,0 (38/38) |
| Time between the end of the protocol and estrus; h | 66,0 \pm 2,5 | 67,3 \pm 2,8 |
| Largest follicle diameter; mm | 3,5 \pm 0,2 | 3,1 \pm 0,2 |
| Second largest follicle diameter; mm | 2,4 \pm 0,2 | 2,2 \pm 0,2 |
| Ewes with CL at the end of protocol, % | 79,0 ^a | 7,9 ^b |
| Mean of ovulated follicles; n | 1,5 \pm 0,1 | 1,7 \pm 0,2 |
| P4 serum concentration, n | 2,8 \pm 0,1 ^a | 3,9 \pm 0,1 ^b |

^{a,bb}Difference among treatments (P < 0,05).

The results presented in Table 2 also show a trend of a greater number of females that accept rides

TABLE 2. Incidence of ewes marked by the teaser on treatment with PGF2 α (two injections of PGF2 α nine days apart, n = 38) or MAP + eCG (intravaginal MAP implant maintained for twelve days and 250 IU of eCG applied at the time of implant removal, n = 38), in the intervals of estrus observation every twelve hours from the end of the protocols (percentage)

| | Intervals of estrus observation (h) | | | | | | |
|-------------------------|-------------------------------------|-------------|-----------------|----------------|-----------------|-----------------|---------------|
| | 0-24 | 25-36 | 37-48 | 49-60 | 61-72 | 73-84 | 85-96 |
| PGF2 α ; % (n/n) | 0 (0/38) | 0 (0/38) | 34,2 (13/38) | 10,5 (4/38) | 31,6 (12/38) | 18,4 (7/38) | 5,3 (2/38) |
| P4+eCG; % (n/n) | 2,6 (1/38) | 0 (0/38) | 31,6 (12/38) | 2,6 (1/38) | 31,6 (12/38) | 26,3 (10/38) | 5,3 (2/38) |

The average diameter of the largest follicle and of the second largest follicle observed at ultrasonographic exam after the treatment did not differ among protocols (P > 0.05) (Table 1). CÁRDENAS et al. (2004) observed pre-ovulatory follicles of 7.6 mm after PGF2 α and GnRH administration. LOPEZ-SEBASTIAN et al. (1997), evaluating the follicular dynamics in Merino sheep, observed the sizes of the two largest follicles in the ovaries. The

at night (within 37-48 hours and 61-72 hours). There are few reports in the literature on this kind of observations, ie, the possible effect of circadian rhythm on estrous behavior.

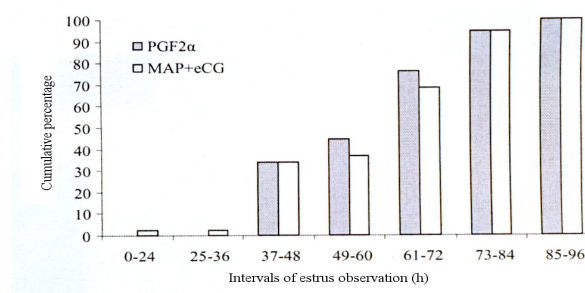


FIGURE 1. Accumulated data of estrus manifestation in twelve-hour observation intervals from the end of PGF2 α protocols (two injections of PGF2 α nine days apart, n = 38) and MAP + eCG protocol (P4 intravaginal implant maintained for twelve days and 250 IU of eCG applied at the time of implant removal, n = 38), showing the grouping of estrus manifestation at each interval until the end of the observation (percentage).

size of the second largest follicle remained constant over the last seven days of the estrous cycle (5.1 to 5.6 mm) but it increased to 6.6 mm on day 0 (day of estrus manifestation). The second largest follicle remained with 3.9 mm. URIBE-VELASQUEZ et al. (2002) observed a maximum follicle diameter of 4.3 mm on the ovulation day, for ewes treated with two doses of PGF2 α within nine days and of 5.4 for ewes treated with P4+eCG. They also observed that the

growth of the dominant follicle in animals treated with P4+eCG was higher than in the ones treated with PGF2 α , attributing this result to the effect of the association between eCG and P4. In this study, there was no difference between protocols, probably because measurements were carried out at the end of protocols, without enough time for eCG activity. Different follicular diameters mentioned in the studies above could have been caused by the fact that the follicles have been measured at least two days before the ewes present estrus. In other studies, they were measured on the ovulation day.

By means of the ultrasonographic exam performed on the last day of the hormonal treatment protocols, the presence of corpora lutea in at least one ovary was observed in 79.0% and 7.9% of the ewes in PGF2 α group and in P4 + eCG group, respectively. This result was expected because, in the evaluation of the MAP+eCG protocol dynamics, P4 remains long enough to luteolysis to happen (twelve days). On the other hand, PGF2 α protocol is based on the presence of a mature corpus luteum at the moment of the medicine second application, to guarantee the efficiency of the synchronization program.

Regardless of the treatment used in this study, a high rate of ovulated follicles was obtained, considering that 100% of ewes submitted to treatments with PGF2 α or MAP + eCG ovulated at least one follicle. In the analysis of the average of follicles ovulated, there was no difference between PGF2 α and MAP + eCG protocols (Table 1). Similar results were reported by RODRIGUES et al. (2004) in woolless sheep whose ovulation was 1.4 ± 0.6 with an eCG dose of 200 IU. These authors stated that woolless sheep from tropical climate need lower doses for estrus synchronization, whereas DIAS et al. (2001) obtained 73.3% of ovulating ewes using the same eCG dose of 200 IU. In this study, there was no difference between the average of follicles ovulated in the right (0.79) and left ovary (0.78) in both protocols.

For P4 serum concentration on day seven of the estrous cycle, MAP + eCG protocol showed

superiority in relation to PGF2 α (Table 1). This result was similar to the one presented by URIBE-VELASQUEZ et al. (2002) in Bergamacia sheep treated with PGF2 α (approximately 4.0 ng / mL) and eCG + P4 (approximately 6.0 ng / mL). A similar result was also presented by EVANS & ROBINSON (1980), who obtained P4 concentrations of 3.3 ± 0.2 ng / mL on day six of the estrous cycle after the P4 implant removal and eCG administration.

EVANS & ROBINSON (1980) demonstrated that eCG can overstimulate ovarian follicles, resulting in corpora lutea with a larger diameter and hence increasing the P4 circulating concentrations. Moreover, eCG has prolonged half-life (MAPLETOFT et al., 2002), providing a stimulating effect on the newly-formed corpus luteum. High P4 circulating concentrations on the first days of the luteal phase in sheep is important for the expression of several growth factors, mainly the ones similar to insulin (IGF), responsible for fetal implantation and growth (OSGERBY et al., 1999), which is fundamental for embryonic development. However, based on the results found in this study, we cannot state that this increase of P4 circulating induced by the MAP+eCG protocol is correlated to the higher rates of conception, as the experiment was not designed to test this hypothesis.

Because both protocols were effective in synchronizing estrus in ewes in the present study, there is an attractive prospect of using a larger scale of PGF2 α protocol, which is less expensive (between 30% and 60% of the cost of MAP+eCG protocol). Although some studies show that PGF2 α protocol may affect sperm transport and interfere in the conception rate (HAWK, 1973), there are others that show similar and satisfactory results in comparison with P4 versus PGF2 α protocols. GODFREY et al. (1997), for example, found no difference (100% and 86%, respectively) in pregnancy rates between treatments.

This study was conducted in the reproductive counter-season (July-October), during months without pasture for livestock feed. So they were kept in confinement, which may have contributed

to the non-interference of photoperiod on the results. Otherwise, they would not have responded to the PGF2 α protocol. In addition, GODFREY et al. (1997) showed that the estrus synchronized with PGF2 α or P4 can be successfully obtained in sheep in the tropics in different seasons. The photoperiod in that place (Virgin Islands) floats two hours in the year and it does not seem to be enough to influence the estrous cyclicity in sheep. Nevertheless, the study by MARTINS et al. (2003) showed that sperm quality and consequently the diagnosis of fertility were affected by seasonal variation in Santa Ines sheep in the region of the Distrito Federal.

Despite its efficacy, there is a negative factor regarding MAP+eCG protocol: many countries no longer allow the use of hormonal treatments in zootechnical-interest animals. For example, in the United States, "U.S. Food and Drug Administration" does not approve the use of the mentioned protocol, which drastically limited the use of such technology (CLINE et al., 2001). Moreover, a long treatment with progestagen is related to a lower follicular renewal, promoting ovulation of persistent dominant follicles and causing a lower pregnancy rate (VIÑOLAS et al., 2001). The repeated use of eCG treatment to synchronize estrus can also reduce fertility due to the growth of anti-eCG (BARIL et al., 1996).

CONCLUSION

Under the conditions of this study, although MAP + eCG protocol presented superior results, because it increased P4 serum concentrations, PGF2 α protocol was as efficient in synchronizing estrus as the former. Besides having a lower cost, it does not cause problems related to the overuse of MAP and eCG. Thus, it is noteworthy the use of PGF2 α as an alternative protocol.

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