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Reproductive performance of ewes treated with an estrus induction/synchronization protocol during the spring season

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Abstract

The aim of this study was to evaluate the reproductive performance of ewes treated with eCG and an exogenous progestagen protocol during the spring season. Forty-eight mixed-breed wool and hair ewes (body condition score of 2.8 ± 0.5 and 41 ± 3 kg) were randomly assigned into two groups (n = 24/group), which received (G-Sync) or not (G-Control) an intravaginal device (Day 0) containing 60 mg of medroxyprogesterone acetate. On Day 7, ewes of the G-Sync were injected with 300 IU of eCG and 30 ug of d-Cloprostenol, im. On Day 9, the device was removed and 12 h later males were introduced into the G-Sync and G-Control groups in a proportion of 1:6. Estrus response observation and mating were performed during Days 10, 11 and 12 from 7 to 9 AM and 4 to 6 PM. After Day 12, males were separated from females for 10 days and later reintroduced into the flock for 45 days. Estrus rates for the G-Control and G-Sync groups during Days 10, 11 and 12 were 4 and 88% ($P < 0.05$), respectively. Pregnancy rates from initial mating on Days 10, 11 and 12 were 0 (G-Control) and 46% (G-Sync; $P < 0.05$). Total pregnancy rates for the whole mating season were 50 (G-Control) and 79% (G-Sync; $P < 0.05$). The exogenous progestagen protocol plus eCG used for estrus induction/synchronization improved the pregnancy rate of mixed-breed wool and hair ewes by about 29% points at the end of the breeding season. Thus, this procedure seems to be appropriate to be implemented as part of the reproductive management of some ovine farms during the non-breeding season.

Keywords: estrus induction/synchronization, ewes, pregnancy, spring season.

Introduction

Mixed-breed ovines have been molded by years of human and natural selection. They have been selected to fit a large range of environmental conditions and human needs. Mixed-breed ewes often possess gene combinations for special adaptations, such as disease resistance, adaptation to harsh conditions or poor-quality food, not found in other breeds. In the State of Parana, Brazil, hardy mixed-breed ovines have been selected for many years and they correspond to approximately 50% of the regional flock. They evolved from numerous different wool breeds such as Suffolk, Ile de France, Texel, Corriedale and Romney Marsh, and hair breeds such as Santa Ines and Morada Nova. Despite their importance for the ovine meat market, there are no data available in the literature about their productive characteristics or about their reproductive activity.

Ewes exhibit seasonal reproductive activity, returning to cyclicity after the summer solstice due to an increase in melatonin secretion by the pineal gland, which is higher during periods of decreasing luminosity (Boland *et al*., 1990; Dogan and Nur, 2006). In the longer days of spring, there is a break in the reproductive period, whereas the shorter days of autumn are associated with the onset of estrus (Dogan and Nur, 2006). Thus, reproductive seasonality is an important factor that limits the productivity of small ruminants (Zarazaga *et al*., 2003).

There are several ways to control the estrous cycle in ewes, such as light manipulation, the ram effect and hormone treatments with progesterone, prostaglandin (PGF), equine chorionic gonadotropin (eCG) and gonadotropin-releasing hormone (GnRH; Boland *et al*., 1990, Keisler and Buckrell, 1997; Wildeus, 2000; Iida *et al*., 2004). Among these hormone treatments, the synchrony of estrus has been highlighted as a tool to improve the reproductive efficiency of herds and flocks (Mazzoni-Gonzalez and Oliveira, 1991; Ozyurtlu *et al*., 2008).

The use of slow-releasing progesterone/ progestagen devices is effective for estrus induction/ synchronization in small ruminants. Intravaginal sponges impregnated with progestagens, such as fluorogestone acetate (FGA) and medroxyprogesterone acetate (MAP), are examples of progesterone/ progestagen devices (Kusakari *et al*., 1995; Mufti *et al*., 1997; Godfrey *et al*., 1999; Ungerfeld and Rubianes, 2002, Kohno *et al*., 2005; Dogan and Nur, 2006). Advantages of this technique include estrus concentration, reduction of days of labor, induction of cyclicity in anestrus females, shortening of the lambing period, appropriate use of males, high pregnancy rates at

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the beginning of the breeding season and the production of homogeneous lots of lambs, which improves the marketing of lamb products. The mentioned advantages lead to an increase in ovine farms reproductive efficiency (Henderson *et al*., 1984).

The use of progesterone releasing devices associated with eCG or follicle stimulating hormone (FSH) in estrus induction/synchronization programs have shown significant effects on estrus response because gonadotropins stimulate ovarian follicular growth of cyclic or acyclic females (Mies Filho *et al*., 1989; Cline *et al*., 2001; Maurel *et al*., 2003). Estrus response and ovulation start earlier and a synchronized ovulation is induced when progestagen is associated with eCG (Cardwell *et al*., 1998). The eCG provides an increase in the diameter of the dominant follicle by acting on the hypothalamic-pituitary-ovarian axis and altering intra-ovarian regulatory mechanisms, besides increasing the maximum diameter and the growth rate of large follicles (Uribe-Velásquez *et al*., 2002). Hormonal treatments during the autumn season provide a good level of synchrony of estrus, resulting in average pregnancy rates of 60% in the first estrus after device withdrawal. Thus, 90% of cyclic ewes can become pregnant in two natural services that can be performed over a period of 21 days (Moraes *et al*., 2002). Reproductive efficiencies of the progestagen treatment at various times during the spring season, however, are still variable (Robinson, 1990; Gordon, 1997; Knights *et al*., 2001; Santos, 2007; Ozyurtlu *et al*., 2008).

The aim of this work was to evaluate the reproductive performance of mixed-breed ewes treated with eCG and an exogenous progestagen protocol used for estrus induction/synchronization during the spring season.

Materials and Methods

Location and nutrition

The experiment was carried out on a farm located in Parana State, in the south of Brazil, 23°18' S, 51°09' W. This location features a subtropical climate, with most rainfall occurring during the summer months. The mating period was chosen to be from September to December (spring season), when average temperatures were 28.7 ± 1.1ºC (range 27.3 to 30ºC). The average daily sunshine for the location during the experimental period was 11:57 h in September, 12:38 h in October, 13:15 h in November and 13:34 h in December. Animals were kept on an 11.4-hectare pasture of *Cynodon plectostachyrus Pilger*, *Brachiaria decumbens* and *Paspalum notatum*. In the morning, the ewes were fed 400 g/day of soybean hulls.

Animals and treatments

Non-pregnant, multiparous, mixed-breed wool and hair ewes (n = 48) were used in the present work. The average body condition score was 2.8 ± 0.5 on a scale of 1 to 5 (Caldeira and Vaz-Portugal, 1998), the average alive body weight was 41 ± 3 kg and the average age was 2 ± 1 years.

The animals were randomly assigned into two experimental groups: G-Sync (n = 24), with hormonal treatment and G-control (n = 24), without hormonal treatment. Therefore, during their estrous cycles, ewes of the G-Sync group received an intravaginal device containing 60 mg of medroxyprogesterone acetate (MAP; Progespon®, Syntex, Argentina; Day = 0). Seven days later (Day 7), the animals were injected with 300 IU of eCG (Novormon®, Syntex, Argentina) and 30 ug of d-Cloprostenol (Prolise®, Arsa SRL, Argentina), im. On Day 9, progesterone devices were removed. For both treated and control groups, 12 hours after device removal, males (n = 8) with proven fertility were introduced into the flock in a proportion of 1:6. The ewes from both groups were exposed to rams in a single group at the same time. Estrus observation and mating were performed during Days 10, 11 and 12 from 7 to 9 AM. and 4 to 6 PM. After Day 12, males were separated from females for 10 days and later reintroduced into the flock for 45 days. To estimate estrus rates, the number of ewes that showed estrus during Days 10, 11 and 12 was considered. Pregnancy diagnosis was performed twice by transrectal ultrasound (Aloka SSD 500, 5 MHz linear transducer), 30 and 85 days after Day 12 (Fig. 1 and 2).

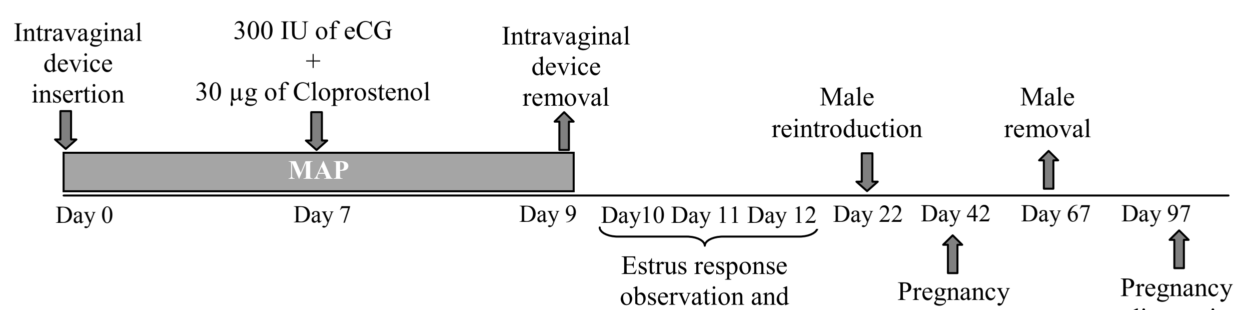

Figure 1. Schematic presentation of eCG treatment and exogenous progestagen protocol for estrus induction/synchronization in mixed wool and hair breed ewes (G-Sync) during the spring season.

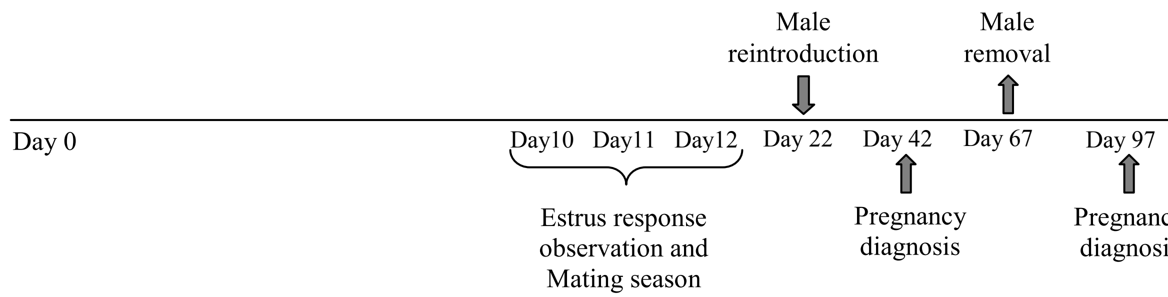

Figure 2. Schematic presentation for mating season in mixed breed wool and hair ewes (G-Control) during the spring season.

Variables

For both groups, parameters analyzed were: rate of onset of estrus, pregnancy rate for mating period on Days 10, 11 and 12, total pregnancy rate for the whole mating season, and prolificacy (number of lambs born per lambing ewe).

Experimental design and statistical analysis

A randomized design was used with 48 animals and two treatment groups. Each animal was considered a single experimental unit. Reproductive performance was analyzed using the chi-square test. The Bioestat 5.0 software was used (Ayres *et al*., 2007). For all analyses, $P < 0.05$ was considered significant.

Results

During the estrus observation period (Days 10, 11 and 12 after intravaginal device insertion), rate of estrus in the G-Sync ewes was 88%. In the G-Control group, only 4% of the ewes showed estrus behavior (Table 1). Pregnancy rates for mating period during Days 10, 11, and 12, total pregnancy rates at the conclusion of the breeding period and prolificacy for G-Control and G-Sync groups are shown (Table 1).

Table 1. Pregnancy rates (%) from mating on Days 10, 11 and 12, total pregnancy rates for the whole mating season and prolificacy for non-synchronized mixed breed wool and hair ewes (G-Control) and ewes treated with estrus induction/synchronization (G-Sync) during the non-breeding season.

	G-Control	G-Sync	P-Value
Number of ewes	24	24	-
Estrus presentation (%)	4 (1)[a]	88 (21)[b]	<0.001
Pregnancy rate from mating (%)	0 (0)[a]	46 (11)[b]	<0.001
Total pregnancy rate (%)	50 (12)[a]	79 (19)[b]	0.04
Prolificacy	1.0 (12)	1.2 (22)	0.1

Day 0 = Intravaginal device insertion. Values with different superscript differ $P < 0.05$between treatments.

By the end of the mating period, the exogenous progestagen protocol used for estrus induction/ synchronization improved the pregnancy rate of mixed-breed wool and hair ewes by about 29% points ($P < 0.05$). Although there was no statistical difference, it is important to report that only ewes in the G-Sync group had twin births (12%).

Discussion

Reproductive seasonality is a limiting factor for small ruminant productivity (Zarazaga *et al*., 2003). However, little information is available about its influence on the reproductive performance of mixed-breed ewes. Several reproductive management strategies can be used to increase the number of lambs produced throughout the year. The estrus induction/ synchronization program has been highlighted as a helpful biotechnology to be used during the breeding and non-breeding seasons to increase the productivity of ovine flocks in scale and frequency, therefore fulfilling consumers demand for sheep meat (Keisler and Buckrell, 1997; Knights *et al*. 2001; Iida *et al*., 2004; Martin *et al*., 2004; Kohno *et al*. 2005).

The use of some drugs, such as progestagen containing devices, eCG, prostaglandin and others, have shown promising results in improving the reproductive performance of ewes in both the breeding and non-breeding seasons (Godfrey *et al*., 1999; Wildeus, 2000; Husein and Kridli, 2003, Iida *et al*., 2004, Kohno *et al*., 2005; Ozyurtlu *et al*., 2008).

In the present study, mixed-breed wool and hair ewes treated with an exogenous progestagen protocol showed an 88% rate of estrus synchrony, aligning with previous studies at different latitudes (range 30^o S to 43^o N) and with different breeds in which 73 to 90% of ewes were observed in estrus after hormonal treatment during the non-breeding season (Crosby *et al*., 1991; Kusakari *et al*., 1995; Viñoles *et al*., 2001; Dogan and Nur, 2006). In another study at 37°55'01' N latitude and also in accordance with current results, Awassi ewes were treated with intravaginal sponges and Controlled Intravaginal Drug Release (CIDR) devices during the non-breeding season. In that study, estrus response for group treated with CIDR devices (n = 20; 90%) did not differ from group treated with intravaginal progesterone sponges (n = 24; 87%), but it was greater than for the control group (n = 18; 17%; Ozyurtlu *et al*., 2008).

In the present work, pregnancy rate for the estrus induced/synchronized group (G-Sync) was 46% for mating period on Days 10, 11 and 12 after intravaginal device insertion, which is similar to that reported by Simonetti *et al*. (2002). These authors carried out an estrus synchronization protocol in Merino ewes using sponges impregnated with 60 mg MAP for 14 days during the non-breeding season and observed that 59 of the 117 ewes (50%) became pregnant. In accordance with the current results, Dogan and Nur (2006), evaluating the effect of different hormonal protocols with MAP, eCG and PGF2α in Kivircik breed at 40°13' N during the non-breeding season, reported that pregnancy rates were between 41 and 76%. Similarly, Ozyurtlu *et al*. (2008), studying the effect of estrus induction/synchronization during the non-breeding season, also observed an increase in pregnancy rate in ewes treated with sponges impregnated with progesterone compared to ewes of the control group (71 and 51%, respectively). Differences in estrus presentation and pregnancy rates among hormonal protocols are probably due to the use of different progesterone and progestagen devices, breed, animal nutritional conditions, latitude and the time of year in which hormonal treatment was implemented (Ozyurtlu *et al*., 2008).

In the current study, control ewes did not show estrus and consequently did not become pregnant in the mating period on Days 10, 11 and 12, suggesting that these animals presented a low rate of cyclicity during this period. In contrast, as stated before, the hormonal treatment (G-Sync) promoted induction/synchronization of estrus in females at the beginning of the spring season, resulting in an approximately 50% pregnancy rate during the first 3 days of the mating period. The concentration of estrus response and mating, and consequently of lambing, may be a great advantage conferred by hormonal treatments because it could allow the production of homogeneous lots of lambs, which could eventually support the demand of the sheep meat market.

Three hypotheses may explain the significant increase in pregnancy rate of mixed-breed ewes in the control group after male reintroduction. First, it can be considered that these animals were not very influenced by the photoperiod, which may be explained by their genetic background (crossbred wool and hair ewes). Other alternatives include the response to sexual stimuli induced by male introduction (ram effect) and the influence of being close to females treated for estrus synchronization. Izard and Vandenberghe (1982) reported positive influences of pheromones from estrus cows on the cyclicity response in females who did not undergo estrus synchronization. It is possible that the interaction between these three effects stimulated the cyclicity response in ewes in the control group.

In the present study, mixed-breed wool and hair ewes treated with exogenous progestagen-eCG protocol presented an improvement of approximately 29% points in the pregnancy rate at the end of the mating season compared to the controls (79 *vs*. 50%). These results align with those from Santos (2007) at 40º N latitude, who observed a 80% pregnancy rate in Merino ewes after hormonal treatment and male reintroduction during the spring season.

In the present study, hormonal treatment did not increase prolificacy in the G-Sync compared to G-Control group. Similarly, Ozyurtlu *et al*. (2008), evaluating the effect of estrus synchronization/induction during the non-breeding season, observed no differences in the prolificacy of ewes treated with hormone protocol (1.2) and ewes in the control group (1.0).

Although there were no statistical differences in the prolificacy over the course of the whole mating period, it is important to report that only ewes treated with the estrus induction/synchronization protocol had twin births, while those in the control group did not.

It is concluded that the implementation of the progestagen-eCG hormonal treatment in mixed-breed wool and hair ewes resulted in a considerable estrus induction/synchronization as measured by a 46% pregnancy rate in the first three days of the mating period. Moreover, the treatment increased pregnancy rate of mixed-breed wool and hair ewes by approximately 29% points over the controls at the end of the mating season. Thus, the eCG treatment and exogenous progestagen protocol used for estrus induction/synchronization seems to be a good procedure to be implemented as part of the reproductive management of some ovine farms during the spring season.

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References

Ayres M, Ayres Jr. M, Ayres DL, Santos AS. 2007. *Bio Estat 5.0*: statistical applications in biological and medical sciences [in Portuguese]. Brasília: Sociedade Civil Mamirauá, CNPq. 138 pp.

Boland MP, Crosby F, O'Callaghan D. 1990. Artificial control of the breeding season in ewes. *Irish Vet J*, 43:2-6.

Caldeira RM, Vaz-Portugal A. 1998. Body condition: concepts, methods of assessment and interest of its use as an indicator for sheep on the farm [in Portuguese]. *Rev Port Ciênc Vet*, 93:95-102.

Cardwell BE, Fitch GQ, Geisert RD. 1998. Ultrasonic evaluation for the time of ovulation in ewes treated with norgestomet and norgestomet followed by pregnant mare's serum gonadotropin. *J Anim Sci*, 76:2235-2238.

Cline MA, Ralston JN, Seals RC, Lewis GS. 2001. Intervals from norgestomet withdrawal and injection of equine chorionic gonadotropin or P.G. 600 to estrus and ovulation in ewes. *J Anim Sci*, 79:589-594.

Crosby TF, Boland MP, Gordon I. 1991. Effect of progestagen treatments on the incidence of oestrus and pregnancy rates in ewe. *Anim Reprod Sci*, 24:109-118.

Dogan I, Nur Z. 2006. Different estrous induction methods during the non-breeding season in Kivircik ewes. *Vet Med*, 51:133-138.

Godfrey RW, Collins JR, Hensley EL, Wheaton JE. 1999. Estrus synchronization and artificial insemination of hair sheep ewes in the tropics. *Theriogenology*, 51:985-997.

Gordon I. 1997. *Controlled Reproduction in Sheep and Goats*. Wallingford, UK: CAB International. 100 pp.

Henderson DC, Downing JM, Beck NFG. 1984. Oestrus synchronization in ewes: a comparison of prostaglandin F2 than salt with a progestagen pessary. *Anim Prod*, 39:229-233.

Husein MQ, Kridli RT. 2003. Effect of progesterone prior to GnRH-PGF2_{α} treatment on induction of estrus and pregnancy in anestrous Awassi ewes. *Reprod Domest Anim*, 38:228-232.

Iida K, Kobayashi N, Kohno H, Miyamoto A, Fukui Y. 2004. A comparative study of induction of estrus and ovulation by tree different intravaginal devices in ewes during the nonbreeding season. *J Reprod Dev*, 50:63-69.

Izard MK, Vandenbergh JG. 1982. Priming pheromones from oestrous cows increase synchronization of oestrus in dairy heifers after PGF-2 alpha injection. *J Reprod Fertil*, 66:189-196.

Keisler DH, Buckrell BC. 1997. Breeding strategies. In: Youngquist RS (Ed.). *Current Therapy in Large Animal Theriogenology*. Philadelphia, PA: WB Saunders. pp. 603-611.

Knights M, Hoehn T, Lewis PE, Inskeep EK. 2001. Effectiveness of intravaginal progesterone inserts and FSH for inducing synchronized estrus and increasing lambing rate in anestrous ewes. *J Anim Sci*, 79:1120-1131.

Kohno H, Okamoto C, Iida K, Takeda T, Kaneko E, Kawashima C, Miyamoto A, Fukui Y. 2005. Comparison of estrus induction and subsequent fertility with two different intravaginal devices in ewes during the nonbreeding season. *J Reprod Dev*, 51:805-812.

Kusakari N, Ohara M, Mori Y. 1995. Seasonal variation in the timing of estrus behavior, LH surge and ovulation following the treatment with progesterone and PMSG in Suffolk ewes. *J Reprod Dev*, 41:212-249.

Martin GB, Milton JT, Davidson RH, Banchero-Hunzicker GE, Lindsay DR, Blache D. 2004. Natural methods for increasing reproductive efficiency in small ruminants: review. *Anim Reprod Sci*, 82/83:231-245.

Maurel MC, Roy F, Herve V, Bertin J, Vaiman D, Cribiu E, Manfredi E, Bouvier F, Lantier I, Boue P, Guillou F. 2003. Immune response to equine Chorionic Gonadotropin used for the induction of ovulation in goats and ewes. *Gynecol Obstet Fertil*, 31:766-769.

Mazzoni-Gonzalez CI, Oliveira VS. 1991. Techniques used for increasing the reproductive efficiency of goats and sheep. In: Abstracts of the 27th Annual Meeting of Brazilian Animal Science Society, 1991, João Pessoa, PB, Brazil. Viçosa, MG: SBZ. pp. 71-102.

Mies Filho A, Endler JO, Moraes JCF. 1989. Estrus and ovulatory response of ewes treated by electric and/or hormonal stimulus. *Rev Bras Reprod Anim*, 13:229-238.

Moraes JCF, Souza CJH, Gonçalves PBD. 2002. Control of the estrous cycle and ovulation in cattle and sheep [in Portuguese]. In: Gonçalves PBD, Figueiredo JR, Freitas VJF (Ed.). *Biotécnicas Aplicadas à Reprodução Animal*. São Paulo, SP: Varela. pp. 25-55.

Mufti AM, Wani GM, Wani NA. 1997. Superovulatory response in Corriedale sheep during different months of the breeding season. *Small Rumin Res*, 25:181-184.

Ozyurtlu N, Kucukaslan I, Cetin Y. 2008. Characterization of oestrous induction response, oestrus duration, fecundity and fertility in awassi ewes during the non-breeding season utilizing both CIDR and intravaginal sponge treatments. *Reprod Domest Anim*, 45:464-467.

Robinson JJ. 1990. Nutrition in the reproduction of farm animals. *Nutr Res Rev*, 3:253-276.

Santos CSA. 2007. *Influence of the ram effect on the estrus synchronization of ewes* [in Portuguese]. Lisboa, Portugal: University of Lisboa. Thesis.

Simonetti L, Ramos G, Gardón JC. 2002. Effect of estrus synchronization and artificial insemination on reproductive performance of Merino sheep. *Braz J Vet Res Anim Sci*, 39:143-146.

Ungerfeld R, Rubianes E. 2002. Short term primings with different progestogen intravaginal devices (MAP, FGA and CIDR) for eCG-estrous induction in anestrus ewes. *Small Rumin Res*, 46:63-66.

Uribe-Velásquez LF, Oba E, Lara-Herrera LC, Souza MIL, Villa-Velásquez H, Trinca LA, Fernandes CAC. 2002. Endocrine and ovarian response associated with the first-wave follicle dominant in sheep synchronized either CIDR or PGF2alpha. *Rev Bras Zootec*, 31:944-953.

Viñoles C, Forberg M, Banchero G, Rubianes E.

2001 Effect of long-term and short-term progestagen treatment on follicular development and pregnancy rate in cyclic ewes. *Theriogenology*, 55:993-1004.

Wildeus S. 2000. Current concepts in synchronization of estrus: sheep and goats. *J Anim Sci,* 77:1-14.

Zarazaga LA, Malpaux B, Chemineau P. 2003. Amplitude of the plasma melatonin nycthemeral rhythms is not associated with the dates of onset and offset of the seasonal ovulatory activity in the Ile-de-France ewe. *Reprod Nutr Dev*, 43:167-177.