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Oestrus synchronization in postpartum autumn-lambing ewes: effect of postpartum time, parity, and early weaning

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Abstract

Three experiments were performed to determine the effect of postpartum time interval, the influence of parity (primimarous *vs* multiparous), and lamb weaning on the response of postpartum ewes to oestrus synchronization/induction treatments during the breeding season. In the Experiment 1 ewes were treated with an 8-days medroxyprogesterone priming and 350 IU of eCG, starting 14-18 (EPP), 24-30 (MPP), or 42-48 (LPP) days postpartum. More EPP than LPP ewes were marked by rams ($p < 0.05$), but conception rate was similar. In Experiment 2, 36 primiparous and 47 multiparous ewes that lambed during autumn received a 12-days medroxyprogesterone treatment followed by the introduction of rams. Ewes that came into oestrus, and that had a corpus luteum on Day 5 was greater in multiparous than in primiparous ewes ($p = 0.04$ and $p = 0.03$ respectively). Pregnancy rates on Day 35 and on Day 90 were similar. Experiment 3 was conducted with 26 ewes that remained with their lambs suckling during all the experiment, and 26 that were weaned ~27 days after birth, 3 days before the introduction of the rams. There were no differences on the percentage of ewes that came into oestrus, or the conception rate. We concluded that in postpartum ewes that had lambed during the breeding season: (1) is possible to induce fertile oestrus during early postpartum; (2) the early response was greater in multiparous rather than in primiparous ewes, although after a 35 d-breeding period differences disappeared and (3) weaning lambs early before stimulation did not improve the response.

Additional key words: intensive breeding; lactation; postpartum anoestrous; progestagen; sheep.

Resumen

Sincronización de celos en ovejas posparto que paren en otoño: efecto del intervalo posparto, la paridad y el destete temprano

Se realizaron 3 experimentos para determinar el efecto del tiempo posparto, de la paridad (primíparas-multíparas) y el destete sobre la respuesta de ovejas posparto a una sincronización/induccción de celos durante la estación reproductiva. En el Experimento 1 se trató a las ovejas con medroxiprogesterona durante 8 días y eCG (350 IU), a los 14-18 (EPP), 24-30 (MPP), o 42-48 (LPP) días posparto. Más ovejas EPP que LPP ($p < 0,05$) manifestaron celo, siendo la tasa de concepción similar. En el Experimento 2 se utilizaron 36 ovejas primíparas y 47 multíparas que parieron durante otoño, y recibieron un tratamiento con medroxiprogesterona seguido de la introducción de carneros. El porcentaje de ovejas en celo, y ovejas que ovularon a los 5 días fue mayor en las multíparas que en las primíparas ($p = 0,04$ y $p = 0,03$ respectivamente); la tasa de preñez fue similar. En el Experimento 3 se utilizaron 26 ovejas que permanecieron con sus corderos lactando durante todo el experimento, y 26 que fueron destetados a ~27 días posparto, 3 días antes de la introducción de los carneros. No hubo diferencias en los porcentajes de ovejas en celo, ni en la tasa de concepción. Se concluyó que en ovejas postparto que parieron durante la estación reproductiva: (1) fue posible inducir celos fértiles durante el posparto temprano; (2) la respuesta temprana fue mayor en ovejas multíparas que en ovejas primíparas, y (3) el destete temprano realizado poco tiempo antes de la introducción de los carneros no mejoró la respuesta reproductiva.

Palabras clave adicionales: anestro posparto; lactación; manejo reproductivo intensivo; ovinos; progestágeno.

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Abbreviations used: BCS (body condition score); BW (body weight); CE-E1 (control ewes used in Experiment 1); CL (corpus luteum); eCG (equine chorionic gonadotrophin); EPP-E1 (early pospartum ewes used in Experiment 1); LH (luteinizing hormone); LPP-E1 (late pospartum ewes used in Experiment 1); ME-E2 (multiparous ewes used in Experiment 2); MPP-E1 (medium pospartum ewes used in Experiment 1); PE-E2 (primiparous ewes used in Experiment 2); SE-E3 (suckling ewes used in Experiment 3); WE-E3 (weaned ewes used in Experiment 3).

Introduction

The interval between parturition and the resumption of reproductive activity in ewes is one of the natural factors that determine the frequency of lambing and the overall lifetime productivity of sheep. Females show a seasonal reproductive pattern that confines lambing activity to the spring months. However, oestrus and ovulation can be induced during the non-breeding season (for review see Smith *et al.*, 1989). Therefore, in those ewes that lambed during autumn, the cyclic activity can be reassumed or induced during the same breeding season.

In postpartum ewes, pregnancy is limited by the time of uterine involution and the interval to reassume the cyclic activity. Uterine involution is completed by the third week (Rubianes & Ungerfeld, 1993) and the first oestrus is observed ~50 days after parturition (Rubianes *et al.*, 1996) when ewes are maintained in a high nutritional plane. Therefore, as not all ewes are cyclic during early postpartum, for accelerated lambing programmes, oestrus induction managements should be included. Oestrus in non-cyclic ewes can be induced with hormonal treatments that mimic the hormonal pattern of an oestrus cycle (Ungerfeld & Rubianes, 2002) or the ram effect (Wright *et al.*, 1984; 1990).

However, there is scarce information in the conditions that affect the response of postpartum ewes to oestrus induction during the breeding season. It is well known that in intensive sheep breeding systems, prolificacy is directly related to parity (Maria & Ascaso, 1999). In this sense, in hair ewes number of lambs/ewe increases until the sixth birth (Sánchez-Davila, 2008). However, in postpartum sheep little is known on the influence of parity on the response to oestrus induction during the breeding season. Although it was reported that more multiparous than primiparous ewes stimulated with rams came into oestrus during the non-breeding season (Pevsner, 2008), there is limited information available on the response of ewes that lambed during the breeding season.

During the early postpartum suckling inhibits luteinizing hormone (LH) secretion (Stellflug *et al.*, 2001), probably through endogenous opioids secretion (Lozano *et al.*, 1998). At the same time, the body weight loss is one of the major limiting factors for oestrous and ovarian activity resumption after parturition (Mbayahaga *et al.*, 1998). Both body condition during pre-mating (Molina *et al.*, 1994) and mating (Gunn *et al.*, 1991) periods affect fertility and prolificacy. Therefore, weaning may be a useful tool to improve the reproductive results in postpartum ewes. However, information differs, as it has been reported that weaning improved (Mandiki *et al.*, 1990; Camacho-Ronquillo *et al.*, 2008), or has no advantages (Warren *et al.*, 1989; deNicolo *et al.*, 2006) on the reproductive responses of ewes to hormonal treatments.

Therefore, three experiments were designed to determine the influences of postpartum time, parity (primimarous *vs* multiparous), and early lamb weaning on the response to oestrous synchronization/induction treatments in postpartum ewes during the breeding season. Hormonal treatments were used for the first experiment, progestagen administration followed by introduction of rams for the second, and the introduction of rams alone for the third experiment.

Material and methods

The experiments were performed in private farms during the breeding season with ewes that were induced to come into oestrus during the previous non-breeding season through the ram effect, and lambed during autumn.

Experiment 1. Reproductive response in autumn-lambing Corriedale ewes induced to ovulate with progestagens + eCG: Effect of postpartum time

The experiment was performed in a farm located in Colonia, Uruguay (35º S) with 118 Corriedale ewes that remained grazing on improved pastures. Birth date (Day 0) were daily recorded during April (autumn, mid-breeding season), identifying ewes and their lambs. All ewes used had a single lamb. Body condition score (BCS; 1 to 5 scale; 1 = extremely emaciated, 5 = excessively fat) and body weight (BW) were registered on the day of birth. All ewes were managed together and remained with their lambs during all the experiment.

Intravaginal sponges impregnated with medroxyprogesterone (60 mg, Syntex SA, Buenos Aires, Argentina) were inserted in all ewes during early postpartum (EPP-E1 group; Days 14-18; n = 16), mid postpartum (MPP-E1 group; Days 24-30; n = 27) or late postpartum (LPP-E1 group; Days 42-48; n = 34). Eight days later sponges were withdrawn, and all ewes received an intramuscular injection of 350 IU of equine chorionic gonadotrophin (eCG) (Novormón, Syntex SA, Buenos Aires, Argentina). Body weight was evaluated again, and ewes were placed with 8 marking sexually experienced Corriedale rams. Marked ewes were recorded once daily during 5 days, but remained with rams until Day 9. Four weeks after coming into oestrus, pregnancy was determined by transrectal ultrasonography using a Pie Medical 480 equipment with a dual linear probe (5/7.5 MHz).

The other 41 ewes remained untreated and served as a control (CE-E1 group) to determine spontaneous cyclic activity resumption. Blood was collected from the jugular vein by venipuncture on Days 12, 21, 27, 31, 37, 41, 46, 51, 60 and 65. Samples were allowed to clot for 1 hour at room temperature and centrifuged for 10 to 20 min. Serum was kept at –20°C until progesterone levels were measured.

Progesterone was measured using a direct solid-phase ^{125}I radioimmunoassay (RIA) (Cout-A-Count TKPG, Diagnostic Products Corporation, Los Angeles, CA, USA) with a sensitivity of 0.3 nmol L^{-1}. The inter- and intra-assay coefficients of variation were <10%. Results are expressed in nmol L^{-1}. The existence of luteal progesterone concentrations was considered when values exceeded 1.5 nmol L^{-1} in two consecutive samples, or were over 3 nmol L^{-1} in two non-consecutive samples.

Experiment 2. Reproductive response in autumn-lambing Merilin ewes induced to ovulate with progestagens and the ram effect: Effect of parity

The experiment was conducted on a private farm located in Flores, Uruguay (34º S), during the breeding season (March to May; autumn) with 83 Merilin ewes. Although scientific data are not available, the Merilin is a local breed considered as intermediate breed according to its seasonal pattern. Lambing date was recorded in a bigger flock, and the 83 ewes were selected considering a maximum range of 30 days in lambing date. From these, 36 were primiparous (1.5 years old; PE-E2 group) and 47 multiparous (3-5 years old; ME-E2 group). Body weigh at Day –12 (Day 0 = introduction of the rams) was 40.1 ± 3.8 and 46.0 ± 4.5 kg, and BCS was 2.8 ± 0.3 and 3.0 ± 0.3 for PE-E2 and ME-E2 ewes respectively. All ewes were managed together, remained with their lambs, and grazed on improved pastures during all the experiment. All ewes used had a single lamb.

Ewes were isolated from rams since 30 days before lambing (sound, sight and smell, minimum distance >1500 m). On Day –12, the ovaries of 33 ewes were scanned with transrectal ultrasound (Aloka 500 with a 7.5 MHz transducer, Japan) determining that only 3 of them had a corpus luteum (CL). As there were electrical problems, the remaining ewes could not be scanned. Immediately (Day –12), intravaginal sponges impregnated with medroxyprogesterone acetate were inserted in all ewes. On Day 0, when ewes had 30 to 60 days after parturition, the sponges were withdrawn and ewes were joined with seven Merino marker rams. Ewes and rams remained together during 60 days.

The number of marked ewes was recorded daily for 5 days. On Day 5, the presence and number of CLs was determined with ultrasound in all ewes. Pregnancy was determined with ultrasound on Days 35 and 90.

Experiment 3. Reproductive response in autumn-lambing Kathadin ewes induced to ovulate with the ram effect: Effect of early weaning

The experiment was conducted on a private farm located in Higueras, Nuevo León, Mexico (25º N), during the breeding season (September to December; autumn) with 52 Kathadin ewes that lambed between September 21st and October 20. Kathadin ewes have slight changes in pregnancy rates throughout the year, so it is considered as a low seasonal breed (Burke, 2005). During all the experiment ewes grazed Buffel pastures (*Cenchrus ciliaris*), from 9:00 to 15:00, and received 500 g d^{-1} of supplement with 12% of crude protein. Ewes were isolated from rams since 30 days before lambing (sound, sight and smell, minimum distance >500 m).

Lambing date was recorded, and ewes were adjudicated to two experimental groups. While 26 ewes (group SE-E3) remained with their lambs suckling during all the experiment, lambs from the other 26 ewes (group WE-E3) were weaned on Day –3 (Day 0 = introduction of the rams; a mean of 27 days after lambing). Separation in time of weaning and the introduction of the rams was decided to avoid overlap of a stressant management (weaning) with the stimulating management (introduction of the rams). Groups were homogeneous according to parity (2.5 ± 0.2 and 2.4 ± 0.2), body weight (45.8 ± 1.3 and 47.8 ± 1.5), body condition at birth (2.4 ± 0.1 and 2.5 ± 0.1) and number of ewes that lambed twins (4/26 and 4/26) (WE-E3 and SE-E3 groups respectively).

On Day 0 (27.2 ± 1.3 and 27.6 ± 1.6 days postpartum for WE-E3 and SE-E3 groups) three vasectomised rams were joined with the flock, and oestrus was recorded until Day 30. Ewes in oestrus were taken out of the flock, mated by a ram, and returned to the flock. Pregnancy was determined with transrectal ultrasound (Falco-ESAOTE, Pie Medical with a 7.5 MHz transducer; The Netherlands) on Day 80.

Statistical analysis

Frequency of marked ewes, conception rates (ewes pregnant/ewes marked) and pregnancy rates (ewes pregnant/ewes treated) were compared by χ^2 test. Body weight was compared by ANOVA. Analysis was performed with Stata 11 (StataCorp LP, College Station, TX, USA).

Results and discussion

In Experiment 1, ewes' BCS at birth was 1.63 ± 0.04. Body weight decreased from 47.7 ± 4.2 kg (mean ± SD) at lambing, to 46.1 ± 4.0 kg at sponge removal ($p < 0.01$). During the first 5 days after the hormonal treatment, significantly more ewes from the EPP-E1 group than from the LPP-E1 group (Table 1; $p < 0.05$) were marked by rams. Pregnancy rate was not significantly different between groups (Table 1). The accumulated frequency of CE-E1 ewes with luteal progesterone concentrations is presented on Figure 1. Until Day 65 (sampling period), 32 ewes presented luteal progesterone concentrations. The interval from parturition to first luteal progesterone concentrations in ewes that had them was 30.8 ± 14.6 days.

Table 1. Percentage of Corriedale ewes marked during the first 5 days and pregnant after an hormonal treatment (medroxyprogesterone acetate + 350 IU of eCG). Ewes lambed during the breeding season, and the hormonal treatment was applied 14-18 (EPP-E1), 24-30 (MPP-E1), or 42-48 (LPP-E1) days after parturition (Experiment 1)

Group	Ewes marked (%)	Pregnancy rate (%)
EPP-E1	13/16 (81.3) [a]	7/16 (43.8)
MPP-E1	19/27 (70.4) [ab]	8/27 (29.7)
LPP-E1	16/34 (47.1) [b]	16/34 (47.1)

For the same column: [a] vs [b]: $p < 0.05$

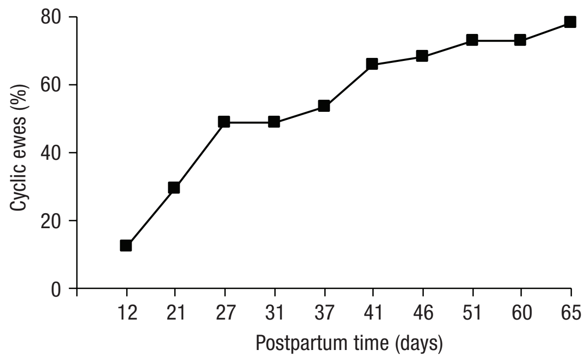

Figure 1. Accumulated percentage of suckling Corriedale ewes that presented luteal phases after parturition during the breeding season.

Our data first confirm and expand previous information on postpartum oestrous induction in postpartum suckling ewes that lambed during the breeding season. Both, the percentage of ewes coming into oestrus and the conception rates were lower to those obtained in non-cyclic Corriedale ewes treated similarly during the non-breeding season (Ungerfeld & Rubianes, 2002). Low results may have been caused by the low BCS that grazing ewes had, probably due to the postpartum and suckling state. In this sense, as hormonal treatments cannot over impose to metabolic restrictions, it would be interesting to determine the relationship between metabolic profiles and reproductive responses in postpartum ewes.

The greater proportion of ewes from the EPP-E1 group that came into oestrus during the first days may be explained by the use of short-term medroxyprogesterone primings. According to data from the control ewes (CE-EE1), probably more EPP-E1 than LPP-E1 ewes were still non cycling at sponge withdrawal. Although short-term primings are effective to induce oestrus in anoestrous ewes (Ungerfeld & Rubianes, 1999, 2002), its application in cyclic ewes determines a higher oestrus dispersion, because many ewes may have functional CLs present at device withdrawal (Viñoles *et al.*, 2001). This probably occurred in LPP-E1 ewes, in which a greater proportion of ewes compared to the other groups was most likely cycling.

The interval from parturition to first progesterone increase phase in untreated ewes (CE-E1 ewes) was similar to our previous observations (Rubianes *et al.*, 1996), and is within the range reported in other breeds (Mandiki *et al.*, 1990; Wright *et al.*, 1990). However, that value corresponds to a small percentage of ewes, as 24.4% of them did not have luteal progesterone concentrations during the observed period (65 days). This low result is not surprising considering the ewes gave birth with a low BCS, and lost BW during the postpartum period. It has been shown that BW is related with the reproductive response of postpartum suckled ewes (Wright *et al.*, 1990).

In Experiment 2, the percentage of ewes that came into oestrus, and that had a CL on Day 5 was greater in ME-E2 than PE-E2 ewes (Table 2). However, the percentage of those that came into oestrus/ovulated did not differ. Pregnancy rates of PE-E2 and ME-E2 were similar on Day 35 and on Day 90 (Table 2). We demonstrated that parity directly influenced the reproductive response, although it is not known the effect of the introduction of the rams itself. As happens with other reproductive managements, the greater earlier response (percentage of ewes coming into oestrus and ovulating) in multiparous than primiparous ewes confirms the hypothesis of differences in favour of the first. In effect, multiparous ewes have greater ovulation rate (Dyrmundsson, 1973). It should be considered that primiparous ewes are subjected to greater energetic requirements, as according to their lighter body weight those ewes were still growing, and at the same time they remained suckling their lambs. In this sense, it has been considered that undernutrition during lactation increases the incidence of anestrus in primiparous autumn-lambing ewes (Dunn & Kaltenbach, 1980). In agreement with our results, in hair sheep, multiparous postpartum ewes come into oestrus earlier than primiparous ewes when stimulated with the ram effect during the early spring and summer (Sánchez-Dávila *et al.*, 2011). This also agrees with what is observed in cattle, in which postpartum multiparous cows rebreed earlier than primiparous cows (Yavas & Walton, 2000). Probably, primiparous ewes might be in a deeper anoestrous condition that may prevent the response to the stimulation treatment.

In Experiment 3, there were no differences on the interval from male introduction to oestrus, the percentage of ewes that came into oestrus, or the pregnancy rate between weaned or suckled ewes (Table 3). Contrary to what we expected, suckling did not influence any of the measured responses in Experiment 3. Although fertility in anoestrous ewes subjected to the ram effect or eCG stimulation is directly related to nutritional status (Folch *et al.*, 1988), it seems that improvement of ewes' metabolic condition probably caused by weaning did not act similar to flushing managements. That difference might be due to the use of an extremely short period between weaning and stimulation. However, it should also be considered that weaning is a stressant event for ewes, and this may have overlapped the positive influence of the end of lactation on metabolic status. Though ewes were weaned 3 days before the introduction of the rams, we cannot discard that an advancement of weaning may improve the response to oestrous synchronization treatments. Similarly, other researchers could not increase the proportion of ewes lambing weaning ewes 3 weeks before oestrous induction in autumn-lambing hair ewes (Knights *et al.,* 2011). In the same direction, no differences were observed after weaning on the resumption of ovarian activity (Pope *et al.*, 1989), or on the response to the ram effect (Poindron *et al.*, 1980) in ewes rearing one or two lambs. It remains to be tested if in this low seasonal breed spontaneous rebreeding of postpartum ewes differs after weaning. Overall, it seems that although weaning might improve reproductive results of oestrus induction in postpartum ewes in some conditions, results may be erratic due to other environmental or physiological limitations with stronger effects than suckling.

In conclusion, in postpartum ewes that had lambed during the breeding season: (1) is possible to induce fertile oestrus during early postpartum; (2) the early response was greater in multiparous rather than in

Table 2. Reproductive response of primiparous or multiparous postpartum Merilin ewes (between parenthesis, percentage) to the introduction of rams during the breeding season (Experiment 2)

	Primiparous	Multiparous	*p*
Marked ewes (Day 5)	27/36 (75.0)	43/47 (91.5)	0.04
Ewes that had a CL (Day 5)	28/36 (77.7)	44/47 (93.6)	0.03
Marked ewes/Ewes that had a CL	27/28 (96.4)	43/44 (97.7)	ns
Pregnancy rate D35	18/36 (50.0)	28/47 (59.6)	ns
Pregnancy rate D90	28/36 (80.0)	40/47 (85.1)	ns

CL: corpus luteum. ns: not significant

Table 3. Reproductive response (between parenthesis, percentage) of Kathadin ewes that were weaned or remained suckling to the introduction of rams during the postpartum period during the breeding season (Experiment 3)

	Weaned ewes	Suckled ewes
Days 0-17		
Ewes in estrus	17/26 (65.4)	19/26 (73.1)
Interval to estrus (d)	7.6 ± 1.4	10. 6± 1.3
Conception rate	9/17 (52.9)	12/19 (63.1)
Days 0-30		
Ewes in estrus	26/26 (100)	25/26 (96.2)
Interval to estrus (d)	12.2 ± 1.6	13.1 ± 1.3
Interval from birth to estrus (d)	40.4 ± 2.0	42.0 ± 1.6
Conception rate	22/26 (84.6)	21/25 (84.0)
Pregnancy rate	22/26 (84.6)	21/26 (80.1)

primiparous ewes, although after a 35 d-breeding period differences disappeared and (3) weaning lambs early before stimulation did not improve the response.

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