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Original article

The effect of progestagen treatment on sheep reproductive performance at different phases of the oestrous cycle

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Abstract — Two experiments were carried out in order to determine the effect of progestagen treatment to synchronise oestrus on sheep reproductive performance at different phases of the cycle, comparing artificial insemination and natural mating. In experiment 1, and after analysis of plasma progesterone concentrations 7 days before and at pessary insertions, animals were divided into four groups: Group F (n = 25), follicular phase at pessary insertion; Group L1 (n = 22), the onset of the luteal phase; Group L2 (n = 18), mid-late luteal phase and Group A (n = 11), anovulatory. The ewes were inseminated 54 h after pessary withdrawal. Mean percentage of fertility was low (30%), and when data from Groups L1 and L2 were pooled, and compared with F+A groups, percentage of fertility was significantly higher ($P < 0.05$) in the latter groups (20% vs. 39%, respectively). Similarly, the number of lambs born per inseminated ewe was twice as high ($P < 0.05$) in these animals as compared with L1+L2 (F+A: 0.50±0.13; L1+L2: 0.25±0.09 lambs born per inseminated ewe). In experiment 2, oestrous cycles of 40 ewes were synchronised with two i.m. injections of 7.5 mg Luprostiol. Seventy-two hours after the second injection (day 0), progestagen pessaries were inserted in 20 ewes (Group 0), when the ewes were in the follicular phase of the cycle. The remaining 20 ewes received the pessaries on day 11, during the luteal phase of the cycle (Group 11). Animals were hand-mated at least twice by fertile rams 48 h later; the ewes were thereafter exposed to rams in the pen overnight. The percentage of fertility was 90% in both groups. No significant differences between groups were observed for litter size and fecundity. In conclusion, an onset of the synchronisation treatment with progestagen in a program of artificial insemination close to the follicular phase of the oestrous cycle can improve both fertility and the number of lambs born per inseminated ewe. If natural service is used, perhaps the stay of the rams with the ewes for at least 24 h might compensate for the asynchrony between ovulation and mating, leading to similar percentages of fertility, whatever the phase of the cycle when the synchronisation treatment began. Further studies involving a higher number of animals are necessary to confirm these results.

sheep / progestagen / oestrous cycle / artificial insemination

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Résumé — L'effet des traitements progestatifs sur la reproduction des ovins. Performances à différentes phases du cycle oestral. Deux expériences ont été réalisées dans le but de déterminer l'effet des traitements progestatifs utilisés pour la synchronisation des oestrus sur les performances de reproduction des ovins à différentes phases du cycle. Dans l'expérience 1 : les animaux ont été répartis en quatre groupes en fonction des concentrations plasmatiques en progestérone déterminées 7 jours avant la pose des éponges vaginales et lors de leur mise en place : Groupe F (n = 25), brebis en phase folliculaire au moment de l'insertion des éponges, groupe L1 (n = 22) brebis au début de la phase lutéale, groupe L2 (n = 18) brebis à la moitié de la phase lutéale et groupe A (n = 11) brebis anovulatoires. Les brebis ont été inséminées 54 heures après le retrait des éponges vaginales. Le taux moyen de fertilité a été faible (30 %). La comparaison entre les deux groupes associés L1+L2 et F+A a montré que le taux de fertilité était significativement plus élevé ($P < 0,05$) dans le groupe associé F+A. De même, le nombre d'agneaux obtenu par brebis inséminée était deux fois plus élevé dans ce groupe comparativement au groupe L1+L2 ($P < 0,05$). Dans l'expérience 2 : la synchronisation des chaleurs de 40 brebis a été induite par deux injections intramusculaires contenant 7,5 mg de luprostiol. Soixante douze heures après la deuxième injection (jour 0), les éponges de progestagènes ont été mises en place chez 20 brebis (groupe 0) en phase folliculaire. Pour les 20 brebis restantes la mise en place a eu lieu le 11e jour lors de la phase lutéale (groupe 11). Les brebis ont été mises à la lutte 48 heures plus tard. Le taux moyen de fertilité obtenu était de 90 % dans les deux groupes. Aucune différence significative n'a pas été observée pour la prolificité et la fertilité entre les deux groupes. En conclusion, les traitements de synchronisation avec des progestagènes au début de la phase folliculaire et dans le cadre d'un programme d'insémination artificielle permet d'améliorer à la fois la fertilité et le nombre d'agneaux par brebis inséminée. Dans les conditions d'une lutte naturelle, la présence des béliers avec les brebis pendant 24 heures au minimum, permettrait de compenser la non-synchronisation des ovulations et conduirait à une fertilité similaire quelle que soit la phase du cycle au moment de l'initiation des traitements progestatifs. Des essais supplémentaires sur un plus grand nombre d'animaux seront nécessaires pour confirmer ces résultats.

brebis / progestagène / cycle oestral / insémination artifielle

1. INTRODUCTION

Since the early 1960s in Australia, progestagen treatments have been applied to synchronise sheep oestrous cycles, using intravaginally inserted pessaries impregnated with progestagen [9]. This method has been introduced in artificial insemination (AI) programs in this species, in order to control the oestrous cycle and achieve acceptable pregnancy rates. So far, the percentage of fertility after AI is not able to reach similar values to that obtained when natural service is performed, especially using frozen semen. A big effort has been done on the study and handling of semen, but the determination of the effect of the particular physiological state of the ewe on fertility and follicular dynamics has been less investigated.

It has been hypothesised that during oestrous synchronisation, ewes are at different days of the cycle, leading to some variation among ewes in the duration of endogenous progesterone secretion [5]. Furthermore, the proportion of endogenous and exogenous progesterone concentrations is able to alter the number of follicular waves and the pulsatile release of LH, and modify the size of the largest follicles. Thus, the timing of oestrus after progestagen withdrawal varies within animals, conditioning the necessary synchrony between ovulation and insemination.

In multiple ovulation and embryo transfer (MOET) programs, the use of oestrous synchronisation and AI procedures are also used. It has been indicated [6] that the use of MOET technology at high latitudes is restricted by the seasonality of breeding in

this species, limiting the number of animals that an embryo transfer team can deal with, within any one year. This situation is different in Mediterranean sheep production systems, where seasonal anoestrus is not an obstacle for these strategies. Firstly, the percentage of cyclicity is relatively high during seasonal anoestrus in these breeds (Rasa Aragonesa, 20–30%) [3], and secondly, AI programs have been introduced at the beginning of seasonal anoestrus, in order to obtain early lambing periods, especially in dairy flocks.

This study was designed to investigate the effect of the phase of the oestrous cycle when progestagen treatments to synchronise oestrus are applied in an AI schedule in a Mediterranean breed of sheep and late in the breeding season, in comparison with a natural service system.

2. MATERIALS AND METHODS

2.1. Experiment 1

In early December, a flock of 97 Rasa Aragonesa ewes, located in Teruel (Spain), were synchronised in oestrus using progestagen (fluorogestone acetate, FGA) pessaries (Chrono-gest, Intervet, Salamanca, Spain). Pessaries were withdrawn 12 d later, and 400 I.U. eCG (Foligon, Intervet, Salamanca, Spain) were then injected. The animals were artificially inseminated 54 h after pessary withdrawal with refrigerated semen, collected from four proven rams, by the veterinarian team of ANGRA (National Association of Rasa Aragonesa Breeders), under its selection scheme. Seven days before and at pessary insertions (day 0), blood samples were collected from the jugular vein and analysed for plasma progesterone concentrations. The animals were divided into four groups according to the phase of the oestrous cycle determined by plasma progesterone levels, considering a threshold of 0.5 $ng \cdot mL^{-1}$ progesterone to determine ovulation followed by normal luteal function, as previously observed in this breed and using the same progesterone assay [3] (Tab. I). Thus, 25 animals were considered to be in the follicular phase of the cycle at pessary insertion (Group F), 22 ewes were at the onset of the luteal phase (Group L1), 18 were at the mid-late luteal phase (Group L2) and 11 ewes, showing basal levels of progesterone on both days were considered as being anovulatory (Group A). Although the rams used already had demonstrated a proven fertility, they were represented within the groups to avoid any difference in fertility due to the ram.

2.2. Experiment 2

This experiment was conducted at the experimental farm of the University of Zaragoza, Spain (latitude 41°40' N), which meets the requirements of the European Community Commission (1986) for Scientific Procedure Establishments.

Table I. Classification of the animals used in experiment 1 considering plasma progesterone (P_4) concentrations of samples collected seven days before and at pessary insertion (day 0) to synchronise oestrus.

Group	n	Phase of the cycle	P_4 day –7	P_4 day 0
F	25	Follicular	Luteal[1]	Basal[2]
L1	22	Onset luteal phase	Basal	Luteal
L2	18	Mid-Late luteal phase	Luteal	Luteal
A	11	Anovulatory ewes	Basal	Basal

[1] $P_4 > 0.50\ ng \cdot mL^{-1}$
[2] P_4 close to 0 $ng \cdot mL^{-1}$

In late January, the oestrous cycles of 40 adult Rasa Aragonesa ewes were synchronised with two i.m. injections of 7.5 mg Luprostiol, an analogous prostaglandin (PG) $F_{2\alpha}$ (Prosolvin, Intervet, Salamanca, Spain), 11 days apart. Seventy-two hours after the second injection (day 0), progestagen (FGA) pessaries to synchronise oestrus (Chrono-gest, Intervet, Salamanca, Spain) were inserted in 20 ewes (Group 0), when the ewes were in the follicular phase of the cycle induced by Luprostiol. The remaining 20 ewes received the pessaries on day 11, during the luteal phase of the cycle (Group 11). Pessaries were withdrawn 12 days after insertion in each group, and 400 I.U. eCG (Foligon, Intervet, Salamanca, Spain) were then injected. Animals were hand-mated at least twice by fertile rams 48 h later; ewes were thereafter exposed to rams in the pen overnight. Blood samples were collected on the two days when Luprostiol was injected and 72 h after the second injection in both groups and on day 11 in Group 11, and analysed for plasma progesterone concentrations.

2.3 Progesterone assay

Progesterone determinations were performed using solid-phase RIA kits based on antibody coated tubes, ^{125}I-labeled progesterone, and rabbit antiserum (CIS bio international, Gif-sur-Yvette, France). The assay sensitivity was 0.05 ng·mL^{-1} progesterone. Intra- and inter-assay coefficients of variation were 13.6% and 15.7%, respectively.

2.4. Statistical analysis

In both experiments, fertility (percentage of ewes lambing), litter size (number of lambs born per lambing ewe) and fecundity (number of lambs born per mated or inseminated ewe) were recorded after parturition, and mean ± s.e. values were calculated. Plasma progesterone concentrations, litter size and fecundity were compared using analysis of variance according to the following fixed effect model: $Y=Xb + e$, where Y is the $N \times 1$ vector of records, b denotes the fixed effects in the model (phase of the oestrous cycle) with association matrix X, and e denotes the vector of residual effects. Percentages of fertility were compared using X^2 tests.

3. RESULTS

3.1. Experiment 1

The mean fertility rate was low (30%), and after a comparison of the four groups, no significant differences were detected. However, when data from the two groups of ewes in the luteal phase after the synchronisation treatment began (L1+L2) were pooled, and compared with those animals showing basal levels of progesterone at the same moment (F+A), the percentage of fertility was significantly higher ($P < 0.05$) in the latter group (Tab. II). Similarly, the number of lambs born per ewe inseminated was twice as high in these animals ($P < 0.05$) as compared with the L1+L2 groups.

3.2. Experiment 2

The animals of both groups presented similar mean plasma progesterone concentrations either at the first (Group 0: 0.286±0.05; Group 11: 0.357±0.07 ng·mL^{-1}) or at the second (Group 0: 0.503±0.05; Group 11: 0.584±0.06 ng·mL^{-1}) Lupostriol injections. The basal levels of progesterone presented by the animals of both groups 72 h after the second injection, and the luteal plasma progesterone concentrations of Group 11 ewes 11 days later (0.623±0.06 ng·mL^{-1}) confirmed the usefulness of the treatment to induce a new sexual cycle.

The percentages of fertility were high and similar in both groups (90%). No significant

Table II. Percentage of fertility and mean (± s.e.) litter size and fecundity of ewes artificially inseminated after a progestagen treatment started when ewes were in the follicular phase of the cycle (F), first (L1) or second (L2) half of the luteal phase, or were in anoestrus (A) in experiment 1.

	n	Fertility	Litter size	Fecundity
F	25	40%	1.40 ± 0.16	0.56 ± 0.15
L1	22	23%	1.40 ± 0.24	0.32 ± 0.14
L2	18	17%	1.00 ± 0.00	0.16 ± 0.09
A	11	27%	1.67 ± 0.33	0.36 ± 0.24
F+A	36	39%[a]	1.46 ± 0.14	0.50 ± 0.13[a]
L1 + L2	40	20%[b]	1.25 ± 0.16	0.25 ± 0.09[b]

[a,b] indicate $P < 0.05$.

differences between the groups were observed for mean litter size (Group 0: 1.39±0.12; Group 11: 1.61±0.12) and fecundity (Group 0: 1.25±0.14; Group 11: 1.45±0.15).

4. DISCUSSION

In the two experiments carried out in this study, it was possible to distinguish between the ewes subjected to both endogenous and exogenous progesterone during the synchronisation treatment (Group F in experiment 1 and Group 0 in experiment 2) from those that were mostly under the effect of the exogenous progestagen treatment (Groups L1 and L2 and Group 11, in experiment 1 and 2, respectively). On the contrary, a fixed-time AI was performed in experiment 1 whereas fertile rams naturally mated ewes in experiment 2.

In spite of the low percentage of fertility recorded in experiment 1, although similar to the values reported by the Rasa Aragonesa Breed Association in the period 1989-1998 (ranging from 19% to 42%, [1]), those ewes which initiated the synchronisation treatment during the follicular phase of the cycle presented the highest levels of fertility, almost twice as the ewes in the luteal phase of the cycle, under the dominance of high plasma progesterone concentrations. Moreover, the slightly higher litter size of these animals led to statistical differences in the number of lambs born per inseminated ewe. The worst reproductive results were reached when the treatment started when endogenous progesterone had begun to decline, so that the ewes were mostly under the effect of the exogenous progestagen treatment.

Leyva et al. [5], treating ewes with sponges containing progestagen at fixed intervals from ovulation (early, middle and late luteal phase), concluded that the extension of the oestrous cycle with these treatments increased the number of follicular waves and modified the size of the largest follicles, this effect being caused by changes in the proportion between endogenous and exogenous progesterone concentrations, which altered the pulsatile release of LH. Furthermore, the ovulatory follicle of ewes treated with progestagen during the late luteal phase had a significantly longer persistence than those from ewes treated during the early or middle luteal phase. On the contrary, beef heifers treated with a synthetic progestagen towards the end of the luteal phase presented an extension of the period of dominance of the ovulatory follicle, with a significant reduction in pregnancy rate [7], due to the ovulation of aged oocytes, because the ovulatory ability of such persistent dominant follicles is maintained [8]. In sheep, and using a model of superovulated animals, it has been reported

that the presence of a large growing follicle at the time of the superstimulatory treatment was associated with lower follicle recruitment and the development of functionally subnormal corpora lutea [10]. Taking all these considerations together, it can be postulated that perhaps the ewes in experiment 1 that received the progestagen treatment during the luteal phase of the cycle, and especially the L2 animals, experimented an alteration of their follicular pattern, with an extension of the dominance of such follicles to give rise to the ovulation of oocytes which were not able to develop an embryo. Moreover, the longer persistence of the ovulatory follicle described in the literature for animals treated with progestagen at the end of the luteal phase [5] could delay ovulation and, considering that a fixed-time insemination was performed, a hypothetical asynchrony between ovulation and insemination could compromise fertility.

In relation to anovulatory ewes, Flynn et al. [2] observed that, in the absence of luteal progesterone, synchronisation with a progestagen sponge for 14 days results in higher LH pulse frequency and ovulation of a follicle with a larger maximum diameter than the controls. This could explain the slightly higher fertility percentages presented by these animals in comparison with those synchronised at the end of the luteal phase.

The evident differences recorded in the first experiment were not confirmed in experiment 2. Apart from the different approach of both experiments concerning the time of pessary insertions (evaluation of the phase of the oestrous cycle using a progesterone assay with hindsight in experiment 1, and previous synchronisation with PG analogous in experiment 2), the main difference lies in the fertilisation procedures used (A.I vs. natural service). Several factors may influence the extent of the interval between the end of progestagen treatment and the start of the controlled oestrous period; this interval may last for 36 h, although some animals may be in oestrus as early as 24 h or as late as 48 h [4]. Since ewes were exposed to rams up to 72 h after pessary withdrawal, the alteration of the normal ovarian pattern described above for animals of Group 11 could have been compensated for by an extension of the mating period. This is the opposite of the fixed-time insemination of experiment 1, giving rise to a better degree of synchrony between ovulation and mating.

In conclusion, an onset of the synchronisation treatment with progestagen close to the follicular phase of the oestrous cycle, in a program of AI at the end of the breeding season, can improve both fertility and the number of lambs born per inseminated ewe. If natural service is used, the stay of the rams with the ewes for at least 24 h might compensate for the asynchrony between ovulation and mating that occurs after progestagen treatments, leading to similar percentages of fertility, whatever the phase of the cycle when the synchronisation treatment began, although further studies involving a higher number of animals are necessary to confirm these results. These conclusions could be an incentive to spread the use of AI in these breeds.

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