Animal Reproduction Science 136 (2013) 268–279

Contents lists available at SciVerse ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Short-lived corpora lutea syndrome in anoestrous ewes following 17β-oestradiol or MAP treatments applied before an allogenic sexual stimulation with rams and oestrous ewes

R.M. Rodríguez Iglesias[a,b,*], N.H. Ciccioli[a,1], J. Ferrería[c], D.A. Pevsner[a], C.A. Rosas[a], M.M. Rodríguez[d], J.R. Pedrueza[e]

[a] Departamento de Agronomía, Universidad Nacional del Sur, B8000 Bahía Blanca, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina
[c] Agencia Nacional de Promoción Científica y Tecnológica, Argentina
[d] Facultad de Ciencias Veterinarias, Universidad Nacional del Centro, Tandil, Argentina
[e] Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, Bahía Blanca, Argentina

ARTICLE INFO

Article history:
Received 3 July 2012
Received in revised form
16 November 2012
Accepted 19 November 2012
Available online 27 November 2012

Keywords:
Ram effect
Seasonality
Short cycles
Corriedale
Corpora lutea

ABSTRACT

When induced to ovulate during anoestrus, ewes, does and cows frequently develop a *short-lived corpora lutea* (SLCL) syndrome associated to lack of previous progesterone. Exogenous progesterone precludes SLCL by blocking oxytocin endometrial receptors, thus inducing *normal life-span CL* (NLCL). Paradoxically, *circa* 50% of unprimed ewes do not develop SLCL. We report results from 3 trials assessing follicular, oestrous, ovulatory, and luteal end-points after 17β-oestradiol or MAP treatments. Oestradiol benzoate (50 μg) induced follicular turnover, provoked ovulation in 40% (24/60) of ewes treated (93% of which developed SLCL), but did not affect the incidence of SLCL (26/53) after an allogenic sexual stimulation (ASS) by rams and oestrous ewes. By the onset of the ASS, most NLCL ewes (26/27) had already experienced turnover of their largest follicle, had smaller largest and second largest follicles, and ovulated their largest follicle more frequently than SLCL ewes did. Most SLCL ewes (19/25) did not ovulate their largest follicles, ovulating instead smaller follicles of identical size to those of NLCL ewes. Priming (40 mg of MAP for 12 days) was partially effective at preventing SLCL even when terminated 14 days in advance of an ASS, but failed at completely preventing SLCL when terminated 6 or more days in advance. The coupling of a timed acquisition of full steroidogenic capability before ovulation with a system of endometrial oestradiol–progesterone–oxytocin receptors linked in an unstable equilibrium controlling the amplification of the luteolytic feed-forward loop of oxytocin and prostaglandin $F_2\alpha$ explains occurrence and relative incidences of both NLCL and SLCL, and links proximate and ultimate causes of the SLCL syndrome.

1. Introduction

Females of a number of ungulate species, including ewes, does and cows, induced to ovulate or spontaneously resuming cyclicity after a period of anovulation, frequently develop a *short-lived corpora lutea* (SLCL) syndrome. That involves the formation of morphologically normal CL after ovulation (usually silent for ewes and cows, preceded by

* Corresponding author at: Departamento de Agronomía, Universidad Nacional del Sur, B8000 Bahía Blanca, Argentina.
Tel.: +54 291 456 6130x4343; fax: +54 291 459 5127.
E-mail address: cerodri@criba.edu.ar (R.M. Rodríguez Iglesias).
[1] Deceased.

0378-4320/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.anireprosci.2012.11.009

oestrus in does), followed by anticipated luteolysis and a second ovulation (still silent in ewes) resulting in the development of CL of normal life-span (ewe: Oldham and Martin, 1978; doe: Camp et al., 1983; cow: Odde et al., 1980). In domestic sheep the SLCL syndrome has been documented at puberty (Berardinelli et al., 1980), at the resumption of cyclicity after seasonal anoestrus (Yuthasastrakosol et al., 1975), and following both GnRH- (McLeod et al., 1982) and ram-induced (Oldham and Martin, 1978) ovulation during seasonal anoestrus.

Some consistent features characterize the SLCL syndrome in ewes. Ewes ovulating either experience the syndrome or develop CL of normal life-span (NLCL); the occurrence of SLCL not followed by a subsequent ovulation and formation of NLCL is rare. In addition, ewes developing SLCL have subsequent reproductive performances comparable to those of ewes forming NLCL at first ovulation (Oldham, 1980; Irazoqui et al., 1992). Those two features suggest that the SLCL syndrome may be part of the 'normal' female reproductive repertoire, rather than some defective way of resuming cyclicity. Two other regular features are the remarkably constant life-span of SLCL (3–4 days; Hunter, 1991) and the fact that NLCL rarely coexist with SLCL in multiple ovulations (Chemineau et al., 2006). The quite invariant life-span of SLCL suggests that the syndrome is probably the consequence of anticipated luteolysis triggered as soon as CL become sensitive to $PGF_2\alpha$, which in sheep occurs as early as 3–4 days after ovulation (Acritopoulou and Haresign, 1980; Rubianes et al., 2003). Thus, anticipated luteolysis seems to be the proximate cause of the SLCL syndrome and this is supported by its complete abolishment following post-ovulatory hysterectomy (Chemineau et al., 1993).

Progesterone priming offers a less radical way of preventing SLCL. A 5 mg dose injected i.m. immediately before a ram exposure (Cognié et al., 1982) precludes the syndrome although a 20 mg dose is generally favoured on the basis of better synchronization of oestrus at the subsequent cycle (Oldham et al., 1985). This standard 20 mg dose abolishes SLCL even when applied 5 days in advance of an ovulation-inducing protocol (Pearce et al., 1987). The effectiveness of these P_4 treatments and the invariable occurrence of NLCL after an SLCL episode indicates that minimum pre-exposure to P_4, both in concentration and time-span, suffices to ensure CL normalcy in the ensuing cycle. In line with this pharmacological and physiological evidence, lack of exposure to P_4 from a previous cycle was originally suggested (Oldham and Martin, 1978) as the cause of SLCL. However, that leaves unexplained the paradoxical fact that a sizable proportion (30–70%) of ewes successfully induced to ovulate after a ram exposure may not experience the syndrome in spite of lacking previous CL. Clearly, lack of P_4 from a previous cycle may be a factor, but it cannot be the ultimate cause of the syndrome. Inadequate follicular development or the ovulation of either 'immature', 'aged' or somehow defective follicles in some ewes along with 'normal' ones in other ewes have also been proposed as alternative explanations for the coexistence of both types of luteal response (e.g. Martin et al., 1986; Legan et al., 1985; Chemineau et al., 2006). On the other hand, even though both proximate and ultimate presumptive causes of the syndrome have been advanced, the chain of events connecting them remains mostly unknown (although see Chemineau et al., 2006 for a recent proposal of a causative mechanism).

In a variety of ungulates, females may be induced to ovulate during seasonal anoestrus by suddenly exposing them to *allogenic sexual stimuli* (ASS), i.e. socio-sexual stimuli, generally originating from social partners, but not directly involving the subject. Previous research in moderately seasonal sheep established that ASS are best provided by a small group of sexually active males and females (Rodríguez Iglesias et al., 1991). In the first of three experiments we attempted to manipulate follicle size patterns before an ASS exposure with the objective of testing the possibility that stage of follicle development by the time of ovulation could be the putative causative factor in the occurrence of SLCL. Although a previous report (Ungerfeld et al., 2004) suggested otherwise, (1) the limited number of ewes used in that trial could have compromised the detection of even large effects and (2) oestrous and ovulatory responses reported for that study did not fit expected patterns of ewes successfully induced to ovulate by rams.

In two other experiments we aimed at identifying a time frame for the P_4 priming effect. Such information is lacking for treatments applied more than 5 days in advance of a ram exposure. Five days is a critical time boundary because it approximates the average length of a follicular wave in ewes. Any effects of priming in advance of such a boundary would be suggestive of P_4 not directly acting upon gonadotrophin-dependent follicles and rather supportive of a role for P_4 in preventing $PGF_2\alpha$ release from the uterine endometrium, a process controlled by the interplay of E_2, P_4 and OT uterine receptors (McCracken et al., 1999). As P_4 priming is a critical component of most out-of-season breeding protocols, time-enveloping its effects also has direct practical implications.

2. Materials and methods

2.1. Location, animals, and management

The experiments were conducted at the Argerich Experiment Station (latitude: 38°44′S) during two consecutive anoestrous seasons. Experiments 1 and 2 were run concurrently on the first year of the study, followed by Experiment 3 in the subsequent year. Management and protocols followed standards for the humane use and care of experimental animals (Consortium for Developing a Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching, 1999). In both years, a Corriedale flock kept on a once-a-year lambing schedule (early autumn mating) was sampled for adult ewes (2- to 6-year-old) to be used in the experiments. Ewes are managed year round under range conditions except during late gestation and early lactation when they graze winter crops. All ewes were kept isolated (sight, sound, smell) from adult males since the end of their previous mating period and were either dry (Experiment 1) or recently weaned (Experiments 2 and 3) by the time they were used.

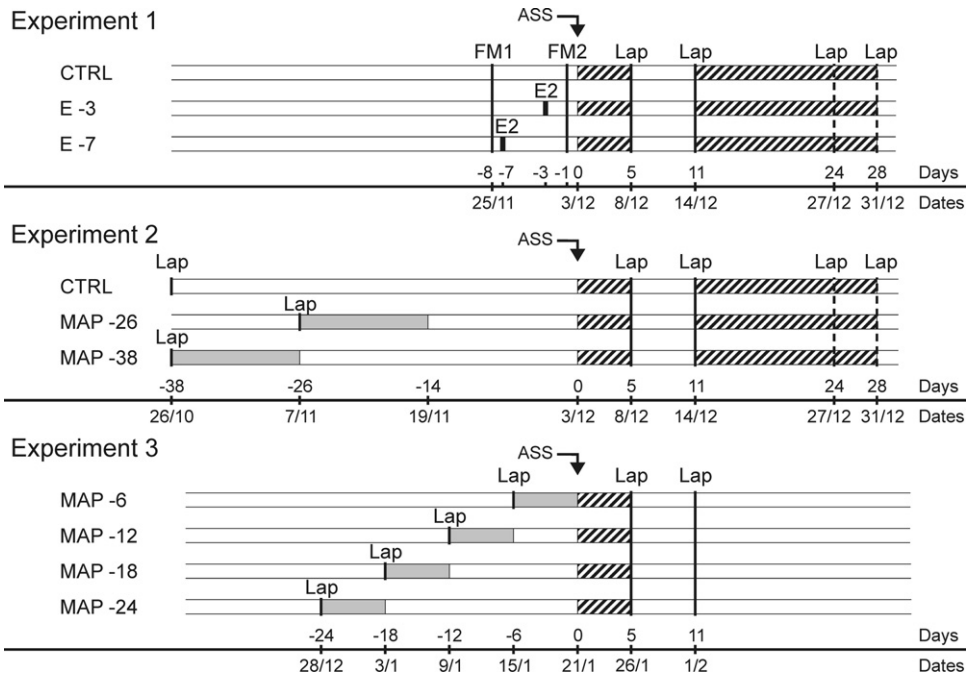

Fig. 1. Timelines (dates and days elapsed relative to the onset of allogenic sexual stimulation: 'ASS') displaying main events for Experiments 1, 2, and 3: Follicle mappings ('FM'), exploratory laparoscopies ('Lap') and oestradiol injections ('E2'). Greyed and striped segments indicate periods of sponge insertion and control of rump marks, respectively. See text for treatment descriptions.

2.2. Design and experimental procedures

Mature Corriedale ewes and vasectomized sexually experienced Milchschaf X Corriedale rams wearing marking harnesses (20 in Experiments 1 and 2, which were run concurrently, 9 in Experiment 3) were used as ASS for the experimental females. Ewes in the ASS groups were brought into standing oestrus as required by applying intramuscular injections of 500 μg of oestradiol propionate following a 6-day progestagen priming period (intravaginal sponges impregnated with 40 mg of medroxyprogesterone acetate, MAP).

Mid ventral laparoscopies were applied during the course of the studies to assess follicular, ovulatory, and luteal responses. Laparoscopies were performed under local anaesthesia and by a highly trained operator. Verifying the occurrence of ovulation, as determined by the presence of corpora lutea, usually demanded less than a minute; follicle mapping (Experiment 1, see below) took between 1 and 3 min.

2.2.1. Experiment 1

Ninety anovular ewes, chosen out of a group of 100 dry ewes screened by laparoscopy in late November (92/100 found acyclic), have their ovaries mapped (diagrammatic recording on paper) for the presence of follicles larger than 1 mm in diameter (FM1, follicle mapping 1, performed on Day −8 relative to the start of the ASS on Day 0 = December 3; Fig. 1, Experiment 1). This was achieved with the help of a stainless steel probe (3 mm in diameter) with a 1.5-cm-long scale engraved at the tip and marked at 1 mm intervals. The probe, normally handled for manipulating uterine horns and ovaries, was used for the comparative assessment of follicle dimensions to the closest mm. Mapping was performed for follicles sized 2 mm or larger; the presence of smaller follicles (1 mm or less) on a given ovarian region was recorded but not mapped. The entire surface of each ovary was diagrammatically depicted in 2D as two ellipses conjoined along the distal edge of the sagittal plane of the ovary. Digital photography was also used to better document the appearance of ovarian surfaces, particularly when a high number of follicles required mapping.

After the initial screening, ewes were randomly assigned to three experimental groups of 30 anovular ewes stratified by age (4 ± 1.8 years; mean ± s.d.), live weight (57 ± 8.1 kg; mean ± s.d.), body condition score (2.7 ± 1.0, mean ± s.d.; scale 1 [emaciated] to 5 [obese]; Jefferies, 1961), and follicle size distribution. Treatments applied to these groups (Fig. 1, Experiment 1) were: i.m. injection of 50 μg of oestradiol benzoate (Burnet Laboratories, Buenos Aires, Argentina) applied 7 (E_2 −7) or 3 (E_2 −3) days before ASS exposure, or sham-injection (CTRL; untreated control). The E_2 dose was chosen on the basis of its apparent ability for altering follicular wave patterns while not inducing ovulation (0 ovulations out of 11 treated ewes in Meikle et al., 2001; 0 out of 7 in Ungerfeld et al., 2004; both studies using Corriedale ewes). The 90 anovular ewes were exposed to 20 rams and 20 oestrous ewes (10 present at the onset of ASS, another 10 added 24 h later) along with the 121 anovular ewes involved in Experiment 2 (see below). Thus, the ratio of ASS animals relative to the total number of anovular ewes exposed was approximately 1:5.

A second follicle mapping session (FM2) was performed on the day before the onset of the ASS exposure period

(Day −1, Fig. 1, Experiment 1). Both follicle mappings were treatment-blind, but FM1 mappings were used as supporting information for FM2. Follicles mapped in both sessions were classified (within subject and session, and on the basis of diameter) to determine the largest, second largest, and third largest follicle observed. Comparisons between experimental groups were later performed for those three classes.

By comparing relative sizes and locations of follicles mapped in both sessions, we identified ewes in which a single follicle, larger than the rest, was mapped occupying different locations in both mapping sessions thus implying turnover of a potentially ovulatory follicle. We also identified ewes in which the largest follicle mapped on FM2 was at the same location as on FM1; differences in relative size between mappings were checked for consistency with the notion that such a follicle was presumably the same.

Laparoscopies were also performed to assess ovulation response (on Day 5), incidence of SLCL (on Day 11), and occurrence of ovulation in ewes showing oestrus after either a cycle of normal length (on Day 24) or a short cycle followed by a normal length cycle (on Day 28). Ewes showing corpora haemorrhagica or recently formed corpora lutea (shinny, deeply red surface, Oldham and Lindsay, 1980) on Day 11 following a record of an initial silent ovulation detected on Day 5, were considered as having experienced a short luteal phase.

Ram exposure lasted for 28 days. During that period animals were housed in a 1.5 ha enclosure and were daily screened for oestrus occurrence (as indicated by rump marks) from Days 0 to 5 and 11 to 28.

2.2.2. Experiment 2

Recently weaned adult ewes ($n = 121$) were randomized (after stratifying by age [4 ± 1.6 years; mean ± s.d.], live weight [54 ± 5.8 kg; mean ± s.d.], and body condition score [2.4 ± 1.1, mean ± s.d.]) to three experimental groups (Fig. 1, Experiment 2) that received intravaginal sponges (40 mg of MAP, Gador Laboratories, Buenos Aires, Argentina) for 12 days starting either 38 (MAP −38; $n = 38$) or 26 (MAP −26; $n = 42$) days before ASS exposure, or acted as untreated controls (CTRL; $n = 41$). Anovulation in these ewes was confirmed by the absence of any recent luteal structures visible on the surface of the ovaries on the day of sponge insertion. Ewes in the CTRL group were subjected to laparoscopy with the same purpose on the day of sponge insertion to the MAP −38 group. The experimental groups were managed as a single mob grazing native pastures until the start of the exposure period (Fig. 1, Experiment 2) when they were merged with the anovular ewes in Experiment 1 ($n = 90$) and exposed to the same ASS group of 20 rams plus 20 oestrous ewes (see above). Thus, the ratio of ASS animals relative to the combined number of anovular ewes in both experiments was approximately 1:5. Oestrus monitoring and additional laparoscopies were performed as described for ewes in Experiment 1.

2.2.3. Experiment 3

Recently weaned adult ewes ($n = 138$) were randomized (after stratifying by age [4 ± 1.8 years; mean ± s.d.], live weight [50 ± 4.1 kg; mean ± s.d.], and body condition score [1.8 ± 0.4, mean ± s.d.]) to four experimental groups (Fig. 1, Experiment 3) that received intravaginal sponges (40 mg of MAP, Gador Laboratories, Buenos Aires, Argentina) for 6 days starting 24 (MAP −24; $n = 34$), 18 (MAP −18; $n = 35$), 12 (MAP −12; $n = 34$), or 6 days (MAP −6; $n = 35$) before an ASS exposure. The MAP −6 group acted as a positive control with a 0% expected incidence of SLCL (Oldham et al., 1985). As in Experiment 2, anovulation (absence of any luteal structure) was verified on the day of sponge insertion to each experimental group. Ewes were joined to rams ($n = 9$) and oestrous ewes ($n = 10$) on Day 0; in this experiment the ratio of ASS animals relative to the number of anovular ewes was approximately 1:7. Experimental ewes were then monitored for oestrus occurrence until Day 5, and examined by laparoscopy on days 5 and 11 (Fig. 1, Experiment 3) to determine incidence of SLCL as described above.

2.3. Statistical analyses

In the three experiments Chi-square tests were used on categorical variables for the assessment of both overall effects and pre-planned comparisons of differences between treatments; Fisher's exact test was applied instead when the expected count for any cell was less than 5. In Experiment 1 fixed-effect ANOVAs were applied to test for differences in follicle sizes; confidence intervals (0.05 family-wise error rate) were calculated to separate paired differences using the Bonferroni method. After testing for treatment effects, ewes were classified according to their luteal responses (SLCL or NLCL) irrespective of treatment. The resulting data set was explored using ANOVA for continuous variables and generalized linear models with binomial errors and logit link function (McCullagh and Nelder, 1989) for the binary response variable 'occurrence of NLCL or SLCL'. Results for continuous variables are presented as mean ± s.e. In Experiment 3, linear and quadratic terms were fitted through linear regression to evaluate a time trend in the occurrence of SLCL. Analyses and graphics were programmed using S-Plus 2000 (MathSoft Inc., 2000).

3. Results

3.1. Experiment 1

Mean sizes of the three largest follicles classes mapped at FM1 (Day −8) were similar across experimental groups ($P = 0.51$–0.86; Table 1). A sizeable number of ewes had recently formed corpora lutea by the time FM2 was performed. Ewes ovulating in between FM1 and FM2 came mostly ($P = 0.001$) from the E_2 treated groups (24/60) rather than the control (2/30), and occurred in a higher proportion ($P = 0.003$) among early (E_2 −7: 18/30) than late (E_2 −3: 6/30) treated ewes. These recently ovulated ewes were kept in the flock, and their luteal lifespans were assessed (see below), but they were not considered for further analyses.

The size of the largest, second largest, and third largest follicles present on Day −1 in the remaining ewes (12 in E_2 −7, 24 in E_2 −3, 28 in CTRL), was affected by the treatments ($P = 0.043$–0.001, Table 1). Confidence intervals (95%)

Table 1
Experiment 1. Size-ranked follicle sizes, and largest follicle turnover assessment in ewes injected with 50 μg of oestradiol benzoate 7 (E_2 −7) or 3 (E_2 −3) days before an allogenic sexual stimulation (ASS), or sham-injected controls (CTRL).

	E_2 −7		E_2 −3		CTRL		*P*	
	Day −8[a]	Day −1	Day −8	Day −1	Day −8	Day −1	Day −8	Day −1
n	30	12	30	24	30	28		
Follicle sizes (mm ± s.e.)								
Largest	4.0 ± 0.22	5.2 ± 0.47	4.2 ± 0.22	3.7 ± 0.35	4.2 ± 0.22	3.9 ± 0.31	0.727	0.043
Second largest	2.9 ± 0.18	3.7 ± 0.27	2.8 ± 0.19	2.6 ± 0.19	2.6 ± 0.18	2.6 ± 0.17	0.510	0.002
Third largest	2.3 ± 0.13	3.2 ± 0.23	2.4 ± 0.13	2.0 ± 0.17	2.3 ± 0.13	2.2 ± 0.15	0.856	0.001
Ewes in which location of largest follicle changed from Day −8 to Day −1 (%)		12/12 (100.0)		15/23[b] (65.2)		20/28 (71.4)		0.057

[a] Relative to onset of ASS.
[b] Incomplete recording of follicle location prevented turnover assessment in one E_2 −3 ewe.

separated E_2 −7 from the control group in the three follicle size classes; in contrast, CTRL and E_2 −3 ewes showed similar mean follicle sizes (Table 1). The proportion of ewes in which the largest follicle observed in FM2 was located in a different position than in FM1, i.e. ewes in which it could be safe to assume that turnover of the largest follicle had occurred, tended to differ among groups ($P = 0.057$; Table 1). This overall trend was traced to a higher ($P = 0.027$) turn over incidence in E_2 −7 (12/12) than in the other two groups (35/51).

Comparable proportions of ewes ovulated across treatments within 5 days of contact with the ASS group (Table 2). None of those ewes displayed oestrus. Four ewes in E_2 −7, two in E_2 −3, and one in CTRL developed unruptured follicles which were clearly filled with luteal tissue by the time ovulation response was assessed on Day 5. In one E_2 −7 and one E_2 −3 ewe the *luteinized unruptured follicle* (LUF) accompanied a morphologically normal CL. Most LUFs developed from follicles which were substantially larger than average when last observed on Day −1: 6, 6, 8, 8 (E_2 −7), 5, 4 (E_2 −3), and 8 (CTRL) mm. The incidence of LUFs differed ($P = 0.032$) across treatments (Table 2) and was higher ($P = 0.022$) in E_2 −7 (4/12) than in the CTRL group (1/28). The combined luteal response calculated by merging CL and LUFs responses was similar ($P = 0.55$) across treatments (Table 2). No ewes were detected in oestrous before Day 14 of ram exposure. The proportion of ewes marked over the 28 days of contact with the rams did not differ ($P = 0.67$) among treatments (Table 2); time to first oestrus detection was also similar.

The proportion of ewes ovulating the largest follicle present on Day −1 did not differ across treatments ($P = 0.12$) but the mean size of such follicles did ($P = 0.029$, Table 3). This overall effect was due in part to smaller follicle sizes (95% Bonferroni CI of the differences excluding zero) in E_2 −3 than in CTRL ewes (Table 3). In contrast, the mean sizes of the three largest non-ovulated non-luteinized follicles were homogeneous across treatments ($P = 0.29$–0.49, Table 3).

The incidence of short luteal phases either associated exclusively to SLCL or including the 4 LUF cases not associated with concurrent CL (3 short-lived, 1 lasting for the expected period), was not affected by the treatments ($P = 0.93$ and $P = 0.66$, respectively; Table 3). Limiting the comparison to ewes in which turnover of the largest follicle occurred before ASS exposure still did not reveal treatment effects ($P = 0.10$, Table 3) and further narrowing the comparison to only ewes developing CL also failed ($P = 0.37$) at exposing any treatment differences. Short-lived CL, in turn, were the norm (10/11) among ewes not experiencing follicular turnover, with comparable incidences in both E_2 −3 and CTRL ewes (Table 3) although none of the persistent largest follicles present in those 10 ewes on Day −1 was ovulated.

Out of the 16 ewes in which the largest follicle recorded in both mapping sessions was presumably the same (8 CTRL and 8 E_2 −3 ewes, Table 1), 4 (all CTRL ewes) did not respond to the ASS, 11 ovulated, and 1 developed an LUF. Thus, ewes not exhibiting follicular turnover before ASS exposure showed a lower ($P = 0.032$) incidence of luteal response

Table 2
Experiment 1. Ovulation and oestrous responses of ewes injected with 50 μg of oestradiol benzoate 7 (E_2 −7) or 3 (E_2 −3) days before an allogenic sexual stimulation (ASS), or sham-injected controls (CTRL).

	E_2 −7	E_2 −3	CTRL	*P*
Ewes ovulating (%)[a]	8/12 (66.7)	22/24 (91.7)	23/28 (82.1)	0.193
Ewes developing LUFs[b] (%)	4/12 (33.3)	2/24 (8.3)	1/28 (3.6)	0.032
Total luteal responses (%)[c]	11/12 (91.7)	23/24 (95.8)	24/28 (85.7)	0.553
Ewes marked (%)	10/12 (83.3)	17/24 (70.8)	23/28 (82.1)	0.668
Interval to oestrus (Days ± s.e.)[d]	20.8 ± 1.14	18.6 ± 0.90	19.9 ± 0.77	0.287

[a] Within 5 days of contact with the ASS group.
[b] Luteinized unruptured follicles.
[c] Ewes forming CLs plus ewes forming LUFs. One E_2 −7 and one E_2 −3 ewe developing both CL and LUF were counted once for total response.
[d] Days elapsed from onset of ASS to first detection of rump marks.

Table 3
Experiment 1. Retrospective follicle patterns and luteal lifespan responses in ewes injected with 50 μg of oestradiol benzoate 7 (E_2 −7) or 3 (E_2 −3) days before an allogenic sexual stimulation (ASS), or sham-injected controls (CTRL).

	E_2 −7	E_2 −3	CTRL	*P*
Ewes ovulating the largest follicle present on Day −1 (%)[a]	2/8 (25.0)	6/21[c] (28.6)	13/23 (56.5)	0.123
Size of largest ovulated follicle when measured on Day −1 (mm ± s.e.)[b]	3.1 ± 0.35	2.2[c] ± 0.21	3.0 ± 0.21	0.029
Size of non-ovulated follicles when measured on Day −1 (mm ± s.e.)[d]				
Largest	3.9 ± 0.55	3.6[c] ± 0.34	3.2 ± 0.32	0.486
Second largest	2.7 ± 0.25	2.4[c] ± 0.16	2.3 ± 0.15	0.322
Third largest	2.4 ± 0.21	2.0[c] ± 0.13	2.0 ± 0.12	0.293
Short lived CL (%)	4/8 (50.0)	10/22 (45.5)	12/23 (52.2)	0.931
Total short luteal phases (%)[e,f]	7/11 (63.6)	10/22 (45.5)	12/24 (50.0)	0.657
Total short luteal phases after largest follicle turnover (%)[e,f]	7/11 (63.6)	3/14 (21.4)	8/20 (40.0)	0.099
Total short luteal phases in ovulating, non-turnover ewes (%)[e]	–	6/7 (85.7)	4/4 (100.0)	1.000

[a] Ewes with CL mapped on Day 5 in the same location previously occupied by the largest follicle identified on Day −1.
[b] Size of largest follicle mapped on Day −1 in the same position afterwards occupied by a CL on Day 5.
[c] Incomplete recording of follicle location prevented assessment in one E_2 −3 ewe.
[d] Limited to ewes with CL and no LUFs on Day 5.
[e] Includes luteal phases originating from LUFs not associated with concurrent CL (3 in E_2 −7, 1 in CTRL); excludes one E_2 −3 ewe developing a LUF that persisted for the duration of the experiment.
[f] Two ewes experienced follicular turn-over but did not respond to the ASS (1 in E_2 −7, 1 in E_2 −3).

(75.0%, 12/16) than ewes in which follicle turnover did occur (95.7%), i.e. in 45 (Table 3) out of 47 (Table 1) ewes, with only 2 ewes not responding to the ASS, 1 in E_2 −7 and 1 in E_2 −3.

When ovulating ewes were classified according to luteal response (NLCL or SLCL) irrespective of treatment (Table 4) an association was apparent ($P = 0.018$) between the type of response and the size of the largest follicle present on Day −1. A similar trend ($P = 0.076$) was found for second largest follicles. Further exploration of the size-response association revealed a non-linear relationship between the probability of occurrence of a luteal response of expected duration and the size of the largest follicle present before ASS. Both linear ($P = 0.001$ or $P = 0.016$) and quadratic ($P = 0.002$ or $P = 0.002$) terms were required in the linear predictor to best describe the relationship either with (results not shown) or without (Fig. 2) the inclusion of LUFs with an informative lifespan record. Quite similar fits were obtained in both cases with 25 and 21% of the null deviance (the generalization of a total variance for non-normal error distributions) explained by follicle size alone, with or without the inclusion of LUFs, respectively. The shape of the relationship (Fig. 2) was primarily accounted for by a sharp drop in the probability of expected lifespan for large-sized follicles. In fact, the probability of expected lifespan was similar ($P = 0.54$) for follicles of 2, 3, or 4 mm in diameter as measured on Day −1.

The development of a CL of expected lifespan was strongly associated to turnover of the largest follicle before Day −1 (26 out of 27 cases, Table 4). Short-lived CL, in contrast, developed in comparable proportions in ewes both experiencing and not experiencing turnover (15 out of 25 cases); these two response patterns differed ($P = 0.002$, Table 4). Relative to NLCL, SLCL were also associated to a lower ($P = 0.026$) frequency of ovulation of the largest follicle present. Largest ovulating follicles originating either SLCL or NLCL were of similar mean size ($P = 0.85$, Table 4). In contrast, the size of the largest and second largest non-ovulated follicles differed ($P = 0.018$ and $P = 0.058$) according to CL lifespan. When the analysis was restricted to ewes experiencing turnover before Day −1 (26 developing NLCL and 15 developing SLCL), trends for larger sizes associated to SLCL were still detected for the largest follicle present on Day −1 (4.1 ± 0.33 vs. 3.3 ± 0.25, $P = 0.065$)

Table 4
Experiment 1. Follicular end-points and interval to oestrus in ewes developing CL of expected or short lifespan irrespective of treatment. Treatments were injection of 50 μg of oestradiol benzoate 7 (E_2 −7) or 3 (E_2 −3) days before an allogenic sexual stimulation (ASS), or sham-injection (CTRL).

	Corpora lutea		*P*
	Expected lifespan	Short-lived	
n	27	25[a]	
Follicles sizes on Day −1 (mm ± s.e.)			
Largest	3.4 ± 0.27	4.4 ± 0.29	0.018
Second largest	2.5 ± 0.15	2.9 ± 0.16	0.076
Third largest	2.2 ± 0.14	2.4 ± 0.14	0.211
Ewes in which location of largest follicle changed from Day −8 to Day −1 (%)	26/27 (96.3)	15/25 (60.0)	0.002
Ewes ovulating the largest follicle present on Day −1 (%)	15/27 (55.6)	6/25 (24.0)	0.026
Size of largest follicle ovulated when measured on Day −1 (mm ± s.e.)	2.7 ± 0.20	2.7 ± 0.21	0.855
Size of largest non-ovulated follicles when measured on Day −1 (mm ± s.e.)			
Largest	3.0 ± 0.28	4.0 ± 0.29	0.018
Second largest	2.2 ± 0.13	2.6 ± 0.14	0.058
Third largest	2.0 ± 0.11	2.1 ± 0.12	0.617
Interval to oestrus (Days ± s.e.)	16.5 ± 0.30	22.7 ± 0.33	0.001

[a] One E_2 −3 ewe developing a short-lived CL had an incomplete follicle record.

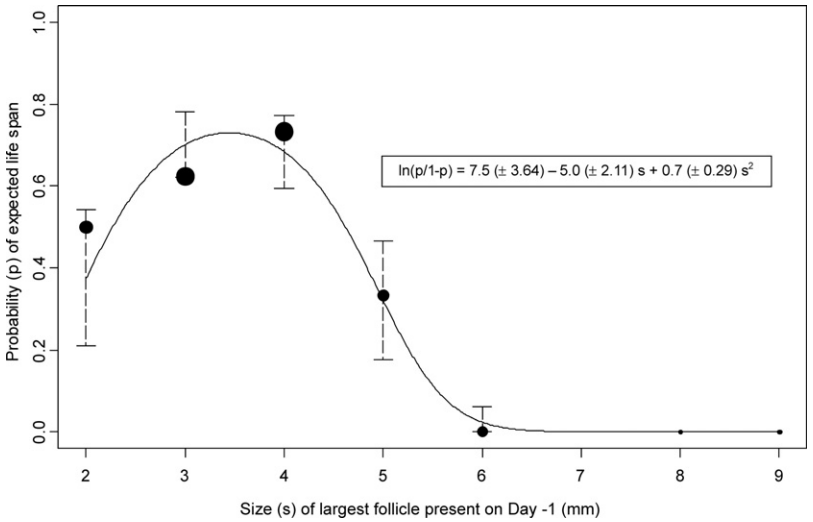

Fig. 2. Probability (p) of occurrence of corpora lutea of expected life-span as a function of the size (s) of the largest follicle present in the ovaries on the day before the onset of an allogenic sexual stimulation with rams and oestrous ewes. Dot sizes are proportional to the total number of follicles in each size class. Vertical lines indicate 2 standard errors around predicted values.

and for the largest non-ovulated follicle (3.6 ± 0.31 vs. 2.9 ± 0.23, $P = 0.09$). Mean sizes of largest ovulated follicles (2.9 ± 0.28 vs. 2.7 ± 0.21, $P = 0.49$) remained similar ($P = 0.49$) between ewes developing NLCL (2.7 ± 0.21) or SLCL (2.9 ± 0.28).

As expected from ovulation response patterns, the interval to first oestrus in ewes experiencing the SLCL syndrome was delayed ($P = 0.001$) by approximately 6 days; onset of oestrus varied from 21 to 25 days in SLCL ewes and from 14 to 20 days in NLCL ewes.

As indicated above, ewes ovulating in between the first and second mapping sessions were again screened by laparoscopy on Day 5 to determine incidence of SLCL. The combined incidence of SLCL among these ewes (15/16, 93.8%; 2/2 in CTRL, 5/6 in E_2 −3, and 8 out of a random sample of 8 taken from the original 16 E_2 −7 ewes ovulating) was higher ($P = 0.003$) than that later recorded in their flockmates after ASS exposure (26/53; 49.1%).

3.2. Experiment 2

The proportion of ewes ovulating within 5 days of ASS exposure was similar ($P = 0.75$) for MAP-treated and control ewes (Table 5). The incidence of SLCL differed across treatments ($P = 0.003$); SLCL were less frequent ($P = 0.022$) in ewes exposed to MAP from 26 to 14 days before stimulation (11/34) than in the untreated controls (22/35). No effect of MAP was apparent when a similar treatment was discontinued 26 days in advance of the ASS exposure ($P = 0.53$ for the comparison between MAP −38 and CTRL). No ewes were detected in oestrus during the first 5 days of contact with the rams but all ewes successfully stimulated were eventually marked by the rams. Ewes experiencing oestrus after NLCL or SLCL clustered around 17 ± 0.4 or 23 ± 0.3 days after the start of the ASS, respectively.

3.3. Experiment 3

As in Experiment 2, timing of MAP treatment did not affect ($P = 0.39$) the occurrence of ovulation within 5 days of the ASS (Table 6). A high proportion of ewes exposed to MAP for the 6 days preceding the ASS (30/32) experienced oestrus and were marked by the rams within 5 d; that was a higher ($P = 0.001$) proportion than observed in the other 3 groups combined (13/100). Unexpectedly, the interval to oestrus was heterogeneous ($P = 0.001$) across treatments; 95% CI separated MAP −6 from each of the other three groups. Approximately half of the ewes showing oestrus in MAP −24 (2/2), MAP −18 (4/8), and MAP −12 (1/3) developed SLCL.

The occurrence of SLCL was affected by the treatments ($P = 0.001$); comparisons of individual treatments against the positive control (MAP −6) were all significant ($P = 0.001$). A decline ($P = 0.001$ for a linear trend, $P = 0.058$

Table 5
Experiment 2. Ovulation and oestrous response of ewes primed with progestagen (40 mg of medroxyprogesterone acetate) for 12 days starting 38 (MAP −38) or 26 (MAP −26) days before an allogenic sexual stimulation (ASS) in late spring, or untreated controls (CTRL).

	MAP −38	MAP −26	CTRL	P
Ovulating ewes (%)[a]	30/38 (78.9)	34/42 (80.9)	35/41 (85.4)	0.749
Short luteal phases (%)	22/30 (73.3)	11/34 (32.3)	22/35 (62.8)	0.003
Oestrus and ovulation (%)[b,c]	27/27 (100.0)	31/31 (100.0)	34/34 (100.0)	1.000

[a] Within 5 days of contact with the ASS group.
[b] Ewes were detected in oestrus from Day 16 to Day 25 after the onset of the ASS.
[c] Seven ewes were not screened for ovulation due to excessive rumen fill.

Table 6
Experiment 3. Ovulation, oestrous, and luteal lifespan responses of anovular ewes to a 6-day priming with 40 mg of medroxyprogesterone acetate started 24 (MAP −24), 18 (MAP −18), 12 (MAP −12) or 6 (MAP −6) days before an allogenic sexual stimulation (ASS) in early summer.

	MAP −24	MAP −18	MAP −12	MAP −6	*P*
Ovulating ewes (%)[a]	33/34 (97.1)	35/35 (100.0)	32/34 (94.1)	32/35 (91.4)	0.390
Marked ewes (%)[b]	2/33 (6.1)	8/35 (22.9)	3/32 (9.4)	30/32 (93.8)	0.001
Interval to oestrus (Days ± s.e.)	5.0 ± 0.51	3.9 ± 0.26	4.3 ± 0.42	2.4 ± 0.13	0.001
Short luteal phases (%)	24/33 (72.7)	21/35 (60.0)	11/32 (34.4)	0/32 (0.0)	0.001

[a] Within 5 days of contact with the ASS group.
[b] All ewes were detected in oestrus within 5 days of the onset of ASS exposure.

for and additional quadratic term) in SLCL incidence was detected as the period of treatment approached the day of exposure to the ASS. However, complete suppression of the SLCL syndrome was achieved only when the MAP treatment was discontinued immediately before the start of the ASS.

4. Discussion

Ovulation and oestrus results from the 3 trials agreed with known responses of anovular Corriedale ewes to an ASS exposure in spring-summer (e.g. Rodríguez Iglesias et al., 1991, 1992, 1996; Pevsner et al., 2010); ovulation (88.6% overall average) and proportion of ewes marked by the rams (92.0% overall average) were consistently high across trials. The incidence of SLCL across treatments approached 50% for dry ewes in Experiment 1, in line with the typical response in moderately seasonal breeds (Martin et al., 1986). In the other two trials SLCL occurred more frequently in ewes either unprimed or primed well in advance of the ASS which agrees with previous observations (Pevsner et al., 2010). Oestrous distributions followed the expected biphasic pattern with peaks around days 16–17 (NLCL ewes) and 22–23 (SLCL ewes) after the onset of the ASS (Martin et al., 1986). Also as expected, a 6-day progestagen priming discontinued immediately before the ASS (MAP −6, Experiment 3) induced synchronized oestrus and was 100% effective at preventing SLCL (Rodríguez Iglesias et al., 1996). In contrast, ewes ovulating with oestrus after a P_4 priming terminated 6 to 18 days before the ASS (Experiment 3) were a novel finding. Unprimed ewes induced to ovulate during seasonal anoestrus rarely exhibit oestrus (Schinckel, 1954) and the responses observed in Experiment 3 (up to 23% in MAP −18) largely exceeded what has been occasionally reported in the literature (Martin et al., 1986). Two possible explanations are a conserved effect of early MAP priming promoting oestrus behaviour in some ewes, or the occurrence of GnRH-supported rather than E_2-supported oestrus (Caraty et al., 2002). Although there is no supporting evidence for any of those two possibilities, most oestrous ewes (11/13) were marked by the rams *after* the expected time of ovulation (i.e. around 50 h after the onset of stimulation, Martin et al., 1986) which suggests a possible involvement of the residual GnRH release normally following an LH surge (Caraty et al., 2002). Caraty et al. (2002) characterized the phenomenon as a sequential pattern of GnRH-supported following E_2-supported oestrus with P_4 involved in both mechanisms. The GnRH secretion support provided by the continuous presence of the ASS group and the timing of the MAP treatments may have triggered GnRH-supported oestrous in some ewes thus explaining the incidence and timing of our observations.

In contrast to previous reports (Meikle et al., 2001; Ungerfeld et al., 2004), we observed a significant proportion of ewes ovulating after E_2 injections, which is in agreement with other attempts at using E_2 on anovular ewes. Beck and Reeves (1973) reported 24% (6/25) of ovulations and Barret et al. (2008) found 5 luteal responses out of 12 treated ewes (i.e. a 42% incidence). Barret et al. (2008) used a relatively high E_2 dose (350 μg), but Beck and Reeves (1973) and Reeves et al. (1974) showed that varying E_2 doses from 12.5 to 200 μg had very similar effects on LH and FSH secretion patterns. As the ability of E_2 to induce ovulation is related to its effect on LH and FSH secretion (Beck and Reeves, 1973; Reeves et al., 1974), it is likely that the response threshold of the ewes used by Meikle et al. (2001) and Ungerfeld et al. (2004) was relatively high due to either chronic ram presence (Meikle et al., 2001) or low ram libido, transportation stress, and lack of adaptation (Ungerfeld et al., 2004).

In our study exogenous E_2 may have head-started an LH-E_2 feed-forward loop ultimately triggering an LH surge in ewes that were close to endogenously emerge from seasonal anoestrus. The high incidence of SLCL recorded in E_2-injected ewes ovulating before the ASS (13/14, 93%) is discussed below in relation to the possible mechanism of the SLCL syndrome.

Oestradiol-induced ovulations were a nuisance and may also have affected the recorded responses of E_2 −3 ewes. Some E_2 −3 ewes might have been in course to ovulation under the effect of injected E_2 by the time they were exposed to the ASS group. However, the similarity of follicular size patterns in E_2 −3 and CTRL ewes at FM2 suggests that any effect of E_2 on follicle size distributions would probably take more than 2 but probably less than 6 days (as in E_2 −7) to develop. In addition of inducing ovulation in some ewes, E_2 had a marked effect on the mean size of the three largest follicle classes in E_2 −7 ewes. Assuming a new follicular wave emerges 2.5–3 days after an E_2 injection (Meikle et al., 2001; Ungerfeld et al., 2004; Barret et al., 2008), and considering a combined average length of about 5 days for the growing and static phases of an average follicular wave during anoestrus (Bartlewski et al., 1998), the largest follicles in E_2 −7 ewes should have reached their maximum size by the time of FM2. Most ewes in E_2 −3, in contrast, should have had a cohort of follicles just starting their growing phase under the effect of E_2; some others may have being well into the regressing phase of a previous wave. Ewes in the CTRL group, in turn, would be expected to

display a random, non-synchronized pattern. Under these assumptions, larger mean follicle sizes would be expected for E_2 −7 rather than for E_2 −3 or CTRL ewes and that was what we observed, thus supporting an E_2 effect on follicular wave emergence. The effect of E_2 treatments on our estimate of largest follicle turnover over the period elapsing from FM1 to FM2 also supports the notion of E_2-induced follicular turnover in E_2 −7 ewes. Turnover in the other two groups was somewhat lower than expected (65% and 71%) considering the average duration of a follicular wave in seasonally anovular ewes (Bartlewski et al., 1998). This may have been due to the limitations imposed by the experimental protocol (i.e. continuous follicle monitoring was beyond consideration given the number of ewes involved and the likely discomfort to be imposed on the subjects) and to the conservative nature of the estimated turnover.

Behavioural oestrus and ovulation, and incidence of SLCL were not affected by the E_2 treatments. The luteal response, either limited to CL or including LUFs, was homogeneous across experimental groups although the incidence of LUFs differed between treatments. Luteinized unruptured follicles are uncommon findings after successful stimulation of seasonally anovular ewes (e.g. only 1 out of 288 ewes not treated with E_2 and exposed to ASS in our 3 trials combined) although they are frequently observed associated to poor responses to both ASS (e.g. Ungerfeld et al., 2004) and GnRH (e.g. Rubianes et al., 1997) protocols. Two common factors linked to LUF occurrence in Experiment 1 were E_2 treatment (6 out of 7 cases, Table 2) and presence of a large follicle at the onset of the ASS. These two factors, in turn, appeared associated in a seemingly causative manner in the E_2 −7 treatment which induced comparatively larger follicle sizes by the time ewes were exposed to the ASS and showed the highest frequency of LUFs across treatments. Thus the E_2 treatment most likely augmented the incidence of LUFs in E_2 −7 ewes via a follicle development effect which was timed by design to the start of the period of increased LH pulsatility triggered by the ASS. Large, antral, morphologically normal but hormonally impaired follicles may persist on the ovaries (Souza et al., 1997) and respond to a luteinizing stimulus. This may explain the puzzling high incidence of LUFs reported by Rubianes et al. (1997) when anovular Corriedale ewes with almost exclusively large (6 mm) follicles present were GnRH-challenged during seasonal anoestrus.

As assessed on Day −1, E_2 treatments induced different mean sizes of largest follicles eventually ovulated (Table 3). The comparatively smaller size of the largest ovulated follicles in E_2 −3 supports the turnover effect of E_2 discussed above. Follicular turnover before the ASS affected the occurrence of short luteal phases. However, modifying the pattern of follicle development did not affect the incidence of SLCL, and post hoc comparisons between ewes with contrasting histories of follicular dynamics (e.g. ovulation of largest vs. smaller follicles) did not reveal any patterns. Ovulation or luteinization of the comparatively larger follicles present in E_2 −7 on Day −1 effectively removed any differences in follicle sizes across treatments. In other words, after accounting for size differences associated to ovulation and LUF formation, populations of follicles 2 mm and larger originating before Day −1 were comparable across treatments.

Experiments 2 and 3 were aimed at approximating a time frame for exogenous progestagens to prevent SLCL occurrence. A 12-day treatment terminated 14 days before an ASS (MAP −26, Experiment 2) prevented SLCL in a significant proportion of ewes. Anticipating the treatment by 12 more days (MAP −38, Experiment 2) or shortening it to 6 days (MAP −18, Experiment 3) failed at preventing the syndrome. Although partially successful, anticipated treatments (MAP −26 in Experiment 2, MAP −12 in Experiment 3) did not prevent SLCL completely as the conventional short-term treatment did (MAP −6, Experiment 3). Assuming that circulating MAP will rapidly dwindle after sponge removal, MAP −26 (Experiment 2) and MAP −12 (Experiment 3) treatments bound partially successful progestagen treatments below an upper limit of 14 days (some SLCL prevented) and a lower limit of 6 days (highly, but not completely effective) in advance of an ovulation-inducing treatment. Those boundaries would be additionally dependent upon the duration of progestagen exposure. At the lower end, complete prevention of SLCL was only achieved when MAP support was kept in place right up until the onset of the ASS protocol (MAP −6, Experiment 3). Although there are no previous reports on short-term progestagen treatments discontinued in advance of an ASS, a single dose of 20 mg of P_4 injected 5 days before inducing ovulation was fairly effective at preventing SLCL (0 out of 10 ewes exposed to rams by Pearce et al. (1987); 2 out of 10 ewes treated with GnRH by Mann and Haresign (2001)). Those findings, and the sharp decline in SLCL prevention as MAP treatment was progressively anticipated in Experiment 3, suggest that around 5 days in advance of an ovulation-inducing treatment lies some critical temporal boundary for the therapeutic effect of MAP. Beyond that boundary beneficial effects still occur until a second boundary is reached, somewhere within a 14- to 26-day bracket before an ovulation-inducing treatment.

Progesterone priming is associated with a decreased $PGF_2\alpha$ response to an OT challenge (Beard and Hunter, 1996) thus suggesting that the presence of functional uterine OT receptors early in the induced cycle plays a critical role in the advanced luteolysis mechanism responsible for SLCL and that P_4 acts primarily by blocking that process in the uterus (Beard and Hunter, 1996; Wathes et al., 1996; Kombé et al., 2003). On the other hand, upregulation of OT uterine receptors by exogenous E_2 develops very quickly (Vallet et al., 1990). If that were the case for endogenous E_2, the emergence of a single wave of gonadotrophin-dependent follicles after termination of a P_4 priming would drastically downgrade any priming effect. That may explain the increased incidence of SLCL from MAP −6 (0%) to MAP −12 (34%). The occurrence of successive follicular waves would eventually erode any benefit of anticipated priming.

In comparison with ewes subsequently developing the SLCL syndrome, by the onset of the ASS ewes later developing NLCL had mostly (26/27) experienced a recent turnover of their largest follicle, had comparatively smaller largest and second largest follicles present, and eventually ovulated their largest follicle in a higher proportion than SLCL ewes did. In contrast, in most SLCL ewes (19/25) the largest

follicles present on Day −1 were not ovulated, smaller follicles of an average size similar to those in the NLCL group were instead selected for ovulation, and still these ewes developed the SLCL syndrome. Thus our results seem to support the observation that, in anoestrous ewes, 'follicles of similar size and [*presumably*] age can produce divergent responses to a GnRH stimulus' (Bartlewski et al., 2001). However, the contrasting follicle dynamics in those two response groups strongly suggests that a timing effect may be the ultimate cause of the SLCL syndrome. Almost invariably (10/11), lack of recent follicle turnover before the ASS was associated to SLCL. However, the syndrome developed *in spite* of smaller, presumably younger follicles being selected for ovulation. The simplest explanation for these effects would be that a minimum and timed gonadotrophin exposure may be required for follicles to achieve a maturational stage critical for developing into NLCL. Large follicles could be replaced either naturally or through induced follicle wave turnover (e.g. Meikle et al., 2001; Ungerfeld et al., 2004; Barret et al., 2008) but that would not guarantee that at least one follicle in the new cohort will develop to synchronize to an eventual LH surge triggered by an exteroceptive stimulus (e.g. exogenous E_2 or GnRH, or ASS-triggered GnRH).

Although minimum exposure to physiological P_4 released by accident (e.g. Beard and Hunter, 1996) or to exogenous P_4 or progestagens applied by design (5 mg of P_4, Cognié et al., 1982; 2.5 mg of MAP, Rodríguez Iglesias et al., 1992) can be 100% effective at preventing SLCL, it is clear that early P_4 production by the newly formed CL, indistinguishable for NLCL and SLCL (e.g. Legan et al., 1985; Hunter et al., 1988; Chemineau et al., 1993; Beard and Hunter, 1994, 1996; Mann and Haresign, 2001; Bartlewski et al., 2001), cannot explain the course of events conducive to anticipated luteolysis. Progestagen treatment after ovulation (e.g. Keisler and Keisler, 1989) does not decrease the incidence of SLCL thus reinforcing the concept that any therapeutic effect of exogenous P_4 should occur *before* rather than *after* ovulation takes place.

Concentrations of follicular receptors and E_2, and steroidogenic function have been reported to be altered in follicles destined to form SLCL in both ewes and cows (Hunter, 1991). Out-of-synchrony follicles, induced by insufficient exposure to gonadotrophins before the occurrence of an LH surge would be unable to develop into steroidogenically normal follicles (White et al., 1987; McNeilly et al., 1981; Murdoch and Van Kirk, 1998) capable of sustaining follicular P_4 production *before* ovulation. Follicular P_4, in turn, would be critical to downregulate the population of OT receptors in the endometrium thus avoiding the anticipated luteolysis triggered by oestrogenic stimulation of the normal luteolytic mechanism (Beard and Hunter, 1994). Thus, we conjecture that, given that follicular P_4 does not reach peripheral circulation in significant quantities (Murdoch and Dunn, 1982), local counter-current links between ovary and uterus (Weems et al., 1989; McCracken et al., 1999) should be the pathways allowing for follicular P_4 to reach the endometrium and to act upon OT receptors *before* ovulation takes place. Both genomic and nongenomic inhibition of OT binding may be effected by follicular P_4 after reaching the endometrium (Dunlap and Stormshack, 2004; Bishop and Stormshack, 2006). This is a critical link to be confirmed in order to validate our hypothesis, but several lines of seemingly unrelated evidence point towards a timing effect as the most parsimonious explanation for SLCL syndrome.

In the first place, the only way to avoid an anticipated CL lysis seems to be the pre-ovulatory downregulation of endometrial OT receptors up to a point in which the establishment of an OT-PGF2α feed-forward loop involving uterus and CL (McCracken et al., 1999) cease to be possible. If that is not achieved, the amplifying effect of the feed-forward loop will unavoidably seal the fate of the CL. Thus, the exposure to a gonadotrophin milieu conducive to fully functional follicular steroidogenesis in advance of an ovulatory stimulus, a temporally random process in anoestrous ewes, is coupled to a basically chaotic system whose final outcome (CL survival or lysis) will be determined by minimal initial differences in the balance of E_2 and P_4 uterine receptors regulating, in turn, the population of OT receptors in the endometrium (McCracken et al., 1999). In a system driven by endometrial receptors linked in such an unstable equilibrium, and in the absence of overriding exogenous factor(s) like a GnRH bolus injection, the expected outcome would be a random oscillation around a 50% of expected lysis events. Thus, the puzzling, widely reported circa 50% incidence of SLCL in anovular ewes exposed to rams (Martin et al., 1986) may turn out to be the most probable outcome of a fail-safe CL life-span control system with coupled ovarian and uterine components.

Additional evidence that timing of the ovulatory event relative to the previous endocrine environment is a critical factor comes from the widely reported differences between the outcome of a single GnRH bolus injection (producing almost exclusively SLCL) vs. that of a variety of protocols designed to mimicking a pulsed gonadotrophin regime followed by a bolus injection (mostly producing NLCL). Our own results in the group of ewes ovulating soon after the E_2 injection (93% SLCL) also fit the pattern of timing-induced failure. In these two situations (GnRH bolus or E_2 injection) the chances for endogenous endocrine processes buffering timing gaps (functional in the ASS model) approaches zero. Consequently, the occurrence of SLCL in those two situation should be expected to approach certainty.

A bolus GnRH injection inducing an anticipated LH surge and ovulation in ewes primed with P_4 before a ram exposure restores the incidence of SLCL to the level observed in unprimed ewes (Pearce et al., 1985). This result was originally interpreted as suggestive that 'progesterone assures normality of corpora lutea by lengthening the period of gonadotrophin priming of follicles before ovulation' (Pearce et al., 1985). Although the effect of P_4 priming is probably a different one (i.e. to preclude anticipated luteolysis at the point of action of OT in the endometrium), arbitrarily shortening the period of gonadotrophin follicle priming indeed had an effect on the life-span of induced CL, as predicted from our *ex post* hypothesis.

At the temporal scale of an oestrous cycle, the proposed unstable equilibrium system would be acting as a fail-safe sieve assuring economy of resources by discontinuing the reproductive process whenever it shows a less than ideal prospect of achieving success.

From an evolutionary perspective, the system could be part of a mechanism of inter-oocyte competition ultimately enhancing the chances of successful reproduction. Growth factors secreted by the oocyte play key roles in folliculogenesis, granulosa cell differentiation in particular (Gilchrist et al., 2004, 2008). Oocytes inside follicles unable to fully and timely develop functional steroidogenesis before ovulation would be eliminated whenever follicles of a subsequent follicular wave trigger an anticipated CL lysis via their own E_2 release in response to the first post-ovulatory FSH wave (Hunter, 1991). Oocytes inside these latter follicles would thus have a chance to pass on their genes. Although that would rarely be the case in domestic ewes (because first and second ovulations are normally silent), it is still possible in does, and it might have been possible in their common ancestor. For animals with year-round sexual segregation except during the rutting season, like most ungulates, developing early synchronous reproduction may have been a critical factor for evolutionary success.

Two key differences between Chemineau et al. (2006) model of SLCL occurrence and our *ex post* hypothesis are that (1) follicle failure would not be due to a systemic lack of 'sustained long-term gonadotrophin activity during anoestrus' but rather to a very short-term timing effect possibly modulated by oocyte action and (2) although effected *after* ovulation, the fate of both ovulated oocyte and developing CL would be sealed *before* ovulation takes place. Although minor, those differences offer a better fit to well known features of the syndrome, and may explain its evolution as a way of optimizing reproductive effort. On the other hand, if our hypothesis were correct, 100% NLCL responses without P_4 priming would be difficult to achieve in sheep, a species that, unlike cattle, seem to lack a clear mechanism of follicular dominance (Duggavathi et al., 2005), a prerequisite to induce follicular synchrony across individuals.

We conclude that the coupling of a timing-dependent process, namely the acquisition of full steroidogenic capabilities in advance of an ovulation stimulus, with a system of uterine receptors for E_2, P_4 and OT linked in an unstable equilibrium and controlling the amplification of a luteolytic feed-forward loop involving OT and $PGF_2\alpha$ (1) explains the natural occurrence of both NLCL and SLCL, (2) links proximate and ultimate causes of the SLCL syndrome, (3) resolves the issue of seemingly similar follicles producing divergent responses to ovulation-inducing factors, and (4) clarifies the puzzling circa 50% incidence of SLCL in sheep breeds of moderate seasonality induced to ovulate by ASS during anoestrus. A critical component of the hypothesis, a pathway for follicular P_4 to reach the endometrium before ovulation, remains to be experimentally validated.

Acknowledgements

The studies were partially supported by grant PICT 08-12411 from ANPCyT, Argentina. Technical assistance from Héctor Larrea and Carlos Colitripai is gratefully acknowledged. Burnet Labs and Gador Labs (Buenos Aires, Argentina) generously provided the oestradiol and MAP used in the experiments.

References

Acritopoulou, S., Haresign, W., 1980. Response of ewes to a single injection of an analogue of $PGF_2\alpha$ given at different stages of the oestrous cycle. J. Reprod. Fertil. 58, 219–223.

Barret, D.M.W., Bartlewski, P.M., Duggavathi, R., Davies, K.L., Huchkowsky, S.L., Epp, T., Rawlings, N.C., 2008. Synchronization of follicular wave emergence in the seasonally anestrus ewe: the effects of estradiol with or without medroxyprogesterone acetate. Theriogenology 69, 827–836.

Bartlewski, P.M., Beard, A.P., Chapman, C.L., Nelson, M.L., Palmer, B., Aravindakshan, J., Cook, S.J., Rawlings, N.C., 2001. Ovarian response in gonadotrophin-releasing hormone-treated anoestrus ewes: follicular and endocrine correlates with luteal outcome. Reprod. Fertil. Dev. 13, 133–142.

Bartlewski, P.M., Beard, A.P., Cook, S.J., Rawlings, N.C., 1998. Ovarian follicular dynamics during anoestrus in ewes. J. Reprod. Fertil. 113, 275–285.

Beard, A.P., Hunter, M.G., 1994. Effects of bovine follicular fluid and exogenous oestradiol on the GnRH-induced short luteal phase in anoestrous ewes. J. Reprod. Fertil. 100, 211–217.

Beard, A.P., Hunter, M.G., 1996. Effects of exogenous oxytocin and progesterone on GnRH-induced short luteal phases in anoestrous ewes. J. Reprod. Fertil. 106, 55–61.

Beck, T.W., Reeves, J.J., 1973. Serum luteinizing hormone (LH) in ewes treated with various dosages of 17β-estradiol at three stages of the anestrous season. J. Anim. Sci. 36, 566–570.

Berardinelli, J.G., Dailey, R.A., Butcher, R.L., Inskeep, E.K., 1980. Source of circulating progesterone in prepubertal ewes. Biol. Reprod. 22, 233–236.

Bishop, C.V., Stormshack, F., 2006. Nongenomic action of progesterone inhibits oxytocin-induced phosphoinositide hydrolysis and prostaglandin F2α secretion in the ovine endometrium. Endocrinology 147, 937–942.

Camp, J.C., Wildt, D.E., Howard, P.K., Stuart, L.D., Chakraborty, P.K., 1983. Ovarian activity during normal and abnormal length estrous cycles in the goat. Biol. Reprod. 28, 673–681.

Caraty, A., Delaleu, B., Chesneau, D., Fabre-Nys, C., 2002. Sequential role of E2 and GnRH for the expression of estrus behavior in ewes. Endocrinology 143, 139–145.

Chemineau, P., Daveau, A., Locatelli, A., Maurice, F., 1993. Ram-induced short luteal phases: effects of hysterectomy and cellular composition of the corpus luteum. Reprod. Nutr. Dev. 33, 253–261.

Chemineau, P., Pellicer-Rubio, M.T., Lassoued, N., Khaldi, G., Monniaux, D., 2006. Male-induced short oestrous and ovarian cycles in sheep and goats: a working hypothesis. Reprod. Nutr. Dev. 46, 129–417.

Cognié, Y., Gray, S.J., Lindsay, D.R., Oldham, C.M., Pearce, D.T., Signoret, J.P., 1982. A new approach to controlled breeding in sheep using the 'ram effect'. Proc. Aust. Soc. Anim. Prod. 14, 519–522.

Consortium for Developing a Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching, 1999. Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. Urbana, IL.

Duggavathi, R., Bartlewski, P.M., Agg, E., Flint, S., Barrett, D.M., Rawlings, N.C., 2005. The effect of the manipulation of follicle stimulating hormone (FSH)-peak characteristics on follicular wave dynamics in sheep: does an ovarian-independent endogenous rhythm in FSH secretion exist? Biol. Reprod. 72, 1466–1474.

Dunlap, K.A., Stormshack, F., 2004. Nongenomic inhibition of oxytocin binding by progesterone in the ovine uterus. Biol. Reprod. 70, 65–69.

Gilchrist, R.B., Lane, M., Thompson, J.G., 2008. Oocyte-secreted factors: regulators of cumulus cell function and oocyte quality. Hum. Reprod. Update 14, 159–177.

Gilchrist, R.B., Ritter, L.J., Armstrong, D.T., 2004. Oocyte-somatic cell interactions during follicle development in mammals. Anim. Reprod. Sci. 82-83, 431–446.

Hunter, M.G., 1991. Characteristics and causes of the inadequate corpus luteum. J. Reprod. Fertil. Suppl. 43, 91–99.

Hunter, M.G., Southee, J.A., Lamming, G.E., 1988. Function of abnormal corpora lutea in vitro after GnRH-induced ovulation in the anoestrous ewe. J. Reprod. Fertil. 84, 139–148.

Irazoqui, H., Rodríguez Iglesias, R.M., Ciccioli, N.H., 1992. Introducción repentina de machos y tratamiento con progesterona en ovejas Corriedale anovulatorias encarneradas en primavera. Rev. Arg. Prod. Anim. 12, 177–183.

Jefferies, B.C., 1961. Body condition scoring and its use in management. Tasmanian J. Agric. 32, 19–21.

Keisler, D.H., Keisler, L.W., 1989. Formation and function of GnRH-induced subnormal corpora lutea in cyclic ewes. J. Reprod. Fertil. 87, 265–273.

Kombé, A., Sirois, J., Goff, A.K., 2003. Prolonged progesterone treatment of endometrial epithelial cells modifies the effect of estradiol an their sensitivity to oxytocin. Steroids 68, 651–658.

Legan, S.J., l'Anson, H., Fitzgerald, B.P., Akaydin Jr., M.S., 1985. Importance of short luteal phases in the endocrine mechanism controlling initiation of estrous cycles in anestrous ewes. Endocrinology 117, 1530–1536.

Mann, G.E., Haresign, W., 2001. Effect of oestradiol treatment during GnRH-induced ovulation on subsequent PGF2α release and luteal life span in anoestrous ewes. Anim. Reprod. Sci. 67, 245–252.

Martin, G.B., Oldham, C.M., Cognié, Y., Pearce, D.T., 1986. The physiological responses of anovulatory ewes to the introduction of rams – a review. Livest. Prod. Sci. 15, 219–247.

MathSoft Inc., 2000. S-PLUS 2000 Professional Edition for Windows, Release 3. MathSoft Inc.

McCracken, J.A., Custer, E.E., Lamsa, J.C., 1999. Luteolysis: a neuroendocrine-mediated event. Physiol. Rev. 79, 263–324.

McCullagh, P., Nelder, J.A., 1989. Generalized Linear Models, 2nd ed. Chapman & Hall, London.

McLeod, B.J., Haresign, W., Lamming, G.E., 1982. The induction of ovulation and luteal function in seasonally anoestrous ewes treated with small-dose multiple injections of Gn-RH. J. Reprod. Fertil. 65, 215–221.

McNeilly, A.S., Hunter, M., Land, R.B., Fraser, H.M., 1981. Inadequate corpus luteum function after the induction of ovulation in anestrous ewes by LH–RH or an LH–RH agonist. J. Reprod. Fertil. 63, 137–144.

Meikle, A., Forsberg, M., Garófalo, E.G., Carlsson, M.A., Lundeheim, N., Rubianes, E., 2001. Circulating gonadotrophins and follicular dynamics in anoestrous ewes after treatment with estrdiol-17β. Anim. Reprod. Sci. 67, 79–90.

Murdoch, W.J., Dunn, T.G., 1982. Alterations in follicular steroid hormones during the preovulatory period in the ewe. Biol. Reprod. 27, 300–307.

Murdoch, W.J., Van Kirk, E.A., 1998. Luteal dysfunction in ewes induced to ovulate early in the follicular phase. Endocrinology 139, 3480–3484.

Odde, K.G., Ward, H.S., Kiracofe, G.H., McKee, R.M., Kittok, R.J., 1980. Short estrous cycles and associated serum progesterone levels in beef cows. Theriogenology 14, 105–112.

Oldham, C.M., 1980. A study of seasonal and ovarian activity in Merino sheep. PhD Dissertation. School of Agriculture, University of Western Australia. Perth, Australia.

Oldham, C.M., Lindsay, D.R., 1980. Laparoscopy in the ewe: a photographic record of the ovarian activity of ewes experiencing normal or abnormal oestrous cycles. Anim. Reprod. Sci. 3, 119–124.

Oldham, C.M., Martin, G.B., 1978. Stimulation of seasonally anovular Merino ewes by rams. II. Premature regression of ram-induced corpora lutea. Anim. Reprod. Sci. 1, 291–295.

Oldham, C.M., Pearce, D.T., Gray, S.J., 1985. Progesterone priming and age of ewe affect the life-span of corpora lutea induced in the seasonally anovulatory Merino ewe by the 'ram effect'. J. Reprod. Fertil. 75, 29–33.

Pearce, D.T., Martin, G.B., Oldham, C.M., 1985. Corpora lutea with a short life-span induced by rams in seasonally anovulatory ewes are prevented by progesterone delaying the preovulatory surge of LH. J. Reprod. Fertil. 75, 79–84.

Pearce, D.T., Oldham, C.M., Haresign, W., Gray, S.J., 1987. Effects of duration and timing of progesterone priming on the incidence of corpora lutea with a normal life-span in Merino ewes induced to ovulate by the introduction of rams. Anim. Reprod. Sci. 13, 81–89.

Pevsner, D.A., Rodríguez Iglesias, R.M., Ciccioli, N.H., 2010. Ram-induced oestrus and ovulation in lactating and weaned Corriedale ewes. Animal 4, 472–479.

Reeves, J.J., Beck, T.W., Nett, T.M., 1974. Serum FSH in anoestrous ewes treated with 17β-estradiol. J. Anim. Sci. 38, 374–377.

Rodríguez Iglesias, R.M., Ciccioli, N.H., Irazoqui, H., 1992. Distribución diaria de celos inducidos mediante "efecto macho" en ovejas Corriedale inyectadas con progesterona o acetato de medroxyprogesterona. Rev. Arg. Prod. Anim. 12, 65–70.

Rodríguez Iglesias, R.M., Ciccioli, N.H., Irazoqui, H., Giglioli, C., 1996. Ovulation rate in ewes after single oral glucogenic dosage during a ram-induced follicular phase. Anim. Reprod. Sci. 44, 211–221.

Rodríguez Iglesias, R.M., Ciccioli, N.H., Irazoqui, H., Rodríguez, B.T., 1991. Importance of behavioural stimuli in ram-induced ovulation in seasonally anovular Corriedale ewes. Appl. Anim. Behav. Sci. 30, 323–332.

Rubianes, E., De Castro, T., Ungerfled, R., Meikle, A., Rivero, A., 1997. Ovarian response after a GnRH challenge in seasonally anoestrous ewes. Can. J. Anim. Sci. 77, 727–730.

Rubianes, E., Menchaca, A., Carbajal, B., 2003. Response of the 1–5 day-aged ovine corpus luteum to prostaglandin F2α. Anim. Reprod. Sci. 78, 47–55.

Schinckel, P.G., 1954. The effect of the presence of the ram on the ovarian activity of the ewe. Aust. J. Agric. Res. 5, 465–469.

Souza, C.J., Campbell, B.K., Baird, D.T., 1997. Follicular dynamics and ovarian steroid secretion in sheep during the follicular and early luteal phases of the oestrous cycle. Biol. Reprod. 56, 483–488.

Ungerfeld, R., Dago, A.L., Rubianes, E., Forsberg, M., 2004. Response of anestrous ewes to the ram effect after follicular wave synchronization with a single dose of estradiol-17beta. Reprod. Nutr. Dev. 44, 89–98.

Vallet, J.L., Lamming, G.E., Batten, M., 1990. Control of endometrial oxytocin receptor and uterine response to oxytocin by progesterone and oestradiol in the ewe. J. Reprod. Fertil. 90, 625–634.

Wathes, D.C., Mann, G.E., Payne, J.H., Riley, P.R., Stevenson, K.R., Lamming, G.E., 1996. Regulation of oxytocin, oestradiol and progesterone receptor concentrations in different uterine regions by oestradiol, progesterone and oxytocin in ovariectomized ewes. J. Endocrinol. 151, 375–393.

Weems, C.W., Weems, Y.S., Lee, C.N., Vincent, D.L., 1989. Progesterone in uterine and arterial tissue and in jugular and uterine venous plasma of sheep. Biol. Reprod. 40, 1–6.

White, L.M., Keisler, D.H., Dailey, R.A., Inskeep, E.K., 1987. Characterization of ovine follicles destined to form subfunctional corpora lutea. J. Anim. Sci. 65, 1595–1601.

Yuthasastrakosol, P., Palmer, W.M., Howland, B.E., 1975. Luteinizing hormone, oestrogen and progesterone levels in peripheral serum of anoestrous and cyclic ewes as determined by radioimmunoassay. J. Reprod. Fertil. 43, 57–65.