Reprod Nutr Dev (1993) 33, 253-261

Original article

Ram-induced short luteal phases: effects of hysterectomy and cellular composition of the corpus luteum

P Chemineau, A Daveau, A Locatelli, F Maurice

INRA, Reproductive Physiology, 37380 Nouzilly, France

(Received 7 October 1992; accepted 16 April 1993)

Summary — Two experiments were conducted on Préalpes ewes to test 2 complementary hypotheses which may explain the short lifespan of corpora lutea observed in some cases after ram-induced ovulation: i) the possible role of the uterus was tested by determining the effects of hysterectomy on the duration of luteal phases after the ram effect (RE); ii) the possible difference due to characteristics of follicles before ovulation was tested by determining the cellular composition and characteristics of corpora lutea (CL) induced by the RE compared to CL of the breeding season (BS) when ovulation is synchronized by FGA-impregnated sponges. In the first experiment, 9 ewes were hysterectomized (Hys) and introduced to rams at the same time as 10 control ewes. Plasma progesterone (P4) was analyzed each day for 17 consecutive d after the introduction of rams. The number of females ovulating was not different for the 2 groups (7/9 *vs* 9/10, respectively), but no Hys ewes experienced short cycles compared to 5 of the 9 control ewes ($P = 0.029$). The second experiment involved 16 ewes subjected to the RE in June, and 5 cyclic ewes in January. The ewes were ovariectomized 82 h after the preovulatory LH surge, the CL were separated, weighed and the luteal cells enzymatically dissociated to count the relative proportions of small (< 20 μ diameter) and large cells and assess *in vitro* P4 secretion both with and without stimulation with 100 ng ovine LH. Plasma P4 concentration increased significantly more slowly in RE than in BS ewes ($P < 0.05$). The weight of twin RE CL did not differ from that of twin FGA-BS CL (m ± SD; 142.1 ± 48.7 *vs* 139.1 ± 23.0 mg). The percentage of large luteal cells was lower in RE than in FGA-BS CL (10.7 ± 4.6 *vs* 25.0 ± 6.6%; $P < 0.001$). The quantity of P4 secreted *in vitro* without LH stimulation was lower in RE than in FGA-BS CL (7.3 ± 6.6 *vs* 13.8 ± 9.8 ng/105 luteal cells/3 h; $P < 0.05$). The incubation of luteal cells for 3 h with 100 ng oLH significantly increased P4 secretion in RE but not in FGA-BS CL (+73% *vs* +9%; $P < 0.05$). It is concluded that the uterus is essential for determining the lifespan of RE-induced CL and that the cellular composition and characteristics of the RE CL are very different from those of FGA-treated breeding season CL.

ram effect / corpus luteum / hysterectomy / luteal cell / ewe

Résumé — Phases lutéales courtes induites par le bélier : effets de l'hystérectomie et composition cellulaire du corps jaune. *Deux expériences ont été conduites sur des brebis de race Préalpes afin de mettre à l'épreuve 2 hypothèses complémentaires pouvant expliquer la courte durée des corps jaunes qui suivent, dans certains cas, l'ovulation induite par l'effet bélier (EB). Le rôle*

éventuel de l'utérus dans la lutéolyse précoce a été recherché en déterminant les effets de l'hystérectomie sur la durée de la phase lutéale induite par l'effet bélier. Une différence possible dans les capacités de lutéinisation des follicules a été appréciée en comparant la composition et les caractéristiques cellulaires des corps jaunes induits par EB, à celles des corps jaunes de la saison sexuelle (SS) (après synchronisation par un traitement FGA). Dans la première expérience, 9 femelles ont été hystérectomisées puis soumises à l'effet bélier au même moment que 10 brebis témoins. Le nombre de femelles qui ont ovulé ne diffère pas entre les groupes (7/9 vs *9/10, respectivement). L'analyse quotidienne de la progestérone plasmatique pendant 17 j après l'introduction des béliers, pour déterminer la durée de vie des corps jaunes induits, montre qu'aucune brebis hystérectomisée ne manifeste un corps jaune de courte durée, au contraire de 5 des 9 brebis témoins (*P = *0,029). Dans la seconde expérience, 16 brebis ont été soumises à EB en juin et 5 brebis cycliques ont été synchronisées avec une éponge contenant du FGA, en janvier. Les brebis ont été ovariectomisées 82 h après le pic préovulatoire de LH, les corps jaunes ont été disséqués, pesés et les cellules lutéales dissociées enzymatiquement pour compter la proportion relative de petites (< 20 μ de diamètre) et de grandes cellules lutéales et mesurer* in vitro *la sécrétion de progestérone avant et après stimulation par 100 ng de LH ovine. La progestérone plasmatique augmente plus lentement chez les brebis EB que chez les brebis SS (*P < *0,05). Le poids frais des corps jaunes issus d'ovulations doubles n'est pas différent entre les brebis EB et les brebis SS (m ± écart type; 142,1 ± 48,7* vs *139,1 ± 23,0 mg). Le pourcentage de grandes cellules lutéales est plus faible dans les corps jaunes EB que dans les corps jaunes SS (10,7 ± 4,6* vs *25,0 vs 6,6%;* P < *0,001). La quantité de progestérone sécrétée* in vitro *sans stimulation par la LH est plus faible chez les corps jaunes EB que chez les corps jaunes SS (7,3 ± 6,6* vs *13,8 ± 9,8 ng/10^5 cellules lutéales/3 h;* P < *0,05). L'incubation des cellules lutéales pendant 3 heures avec la LH ovine augmente significativement la sécrétion de progestérone des corps jaunes EB mais pas celle des corps jaunes SS (+73* vs *+9%;* P < *0 ,05). Il est conclu que l'utérus détermine la durée de vie des corps jaunes induits par EB et que la composition cellulaire et les caractéristiques des corps jaunes induits par EB sont différentes de celles des corps jaunes de SS, après synchronisation par FGA.*

effet bélier / corps jaune / hystérectomie / cellules lutéales / brebis

INTRODUCTION

The introduction of males among previously isolated, anovular ewes and goats rapidly induces synchronous ovulations followed by 1 of 2 types of corpora lutea (CL): either CL of normal duration or of short duration of ≈ 5–6 d (Martin *et al*, 1986). Some factors such as nutrition, season and breed, moderate the frequency of short ovarian cycles relative to normal cycles (goat: Chemineau, 1983; ewe: Khaldi, 1984 ; Lassoued and Khaldi, 1990) but the duration of short cycles is surprisingly very constant. The relative proportions of short and normal cycles are modified by these modulating factors but never the cycle duration. Most authors have attributed this to an insufficient stimulation of the preovulatory follicles by the gonadotropins prior to luteinization (for review see : Martin and Scaramuzzi, 1983), but no satisfactory explanation has been given for the observation of short cycles and for their constant duration.

In the experiments described here we therefore decided to test 2 complementary hypotheses which may at least in part explain the short lifespan of some CL which follow ram-induced ovulations during the anestrous season:

– is the uterus involved, as in the normal cyclic CL, in the luteolytic mechanisms of the short-duration CL ? We therefore planned to determine the effects of removal of the uterus prior to ram introduction on the lifespan of CL;

– are CL formed after ram-induced ovulation different from those observed after

progestogen priming during the sexual season ? We therefore determined the cellular composition and characteristics of ram-induced CL in comparison with CL collected after progestagen synchronization during the breeding season in cyclic ewes (FGA-BS).

MATERIAL AND METHODS

Experiment 1

Animals

Nine Préalpes ewes were hysterectomized under general anesthesia during the anestrous season (Hys) and maintained for 1 month with 10 other Préalpes ewes (controls) completely isolated from rams. Two rams of the same breed were introduced among the females for 17 d on 18 June.

Blood sampling and progesterone assay

All ewes were blood-sampled daily by jugular venepuncture for 17 d after the introduction of the rams. The blood was immediately centrifuged and stored at –20°C until assayed for progesterone (Saumande *et al,* 1985), the method having a detection limit of 0.1 ng/ml and intra-assay and inter-assay coefficients of variation of 11 and 12 %, respectively. Ewes were separated into 2 groups relative to their progesterone levels in plasma: those in which progesterone had decreased to basal levels on d 7 and increased thereafter (indicating a CL of short duration), and those in which progesterone continued to increase (indicating a CL of normal duration).

Experiment 2

Animals

The experiment involved 16 adult Préalpes ewes in June (ram effect ewes, RE), and 5 in January (breeding season ewes, BS). In order to induce ovulations during seasonal anestrus *via* the ram effect, females were completely isolated from males for 1 month, after which the rams were re-introduced. Cyclic females were identified by assaying plasma progesterone twice weekly (Terqui and Thimonier, 1974) during the month of isolation from the rams. In January, during the sexual season, ovulation was synchronized in cyclic females by using FGA-impregnated vaginal sponges (30 mg) left in place for 11 d.

LH surge, ovariectomy, plasma progesterone

Blood samples were taken every 4 h, from 12 until 100 h after the introduction of males in June and from 21 to 72 h after sponge removal in January in order to detect the onset of the preovulatory LH surge in the plasma (Pelletier *et al,* 1982). This LH surge was detected by daily rapid radioimmunoassay (Cahill, 1978); the females were castrated 3 to 4 d after the surge.

Ovariectomies were performed under general anesthesia and CL were separated from the ovary immediately after collection. In June, 5 females bearing a CL on each ovary were hemiovariectomized (3 left and 2 right ovaries) in order to determine the activity of the remaining CL.

Twice-daily blood samples (8:00–20:00) were taken from the LH surge until just before ovariectomy or until d 12 for hemiovariectomized females in order to follow luteal activity by assaying plasma progesterone levels (Saumande *et al,* 1985).

Preparation of luteal cells

Immediately after dissection, CL were weighed and dissociation of luteal cells was performed (Stouffer *et al,* 1976; Lemon and Loir, 1977; Fitz *et al,* 1982; Rodgers and O'Shea, 1982): CL were sliced with razor blades before being introduced into 5 ml Dulbecco's Modified Eagle's Medium (DMEM; GIBCO) containing 400 units of collagenase (type IV, SIGMA) per ml. They were incubated in a shaking water bath (37°C) for 1.5 h, after which another 5 ml fresh collagenase/DMEM solution was added before a further 1.5 h incubation. At the end of the 3-h incubation, after filtration through a nylon bag, the solu-

tion containing the suspension of cells was centrifuged (5 min at 180 *g*), the supernatant removed and the cells resuspended in fresh DMEM without collagenase. An aliquot was collected and assessed for measurements of cell diameters (ocular micrometer) which permitted their classification into small (< 20 μ diameter) or large luteal cells (≥ 20 μ diameter), and assessment of cell viability *via* the Trypan blue exclusion test (which showed that cell viability was always > 90%) (Rodgers and O'Shea, 1982). A further centrifugation was performed, the supernatant removed and the cells resuspended in fresh DMEM. Two aliquots were immediately dispensed in duplicate into glass tubes to give a final concentration of 10^5 cells/ml after the addition of 1.0 ml DMEM containing only DMEM to one replicate and 100 ng/ml pure ovine LH to the other. After 3-h incubation in a shaking water bath (37°C) the tubes were centrifuged (20 min, 400 g) and the supernatant was frozen (-20°C) until progesterone assay (Saumande *et al*, 1985).

Statistical analysis

Data were analysed using χ^2 and Student's *t*-tests as appropriate and analysis of variance after arcsine transformation in the case of percentages (Dagnélie, 1970).

RESULTS

Experiment 1

The number of ewes ovulating after the introduction of rams did not differ in the 2 groups (Hys: 7/9 *vs* control: 9/10). No short luteal phases were detected in the hysterectomized ewes, whereas 5 control ewes experienced short cycles (P = 0.029). Until d 11 the plasma progesterone concentration of control ewes with normal cycles was not significantly different from that of hysterectomized ewes (fig 1). In contrast, the plasma progesterone levels in control ewes experiencing short cycles showed a transitory maximum on d 4–5 then decreased to zero on d 7 before increasing thereafter, which demonstrated the existence of corpora lutea of short duration followed by a normal ovulation after the introduction of the rams.

Experiment 2

Ovulations

In June, 1 female was cyclic before the introduction of the rams and consequently was excluded from the experiment. Four ewes did not experience an LH preovulatory surge and were consequently not included in the experiment. Eleven females (=73%) experienced a preovulatory LH surge on average (middle of the surge) 46 h (± SD 12) after the rams were introduced and subsequently ovulated. Ovariectomies were performed 82 h (± 9) after the LH preovulatory surge. Fifteen CL were surgically removed from the 11 females and 5 CL from twin ovulations on different ovaries were left in place in 5 females. Among the 15 CL removed which were used for *in vitro* measurements, 5 CL came from twin ovulations on different ovaries; 8 came from twin ovulations on the same ovary and 2 were from single ovulations.

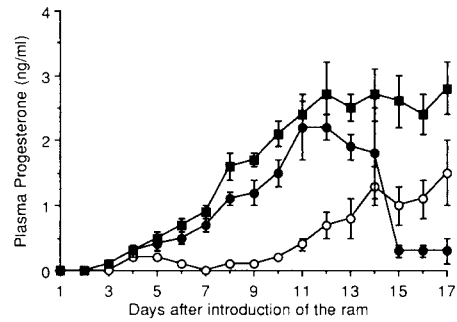

Fig 1. Plasma progesterone concentration after the ram effect in control and hysterectomized (d-30) Préalpes ewes. m ± SEM. Control ewes: normal cycle (●); short cycle (O); hysterectomized ewes (■).

In January all females experienced a preovulatory LH surge on average 56 h (± 4) after sponge removal and subsequently ovulated. Ovariectomies were performed 82 h (± 3) after the LH preovulatory surge. Ten CL were removed, all of which were from twin ovulations; 3 females bore both CL on one ovary and the other 2 bore one CL on each ovary.

Plasma progesterone concentrations

The increase in plasma progesterone concentration was significantly slower in RE ewes than in BS ewes (significant interaction group x time at $P < 0.01$). At the time of ovariectomy, the plasma progesterone concentration of RE ewes was significantly lower than that of BS ewes (0.41 ± 0.14 *vs* 0.57 ± 0.18 ng/ml, respectively $P = 0.039$; fig 2).

Characteristics of corpora lutea (table I)

The mean weight of RE CL was not significantly different from that of FGA-BS CL. When considering only CL from twin ovulations, the mean weights were very similar from one group to another. The percentage of large luteal cells was significantly higher in the FGA-BS CL compared to RE CL. More than twice as many large luteal cells were counted in FGA-BS CL as in RE CL. No difference was observed between CL collected from females in which the twin CL were left intact resulting in CL of

Table I. Comparison of weight and percentage of large secretory cells in corpora lutea from Préalpes ewes after ram-induced ovulation *versus* after FGA-synchronized ovulation during the breeding season.

	Ram-induced CL	*Breeding season CL*
Weight of all CL	156.1[a] ± 58.3 (n = 13 twins + 2 singles)	139.1[a] ± 23.0 (n = 10 twins)
	Total	
Weight of twin CL	142.1[a] ± 48.7 (13)	139.1[a] ± 23.0 (10)
	Duration of twin CL left in place	
	Short	*Normal*
Weight of twin CL removed from ewes in which the remaining CL was left in place	185.3 (3)	141.7 (2)
	Total	
Percentage of large cells (≥ 20 μ diam)	10.7[a] ± 4.6 (13)	25.0[b] ± 6.6 (10)
	Duration of twin CL left in place	
	Short	*Normal*
Percentage of large cells in twin CL removed from ewes in which the remaining CL was left in place	7.6 (3)	8.5 (2)

Weight in mg; m ± SD. Values with different superscripts are significantly different (a = b; $P < 0.001$).

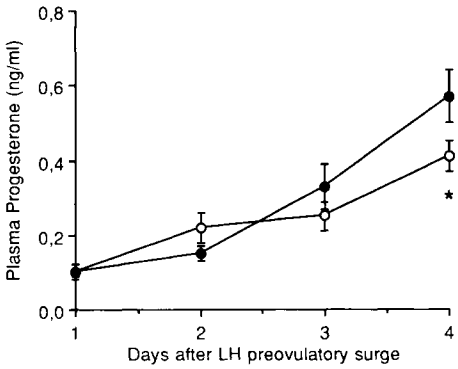

Fig 2. Plasma progesterone concentration after the preovulatory LH surge in Préalpes ewes induced to ovulate either by the introduction of rams during the non-breeding season or by synchronization with FGA-impregnated sponges during the breeding season m ± SEM. Ram effect (O); breeding season (●); * $P < 0.05$.

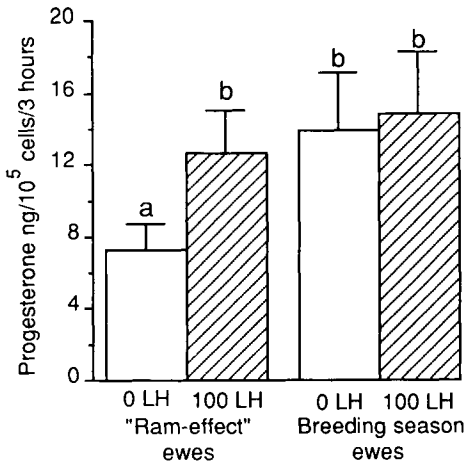

Fig 3. Progesterone secretion by dispersed luteal cells after 3-h incubation with or without 100 ng/ml oLH. The luteal cells were from the corpora lutea collected from Préalpes ewes induced to ovulate either by the introduction of rams during the non-breeding season or by synchronization with FGA-impregnated sponges during the breeding season. m ± SEM; values with different superscripts are significantly different (at $P < 0.05$).

short duration and those collected from females in which the CL were left in place resulting in CL of normal duration.

In vitro progesterone secretion

In the absence of stimulation with oLH, progesterone secretion by luteal cells from RE CL was significantly lower than by cells from FGA-BS ewes (7.3 ± SEM 1.7 *vs* 13.8 ± 3.1 ng; $P < 0.05$). Incubation with 100 ng oLH induced a significant increase in progesterone secretion by luteal cells from RE CL (+73%) but not by those from FGA-BS CL (+7%) (fig 3).

After stimulation by the ram effect in June (RE CL), there was less progesterone secretion by CL collected from females in which the twin CL left intact were of short duration than by CL collected from females in which the remaining CL were of normal duration (2.9 *vs* 10.3, and 12.7 vs 17.15 ng, for short *vs* normal and 0 and 100 oLH, respectively).

DISCUSSION

Removal of the uterus prior to ram introduction did not change the percentage of ovulating ewes but provoked the complete disappearance of short-lifespan corpora lutea. Such a result indicates that the fixed duration of the short ovulatory cycle induced by the introduction of rams is dependent on the presence of the uterus as it is for cycles of normal duration. It could be postulated that uterine prostaglandin secretion is responsible for the short-lifespan CL. It has been demonstrated that ovine CL are sensitive to prostaglandin after d 5 of the cycle (Acritopoulou and Haresign, 1980), and that specific inhibition of prostaglandin synthesis by intra-uterine injections of indomethacin from d 2 to 4 after the introduction of rams tends to reduce the pro-

portion of short ovulatory cycles (Lassoued and Khaldi, 1989). By using GnRH instead of the ram effect to produce short-lifespan CL in anestrous ewes, Southee *et al* (1988) showed a similar pattern of retention of CL after hysterectomy. These similarities between the 2 experiments indicate that the luteolytic mechanisms after ram effect and after GnRH treatment are possibly similar, but raise the question of determining why this luteolytic effect appears in some animals and not in others.

In the second experiment described here, it is clearly apparent that RE CL did not have the same cellular composition as CL from ewes synchronized with FGA during the breeding season. There was a lower percentage of large luteal cells in RE CL than in FGA-BS CL. It has been demonstrated that in normal CL, large luteal cells are from the granulosa cells of the follicle and small luteal cells from the thecal cells of the follicle (Alila and Hansel, 1984). The observation of such a difference in the relative proportion of large and small luteal cells in 3.5-d-old CL from RE and BS ewes strongly suggests that the follicles that are induced to ovulate by the presence of rams do not have the same characteristics as those that are going to ovulate during the breeding season after FGA treatment.

In the 2 CL groups, the *in vitro* secretion of progesterone before and after gonadotropin stimulation was consistent with the relative proportion of small and large luteal cells. Rodgers *et al* (1983) have already shown that, when separated into 2 groups by cell sorting, ovine large luteal cells secrete large quantities of progesterone without stimulation but do not respond to LH stimulation; in contrast, small luteal cells contribute much less to progesterone production if not stimulated, but are very sensitive to LH stimulation. In the present experiment lower basal secretion of progesterone was observed in RE CL, in which a lower proportion of large luteal cells was detected, than in FGA-BS CL; but these RE CL secreted more progesterone after LH stimulation.

Such a difference in the cellular composition and characteristics between these 2 types of CL also explains the difference in plasma concentration of progesterone found on d 4 between ram-induced and breeding season ewes. FGA-treated breeding season ewes had higher proportions of large luteal cells, which enhanced progesterone plasma concentrations compared to ram-induced ewes.

However, in the present experiment, because of the necessity to strictly synchronize the LH preovulatory surge during the breeding season to give a precise timing for ovariectomy, the comparison between the 2 types of CL is confounded by at least 2 factors: 1) the CL being compared were produced at 2 different seasons of the year; 2) the FGA-BS CL were produced after progestogen priming (endogenous progesterone plus exogenous FGA), whereas the RE CL were not. However, it has been demonstrated that progesterone or progestogen priming is able to completely suppress ram-induced short luteal phases (Oldham *et al,* 1978/1979; Lindsay *et al,* 1982; Pearce *et al,* 1985). In the same way, progesterone pretreatment has a direct effect on GnRH-induced preovulatory follicles (Hunter *et al,* 1986; Hunter, 1991). These findings may indicate that progesterone or FGA during the breeding season may act directly on follicles which were collected to determine their ability to develop into normal corpora lutea (Martin *et al,* 1986).

Taken together, these results suggest that the sequential mechanisms involved in the control of CL after introduction of rams during anestrus could be the following:

– follicles induced to ovulate are of poor quality because of the unsustained gonadotropin activity during anestrus,

– CL developed from these follicles have an insufficient proportion of large luteal cells, which usually secrete large quantities of progesterone,

– the plasma progesterone is consequently insufficient to block the gonadotropin activity on d 3–5 after LH surge, allowing new follicles to grow and secrete estrogens which stimulate prostaglandin secretion by the uterus, thus causing luteolysis.

ACKNOWLEDGMENT

The authors wish to thank J Thimonier for help in the preparation of this manuscript.

REFERENCES

Acritopoulou S, Haresign W (1980) Response of ewes to a single injection of an analogue of PGF-2α given at different stages of the oestrous cycle. *J Reprod Fertil* 58, 219-223

Alila HW, Hansel W (1984) Origin of different cell types in the bovine corpus luteum as characterized by specific monoclonal antibodies. *Biol Reprod* 31, 1015-1025

Cahill LP (1978) Studies of folliculogenesis in the sheep. Thèse Doct ès Sciences, Univ Paris VI, 141 pp

Chemineau P (1983). Effect on oestrus and ovulation of exposing Creole goats to the male at three times of the year. *J Reprod Fertil* 67, 65-72

Dagnélie (1980) *Théorie et Méthodes Statistiques*. Presses Agron Gembloux, Gembloux, Belgium, vol 2, p 451

Fitz JA, Mayan MH, Sawyer HR, Niswender GD (1982) Characterization of two steroidogenic cell types in the ovine corpus luteum. *Biol Reprod* 27, 703-711

Hunter MG, Southee JA, McLeod BJ, Haresign W (1986) Progesterone pretreatment has a direct effect on GnRH-induced preovulatory follicles to determine their ability to develop into normal corpora lutea in anoestrous ewes. *J Reprod Fertil* 76, 349-363

Hunter MG (1991) Characteristics and causes of the inadequate corpus luteum. *J Reprod Fertil* suppl 43, 91-99

Khaldi G (1984) Variations saisonnières de l'activité ovarienne, du comportement d'oestrus et de la durée de l'anoestrus *postpartum* des femelles ovines de race Barbarine: influences du niveau alimentaire et de la présence du mâle. Thèse Doct ès Sciences, USTL, 168 pp

Lassoued N, Khaldi G (1989) Influence de l'indométacine sur la durée du cycle ovarien induit par effet mâle chez la brebis. *Ann INRAT (Tunis)* 62(9), 3-14

Lassoued N, Khaldi G (1990) Influence du niveau alimentaire avant et après la mise-bas sur la réponse des brebis de race Barbarine à l'effet mâle. *Ann INRAT (Tunis)* 63(2), 1-15

Lemon M, Loir M (1977) Steroid release *in vitro* by two luteal cell types in the corpus luteum of the pregnant sow. *J Endocrinol* 72, 351-359

Lindsay DR, Cognié Y, Signoret JP (1982) Méthode simplifiée de maîtrise de l'oestrus chez la brebis. *Ann Zootech* 31, 77-82

Martin GB, Scaramuzzi RJ (1983) Induction of oestrus and ovulation in seasonally anovular ewes by exposure to rams. *J Steroid Biochem* 19, 869-875

Martin GB, Oldham CM, Cognié Y, Pearce DT (1986) The physiological responses of anovulatory ewes to the introduction of rams – a review. *Livest Prod Sci* 15, 219-247

Oldham CM, Martin GB, Lindsay DR (1978/1979) Stimulation of seasonally anovular Merino ewes by rams. I. Time from introduction of the rams to the preovulatory LH surge and ovulation. *Anim Reprod Sci* 1, 283-290

O'Shea JD, Rodgers RJ, Wright PJ (1984) Morphometric analysis and function *in vivo* and *in vitro* of corpora lutea from ewes treated with LHRH during seasonal anoestrus. *J Reprod Fertil* 72, 75-85

Pearce DT, Martin GB, Oldham CM (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

Pelletier J, Garnier DH, de Reviers MM, Terqui M, Ortavant R (1982) Seasonal variation in LH and testosterone release in rams of two breeds. *J Reprod Fertil* 64, 341-346

Rodgers RJ, O'Shea JD (1982) Purification, morphology and progesterone production and content of three cell types isolated from the corpus luteum of the sheep. *Aust J Biol Sci* 35, 441-455.

Rodgers RJ, O'Shea JD, Findlay JK (1983) Progesterone production *in vitro* by small and large ovine luteal cells. *J Reprod Fertil* 69, 113-124

Saumande J, Tamboura D, Chupin D (1985) Changes in milk and plasma concentrations of progesterone in cows after treatment to induce superovulation and their relationships with number of ovulations and of embryos collected. *Theriogenology* 23, 719-731

Southee JA, Hunter MG, Law AS, Haresign W (1988) Effect of hysterectomy on the short life-cycle corpus luteum produced after GnRH-induced ovulation in the anoestrous ewe. *J Reprod Fertil* 84, 149-155

Stouffer RL, Nixon WE, Gulyas BJ, Johnson,DK, Hodgen GD (1976) *In vitro* evaluation of corpus luteum function of cycling and pregnant rhesus monkeys: progesterone production by dispersed luteal cells. *Steroids* 27 (54), 543-551

Terqui M, Thimonier J (1974) Nouvelle méthode radioimmunologique rapide pour l'estimation du niveau de progestérone plasmatique. Application pour le diagnostic précoce de la gestation chez la brebis et chez la chèvre. *CR Hebd Séanc Acad Sci, Paris D* 279, 1109-1112