PGF$_{2\alpha}$-induced milk ejection in ewes having cyclic or pregnant corpora lutea

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Summary. The production of luteal oxytocin in ewes, resulting from the intrajugular injection of 200 µg of PGF$_{2\alpha}$, could be determined by the increase in intramammary pressure. This simple indirect method of measuring the activity of the corpus luteum enabled easy detection of renewed post-partum ovulation or the onset of pregnancy. The response was monitored every two days between Days 0 and 25, then every 4 days between Days 25 and 59 in : — 9 cyclic ewes (group B) ; — 9 cyclic ewes treated with three daily intramuscular injections of 25 mg of Trilostane, a steroid synthesis inhibitor, between Days 7 and 25 (group A) ; — 11 prenant ewes (group C).

Progesterone levels were determined each day from blood sampled in the jugular vein. Trilostane produced a decrease in plasma progesterone, not a total suppression (fig. 3), but did not significantly modify the intramammary pressure variations resulting from PGF$_{2\alpha}$ injections. These were identical in both cyclic and pregnant ewes during the first 15 days : they increased from D0 to D7 and decreased between D12 and D15 (fig. 4).

After D15, the increase in intramammary pressure progressively weakened and became 0 at D17 in the cyclic ewes, whereas in the pregnant animals there was a renewed increase in intramammary pressure until D20 ; this regressed progressively afterwards and disappeared towards D45. This transitory, renewed activity between D15 and D20 might be an indirect or direct result of the message delivered by the embryo to maintain the corpus luteum. Several hypotheses are discussed with a view to explaining this phenomenon.

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Introduction.

In cows (Labussière *et al.,* 1982) and ewes (Labussière *et al.,* 1983), the intrajugular administration of a synthetic analogue of PGF$_{2\alpha}$, (Dinolytic Upjohn), induces milk ejection which is directly correlated to plasma progesterone levels. Around the time of oestrus, PGF$_{2\alpha}$ has little or no effect. However, when a similar dose is administered during luteal phase there is a large increase in intramammary pressure (IMP).

In numerous species, and in particular in ewes, the corpus luteum contains and produces large amounts of oxytocin (Wathes and Swan, 1982 ; Wathes *et al.,* 1986) which are found in the ovarian vein in response to an intravenous injection of PGF$_{2\alpha}$ (Flint and Sheldrick, 1982). The observed response of the mammary gland could be due to this liberation of luteal oxytocin (and not to neuro-hypophysial oxytocin) because intrajugular injection of PGF$_{2\alpha}$ does not induce

milk ejection after bilateral ovariectomy (Labussière, Eyi Ngui and Combaud, 1986) or after temporary occlusion of the ovarian veins and arteries (Labussière and Combaud, 1988).

Recording the changes in intramammary pressure after injection of $PGF_{2\alpha}$ is an indirect but simple method for measuring luteal activity, thus facilitating the detection of the reinitiation of ovulation at the end of the anoestrus post-partum period or early diagnosis of pregnancy.

In the present study, we used this method to measure the response of the corpus luteum at regular intervals after ovulation in pregnant ewes (group C), cyclic ewes (group B) or cyclic ewes treated with Trilostane (Winthrop) (group A), an inhibitor of progesterone synthesis.

Material and methods.

Experimental procedure. — Twenty-nine Lacaune ewes lambed between the 5th January and 22nd February 1985 ; they were separated from their lambs 48 h after parturition and were then milked by machine twice a day at 7 : 30 a.m. and 4 : 15 p.m.

Towards the end of the third month of lactation, a polyurethane sponge containing 40 mg of fluorogestone acetate (Intervet) was placed in the vagina of each ewe at 5 p.m. The sponge was removed 14 days later at 5 p.m. and an intramuscular injection of 600 IU of PMSG was given at the same time.

The oestrus brought on by this treatment began a little more than 30 h after sponge removal, that is at about 1 a.m. two days later (Day 0) (Cognié, Mariana and Thimonier, 1970).

The 29 ewes were then divided into 3 groups (A, B and C) (fig. 1) of similar age (about 40 % first lactation and 60 % adults) and with a similar milk production of about 750 ml/day.

Group A. Nine cyclic ewes received three intramuscular injections of 25 mg of Trilostane daily at 7 : 30 a.m., 3 p.m. and 10 : 30 p.m., diluted in 5 ml of a vehicle solution of dimethyl sulphoxide with glucose added (DMSO 800 ml, glucose 40 g, H_2O qsp 1 000 ml) between Days 7 and 25. The decrease in blood progesterone brought about by Trilostane could produce a parasitic ovulation (undesirable before D16-D17) so a polyurethane sponge impregnated with fluorogestone acetate (FGA) was placed in the vagina of each ewe between D7 (5 p.m.) and D15 (5 p.m.). Sponge removal was accompanied by an intramuscular injection of 600 IU of PMSG. Between D0 and D25 blood samples were taken daily at 2 p.m. to measure progesterone levels (see below) ; variations in intramammary pressure (IMP) resulting from the intrajugular injection of 200 µg of Dinolytic (Upjohn) every 2 days were recorded before the morning milking. This was done to indirectly measure luteal oxytocin production (see below).
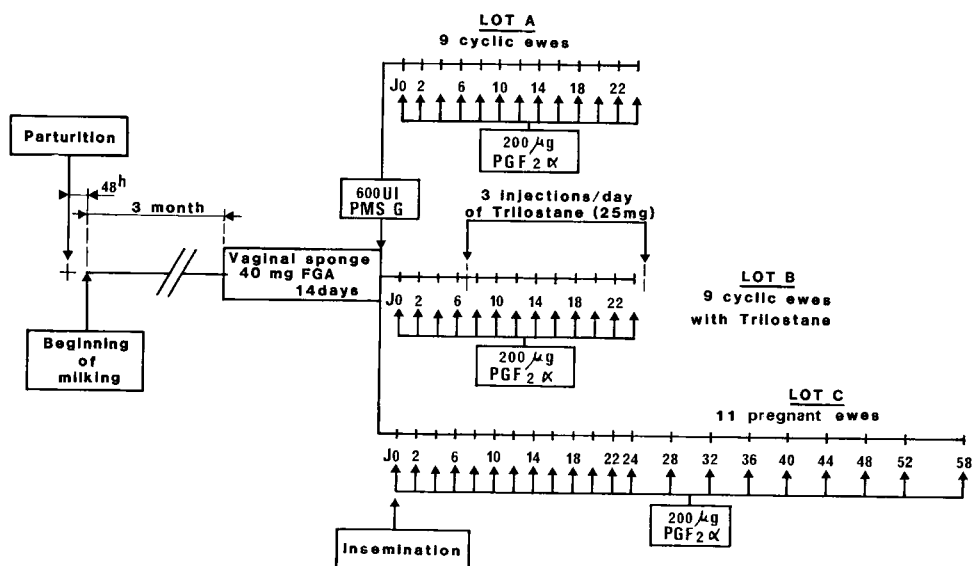
FIG. 1. — *Diagram showing the organisation of the experiment.*

Group B. Nine control cyclic ewes were not treated with Trilostane. However, they were given three 5-ml injections daily (at 7 : 30 a.m., 3 p.m. and 10 : 30 p.m.) of the vehicle solution of DMSO between D7 and D25. Otherwise, FGA treatment between D7 and D25, blood sampling (progesterone levels) and IMP measurements between D0 and D25 were carried out exactly as described for group A.

Group C. Eleven pregnant ewes were bred at D0. The endocrine activity of the corpus luteum was studied in exactly the same conditions as those of groups A and B, *i.e.* progesterone was sampled daily and oxytocin every 2 days ; after D24, IMP was measured every 4 days. The methodology used for the FGA treatment between D7 and D15 and for the 3 daily injections of the vehicle solution of DMSO without Trilostane, between D7 and D25, was identical to that of group B.

Evaluation of plasma progesterone levels. — Blood samples were taken from the jugular vein of each ewe once a day at 2 p.m. using heparinized vacutainers. The tubes were centrifuged for 15 min at 3,000 × g and the supernatant frozen at — 20 °C. Progesterone concentration was evaluated by radioimmunoassay using Saumande's technique (1984, personal communication).

Injection of $PGF_{2\alpha}$ and recording of intramammary pressure variations. — The morning milking was slightly delayed as the effects of $PGF_{2\alpha}$ on intramammary pressure were measured between 8 : 30 and 10 : 30 a.m. 200 µg of Dinolytic were diluted in 2 ml of a sterile solution of 0.9 % NaCl and then injected into one of the two jugular veins. Before this, 2 to 3 cm of a polyethylene catheter (type PE 190 Intramedic, Clay Adams ; length 100 cm, external diameter 1.70 mm,

internal diameter 1.19 mm) were introduced into the teat canal. The other end of the catheter was connected to a Hewlett Packard pressure transducer (type 1280C), placed at the same height as the cistern. Pressure variations were recorded with a Hewlett Packard recorder (type 7754A) equipped with a 8805B amplifier.

Figure 2 shows the two parameters used to characterize these variations. The deflection amplitude was the difference in pressure (mm/Hg) between the height of the graph before $PGF_{2\alpha}$ injection and the maximum reached after injection. The surface area under the curve measured during the first 4 min of response combined pressure amplitude and persistance.

Statistical analysis. — Curve height and profile, showing changes in the representative parameters of luteal function (progesterone, amplitude and surface of IMP deflection) every 2 days, were compared using Morrisson's method (1967) which takes into account the correlation between successive measurements.

Results.

Plasma progesterone.

Figure 3 shows that the blood progesterone levels of groups B and C responded in the usual way in cyclic and pregnant ewes. From D7, Trilostane administration to group A animals was followed immediately by a non-significant reduction in plasma progesterone (Morrisson, 1967), the level of which remained higher than 1.25 ng/ml.

Intramammary pressure variation resulting from $PGF_{2\alpha}$ injection.

The Trilostane administration did not seem to affect the milk ejection response brought about by Dinolytic injection. Morrisson's test (1967) revealed no differences between group A and group B (D0 to D17) for either the maximal deflection amplitude of intramammary pressure (fig. 4a) or for the surface area under those curves during the first 4 min of response (fig. 4b). During the first two weeks after oestrus in the pregnant ewes (group C), these two parameters (amplitude and surface area) followed the same trend as those recorded in the cyclic ewes of groups A and B, *i.e.* milk ejection responses were greatly reduced between D11 and D15. In contrast to the cyclic animals, these responses never completely disappeared on D16 or D17 in the pregnant ewes ; they even increased up until D20, then gradually decreased and disappeared towards D45. All eleven pregnant ewes showed this renewed activity ; figure 5 shows the 20 recordings obtained from one ewe between D0 and D60.
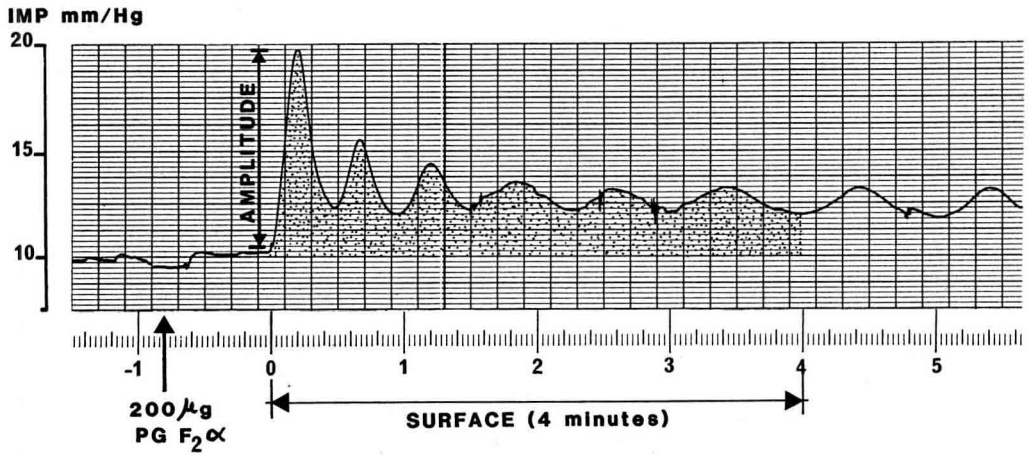
IMP mm/Hg

FIG. 2. — *The two parameters used to show variations of intramammary pressure :* — *Amplitude :* height of the deflection measured (in mm/Hg) between pressure before injection and that measured at maximal response. — *Surface area* (cm$_2$) under the curve measured during the first 4 min of response (shaded).
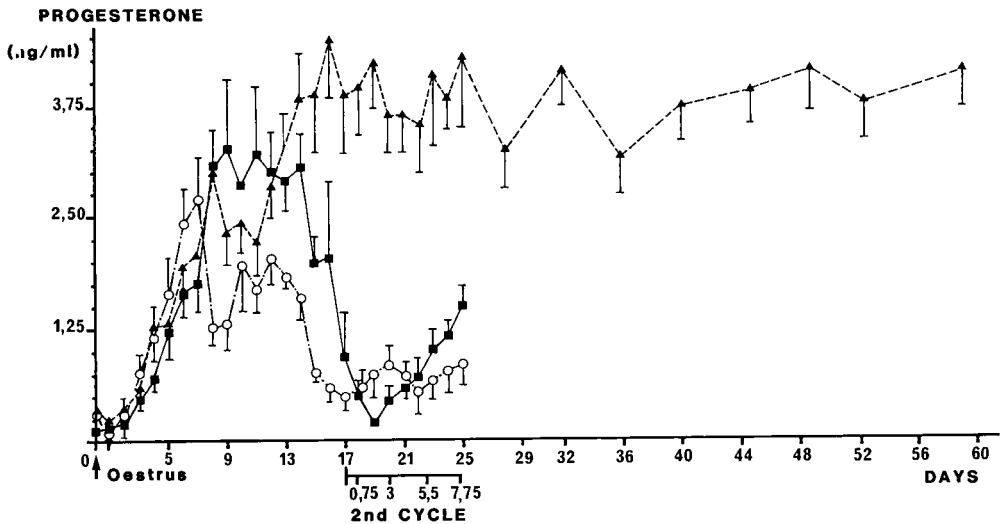

FIG. 3. — *Progesterone level change in jugular vein blood of ewes of* Group A (○), B (■) and C (▲).
— Average values with S/$\sqrt{n}$. — Polyurethane vaginal sponges (30 mg FGA) were placed in the vagina of each ewe between D7 and D15. Removal at D15 was accompanied by an (IM) injection of PMSG (600 IU). — 9 cyclic ewes from Group A received 3 injections per day (IM) of 25 mg of Trilostane between D7 and D25.
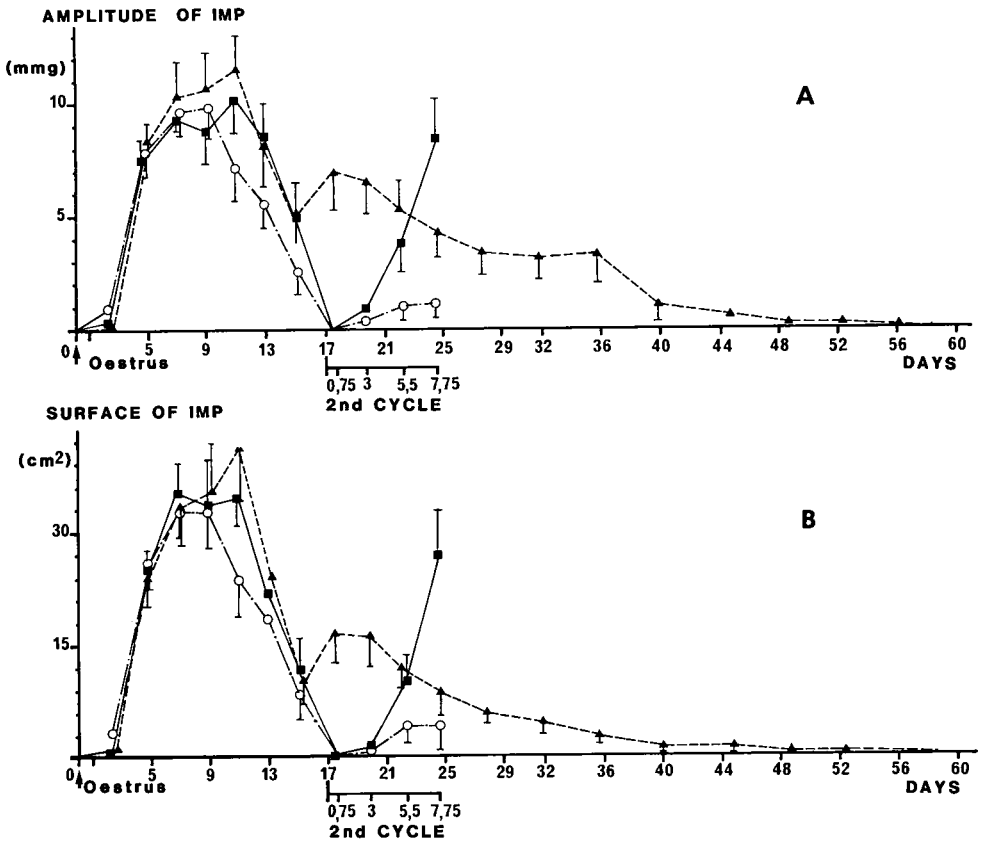
FIG. 4. — *Effects of intrajugular injection of 200 µg of Dinolytic on intramammary pressure in ewes of* Group A (○), B (■) and C (▲).

FIG. 4a. — *Maximal amplitude deflection* (mm/Hg) *before and after injection and after injection.*

FIG. 4b. — *Surface area under the curve showing intramammary pressure during the first 4 min* (planimetric calculation).

— Average values with S/√n. — A polyurethane vaginal sponge (30 mg FGA) was placed in the vagina of each ewe between D7 and D15. Removal at D15 was accompanied by an injection (IM) of PMSG (600 IU). — The cyclic ewes from Group A received 3 injections/day (IM) of 25 mg of Trilostane between D7 and D25.

It should be noted that a small intramammary pressure response was not only characterized by a small deflection but also by a series of peaks of short duration. During the first few days of luteal phase, these small peaks tended to join and could be confused with a long, fused tetanus response when they became stronger. The peaks, which lasted about 20 sec, reappeared temporarily around D15, joined once more between D16 and D20, and finally became progressively apparent and further apart between D25 and D40.

Calculation of the surface area under the IMP curve for the first 4 min of response may thus be a better criterion for judging the mode of oxytocin release and the persistance of its effect on the mammary gland than simple measurement of maximal curve deflection.
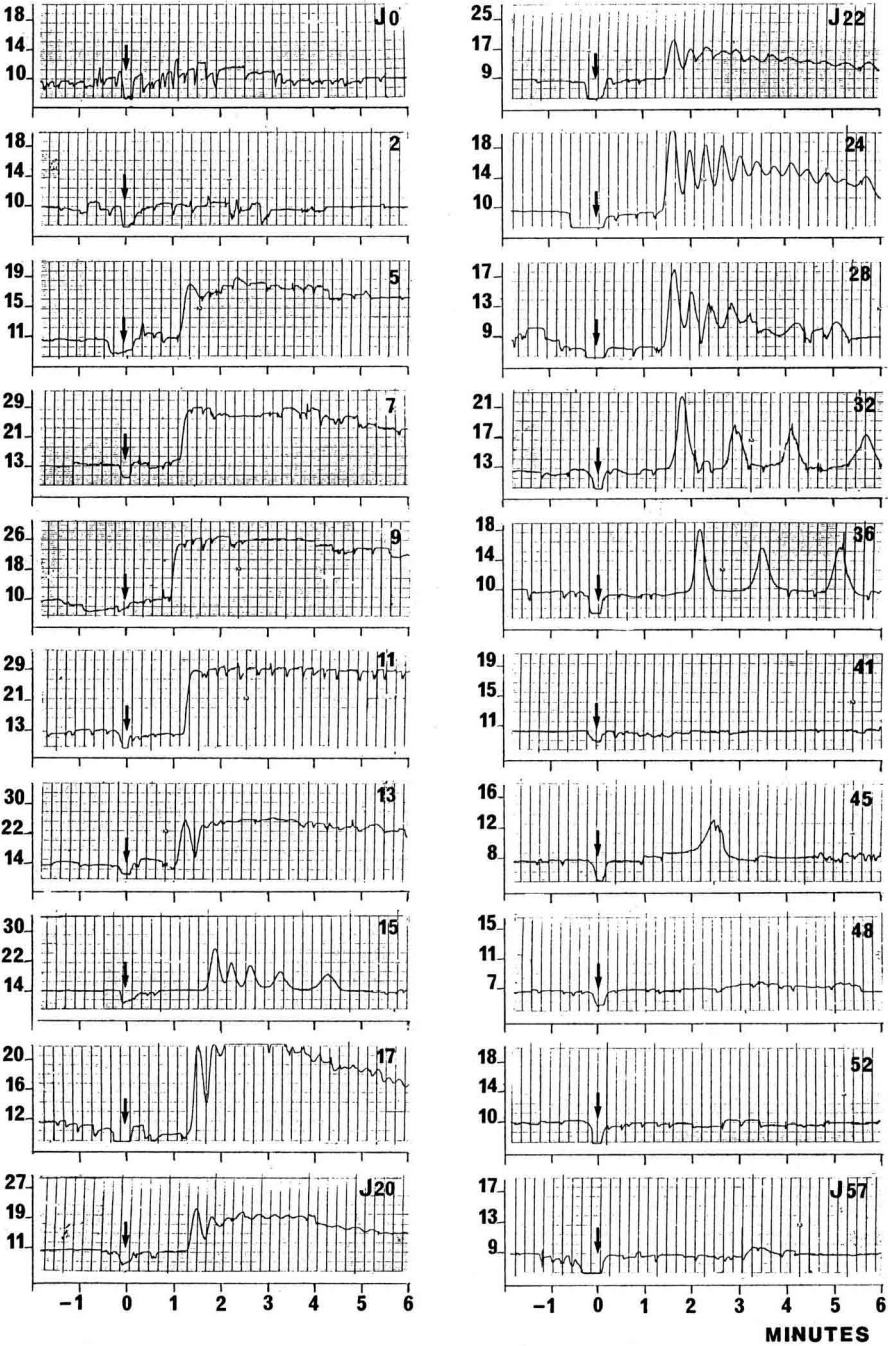
INTRAMAMMARY PRESSURE (mmHg)

FIG. 5. — *The effects of intrajugular injection of 200 µg of Dinolytic on the intramammary pressure of ewe 888*. 20 recordings obtained during the first 60 days of gestation are shown. ↓ moment of injection.

Discussion.

In bovines (Schams, Kruip and Koll, 1985) as in ovines (Wathes et al., 1986), it is possible to detect the presence of oxytocin in the wall of large preovulatory follicles and, by immunocytochemistry, in the granulosa cells (Kruip et al., 1985 ; Wathes et al., 1986).

Ivell et al. (1985) showed that mRNA coding oxytocin increased greatly in cow corpus luteum, particularly at the beginning of ovulation and in the 3 following days. In this species, the concentration of the hormone in luteal tissue is maximal about the end of the first week (Wathes, Swann and Pickering, 1984). It is approximately the same in cyclic and pregnant ewes (Sheldrick and Flint, 1983a ; Lacroix, 1986) which show an abundant stock of secretory granules in the large granulosa cells. All authors have demonstrated in both bovines and ovines that, from this stage onwards, there is first a progressive decline in tissue oxytocin, then a sudden drop whether the corpus luteum is regressing (cyclic ewes), whether the animals remain intact due to pregnancy, or whether they are hysterectomized (Sheldrick and Flint, 1983b ; Lacroix, 1986).

The pattern of plasma oxytocin levels in ewes (in the ovarian vein or in the general circulation) shows a low during the period around oestrus (Webb et al., 1981 ; Schams, Lahlou-Kassi and Glatzel, 1982) and a rapid increase during the first 6 to 7 days, followed by a decrease in cyclic ewes between D13 and D15 and a little earlier in pregnant ewes (Mitchell et al., 1982 ; Schams and Lahlou-Kassi, 1984 ; Sheldrick and Flint, 1981, 1983a ; Hooper and Thorburn, 1987).

Finally, the recent work of Hooper, Watkins and Thorburn (1986) shows that pregnancy in sheep does not significantly modify the frequency of oxytocin pulses in the utero-ovarian vein during luteolysis, but that the basal levels of the hormone are nevertheless more elevated in this vein when the corpus luteum is maintained (Hooper, Watkins and Thorburn, 1986).

Conclusions.

Intravenous injections of low doses of Dinolytic were given every 2 days beginning at oestrus to stimulate luteal oxytocin release, which is simple to measure by the increase in intramammary pressure. Although these injections did not strictly duplicate the effects of $PGF_{2\alpha}$ endometrial pulses (Moore et al., 1986), our work :

1) confirms the essentials of the studies cited, because the change in milk ejection response parameters was the same in both the cyclic and the pregnant ewes. However, in the latter, earlier decreases in IMP, which could have been predicted from the oxytocin levels (Schams and Lahlou-Kassi, 1984 ; Sheldrick and Flint, 1981, 1983a), were not observed ;

2) shows that in all the pregnant ewes there was an increase in IMP after D15, probably resulting from an oxytocin release which could be a direct (or indirect ?) consequence of the embryo. It is interesting to note that this recovery coincides perfectly with the increase in either luteal oxytocin or plasma oxytocin

observed between D15 and D17 by Sheldrick and Flint ([1]) (1983a, 1984) and by Hooper and Thorburn (1987) in pregnant ewes. Each of the 11 ewes tested exhibited this intensified PGF$_{2\alpha}$ sensitivity. These new marked responses of intramammary pressure persisted for a little less than one week and afterwards decreased progressively between D20 and D40 of pregnancy. They disappeared at about the same time, as there seemed to be no oxytocin left in the corpus luteum (Sheldrick and Flint, 1983a).

It is possible that the sudden decrease in milk ejection noted from D12 in pregnant ewes resulted from low luteal oxytocin levels. Sheldrick and Flint (1984) suggest that, at this stage, the abundant secretion of uterine PGE$_2$ (Lewis *et al.*, 1978 ; Ellinwood, Nett and Niswender, 1973 ; Lacroix and Kann, 1982) and embryonic PGE$_2$ (Marcus, 1981 ; Lacroix and Kann, 1982) could act not only as a luteinizing factor ([2]) (Speroff and Ramwell, 1970 ; Sellner and Wickersham, 1970 ; Pratt, Butcher and Inskeep, 1977) but also as a liberating agent for oxytocin (Sheldrick and Flint, 1984 ; Labussière and Combaud, 1988). PGE$_2$ would be capable of temporarily « emptying » the corpus luteum and of disconnecting the positive feedback system between the oxytocin pulses and those of uterine PGF$_{2\alpha}$ which would normally lead to luteolysis ([3]).

The partial restoration of luteal reserves after this temporary perturbation would explain the renewed activity after D15, but this was understood to be only transitory and did not continue after 1 1/2 months, considering the aging and disappearance of large luteal cells rich in secretory granules and their replacement by thecal cells (Alila and Hansel, 1984). In addition, the embryo might secrete oxytocin after D15 of pregnancy ; Lacroix (1986) has shown the presence of an oxytocin-like substance in the embryonic tissue of ewes and there was an increase in the concentration of this substance between D15 and D21 of pregnancy.

No matter which hypothesis is accepted, our results suggest that the ability of the corpus luteum to release oxytocin declines after D13 of pregnancy, although plasma progesterone is not affected. Inversely, Trilostane did not significantly (Morrisson, 1967) affect milk ejection resulting from PGF$_{2\alpha}$ administration. As steroid synthesis was not totally suppressed, it cannot be concluded that progesterone played no role in the oxytocin concentration of the corpus luteum. Tan, Tweedale and Biggs (1982) showed that oxytocin could augment progesterone production in cells throughout the bovine corpus luteum and, in the same species, the synthesis of the two hormones in cultures of granulosa cells is also closely correlated (Geenen *et al.*, 1985). Otherwise, it is known that

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([1]) Sheldrick and Flint (1986) did not confirm these renewed oxytocin stocks in luteal tissue and thought their preliminary results might have been misleading due to the use of an antibody which was not specific enough.

([2]) According to Harrisson, Kenny and Niswender (1987), PGE$_2$ not stimulate progesterone secretion.

([3]) Thorburn *et al.* (1973), Barcikowski *et al.* (1974) and Lacroix and Kann (1986) showed that embryo presence at this stage suppressed endometrial pulsative PGF$_{2\alpha}$ surges.

progesterone injection in the first days of the ewe cycle favours uterine production of $PGF_{2\alpha}$ (Scaramuzzi et al., 1977) and advances luteolysis. However, on the contrary, Vincent and Inskeep (1986) noted that after D12, ovariectomy or lutectomy resulted in an augmentation of plasma $PGF_{2\alpha}$ levels in the utero-ovarian vein.

In *conclusion*, this original research is based on the use of milk ejection as an easy method for determining the response amplitude of oxytocin from the corpus luteum of cyclic or pregnant ewes (or even from the embryo ?) to weak doses of $PGF_{2\alpha}$. In order to validate this method, we now wish to associate the increase in IMP, brought about by prostaglandins, with the synchronous augmentation of plasma oxytocin in the ovarian vein.

Résumé. *Ejection du lait provoquée par PGF$_{2\alpha}$ sur des brebis possédant un corps jaune cyclique ou un corps jaune de gestation.*

Chez la brebis, la décharge d'ocytocine lutéale induite par l'injection intrajugulaire de 200 µg de $PGF_{2\alpha}$ peut être mise en évidence par une augmentation de la pression intramammaire. Cette méthode d'approche indirecte, mais simple, de l'activité du corps jaune offrant la possibilité de détecter facilement une reprise d'ovulation post-partum ou un début de gravidité, nous avons jugé utile de décrire les réponses obtenues tous les 2 jours (entre J0 et J25) puis tous les 4 jours (entre J25 et J59) chez : — 9 brebis cycliques (lot B) ; — 9 brebis cycliques traitées entre J7 et J25 par 3 injections intramusculaires quotidiennes de 25 mg de trilostane (inhibiteur de synthèse stéroïdienne) (lot A) ; — 11 brebis gestantes (lot C).

La progestérone est dosée chaque jour dans le sang de la veine jugulaire. Le traitement au trilostane provoque une diminution (et non une abolition) des taux plasmatiques (fig. 3) mais ne modifie pas significativement les variations de pression intramammaire induites par l'injection de $PGF_{2\alpha}$; celles-ci sont identiques chez les brebis cycliques et gestantes pendant les 15 premiers jours (elles augmentent de J0 à J7 et diminuent entre J12 et J15) (fig. 4).

Au-delà de J15, l'augmentation de pression intramammaire devient de plus en plus faible puis s'annule à J17 chez les femelles cycliques alors que l'ampleur des réponses s'accroît à nouveau jusqu'à J20 chez toutes les femelles en gestation ; elles régressent ensuite progressivement et disparaissent vers J45.

Il est possible que cette reprise d'activité transitoire entre J15 et J20 soit le reflet direct ou indirect, du message que délivre l'embryon pour maintenir le corps jaune. Plusieurs hypothèses sont discutées en vue d'expliquer ce phénomène.

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