LUTEOTROPIC FACTORS IN THE SHEEP

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Robert Denamur died suddenly on 25th April 1973 at the age of 48. He was a great scientist, who retained a deep sense of personal humility; he was always a prey to nagging doubts about the validity of his own experimental observations. This unique ability to indulge more in self-criticism than in the criticism of others made him a perfectionist, and prevented him from rushing into print until he had checked, re-checked and repeated all his experiments. Thus, the greater part of his researches will now remain unpublished; the excellence of his existing contributions in the fields of lactational and ovarian physiology give us some indication of what the scientific community has lost by his untimely death.

This paper, full of 'unpublished observations' that must now remain for lesser men to confirm and document, was the last that he wrote, and it was published posthumously in 1973 in the Proceedings (pp. 213-223) of a conference on 'Le Corps Jaune', held in Paris in July 1973. This paper is reproduced at the wish of the members of the Society for the Study of Fertility and with the permission of Masson et Cie. I have taken the liberty of translating the article somewhat freely, and I hope that it may serve as a memorial to a close friend, a gentleman of Science, and above all a gentle man of Science.

R.V.S.

In the sheep, the formation of the corpus luteum and its subsequent secretory activity are the result of the trophic action of a number of pituitary hormones; during gestation, there is an additional feto-placental trophic influence. It is difficult to discuss these luteotrophic effects without taking into account the luteolytic factors, which are principally of uterine origin in this species (McCracken & his co-authors, 1972; Goding, 1973). In most physiological situations, the secretory activity of the corpus luteum is the net result of an interplay between these trophic and lytic factors; thus the maintenance, regression or stimulation of the corpus luteum can result from a change in balance between these trophic and lytic effects. It therefore follows that the measurement of the plasma concentrations of luteotrophic hormones can only be of relative physiological significance. Bearing in mind this limitation, we will discuss the sequence of trophic effects during the oestrous cycle, and the changes that take place at the end of the oestrous cycle during luteolysis, and at the beginning of pregnancy.

I. THE PITUITARY CONTROL OF THE CORPUS LUTEUM IN THE CYCLE

The average length of the oestrous cycle of the sheep is about 17 days; four-fifths of this time is occupied by the luteal phase. The pro-oestrous phase is extremely short, and oestrus itself, which lasts from 28 to 30 hr, is followed soon after by ovulation (Hansel & Echterenkamp, 1972).

The weight of the corpus luteum, its progesterone content, and the concentration of progesterone in the peripheral plasma increase rapidly in the immediate postovulatory phase, then remain relatively constant until the 14th day of the cycle before declining precipitously at the time of luteolysis (Plotka, Erb & Harrington, 1970; Denamur, 1972; Geschwind, 1972; Lemon & Thimonier,
1973). Estimates of the concentration of progesterone in ovarian vein plasma, measurement of nucleic acids and of the rate of progesterone secretion, and numerous morphological, histochemical and ultrastructural studies of ovine corpora lutea are in accord with this. Finally, the peripheral progesterone concentration is significantly elevated when the animal possesses an increased number of functional corpora lutea (Thorburn, Bassett & Smith, 1969).

The development and secretory activity of the corpus luteum depends on various trophic factors during the oestrous cycle, the most important of which are the following:

(1) *Hormonal discharge during oestrus.* The changes in FSH have recently been reviewed by Geschwind (1972), Kerdelhué, Kann & Jutisz (1972) and L’Hermite, Niswender, Reichert & Midgley (1972); the changes in LH by Geschwind (1972) and prolactin by Reeves, Arimura & Schally (1970), Bryant, Greenwood, Kann, Martinet & Denamur (1971), Kann (1971) and Cumming, Brown, Goding, Bryant & Greenwood (1972). The relative importance of the discharge of these three hormones at oestrus on the subsequent fate of the corpus luteum has still not been precisely determined; it appears, however, that the secretion of prolactin can be considerably reduced (by treatment with ergocryptine) without altering the normal course of the oestrous cycle in intact animals (Niswender, 1972; G. Kann and R. Denamur, unpublished work). This ovulatory discharge of hormones allows a limited development of the corpus luteum in sheep subsequently hypophysectomized 40 hr after the beginning of oestrus; by the 12th day of the cycle they show 30 to 40% of the degree of morphological luteal development seen in normal controls (Denamur, 1968, and unpublished work). The secretory activity of these corpora lutea after hypophysectomy is also of shorter duration and reduced intensity (lower progesterone concentrations in peripheral blood and lower ribonucleic acid concentrations in luteal tissue) than in intact controls.

Following hypophysectomy on Day 2 of the cycle and the simultaneous administration of anti-LH serum and anti-prolactin serum, the development of the corpus luteum is not impaired any further in the ensuing 9 days. The trophic stimulus initiated by the hormonal discharge at ovulation (principally LH and FSH) therefore continues to exert its effect during the luteal phase; this is a real effect, and the results cannot be explained by incomplete hypophysectomy. This interpretation is in agreement with the observations of Channing (1970a, b), Kammerman, Canfield, Kolena & Channing (1972) and Channing & Kammerman (1973), who showed that the granulosa cells of the pig and the monkey gradually acquired LH-receptor sites before ovulation and that the subsequent capacity of these cells to synthesize progesterone is in part programmed at the time of oestrus.

(2) *Luteinizing hormone.* In contrast to the variations in the secretion rate of progesterone in sheep, the plasma concentrations of LH remain extremely low throughout the luteal phase, though some fluctuations may occur (Geschwind, 1972; Denamur, 1972). This lack of correlation between the plasma concentrations of progesterone and LH is explained by the absence of a feedback of the steroid on the basal rates of LH secretion (Goding, Blockey, Brown, Catt & Cumming, 1970; Scaramuzzi, Tillson, Thorneycroft & Caldwell, 1971).
However, LH possesses important trophic actions on the cyclical corpora lutea; it can augment the synthesis of progesterone during the course of incubations of luteal slices in vitro (Kaltenbach, Cook, Niswender & Nalbandov, 1966), and infusion into the ovarian artery stimulates progesterone secretion (McCracken, Baird & Goding, 1971). Luteinizing hormone also increases the secretion of progesterone and the weight of the corpus luteum in sheep hypophysectomized on the 2nd day of the oestrous cycle (R. Denamur, unpublished work).

Finally, the repeated administration of antiserum to LH will result in partial regression of the cyclical corpora lutea in intact animals (weight of corpus luteum following administration of normal serum, 620 mg±45; weight of corpus luteum following anti-LH serum, 388 mg±39) or in animals in which the pituitary stalk has been sectioned (weight of corpus luteum following normal serum, 542 mg±32; weight of corpus luteum following anti-LH serum, 320±33) (R. Denamur, unpublished work).

(3) Prolactin. The concentration of prolactin in the plasma is only slightly elevated in the luteal phase of the sheep oestrous cycle (Davis, Reichert & Niswender, 1971; Kann, 1971; Cumming et al., 1972), and like that of LH, it is not apparently related to the secretion of progesterone. Furthermore, prolactin cannot stimulate the secretion of progesterone by the corpora lutea of intact cycling animals either in vitro or in vivo (McCracken et al., 1971). The luteotrophic properties of prolactin can, however, be demonstrated in cycling sheep after hypophysectomy on the 2nd day of the cycle, when it can stimulate the weight and the secretory activity of the corpora lutea (weight = 407 mg±91 after prolactin treatment as compared to 247 mg±23 in untreated controls). The consequences of pituitary stalk section on Day 3 of the cycle also favour a luteotropic rôle for prolactin during the cycle. This surgical intervention causes a considerable diminution in the secretion of LH, which can no longer be detected in the peripheral blood by radioimmunoassay (Kann & Denamur, 1973), whereas prolactin can still be measured quite easily (Bryant et al., 1971); stalk section also allows the development of relatively normal corpora lutea up to the 12th day of the cycle (Denamur, Martinet & Short, 1966, 1970). If any residual secretion of LH following pituitary stalk section is eliminated by injection of an LH antiserum, the corpora lutea are nevertheless better developed than after hypophysectomy (320 mg±33 versus 247 mg±23).

(4) Follicle-stimulating hormone. The plasma concentrations of FSH have recently been measured during the luteal phase of the oestrous cycle (Kerdelhué et al., 1972; L'Hermite et al., 1972). However, FSH neither stimulates progesterone secretion in vitro nor in vivo (McCracken et al., 1971) and any effect it may have on the corpus luteum is still unknown.

II. THE RELATIONSHIP BETWEEN LUTEOLYSIS AT THE END OF THE CYCLE AND THE PLASMA CONCENTRATIONS OF LH AND PROLACTIN

(1) Normal luteolysis. The declining secretory activity of the corpus luteum first becomes apparent on the 13th or 14th day of the cycle (Thorburn, Cox, Currie, Restall & Schneider, 1972). The concentrations of prolactin in the peripheral plasma are not significantly altered at the beginning of luteolysis;
they still show several peaks coinciding with rises in the oestradiol concentration. Is prolactin involved in the cyclical regression of the sheep corpus luteum as Wuttke & Meites (1971) have postulated for the rat? Large amounts of prolactin are not necessary for luteolysis in the sheep because it can still occur on Days 14 to 17 of the cycle following section of the pituitary stalk on Day 3 or Day 10. Furthermore, ergocryptine does not interfere with cyclical luteolysis (Niswender, 1972; G. Kann and R. Denamur, unpublished work).

The content of LH-RH in the hypothalamus declines between the 13th and 15th days of the cycle in the sheep (Jackson, Roche, Foster & Dziuk, 1971). The plasma concentrations of LH do not, however, show any significant variations before or during luteolysis in the sheep; but they do show a tendency to increase during the pro-oestrous phase (Geschwind, 1972).

Thus it appears unlikely that cyclical luteolysis could be a result either of a deficiency of LH or of prolactin, and in any case such an hormonal mechanism would not explain the local influence of the uterus on luteolysis. The dominant rôle of the uterus in luteolysis is also emphasized by the fact that hormone mixtures (250 i.u. prolactin plus 0.5 mg LH daily) which are extremely luteotrophic in hysterectomized hypophysectomized sheep (Denamur et al., 1973) cannot prolong the corpora lutea after hypophysectomy alone on the 10th day of the cycle (R. Denamur, unpublished work).

(2) Induced luteolysis. The cyclical regression of the corpus luteum is markedly retarded by X-irradiation of the Graafian follicles; on the other hand, it can be precipitated by the administration of oestradiol towards the end of the luteal phase in intact sheep or those in which the pituitary stalk has been sectioned (Denamur & Kann, 1973). Regression can still take place in spite of high plasma prolactin levels, as seen in normal sheep or following stalk section (Kann & Denamur, 1973). The concentrations of LH in the plasma of intact sheep receiving oestradiol are also slightly elevated for a short while, although LH is not measurable in animals which have undergone stalk section (Kann & Denamur, 1973). Thus precocious luteolysis is not related in any simple manner to gonadotrophic hormone secretion. We have also shown that the luteolytic action of oestradiol is mediated through the uterine luteolysis (Denamur & Kann, 1973), as is the luteolysis induced by the presence of an IUD (Spilman & Duby, 1972). The increase in plasma concentrations of prolactin and LH following a luteolytic dose of oestradiol in normal animals is accompanied by an even more significant increase in the secretion of prostaglandin F2α (R. Denamur, unpublished work). In sheep in which the pituitary stalk has been sectioned, it is probable that variations in the concentrations of trophic and lytic factors following the injection of oestradiol are of more limited amplitude, but the ultimate result is a luteolytic effect. The importance of the balance which must exist between the two pituitary luteotrophic hormones and the uterine lytic factor are emphasized by the fact that it is possible to augment the trophic influence and so maintain the corpus luteum in spite of the administration of oestradiol. Exogenous LH can prevent either normal or induced luteolysis in intact animals, whereas prolactin, even when injected in enormous quantities, cannot prolong the life of the cyclical corpus luteum in intact or hypophysectomized sheep (Denamur & Mauléon, 1963; Karsch, Cook, Ellicott, Foster,
Jackson & Nalbandov, 1971). Pharmacological doses of LH (2.5 mg/day), on the other hand, were able to maintain the corpus luteum for a limited time in 60% of cycling sheep (Karsch, Roche, Noveroske, Foster, Norton & Nalbandov, 1971). Furthermore, the luteolytic consequences of an IUD (Stormshak, Lehman & Hawk, 1967), or injections of progesterone (Lewis, Taylor & Inskeep, 1968) or oestradiol (Kann & Denamur, 1973) in intact sheep, are in part prevented by the administration of extremely large quantities of HCG. The physiological significance of these experiments is limited because the normal rate of LH secretion following a luteolytic injection of oestradiol is always less than 2.5 mg LH per day. Thus it appears that normally the uterine luteolysin is secreted in sufficient quantities to dominate the trophic effects of prolactin and endogenous LH.

III. THE LUTEOTROPHIC FACTORS ALLOWING THE ESTABLISHMENT OF PREGNANCY

The corpus luteum is essential for the maintenance of pregnancy for the first 50 days (Denamur & Martinet, 1955) and the injection of progesterone in ovariectomized sheep during this period allows the maintenance of gestation (Foote, Gooch, Pope & Casida, 1957; Moore & Rowson, 1959; Bindon, 1971). The secretory activity of the corpus luteum of pregnancy does not appear to be increased above that of the corpus luteum of the cycle during these first 50 days (Edgar & Ronaldson, 1958; Short & Moore, 1959; Harrison & Heap, 1968; Bassett, Oxborrow, Smith & Thorburn, 1969; Fylling, 1970). Thus whatever may be the nature of the luteotrophic stimulus, it appears to act initially by increasing the life-span of the corpus luteum.

(1) Plasma concentrations of LH and prolactin at the beginning of pregnancy

The concentrations of LH and prolactin in the peripheral plasma do not show any increase at the beginning of gestation (Denamur, Kann & Short, 1973), therefore it is unlikely that the embryo stimulates gonadotrophin production by the maternal pituitary. A pregnancy can even become established in spite of extremely low concentrations of LH and prolactin in the plasma; section of the pituitary stalk on the 10th day of gestation allows the early stages of embryonic development to continue in spite of an abrupt fall in the plasma concentrations of prolactin and especially of LH.

(2) The necessity for LH and prolactin in the establishment of pregnancy

Hypophysectomy carried out on Day 3 or Day 10 of gestation brings about complete luteal regression by Day 20 and the termination of pregnancy. Hypophysectomy carried out on Day 30, which is after placental attachment (Amoroso, 1952; Bjorkman, 1965; Davies & Wimsatt, 1966; Boshier, 1969), also leads to regression of the corpus luteum and abortion (Denamur, 1968; R. Denamur, unpublished work).

The administration of either prolactin (250 i.u./day) or LH (0.5 mg/day) following hypophysectomy on the 10th day of pregnancy permits some degree of luteal maintenance up to Day 20, although abortion eventually takes place a few days later. By contrast, a mixture of prolactin and LH (250 i.u.+0.5 mg/day) allows normal luteal development up to Day 20 and maintenance of
a normal embryo (R. Denamur, unpublished work). A mixture of anti-LH and anti-prolactin serum given daily to normal sheep from Day 10 to Day 20 of gestation causes regression of the corpus luteum and abortion. After pituitary stalk section on the 10th day of gestation, injection of antiserum either to LH or to prolactin can bring about regression of the corpus luteum and abortion (R. Denamur, unpublished work).

(3) Does the embryo have a luteotrophic action or only an anti-luteolytic one?

Moor & Rowson (1966a, b, c, d) have shown that the embryo must be present in the uterus on Day 12 to 13 if the corpus luteum of the cycle is to be converted into one of pregnancy. Although the concentrations of LH and prolactin do not appear to change at this time, the embryo could augment the maternal luteotrophic activity, or it could diminish the uterine lytic influence and thus tip the balance in favour of luteal maintenance.

(a) Arguments in favour of a fetal luteotrophin. The evidence is only indirect during the first 50 days of gestation and rests on the following experiments.

If a fetal luteotrophin is produced between Days 10 and 20 or Days 30 and 42 of gestation, it is certainly incapable of maintaining the secretory activity of the corpus luteum following hypophysectomy. Furthermore, even if the embryo is maintained by progesterone therapy after hypophysectomy, the corpus luteum still regresses (R. Denamur, unpublished work).

Also, a comparison of the effects on the corpus luteum of LH (0.5 mg/day) or prolactin (250 i.u./day), or a mixture of the two hormones given either to non-pregnant sheep hypophysectomized on Day 10 of the cycle (no luteal maintenance to Day 20, except if LH is injected in pharmacological quantities together with prolactin), or given to pregnant sheep hypophysectomized on Day 10 (excellent maintenance of corpora lutea to Day 20 with a mixture of prolactin and LH) does not allow one to decide between an anti-luteolytic or a luteotrophic effect of the fetus. The same is true of sheep which receive LH (0.5 mg/day) following pituitary stalk section on Day 10 of the cycle (no maintenance of the corpus luteum) or on Day 10 of gestation (maintenance of the corpus luteum to Day 20) (R. Denamur, unpublished work). On the other hand, the striking difference between our previously published results in cycling sheep which were hypophysectomized and hysterectomized on the 10th day of the cycle (LH alone has no luteotrophic action; prolactin is luteotrophic in large doses; a mixture of prolactin and LH is fully luteotrophic), with those obtained in pregnant sheep hypophysectomized on Day 10 or Day 30 (LH alone has some effect in the Day-10 animals and is extremely luteotrophic in Day-30 animals—R. Denamur, unpublished work) suggests that the early embryo could well be secreting a luteotrophic factor. These observations are in agreement with the results of Rowson & Moor (1967), who showed a luteotrophic effect of homogenates of 14- and 15-day embryos, and with the results of Forsyth (1973).

Section of the pituitary stalk on Day 10, 20 or 30 of gestation allows the pregnancy to continue for several weeks (Denamur, 1968; R. Denamur, unpublished work). The same operation carried out on the 10th day of the cycle in hysterectomized sheep, which are therefore deprived of any luteolytic
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influence, only allows luteal maintenance for 15 days (Denamur et al., 1966). These different effects of pituitary stalk section can only be explained by the early presence of some feto-placental luteotrophin.

In the sheep after pituitary stalk section, antiserum to LH or prolactin given between Days 10 and 20 will cause abortion in 100% of the treated animals. Between the 30th and 42nd day, prolactin antiserum is completely ineffective and LH antiserum will only induce abortion in 50% of sheep (R. Denamur, unpublished work). The concentrations of endogenous LH and prolactin are always reduced following pituitary stalk section.

Hypophysectomy on the 60th day of gestation permits the maintenance of pregnancy for at least 12 days. The weight of the corpus luteum is about 75% of that in control animals (Denamur, 1968).

All these results are therefore in support of the concept of a progressive increase in secretion of a feto-placental luteotrophin.

(b) Arguments in favour of an anti-luteolytic effect of the embryo. Once again the evidence is indirect, but there are many experiments to show that the embryo does exert a local action on the ipsilateral corpus luteum (Moor & Rowson, 1966a, b; Moor, Rowson, Hay & Caldwell, 1969). The simplest explanation for this is that the conceptus somehow overcomes the local luteolytic action of the uterus (Caldwell, Rowson, Moor & Hay, 1969; Short, 1969; Rowson, 1970). The measurement of prostaglandin $F_2\alpha$ in utero-ovarian plasma has not yet provided any support for this possibility because the results so far obtained are few, and contradictory. Thus the secretion of PGF$_2\alpha$ paradoxically appears to increase at the moment of recognition of pregnancy according to Wilson, Butcher & Inskeep (1972), although it declines markedly according to Thorburn et al. (1972). More research is essential in order to discover whether the secretion of prostaglandin $F_2\alpha$ is really decreased, or whether it is normally liberated into the uterine venous blood at the time of maternal recognition of pregnancy, but is unable to exert a luteolytic effect.

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Robert Denamur


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