THE DEMONSTRATION THAT $\text{PGF}_{2\alpha}$ IS THE UTERINE LUTEOLYSIN IN THE EWE

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An 'in memoriam' notice to James R. Goding, who died on 27th June 1973, has been published in the *Journal of Reproduction and Fertility*, Vol. 37, pp. 435–436 (1974) as a Foreword to the Proceedings of the Australian Society for Reproductive Biology.

At the Annual General Meeting of the Society for the Study of Fertility in July 1973, the members of the Society expressed the wish that the paper by J. R. Goding given posthumously at the Conference on 'Le Corps Jaune' held in Paris in July 1973 should be published in this Journal as an additional mark of respect. The paper is reproduced from the Proceedings of this Conference (1973, pp. 312–323) by permission of Masson et Cie. Ed.

The title of this paper was not proposed by its author, but by the organizers of this conference. Having decided to accept the implied challenge, this paper has been written in the form of a review of the evidence, both for and against the proposition, under three main headings:

The evidence that a uterine luteolysin exists.

The evidence that $\text{PGF}_{2\alpha}$ is 'a' uterine luteolysin.

The evidence that $\text{PGF}_{2\alpha}$ is 'the' uterine luteolysin:

—the only PG in the uterus and uterine vein blood is $\text{PGF}_{2\alpha}$.

—the uterine secretion of $\text{PGF}_{2\alpha}$ is quantitatively sufficient to account for luteolysis.

—no satisfactory alternative explanation for luteolysis exists.

EVIDENCE THAT THERE IS A LUTEOLYSIN OF UTERINE ORIGIN

Before proceeding to a detailed analysis in this section, the possible causes of physiological luteolysis will be considered:

—the corpus luteum may regress because it has reached the end of its biological life-span. This explanation would make redundant any external system for the termination of luteal function.

—the corpus luteum may regress because of withdrawal of hormones necessary for its continued functioning.

—the corpus luteum may regress because of the action of a luteolysin.

The first possibility (intrinsic life-span of the corpus luteum) is mentioned solely because it is frequently held to be the correct explanation for luteolysis in man. However, it is really a "faute de mieux" explanation, which is acceptable only in the absence of evidence of a more definite kind. However, although hysterectomy causes profound effects on luteal maintenance in the ewe, no such consequence is found in man. Hence, such an explanation may yet prove to be correct for this species. In the remainder of this paper, evidence will

be adduced from the ovine species, hence its relevance in absolute terms is also confined to that species.

The second possibility (withdrawal of hormone support, no luteolysin being required) has been put forward *in extenso* by Nalbandov and his colleagues [1]. They have argued vigorously that evidence based on pelvic surgery—particularly on the uterus—must be rejected as artifactual; on the other hand, the most important part of their case is based on the results of surgery, i.e. hypophysectomy with or without pituitary hormone replacement. It would appear obvious that all surgical interference carries the possibility of creating artifactual or non-specific phenomena, and must be carefully controlled. The question of pelvic surgery causing artifact will be considered later, but the evidence concerning the rôle of the pituitary in luteolysis will now be examined.

According to Nalbandov & Cook [2] hypophysectomy is followed by rapid regression of the corpus luteum, unless certain pituitary hormones are replaced, no interruption of over an hour being tolerated [3]. Further, they found that the continuous infusion of LH resulted in prolongation of the life of the corpus luteum in the intact ewe [4]. However, the dose required for this effect (1.25 to 2.5 mg/day) is probably pharmacological, as all the follicles become luteinized. On the basis of these experiments, Nalbandov & Cook (1968) argued that luteolysis in the normal cycle was due to the withdrawal of pituitary hormone action. However, even when the plasma levels of LH, FSH and prolactin were measured at very frequent intervals during the cycle, no fall in gonadotrophin secretion could be observed at the time of luteolysis [5, 6]. Nalbandov also proposed that, as the follicle enlarged and developed large numbers of receptor sites for LH [7], it could divert essential gonadotrophins away from the existing corpus luteum. This hypothesis is unlikely to be true because luteolysis still occurs when the dominant follicle and the corpus luteum are in opposite ovaries.

In summary, although the pituitary is of the greatest importance in luteal maintenance [1], there is no evidence to show that withdrawal of pituitary support is in any direct way concerned with luteal regression during the oestrous cycle in the sheep.

Nalbandov's group have also provided evidence for the direct luteolytic rôle of oestradiol [2, 4, 50]. However, as they have been imbued with an almost fanatical aversion to hysterectomy [2], the possibility of an indirect action by way of the uterus cannot be excluded from their results. This subject will be considered again later.

The evidence against the existence of a uterine luteolysin being relatively unconvincing, it is now proposed to consider the evidence for such a proposition.

Classical evidence for the existence of a uterine luteolysin

- (1) Excision of the uterus results in prolonged luteal maintenance [8].
- (2) Partial excision of the uterus extends the life-span of the corpus luteum, roughly in proportion to the amount removed. This effect is confined to the corpus luteum adjacent to the portion of uterus removed, i.e. it is a local effect [9].

(3) Transplantation of the ovary, leaving the uterus *in situ*, also extends the life-span of the corpus luteum [10, 11].

(4) Transplantation of the uterus, leaving the ovary *in situ*, also results in luteal maintenance [11].

(5) Transplantation of both ovary and uterus together, as a single block of tissue, results in normal cyclical function [12].

(6) Infusion and cross-circulation experiments have demonstrated the presence of a luteolysin in utero-ovarian venous blood at the time of luteolysis [13–15, 54].

(7) Until recently, however, efforts to recover a potent luteolysin from uterine tissue or secretions have proved inconclusive [2, 16].

These findings, suggestive as they were, might have led to an 'impasse' except for a series of fortunate circumstances.

Firstly, as stated above [10, 11], sheep with ovarian transplants fail to show normal cyclical behaviour. The usual situation is that these ovaries contain corpora lutea which are maintained over a long period and are thus excellent *in vivo* preparations for testing the activity of possible luteolysins. An important feature of this preparation for this purpose turned out to be that the ovarian arterial circulation is accessible [10], and hence the preparation can be used for the study of the effects of compounds which are rapidly cleared from the circulation. A number of compounds, such as histamine and adrenaline, were shown to be without luteolytic effect [17].

Secondly, the Karolinska group, and many others, maintained a continuing interest in characterizing and defining the properties of the prostaglandin series of compounds [18–20].

Thirdly, Pharriss & Wyngarden [21] put forward a theory that PGF_{2α} may cause luteolysis in the rat by virtue of its veno-constrictive properties, i.e. by constricting the utero-ovarian vein. As will be seen later, decrease in venous effluent is not a necessary concomitant of luteolysis. But PGF_{2α} did prove to be luteolytic, and this finding set the stage for the empirical testing of prostaglandins for luteolytic activity in sheep.

EVIDENCE THAT PGF_{2α} IS A UTERINE LUTEOLYSIN

Evidence that PGF_{2α} is a luteolysin. Using the ovarian autotransplant preparation, it was easy to demonstrate that PGF_{2α} was a very potent luteolysin. McCracken's group [22] and our group [23, 24] made this discovery independently. McCracken *et al.* (1970) used an initial intra-arterial dose of 100 µg PGF_{2α}/hr which resulted not only in a rapid luteolysis, but also in a clear-cut fall in blood flow through the ovary [22]. This finding, although widely quoted, is not typical of the action of PGF_{2α} at lower dose-rates. We started with an initial intra-arterial dose of 40 µg/hr. Prompt luteolysis resulted without any consistent effect on blood flow [24]. We gradually reduced the infusion rate of PGF_{2α}, in successive experiments, and were able to show that 10 µg PGF_{2α}/hr for 3 to 4 hr always resulted in luteolysis. However, when the dose was reduced to 2 µg/hr, even for 7 to 9 hr, PGF_{2α}, while causing a fall in progesterone secretion, only occasionally caused complete luteal regression with the animal subsequently returning to oestrus.

Hence, an intra-arterial infusion of $2\text{ }\mu\text{g}$ $\text{PGF}_{2\alpha}$ /hr appears to be in the region of the lowest effective dose for luteolysis.

Subsequently, Thorburn & Nicol [25] were able to show that intra-arterial infusions of $25\text{ }\mu\text{g}$ $\text{PGF}_{2\alpha}$ /hr caused luteolysis in the conscious intact ewe. In their study, $\text{PGF}_{2\alpha}$ was infused through a side-branch of the ovarian artery through an indwelling cannula, the ovary remaining *in situ*.

From all these studies, it is clear that $\text{PGF}_{2\alpha}$ is a potent luteolysin. No contradictory evidence has yet been adduced on this point.

PGF_{2α} is a uterine luteolysin. It is a curious fact that, as recently as 1970, nobody had discovered prostaglandins of any sort in the ovine uterus or in its secretions. Yet a prostaglandin-like material had been found in human menstrual fluid years previously by Pickles [26]. The explanation proved to be simply that nobody had studied ovine uterus in this regard.

As soon as definitive work began, positive results were soon forthcoming. The first report was that of Green & Samuelsson, who found $\text{PGF}_{2\alpha}$ in high concentration in uterine venous blood at the time of luteolysis [17]. At about this time also, Bland *et al.* [27] reported $\text{PGF}_{2\alpha}$ to be in uterine vein blood. They used bioassay with gas-liquid chromatography (GLC)/mass spectrometry (see later) and found concentrations of up to about 8 ng/ml [31]. In addition, Wilson *et al.* (1972) [28] reported the discovery of prostaglandin in the uterine endometrium during the ovine oestrous cycle. However, from the quantitative aspect, the record goes to Harrison *et al.* who reported the finding of enormous amounts of $\text{PGF}_{2\alpha}$ (up to 7.6 mg in one case) in the uterine fluid taken from non-pregnant sheep with ovarian autotransplants [29].

Finally, Thorburn *et al.* [30] made a detailed study of the time-course of PGF secretion in normal cyclic sheep, using catheters implanted in the uterine vein. They found that, even in the luteal phase, PGF was secreted in uterine venous blood. This secretion took the form of short spikes which lasted only about 1 to 3 hr. Their frequency and magnitude increased progressively until the time of luteolysis was reached, when an abrupt fall of peripheral progesterone occurred. In a second study, progesterone was measured in the utero-ovarian vein, in addition to PGF . They found a good correlation between the occurrence of spikes of PGF and troughs of progesterone. As the spikes intensified, the troughs gradually coalesced and luteolysis resulted [31].

It thus seems indisputable, from the evidence derived from the transplant preparations and from the intact ewe, that $\text{PGF}_{2\alpha}$ is a uterine luteolysin.

$\text{PGF}_{2\alpha}$ IS THE UTERINE LUTEOLYSIN

PGF_{2α} is the sole PG in the ovine uterus and its secretions. This section will begin with a consideration of the validity of the various methods used to determine prostaglandins in uterine tissues and fluids. In general, three methods have been used: extraction and silicic acid column chromatography followed by bioassay [28] or by bioassay and GLC/mass spectrometry [27, 29]; radioimmunoassay [30, 32–35]; GLC/mass spectrometry using a deuterium method [36]. Of these, the bioassay method appears to be the least suitable for use in ovine plasma, e.g. in one publication the results of bioassay appeared to bear little relation to the presence or absence of $\text{PGF}_{2\alpha}$ as determined by mass spectrometry [27]. It is

doubtless adequate for the estimation of PGs when they are present in large amounts [29]. No currently described radioimmunoassay discriminates between PGF_{1α} and PGF_{2α}, but there does not appear to be any cross-reaction with PGs of the A, B or E series or with known metabolites and precursors. Assays such as those used by Thorburn and his co-authors [30, 31] have low blank values and peak values which compare well with those obtained by the use of more sophisticated methods [15]. The results of Caldwell *et al.* for peripheral blood [32] appear to be relatively high by comparison, and those of Coudert *et al.* [35] very much higher still (see later). There is no such limitation concerning specificity or quantification in the studies of the Swedish group and it seems reasonable to accept from their work that PGF_{2α} is the only PG present in uterine venous plasma [15].

The uterine secretion of PGF_{2α} is quantitatively sufficient to account for luteolysis. The obvious question arises, if PGF_{2α} is virtually completely removed from blood during its passage through the lung [37], how could the uterine secretion of even a large quantity of PG be able to reach the ovary in amounts capable of causing luteolysis? Some time ago, it occurred to me that PGs, being lipophilic, may possibly be able to diffuse rapidly across the walls of blood vessels of the size of the ovarian artery and the utero-ovarian vein, i.e. PGs secreted into the uterine vein, could, by a form of counter-current process, pass down a concentration gradient, but against a pressure gradient to reach the ovarian artery. So I decided to see if separating the ovarian artery from the uterine vein in the ewe could prevent the occurrence of luteolysis at its usual time. I carried out this procedure in a few animals and indeed found that such a procedure would prevent luteolysis [23]. Then, with McCracken & Baird [15, 17], we demonstrated that tritiated PGF_{2α}, when infused into the uterine vein, appeared as such in the ovarian artery in concentrations far higher than those in the adjacent iliac artery. The percentage transfer was low and there was a time-delay of some 15 to 20 min. However, both of these features suggested that we were dealing with a real phenomenon.

These experiments have been repeated on a number of occasions both at the Worcester Foundation and at Baird's Laboratory at Edinburgh, with similar results. Thus, at that time (1971), we were in possession of two independent forms of evidence concerning the method whereby PGF_{2α} could exert a local luteolytic effect, such as had been observed some years before by Moor & Rowson in their uterine excision experiments [9].

These findings stimulated both our group at Werribee [38] and that of Thorburn [25] to undertake further studies on the quantitative aspects of this phenomenon. Both groups found out independently that 20 to 25 μg PGF_{2α}/hr, infused for some 4 to 8 hr into the uterine vein on the same side as a corpus luteum, were sufficient to cause luteolysis and the initiation of a new oestrous cycle.

This requirement of 20 μg PGF_{2α}/hr for luteolysis is in reasonable agreement with the secretion rate of PG observed by direct measurement of PGF_{2α} in uterine venous blood. Thorburn *et al.* [30] reported peak PG concentrations in the uterine vein of some 22 ng/ml while the Karolinska group's figure was somewhat higher [15]. Estimates of the blood flow in one middle uterine vein

vary, and neither of these groups attempted to make direct observations of blood flow when the samples were collected. The figure of 1 litre/hr however, is almost certainly of the correct order of magnitude [39] from which it is possible to estimate the peak secretion rate of $\text{PGF}_{2\alpha}$ to be of the order of 20 to 50 $\mu\text{g/hr}$. This is in reasonable agreement with the rate of infusion required to achieve luteolysis.

In summary, I consider that few of the known hormones have been more closely characterized *in vivo*, in the sense that it has been possible to equate known secretion rates to their observed dose-for-effect. For this reason, as well as all the foregoing less direct evidence, it seems reasonable to conclude that $\text{PGF}_{2\alpha}$ is the uterine luteolysin in the ewe.

This view has been, if anything, strengthened by two series of experiments designed to test the counter-current theory.

Restall *et al.* repeated the experiment of separating the ovarian artery from the uterine vein [40].

They confirmed that the separation procedure did indeed lead to prolongation of luteal function. However, if, in these animals, large doses of $\text{PGF}_{2\alpha}$ (86 $\mu\text{g/hr}$) were infused into the uterine vein, luteolysis still took place in some cases.

It proved that luteolysis did not occur when the infusion was made into the utero-ovarian vein in the region of the separated vessels, but did occur when the infusion was made on the uterine side of the junction with the ovarian vessels. The authors concluded that the separation procedure acted by virtue of the concomitant rupture of the autonomic nervous system in the region. This view was strengthened by the fact that luteolysis could be prevented in some cases by very large doses of a ganglion-blocking drug.

However, the infusion or cross-circulation experiments referred to earlier [13–15, 54] demonstrated that nerve connections were not essential for luteolysis to occur, a finding confirmed by the results of intra-arterial infusions of $\text{PGF}_{2\alpha}$. Recent studies by Baird & Land [41] provide a more likely explanation for the results of Restall *et al.* Inskip & Butcher [42] had previously found that luteal maintenance resulted from ligation of the uterine vein and artery, but not from ligation of the uterine artery alone. Baird & Land ligated the uterine vein(s) proximal to the entry of the ovarian vein, with the intention of preventing $\text{PGF}_{2\alpha}$ from gaining access to that segment of uterine vein where it is in very close apposition to the ovarian artery. In their control group, all connections between the uterus and ovarian pedicle were severed except the middle uterine vein. All ten ewes showed normal luteal regression. In the experimental group, ligation of the uterine vein alone resulted in luteal maintenance in four out of ten ewes only. The explanation for the occurrence of luteolysis after uterine vein ligation proved to be that this operation had resulted in the opening up of a venous arcade along the oviduct. This arcade formed an anastomotic pathway from the uterine venous system to the ovarian veins; interruption of this channel as well as the uterine vein almost invariably resulted in luteal maintenance. It perhaps should be emphasized that in the experimental series, the nervous connections between uterus and ovary were virtually intact. It therefore seems probable that some of the large dose of

PGF_{2α} used by Restall *et al.* [40] may have also gained access to the ovarian venous system by an anastomotic pathway.

In any case, the evidence of Restall *et al.*, although bearing on the mechanism of action of PGF_{2α} as well as the validity of the 'counter-current' theory, does not affect the main conclusion concerning the physiological rôle of PGF_{2α} as the uterine luteolysin.

Some indirect supporting evidence for this rôle has recently been provided by Spilman & Duby [43]. Those workers showed that the oestrous cycle could be shortened by the use of an intra-uterine device, and that this effect could be overcome by Indomethacin, an inhibitor of prostaglandin synthesis. It is surprising that more use of this compound has not been made in the study of the rôle of PGF_{2α} in the sheep.

Certain pieces of evidence casting doubt on the rôle of PGF_{2α} as the uterine luteolysin will now be considered.

Niswender *et al.* [44] found that autotransplantation of the ovine uterus, away from the ovaries to a peritoneal site, did not lengthen the oestrous cycle in most of their sheep. It is possible, however, that uterine secretions could still gain access to the pelvis in those circumstances and further studies are needed to clear up this point.

Wilson *et al.* [45] found larger amounts of PGF in the uterine venous blood of pregnant ewes than of non-pregnant ewes on Day 13 after oestrus.

These results, being based on single samples of blood taken on an unsuitable day (i.e. before luteolysis) and analysed by an unsuitable method (see earlier), require verification. However, the mechanism whereby the embryo contrives the maintenance of the corpus luteum has not been defined. It is possible that it may act directly by overcoming the luteolytic effect of PGF_{2α} on the target tissue, the corpus luteum. Further studies using appropriate methods will be of the greatest importance.

A third dissentient voice has been raised [35]. Although the paper is of little technical merit for reasons to be put forward, the nature of the conclusions drawn appear to require that this publication be considered.

Coudert *et al.* [35] claimed to "cast doubt on the physiologic rôle of PGF as the uterine luteolytic factor". They found that, although PGF in "vena caval" serum rose as the luteal phase progressed, such a rise could not be correlated with the time of fall of progesterone at luteolysis. This paper gave values for PGF by radioimmunoassay, all other parameters, and supporting evidence, being inferential and, in most cases, unpublished.

Among its more obvious defects are the following:

(1) Sampling errors due to positioning of the catheter. The authors are mistaken in believing the utero-ovarian vein joins the inferior vena cava in the sheep. They endeavoured to position the tip of the cannula near this (non-existent) junction. It is likely that large errors would have arisen depending upon whether it was placed proximal or distal to the junction of the utero-ovarian vein and the iliac vein. In any event, if it were desired to verify the work of Thorburn *et al.* [30–31], it would have been immensely preferable to cannulate the uterine vein or the utero-ovarian vein itself; a procedure that Thorburn *et al.*, and others, have shown to be perfectly feasible.

(2) Sampling errors due to frequency of collection of blood. The discontinuous form of the graphs relating PGF secretion to time [30] render almost valueless studies based on once-daily blood sampling, and such practices should now be abandoned.

(3) Analytical errors. The authors used the radioimmunoassay of Caldwell *et al.* [32], but obtained results which were higher even than published data by almost an order of magnitude (see their addendum). It therefore seems probable that all their data are artifactual, for the good reason that PGF was measured in serum and not in plasma [46].

(4) Choice of experimental conditions. The sheep used were given pre-treatment with large doses of synthetic progestagen, and PMSG, and were just at the beginning of the breeding season. Under these conditions, the precise time of luteolysis would have been uncertain. No data on this point were provided. In any case, it would have been preferable to use normally cyclic ewes for such a study.

(5) The supporting statement that “PGF_{2α}-³H does not cross from the utero-ovarian vein to the ovarian artery” was based on unpublished ‘preliminary data’ which is probably of doubtful validity. A similar assertion was made by one of the authors (S.P.C.) at the International Congress of Endocrinology (1972) in the course of discussion of another paper. He stated that, after infusion of [³H]PGF_{2α} into the uterine vein, there was no difference between the ³H content of ovarian arterial blood and that of peripheral blood.

This finding was hardly surprising, as no effort had been made to separate PGF_{2α} from breakdown products in either case. In the experiments conducted by Baird, McCracken & Goding [14], the crude ³H content of ovarian arterial plasma exceeded its peak ³H content as PGF_{2α} by a factor of well over 100:1.

In view of all the foregoing, it seems reasonable to conclude that the present evidence tendered by Coudert *et al.* is, unfortunately, worthless.

Nevertheless, the proponents of the present thesis would welcome any serious attempt to challenge their contention that PGF_{2α} is the uterine luteolysin in the sheep.

Possible alternative explanation for luteolysis. Oestrogens have been put forward by Nalbandov as possible luteolysins. If given early in the cycle, they cause a prolongation of the life of the corpus luteum. The mechanism of this effect has not yet been fully defined, but appears to be the result of an action on the uterus [47, 48, 53]. On the other hand, if given after Day 10, oestrogen causes premature luteal regression. This phenomenon also was shown to be dependent on the presence of the uterus [49, 52, 53] and is, in all probability, mediated by PGF_{2α} for the following reasons: Caldwell *et al.* [32] found that oestradiol (and to some extent progesterone) stimulated the secretion of PG in the castrated ewe. We now have similar evidence in the ewe bearing a utero-ovarian transplant (unpublished observations). On the other hand, the effect of X-irradiation, which causes follicular degeneration but leads to persistence of the corpus luteum, is probably mediated in the same way through oestradiol. Irradiation of the ovaries in sheep with ovarian transplants has been shown to cause the complete cessation of oestradiol secretion [17]. An interesting aspect and unexplained finding was described recently by Karsch *et al.* [50]. Oestrogen was shown to be

luteolytic when injected directly into the adjacent ovarian stroma. This effect was bilateral, suggesting a possible indirect effect. As no group in this series was hysterectomized, an effect mediated by PGF_{2α} could not be excluded.

No review of the luteolytic rôle of PGF_{2α} would be complete without placing the phenomenon of luteolysis itself in perspective. As the result of an elegant and most extensive series of studies, Denamur and his co-workers at Jouy-en-Josas have been able to show that the survival of the corpus luteum is not just simply a matter of the presence or absence of a luteolysin. They investigated the effects of hypophysectomy, with or without hormone replacement, or with and without oestradiol or specific antisera to LH or prolactin as well as similar combinations with pituitary stalk section and X-irradiation; all in the presence or absence of the uterus [48, 51–53].

Their work shows clearly that the survival of the corpus luteum depends upon the outcome of a battle between two opposing forces: on the one hand, those of the pituitary (LH and prolactin) and the embryo (mechanism unknown) acting in the direction of survival; on the other, the uterus and its ally, the follicle (oestradiol and possibly other ovarian agents), acting to cause its dissolution (R. Denamur, in preparation: see *J. Reprod. Fert.* **38**, 251–259).

There are many problems left unsolved. Some of the more obvious are as follows:

—what are the quantitative aspects of the stimulation of PG secretion by oestradiol and by progesterone?

—how is it that pituitary stalk section on Day 3 or Day 10 of the cycle which results in grossly diminished secretion of LH and FSH—as well as the absence of ovarian follicles > 2 mm diameter—is still followed by luteal regression at the normal time, Day 17 [52–53]?

—what is the precise mechanism whereby PGF_{2α} causes luteolysis in sheep?

—how is the PGF_{2α}-luteolytic mechanism defeated in pregnancy in sheep?

—is there any direct luteolytic action of oestradiol on the ovary?

—in what way is the mechanism of luteolysis by PGF_{2α} in sheep applicable to luteolysis in other species?

—how can PGs or their analogues be used to manipulate the reproductive cycles of the various species?

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