UTERINE PROSTAGLANDINS IN THE NON-PREGNANT SHEEP:

STUDIES ON RELEASE AND LYMPHATIC TRANSPORT

BY

SHEIK EL DIN AWAD ABDEL RAHIM

B.V.Sc., Dip.Trop.Anim.H.&P., M.Phil (Edinburgh)

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This thesis was composed by the undersigned and is a report of the work undertaken by him on an original line of research. All sources of information are shown in the text and listed in the references and all assistance given is indicated and acknowledged. None of the work reported has been presented for any other degree or professional qualilfication.

signed

SHEIK EL ABDEL RAHIM

TO

MY MOTHER AND FATHER

WITH LOVE AND GRATITUDE

SHEIK

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Contents PAGE

Title page.....

Acknowledgements.....

Contents.....

Thesis Summary.....

General Introduction..... 1

Part 1

Studies on uterine $PGF_{2\alpha}$ release in non-pregnant sheep.....

General Introduction.....

Housing and Husbandry..... 43

Animals..... 43

Housing and Animal Care..... 43

Detection of Oestrus..... 43

Materials and Methods.....

Animals and general procedures..... 45

Anaesthesia..... 45

Anastomosis of the utero-ovarian vein to the mammary vein.....

Introduction..... 45

Procedure of anastomosis..... 46

Fistulation of Uterine Horn.....

Introduction..... 49

Procedure of uterine horn fistulation... 49

Post-operative Treatment..... 49

Sampling of blood and uterine tissues... 50

Blood sampling from peripheral and mammary vein..... 50

Biopsy procedure for uterine tissue. 50

PAGE

Analysis of Samples..... 51

Extraction of prostaglandins in uterine tissues..... 51

Extraction procedure of prostaglandins in plasma..... 53

$PGF_{2\alpha}$ radioimmunassay..... 53

Solutions used for $PGF_{2\alpha}$ radioimmunassay..... 54

Antiserum..... 54

Method of radioimmunoassay..... 54

Standard curve..... 56

Specificity..... 56

Sensitivity and Precision..... 57

PGE_2 radioimmunassay..... 57

Measurement of progesterone by radioimmunoassay..... 58

Chemicals and solutions used for progesterone radioimmunassay..... 58

Extraction procedure for progesterone..... 58

Radioimmunoassay for progesterone... 59

Standard curve..... 60

Specificity..... 60

Recovery test for progesterone..... 61

Measurement of oestradiol-17β by radioimmunossay..... 61

Antiserum..... 62

Extraction of oestradiol-17β in plasma samples..... 62

Radioimmunoassay for oestradiol-17β.

Recovery estimates..... 64

Accuracy and precision..... 64

PAGE

Sampling Frequency During the Experiment..... 66

Analysis of Data..... 68

Results..... 68

Discussion..... 72

Part 11

Studies on $PGF_{2\alpha}$ Transport:

General Introduction..... 77

Chapter I The Effect of Surgical Seperation of the Uterus and Ovaries and Cannulation of the Uterine Vein on Oestrus Cycle Length in Sheep.

Introduction..... 82

Materials and Methods..... 84

Animals and experimental procedure..... 84

Results..... 86

Discussion..... 87

Chapter 11 Anatomy of Uterine Lymphatic Drainage.

Introduction..... 90

Materials and Methods..... 93

Results..... 97

Discussion..... 97

Chapter 111 Cannulation of the Uterine Lymphatic Vessels and Collection of Samples for Prostaglandin Analysis..... 99

Materials and Methods..... 101

Cannulation and collection of lymph samples..... 101

PAGE

Measurement of prostaglandins in lymph... 102

$PGF_{2\alpha}$ assay..... 103

PGE_2 assay..... 103

Results..... 103

Discussion..... 104

Chapter IV $PGF_{2\alpha}$, PGE_2 and Steroid Concentration in Sequential Samples of Uterine Lymph..... 105

Introduction..... 105

Methods..... 106

Hormone Assays..... 108

Results..... 108

Discussion..... 110

General Discussion..... 112

References..... 126

Appendices

Appendix I.....

Instructions on Nakayama`s Instrument for small vessel anastomosis.

Appendix II.....

Cross-reactivites of progesterone antibody.

Appendix III.....

Cross-reactivities of oestradiol-17β antibody.

Appendix IV.....

Publications resulting from this work.

Thesis Summary

Experiments were conducted to study the stimulus for $PGF_{2\alpha}$ release from the uterus and the utero-ovarian pathway for $PGF_{2\alpha}$ transport in sheep.

Frequent uterine venous blood samples were collected from the mammary vein after anastomosing it with the uterine vein, while simultaneous uterine tissue samples were obtained by fistulation of the uterine horn through the abdominal wall thus exposing the uterine endometrium to the exterior. Uterine venous blood and tissue samples were analysed for $PGF_{2\alpha}$, progesterone and oestradiol-17β and endometrial $PGF_{2\alpha}$ and PGE_2 content and synthesizing ability respectively. There was an increase in $PGF_{2\alpha}$ release towards the end of the cycle with peaks appearing consistently at 30-45 and 3-9 hrs before the decline in progesterone concentrations. Further $PGF_{2\alpha}$ peaks occurred after the initiation of progesterone decline in 4 of 5 cycles studied. Elevation of endometrial $PGF_{2\alpha}$ content and synthesizing ability occurred at similar times to $PGF_{2\alpha}$ release, but did not consistently either precede, coincide or follow it. This suggested that neither an increase in $PGF_{2\alpha}$ content or an increase in the ability of the uterus to synthesize $PGF_{2\alpha}$ are prerequsites for $PGF_{2\alpha}$ release. Oestradiol-17β showed an increase in levels around the time of both periods of $PGF_{2\alpha}$ increase. These elevated levels of oestradiol-17β precede and/or coincide with peaks of $PGF_{2\alpha}$, thus confirming a role for oestradiol in $PGF_{2\alpha}$

release. An increase in oestradiol-17β and $PGF_{2\alpha}$ was also observed after the commencement of progesterone decline but before oestrus.

Investigations into the utero-ovarian pathway for $PGF_{2\alpha}$ indicated that the oestrous cycle of the ewes were extended when the uterine vein was cannulated and all other direct tissue connections between the uterine horn and the ipsilateral ovary were surgically separated. The results demonstrated that blood passing through the uterine vein is not sufficient to account for the luteolytic effect of the uterus on the ipsilateral ovary. Examination of the anatomy of the uterine lymphatic drainage revealed that there was no direct lymph flow between the uterus and ovaries, and that the main uterine lymphatic trunks were in close association with the main utero-ovarian artery. In view of this finding the availability of prostaglandins in lymph drainage from the uterus at different stages of the oestrous cycle was examined. Samples of uterine lymph were collected on different days of the oestrous cycle and analysis showed that $PGF_{2\alpha}$ was present in this lymph in amounts comparable to those in uterine venous blood. Furthermore, the mean concentration remained low until day 12, while increased amounts were present from day 12 onwards. There was no significant variation in PGE_2 in uterine lymph during the oestrous cycle. Sequential uterine lymph samples were obtained by chronic cannulation of a uterine lymph vessel. These sequential

samples were analysed for $PGF_{2\alpha}$, PGE_2, progesterone and oestradiol-17β . The results indicated that the concentration of $PGF_{2\alpha}$ in uterine lymph fluctuated considerably, with a complex series of peaks being apparent especially from the time of initiation of luteal regression (day 12) to the end of the cycle. The results demonstrated that $PGF_{2\alpha}$ is present in uterine lymph in increased quantities at the time of luteal regression. Again there was no significant change in PGE_2 concentration. However there were high levels of oestradiol-17β, and progesterone levels did not decline to low levels that were seen in venous blood. It was concluded that, it is possible that a counter-current transfer system similar to that proposed for the transfer of $PGF_{2\alpha}$ from the utero-ovarian vein to the ovarian artery may occur between the main uterine lymph trunks and the ovarian arterial blood, and so form an additional or even alternative method of transfer of $PGF_{2\alpha}$ from the uterus to the ovary.

General Introduction

The effect of the corpus luteum on the functional life span of the corpus luteum was first shown by Loeb in 1923. Removal of the uterus during the luteal phase of the cycle in the guinea-pig extended the life-span of the corpus luteum. On the basis of these experiments, Loeb suggested that the uterus, in particular its mucosa, possibly produces an internal secretion which exerts a specific abbreviating effect on the life-span of the corpus luteum (Loeb 1927). This suggestion has proved to be true and since that time it has been demonstrated that hysterectomy prolongs luteal function in a variety of other species: sheep (Wiltbank & Casida 1956); pig (Anderson, Butcher & Melampy 1963); cow (Wiltbank & Casida 1956; Anderson, Neal & Melampy 1962); mare (Hughes, Stabenfeldt & Evans 1977); hamster (Duby, McDaniel, Spilman & Black 1969); rabbit (Asdell & Hammond 1933; Chu, Lee & You 1946); rat (Melampy, Anderson & Kragt 1964; Bradbury 1937); and mouse (Crister, Rutledge & French 1980). On the other hand, hysterectomy does not appear to affect luteal function in the monkey (Bur_ford & Diddle 1936) and human (Beling, Marcus & Markham 1970), suggesting that in these latter species the control of the life-span of the corpus luteum is independent of the uterus.

The destructive effect of the uterus on the corpus luteum was firmly established in the late 1960s (see review by Anderson, Bland & Melampy 1969). One of the

striking features of the utero-ovarian relationship in many species is that the luteolytic factor acts primarily on a local basis -each uterine horn exerting its influence over the ipsilateral ovary only. This occurs in the ewe (Moor & Rowson 1966; Inskeep & Butcher 1966; Caldwell, Rowson, Moor & Hay 1969; Goding 1974), guinea-pig (Bland & Donovan 1969; Butcher, Barley & Inskeep 1969) and hamster (Orsini 1968). In the nonpregnant pig, unilateral regression also occurs but only when all but one quarter of one uterine horn has been removed (du Mesnil du Buisson 1961; Anderson, Butcher & Melampy 1961). Removal of the uterus at different stages of the cycle has shown that the luteolytic factor was secreted around the time of luteal regression. However, there is considerable variation among the different species in the exact timing of onset and the duraton of secretion needed to bring about luteal regression.

In 1971 prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) was identified as the uterine luteolytic hormone in the sheep (McCracken Baird & Goding 1971; McCracken, Carlson, Glew, Baird Green & Samuelsson 1972; McCracken Baird & Samuelsson 1973; Goding 1974; Baird, Land, Scaramuzzi & Wheeler 1976), and soon afterwards good evidence was found for a similar role for $PGF_{2\alpha}$ in the cow (Kindahl, Edquist, Bane & Granstrom 1976; Nancarrow, Buckmaster, Chamley, Cumming, Cummins, Drinan, Findlay, Restall, Schneider & Thorburn 1973), the pig (Eddy, Moeljano,

Bazer & Thatcher 1976; Gleeson, Thorburn & Cox 1974), the guinea-pig (Poyser 1976) and probably several other mammalian species (see review by Horton & Poyser 1976).

However, before $PGF_{2\alpha}$ could be shown to be the main uterine luteolytic hormone certain criteria concerning its role in luteolysis had to be met. These criteria were; that exogenous adminstration should induce luteal regresson; $PGF_{2\alpha}$ should be secreted by the uterus in greater amounts towards the end of the cycle than at earlier times; it must act directly on the corpus luteum to terminate its function; and should pass from the uterus to the ovary by a local pathway.

Investigations during the last 10 years have concentrated on examination of these aspects in different species and many of the results of the investigations are summarized in the later chapters of this thesis. Having established that prostaglandin $F_{2\alpha}$ was probably the physiological luteolytic hormone, several studies followed to examine the conditions which effected its production by and release from the uterus. Various investigations have examined the effect of exogenous steroids on $PGF_{2\alpha}$ synthesis and release. The results of these studies indicated that oestrogen in the presence of progesterone (possibly in association wth oxytocin) can increase the production and release of $PGF_{2\alpha}$ towards the end of the oestrous cycle (see review by McCracken, Schramm, Barcikowski & Wilson 1981). However, the effect of these steroids varied with the

method of application, time and species of animal (see Poyser 1981). These studies were followed by measurements of the endogenous levels of these hormones. Limited studies on serial measurement of oestradiol and progesterone indicated an association between these steroids and $PGF_{2\alpha}$ during the later part of the oestrous cycle (Barcikowski, Carlson, Wilson & McCracken 1974; Thorburn, Cox, Currie, Restall&Schneider 1972).

The pathway by whch $PGF_{2\alpha}$ reaches the ovary has been investigated by surgical separation and ligation experiments whch indicated that the luteolytic substance reaches the ovary via the vascular system (Bland & Donovan 1966; Kiracofe, Menzies, Gier & Spies 1966). Examination of the vascular connections between the uterus and ovaries in several species indicated that it would be possible for a substance to diffuse from the uterine vein into the ovarian artery and thereby pass to the ovary. A counter-current mechanism was then proposed (Barret, Blockley, Brown, Cumming, Goding, Mole & Obst 1971) and evidence has been obtained that $PGF_{2\alpha}$ does pass from the utero-ovarian vein by such a mechanism. However, the efficiency of transfer was low (McCracken Barcikowski, Carlson, Green & Samuelsson 1973). The existence of this counter-current mechanism was later disputed (Coudert, Phillips, Faiman, Chernecki & Palmer 1974), and Thorburn and Mattner (1971) failed to interrupt the normal oestrous cycle in the sheep by separating the utero-ovarian vein from the ovarian

artery. Several investigators have suggested that uterine lymph could be a possible route for $PGF_{2\alpha}$ transfer to the ovary (Bland & Donovan 1969; Hansel Concannon & Lukaszeska 1973; Meckley & Ginther 1969 and recently Staples, Fleet & Heap 1982). However, studies on the uterine lymph drainage are few and the functional signficance and the presence of $PGF_{2\alpha}$ in the uterine lymph vessels during the oestrous cycle have not yet been investigated.

Consequently in this thesis, investigations are reported on several areas of utero-ovarian relationships in sheep. The results of these investigations help us to extend our understanding of the uterine control of ovarian function.

The following aims were selected for investigation.
1) What is the stimulus for $PGF_{2\alpha}$ release from the uterus in sheep ?.

A) A detailed study of the relationships between uterine content of $PGF_{2\alpha}$ and PGE_2, the ability of the endometrial tissue to synthesize prostaglandins and the concentration of $PGF_{2\alpha}$ in the uterine venous blood to see at what stage of the release process the stimulus for release acts.

B) Simultaneous measurement of progesterone, oestradiol-17β and $PGF_{2\alpha}$ concentrations in the uterine venous blood to investigate the nature of the release stimulus.

2) What is the utero-ovarian pathway for $PGF_{2\alpha}$ transport

in sheep ?.

A) Study the effect on the oestrous cycle of surgical seperation of the uterus and ovaries with cannulation of the uterine vein, to see if the venous route is sufficient to maintain normal oestrous cycles.

B) Study the anatomy of the lymphatic drainage of the uterus in the non-pregnant sheep to see if a lymphatic pathway is feasible.

C) Cannulation of lymphatic ducts and collection of lymph samples to see if prostaglandins are present in lymph in sufficient concentrations and at the appropriate time to cause luteolysis.

Studies On Uterine $PGF_{2\alpha}$ Release In Non-Pregnant Sheep

Introduction

Prostaglandins are distributed throughout most tissues of the reproductive tract and are involved in several reproductive processes in both the male and the female (Poyser 1973). A great deal of evidence is now available which indicates that the uterine luteolytic hormone in many mammalian species is prostaglandin $F_{2\alpha}$ (see General Introduction). It is commonly regarded that prostaglandins are not stored in tissues and any increase in tissue levels is preceded by synthesis. Prostaglandins are synthesized from arachidonic acid, which has to be released from some bound source before prostaglandin synthesis can take place. Phospholipids are often regarded as the main source of arachidonic acid for prostaglandin synthesis. However, cholesterol esters and triglycerides may also be potential sources of arachidonic acid (Poyser 1973). The physiological stimulus for prostaglandin synthesis probably liberates arachidonic acid from at least one of these sources by stimulating the appropriate enzyme, either phospholipase A_2 (PLA2), cholesterol esterase or triglyceride lipase (Fig. 1).

Figure 1

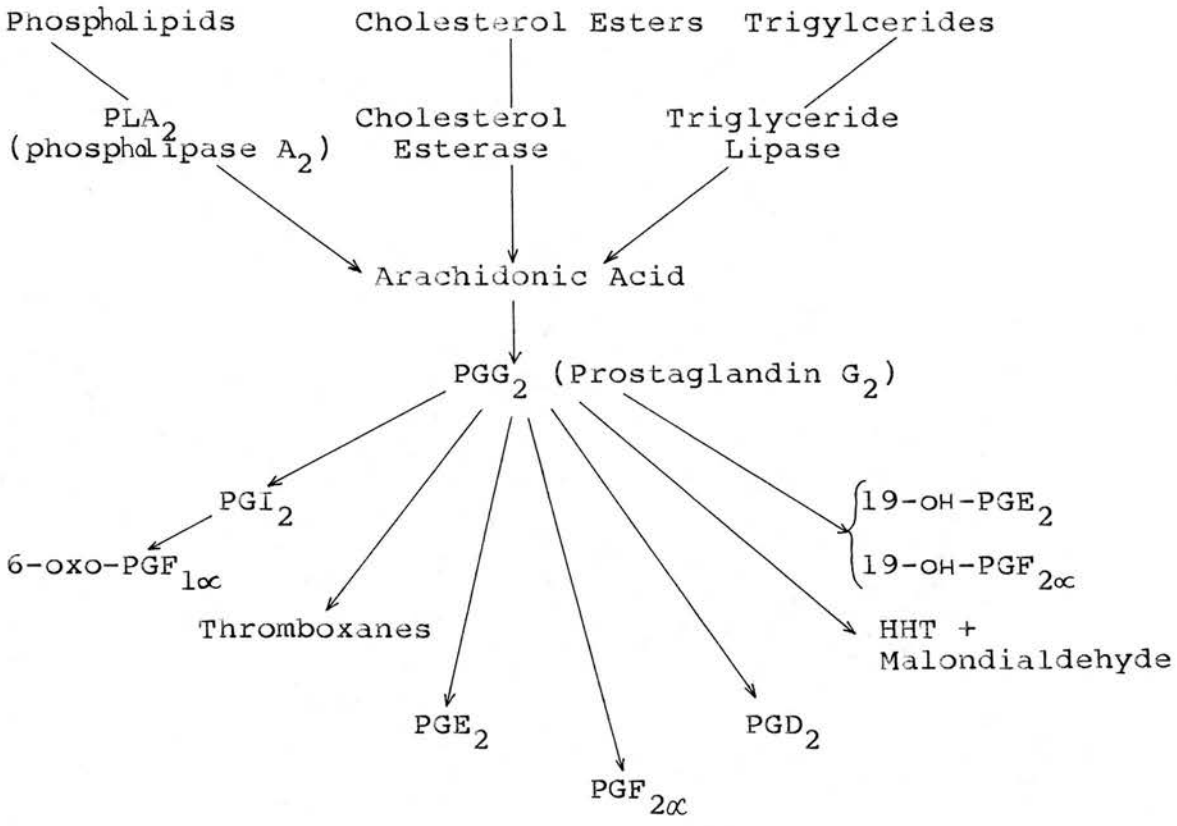


Figure 1 shows potential sources of arachidonic acid for conversion into prostaglandins and possible products formed (adapted from Poyser 1978).

These prostaglandin pathways have recently been reviewed by Poyser (1981). Briefly prostaglandin G_2 (PGG_2) is formed from the arachidonic acid, which is then converted to the main prostaglandin released (e.g. $PGF_{2\alpha}$ from the uterus for luteolysis). However, the precise mechanism controlling which prostaglandin is synthesized is not fully known, though it probably depends upon the tissue content of the catalysing enzymes. For example the presence in the tissues of more of the enzyme which converts PGG_2 into PGE_2 than the

enzyme which converts PGG_2 into prostacyclin (PGI_2) would produce PGE_2 as the main prostaglandin. However, the release of arachidonic acid is generally considered to be the rate-limiting step in prostaglandin synthesis, and the stimulation of a lipase or esterase enzyme by the hormonal stimulus would be a simple way of regulating prostaglandin synthesis by reproductive tissues (Poyser 1978).

In the early 1970s $PGF_{2\alpha}$ was identified as the uterine luteolytic hormone in the sheep (McCracken et al 1972; Goding 1974; Baird et al 1976), soon afterwards evidence was found for a similar role for $PGF_{2\alpha}$ in the cow (Nancarrow et al 1973; Kindahl et al 1976), the pig (Gleeson Thorburn & Cox 1974), the guinea-pig (Blatchley & Donovan 1972), and several other mammalian species (see review by Horton & Poyser 1976). The demonstration that $PGF_{2\alpha}$ is the uterine luteolytic hormone commenced with studies which showed its presence and release from the endometrium at the end of the normal oestrous cycle. Bland, Horton and Poyser (1971) first identified (by gas-liquid chromatography/mass spectrometry) and measured (by bioassay) $PGF_{2\alpha}$ in uterine blood of sheep. High concentrations of $PGF_{2\alpha}$ were detectable only during days 14-16 of the cycle when levels reached a maximum of 8ng/ml. Subsequently, higher levels of $PGF_{2\alpha}$ in uterine venous blood of ewes were reported by other workers (Green, Samuelson, Carlson & McCracken 1971; Thorburn et al 1972), but again the highest values were

recorded at the end of the oestrous cycle. Similar levels of $PGF_{2\alpha}$ in the uterine venous blood have been found towards the end of the oestrous cycle in the pig (Gleeson & Thorburn 1973) and cow (Nancarrow et al 1973; Shemesh & Hansel 1975). Thus elevated $PGF_{2\alpha}$ levels are present in the uterine vein at the end of the normal oestrous cycle.

The pattern of prostaglandin $F_{2\alpha}$ release into the blood was subsequently demonstrated. Thorburn and coworkers (1972,1973), by frequent sampling of blood from the uterine vein detected a complex series of $PGF_{2\alpha}$ peaks between day 13-16 of the oestrous cycle. These peaks were of short duration and increased towards the end of the oestrous cycle. Prostaglandin peaks at day 13 were found to coincide with the beginning of luteal regression and a transient marked decrease in progesterone following each $PGF_{2\alpha}$ peak. Fitzpatrick and Sharma (1973) sampling twice a day through an indwelling catheter introduced via the saphenous vein demonstrated that blood from the posterior vena cava anterior to the junction with the uterine veins contained low concentrations of $PGF_{2\alpha}$ until four days prior to oestrus when it increased significantly. Baird et al (1976) measured $PGF_{2\alpha}$ in the utero-ovarian venous blood collected from ewes with the uterus and ovaries transplanted to the neck. They found the first significant rise of $PGF_{2\alpha}$ occurred on day 12-14 at the time when progesterone declines, although the episodic

peaks of $PGF_{2\alpha}$ were larger ($10ngml^{-1}$) on the day before the onset of oestrus. Futhermore Nett, Staigmiller, Akbar, Dickman, Ellinwood and Niswender (1976) collected blood samples every 3 hours from day 11-17 in both pregnant and non-pregnant ewes using indwelling cannulae introduced into theutero-ovarian vein via a branch of the uterine vein. Measuring prostaglandin $F_{2\alpha}$ by radio-immunoassay they found no signficant difference in mean levels of $PGF_{2\alpha}$ between cycling and pregnant ewes. However, there was more variation ($P<0.01$) in levels of $PGF_{2\alpha}$ in cycling ewes than in pregnant ones; this variation was reflected by a greater number of peaks in cycling ewes than in pregant ones. Kindahl et al (1976) measured the level of the major plasma metabolite of $PGF_{2\alpha}$, namely 15 keto-13,14-dihydro $PGF_{2\alpha}$ in peripheral venous plasma during the reproductive cycle in sheep and cattle. In both sheep and cattle low levels were found during the major part of the oestrous cycle, but during the last days of the cycle high levels of prostaglandin metabolites were found. These high levels coincided with decrease in progesterone production. Recently Alwachi etal (1979) measured $PGF_{2\alpha}$ during the oestrous cycle in the mammary vein of sheep after anastomosing it to the uterine vein. Daily sampling gave the impression of relatively smooth changes in plasma levels, however, more frequent sampling (every 1/2 hr) showed that $PGF_{2\alpha}$ fluctuated episodically and there was a complex series of peaks during the last days of the cycle. The

conclusion from all these data is that, $PGF_{2\alpha}$ is released from the uterus into the uterine vein in an episodic manner and in increased concentrations from the time of luteal regression until the end of the cycle. However, possibly due to the intermittent nature of $PGF_{2\alpha}$ release and the sampling difficulties that accompany such a pattern, a rise in plasma $PGF_{2\alpha}$ preceding the fall in plasma progesterone does not appear in every case reported.

Some other prostaglandins have also been studied. It has been found that both PGE_1 and PGE_2 have no luteolytic effect in sheep (Aldridge, Barrett, Brown, Funder, Goding, Kaltenbach & Mole 1970; Carlson, Rugg, Glew, Barcikowski & McCracken 1972; Inskeep, Smutny, Butcher & Pexton 1975; Henderson, Scaramuzzi & Baird 1977; Mapletoft, Miller & Ginther 1977). On the contrary Pratt, Butcher & Inskeep (1977) reported that intrauterine administration of PGE_2 lengthened the oestrous cycle of ewes by about 2 days. Furthermore Mapletoft et al (1977) found that PGE_2 counteracted the luteolytic effects of $PGF_{2\alpha}$ when both hormones were given by perivascular injection into the mesovarian artery of non pregnant ewes. Likewise PGE_2 infused with $PGF_{2\alpha}$ into the ovarian artery of ovaries transplanted to the ewe's neck prevented the reduction in secretion of progesterone seen when $PGF_{2\alpha}$ was infused alone (Henderson et al 1977). Prostaglandin E_2 has been shown to bind to $PGF_{2\alpha}$ receptors in bovine corpora lutea (Rao

1975) and Henderson and McNatty (1977) suggested that PGE_2 enhances the function of the adenylate cyclase system and overcomes the effects of $PGF_{2\alpha}$. It therefore appears that exogenous PGE_2 functions as an antiluteolysin rather than a luteotrophin. However, prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$) was found to have luteolytic properties when infused into the arterial supply of a transplanted ovary in sheep (Aldridge et al 1970; Carlson et al 1972). However, unlike $PGF_{2\alpha}$ both $PGF_{1\alpha}$ and arachidonic acid failed to show a luteolytic effect in sheep when injected into a large follicle in the corpus luteum- containing ovary (Inskeep et al 1975; Pexton 1975).

Subsequent to the identification of $PGF_{2\alpha}$ in the utero-ovarian vein, extensive experimentation occurred aimed at demonstrating that $PGF_{2\alpha}$ causes regression of the corpus luteum and thus resulted in the fall of progesterone levels. McCracken, Glew and Scaramuzzi 1970) infused $PGF_{2\alpha}$ into a branch of the uterine vein of ewes between day 6-9 of the cycle at a rate of 40 $ug.h^{-1}$ for 6 hrs. Such infusion caused the peripheral plasma progesterone concentration to fall to a low level and the animals returned to oestrus within 60 hrs. However infusion of $PGF_{2\alpha}$, in doses equivalent to those found in the uterine venous blood on day 15, into the ovarian artery on day 6 of the oestrous cycle failed to cause luteal regression but did cause a slight decrease in plasma progesterone (McCracken 1970; Barrett, Blockley,

Brown, Cumming, Goding, Mole & Obst 1971). $PGF_{2\alpha}$ has also been demonstrated to be luteolytic in other species. Blatchley and Donovan (1972) have shown this effect in hysterectomized guinea-pigs. Similar findings have been reported in the pseudo pregnant rat (Duncan & Pharris 1970), and in the rhesus monkey (Kirton, Pharriss & Forbes 1970). Pharris and Wyngarden (1969) demonstrated that intra-uterine or intra-cardiac infusions or subcutaneous injection of prostaglandin F into the rat reduced the duration of pseudo pregnancy. But $PGF_{2\alpha}$ was luteolytic in the rat only when given after day 4 of pseudopregnancy. The conclusion from all these studies is that $PGF_{2\alpha}$ is luteolytic, and that it is present in the uterine venous blood in higher quantities during the period of luteal regression.

The possibility that the uterus was the origin of the luteolytic effect is suggested by Loeb`s work (1923, 1927) on the guinea-pig, but extraction of such a substance from the uterus has met with little success. Various workers (Williams, Johnston, Lauterbach & Fagan 1967; Duby et al 1969; Caldwell et al 1969; Lukaszewaska and Hansel 1970) have extracted a substance from bovine or ovine endometria towards the end of the oestrous cycle which proved to be luteolytic in hamsters or rabbits but could not characterize it. Studies in the pig were more successful (see Anderson et al 1969). Following the identification of $PGF_{2\alpha}$ in uterine venous blood, Wilson, Cenedella, Butcher and Inskeep (1972)

found significantly higher concentrations of $PGF_{2\alpha}$ in ovine uterine tissue on day 14 of the oestrous cycle than on day 3,5 or 11. Similar elevated values of $PGF_{2\alpha}$ on days 14 and 15 have been reported in the guinea-pig (~~Blatchley & Donovan 1972~~; Poyser 1972). The uterine component responsible for prostaglandin production has also been investigated. Louis, Parry, Robinson, Thorburn and Challis (1977) reported that the ovine caruncular tissue contained more $PGF_{2\alpha}$ than the intercaruncular tissue (composed of endometrium and myometrium) and released more $PGF_{2\alpha}$ and 13,14-dihydro-15-oxo $PGF_{2\alpha}$ during incubation in vitro. However, Pexton, Ford, Wilson, Inskeep and Butcher (1975) found that concentrations of $PGF_{2\alpha}$ were similar in caruncular and intercaruncular tissue, but both were considerably higher than in myometrial tissue. The apparently contradictory findings of Louis et al (1977) appear to be due to their intercaruncular tissue containing a mixture of endometrium and myometrium. More recently, Alwachi, Bland and Poyser (1979,1980) measured the amount of $PGF_{2\alpha}$ and PGE_2 in the endometrial tissue of sheep and its ability to synthesize these prostaglandins during the latter part of the oestrous cycle. They found there was no difference between the content of or synthesizing ability of $PGF_{2\alpha}$ or PGE_2 in/by caruncular or non-caruncular endometrial tissue, although like Pexton et al (1975) they found that the myometrium contained and produced significantly less $PGF_{2\alpha}$ than

either part of the endometrium. They failed to confirm an increase in the $PGF_{2\alpha}$ content of the endometrium at the end of the cycle but did find that the uterus had an increased ability to synthesize $PGF_{2\alpha}$ at the time of the progesterone decline.Ellinwood, Nett and Niswender (1979) measured the synthesis of $PGF_{2\alpha}$ and PGE_2 by ovine endometrial tissue in vitro on day 13, 15 and 17 of the oestrous cycle and of pregnancy. There was no difference in the quantity of $PGF_{2\alpha}$ synthesized by tissue collected on day 13 of the cycle or pregnancy. However, synthesis of $PGF_{2\alpha}$ was greater on day 15 and 17 of pregnancy than on similar days of the oestrous cycle. Furthermore, concentrations of $PGF_{2\alpha}$ and PGE_2 in endometrial tissue followed the same pattern as in vitro secretion suggesting that in vitro secretion, in this study, accurately reflects the capacity of the endometrial tissue to synthesize prostaglandins (Ellinwood et al 1979). Thus endometrial tissue synthesis of $PGF_{2\alpha}$ also increases during early pregnancy.

Fenwick (1977) studied prostaglandin production by rat uterine homogenates and found that the highest production of prostaglandins occurred on day 5 of pseudo pregnancy. However the amounts of $PGF_{2\alpha}$ and PGD formed were higher than the amounts of PGE_2 on all days studied. However, these authors also found that 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$) was the major prostaglandin produced following incubation of rat pseudo pregnant uterine homogenates. Similar studies on

guinea-pig and sheep uterine homogenates (Jones, Poyser & Wilson 1977) showed that following incubation of sheep uterine homogenates, 6-oxo-$PGF_{1\alpha}$ was the major product identified, whereas in the guinea-pig more $PGF_{2\alpha}$ than 6-oxo-$PGF_{1\alpha}$ was produced. These results on guinea-pig have recently been confirmed by Poyser (1983) using only the uterine endometrium. He again found that $PGF_{2\alpha}$ was the major prostaglandin synthesized together with lesser quantities of PGE_2 , 6-oxo-$PGF_{1\alpha}$ and thromboxane (TBX). The production of all these four compounds by the endometrium increased from day 11 to day 15, although $PGF_{2\alpha}$ production increased to a greater extent than production of the other three compounds. $PGF_{2\alpha}$ continued to increase after day 15 and up to oestrus.

Recently in vitro incubation of sheep uterine tissue (Alwachi et al 1980) has shown that both caruncular and non-caruncular endometrium can synthesize appreciable quantities of $PGF_{2\alpha}$, PGE_2 and 6-oxo-$PGF_{1\alpha}$, while the the myometrium synthesizes predominantly 6-oxo-$PGF_{1\alpha}$. In homogenates of whole uteri this efficiency can be extended to substrate of endometrial origin also. Similar findings have been reported for human uterine tissue where homogenates of whole uterus produce more 6-oxo-$PGF_{1\alpha}$ than the combined production by homogenates of myometrium and endometrium incubated separately (Abel & Kelly 1979).

Let us now examine how the changes in other female reproductive hormones vary relative to prostaglandin

levels in the cycling female. The hormone most closely associated with $PGF_{2\alpha}$ is Progesterone. It is secreted mainly by the corpus luteum and is an indication of the functional activity of the corpus luteum. Edgar and Ronaldson (1958) first reported the concentration of progesterone in the venous blood from the sheep ovary containing the corpus luteum. Their findings were confirmed and extended subsequently by other workers (Smith & Robinson 1969; Thorburn, Basset & Smith 1969;Poltka, Erb & Harrington 1970; Sarda, Robertson & Smeaton 1973; Bedford, Harrison & Heap 1974; Yuthasastrakosol, Palmer & Howland 1975; Quirke & Gosling 1976; Hopkinson & Fitzpatrick 1977; and Quirke, Hanrahan & Gosling 1979). The main finding was that the concentration of progesterone in ovarian venous and in peripheral blood rises gradually from the time of ovulation to a peak between day 8 and 14. The progesterone plateaus and remains constant until some time between 60 and 30 hrs before the onset of the next oestrus. There was some variation in the rate of progesterone decline between animals (Bassett, Oxborrow, Smith & Thorburn 1969; Fylling 1970; Pant, Hopkinson & Fitzpatrick 1977). Such variation could be the cause of differences in oestrous cycle length between sheep. Progesterone levels have also been measured in luteal tissue and a good correlation between concentration in ovarian blood and that in luteal tissue was found by Deane, Hay, Moor, Rowson and Short (1966). These authors also found

a positive correlation between the peripheral plasma concentration and the number of corpora lutea in the ovaries after stimulation with PMSG in the ewe. Breed differences in ovulation rates may also be reflected in plasma levels of progesterone (Land, Pelletier, Thimonier & Mauleon 1973; Quicke & Gosling 1976). Other workers (Plotka et al 1970; Geschwind 1972) have found that the weight of the corpus luteum, its progesterone content and the concentration of progesterone in peripheral plasma all increased in the post-ovulatory phase & remained relatively constant until about the 12th day of the cycle before declining at the time of luteolysis.

Another steroid hormone thought to be closely associated with $PGF_{2\alpha}$ is oestradiol-17β. This is also of ovarian origin. The results of several earlier workers indicated that serum oestradiol-17β concentrations are low on days 11 and 12, begin to rise on day 13 or 14 and continue to increase until day 15 or 16 (Moor, Hay, Short & Rowson 1969; Scaramuzzi, Caldwell & Moor 1970; Bjersing, Hay, Kann, Moor, Naptolin, Scaramuzzi, Short & Yonglai 1972; Denamur & Short 1973; Warren, Hawk & Bolt 1973). The increase in oestradiol precedes the decline in serum progesterone levels which occurs on day 14 to 15 and coincides with the beginning of the enlargement of the follicles for the next oestrus (Smeaton & Robertson 1971; Holst, Barden & Mattner 1972). Scaramuzzi et al (1970) measured oestradiol-17β

in ovarian venous blood by radioimmunoassay and found that the concentration of oestradiol-17β had started to increase by day 15, in addition they found two oestrogen peaks on days 3-5 and 7-10. Other workers (Cox, Mattner & Thorburn 1971; Seamark & Brown 1971; Mattner & Braden 1972), later confirmed the peak on day 4, while the day 8 peak has been detected in peripheral plasma by Obst, Seamaark and Brown (1971) and coincides with the culmination of the first wave of follicular growth described by Brand and de Jong (1973). Furthermore Mattner and Braden (1973) found that the three surges of oestradiol secretion do not always occur from the same ovary and are not necessarily associated with the ovary containing the corpus luteum, or the ovary from which the next ovulation will occur. Baird (1978) measured the secretion of oestradiol and LH in jugular venous blood in ewes with utero-ovarian transplants to the neck and found that pulses of oestradiol followed those of LH throughout the cycle.

Evidence for a role for oxytocin in luteolysis is still incomplete. Early investigations by Hansel and co-workers (Armstrong & Hansel 1959; Hansel & Wagner 1960; Malven & Hansel 1964), demonstrated that oxytocin induces premature luteal regression in the cow. It was found by these authors that injections of oxytocin during the first week of the oestrous cycle reduced the length of that cycle, while treatment on day 15 of hysterectomized animals did not alter luteal function.

This oxytocin treatment stimulates $PGF_{2\alpha}$ release from the uterus in cows and causes a premature decline in plasma progesterone concentrations (Newcomb, Booth & Rowson 1977; Milrae & Hansel 1980). ~~On the other hand oxytocin did not appear to abbreviate the cycle in sheep (Sharma & Fitzpatrick 1974).~~ ~~However~~, ~~more~~ Recent investigators have found that exogenous oxytocin can stimulate the release of $PGF_{2\alpha}$ from ovine uterine tissue during the late luteal phase of the cycle in vitro (Roberts, Barcikowski, Wilson, Skarness & McCracken 1975), and in vivo (Sharma & Fitzpatrick 1974; Roberts, McCracken, Garagan & Soloff 1976). These results suggest that oxytocin may also be involved in ovine luteolysis. Roberts et al (1976) found that oxytocin stimulates $PGF_{2\alpha}$ release from the sheep endometrium on day 15 but not on day 5 of the oestrous cycle. Infusion of oxytocin into the uterine artery of sheep in the latter part of the cycle was found (Roberts et al 1976) to significantly increase the rate of $PGF_{2\alpha}$ secretion, however Sharma and Fitzpatrick (1974) could not demonstrate an effect of oxytocin alone, but found that oestrogen and oxytocin together caused prostaglandin release. More recently, (Sheldrick, Mitchell & Flint 1980) found that active immunization against oxytocin delayed corpus luteum regression, indicating that oxytocin may form part of the physiological stimulus for prostaglandin release at the end of the oestrous cycle. In keeping with this Roberts et al (1976) found that synthesis of $PGF_{2\alpha}$ by

the ovine uterus may involve interaction between oxytocin and its endometrial receptors, and suggested that oxytocin could play a role in luteal regression by contributing to the regulation of uterine synthesis of $PGF_{2\alpha}$. Fairclough, Moore, McGowan, Peterson, Smith, Teviot & Watkins (1980) measured plasma concentration of neurophysin 1/11 (N-1/11) and 13,14-dihydro-15-keto prostaglandin F (PGFM) in sheep and found that, although peaks of PGFM and N-1/11 occurred intermittently during the last days of the oestrous cycle, on at least one occasion a surge of N-1/11 was observed to coincide with the rise in PGFM concentration. Since oxytocin release is specifically associated with that of neurophysin (Moore & Watkins 1979), Fairclough and coworkers (1980) suggested that the pulsatile nature of oxytocin released from the posterior pituitary gland may be responsible for the pulsatile nature of $PGF_{2\alpha}$ release from the sheep uterus.

Oxytocin has also been shown to stimulate $PGF_{2\alpha}$ release from the endometrium of ovariectomized rats treated with oestradiol (Campos, Liggins & Seamark 1980). Moreover, Soloff (1976) had previously shown that in ovariectomized rats oestrogenic substances lead to enhanced binding of oxytocin by the uterus. Oxytocin also stimulated $PGF_{2\alpha}$ release from the uterus of ovariectomized, oestradiol-treated rabbits; although this effect of oxytocin was blocked by the addition of progesterone. An influence on prostaglandin release

would also explain the findings reported by Sharma and Fitzpatrick (1974). These workers found that oxytocin failed to stimulate the uterus of anoestrus ewes to release $PGF_{2\alpha}$ unless the animal was first primed with oestradiol-17-β. These data indicate that oxytocin may have a physiological role in causing luteolysis. Recent reports (Sheldrick & Flint 1981; Webb, Mitchell, Falconer & Robinson 1981; Schams, Lahlou-Kassi & Galtzel 1982) show a correlation between the secretion of oxytocin and that of progesterone. However within the limitiations of the daily sampling regimen used, these authors did not detect an increase in the plasma concentration of oxytocin at the time of luteal regression. Oxytocin may be produced by the ovary instead of the pituitary gland (Wathes & Swann 1982; Flint & Sheldrick 1982). These authors suggested that luteal oxytocin may be involved in the process of luteal regression either by stimulation of uterine $PGF_{2\alpha}$ secretion or through a local effect on or within the luteal cells. However, in very recent reports, Sheldrick and Flint (1983 a,b) found that a positive feedback may operate between the ovary and the uterus by which oxytocin causes uterine $PGF_{2\alpha}$ release and that luteal synthesis, and/or secretion of oxytocin may be influenced by uterine $PGF_{2\alpha}$. Consequently it is possible that oxytocin in the corpus luteum is not involved in intra-luteal events mediating prostaglandin-induced luteolysis.

Undoubtedly, this identification of the the corpora lutea as the major potential source of oxytocin secretion suggests that ovarian oxytocin secretion in response to the uterine release of $PGF_{2\alpha}$ has a role in the control of luteal regression. Nevertheless, the data is limited and further work is needed especially including other hormones and species of animals.

As regards other hormones, FSH, LH and prolactin were all found to be necessary for the formation of the corpus luteum and the continued maintenance of luteal function and structure during the oestrous cycle. Of these hormones FSH measure by RIA showed a significant increase on the day of oestrus compared to the levels measured during the cycle (Hopkinson & Pant 1973; Salamonsen, Jonas, Burger, Buckmaster, Chamley, Cumming, Findlay & Goding 1973; Pant, Hopkinson & Fitzpatrick 1977). FSH stimulates the growth of the ovarian follicle by promoting mitotic proliferation of the granulosa cells. Forbes and Clegg (1969) found that plasma levels of FSH fluctuated greatly following insertion of an IUD into the anterior portion of both uterine horns of ewes. By contrast LH concentrations during the oestrous cycle show a mean resting concentration of 3 ng/ml in plasma with an abrupt surge commencing 4 to 16 hours after oestrus (Goding, Catt, Brown, Kaltenbach, Cumming & Mole 1969). In each case the concentration returned to the basal level by 10 hours after the start of the surge. Peaks of higher values and duration were obtained after

administration of oestradiol-17β to anoestrus sheep.

Basal circulatory levels of prolactin were measured in ruminants by Joke (1970) who took daily blood samples from heifers and goats and found the average basal circulating levels to be 2.0 to 6.8 ng/ml respectively, with a considerable day to day variation. In addition a wide variation in plasma concentrations during the day was reported by Schams and Karg (1970) and Swanson and Hafs (1971). Reeves, Arimura and Schally (1970) studied the variation in blood prolactin and LH measured simultaneously during the various stages of the oestrous cycle in the ewe. They reported that prolactin levels were significantly higher during pro-oestrus and day 1 of the oestrous cycle than at any other stage. LH levels were elevated on day 1 of oestrus and low in the other stages. Levels ranged between 1.4 to 1.9 ng/ml. These data suggest that the duration of the rise of prolactin was longer and preceded the elevated LH level at oestrus. The results of plasma prolactin measurements reported by Kann(1971) and Bryant, Greenwood, Kann, Martinet and Denamur (1971) showed that massive prolactin secretion occurred at oestrus in the ewe. Cumming, Brown, Goding, Bryant and Greenwood (1972), studied the release of prolactin in more detail over a 47 hour period from the onset of oestrus in the ewe. Surges of prolactin occurred around the time of pre ovulatory LH release which began to rise 4 to 16 hours after oestrus was first detected. The prolactin

concentration reached its peak during the first 10 hours after the onset of LH peak, but no large peaks were observed after the prolactin concentration returned to base line. This pattern of secretion raises the possibility that there could be a common mechanism causing the release of these two hormones. This possibility was reinforced by the findings of Fell, Beck, Cumming and Goding (1972), who reported that oestradiol-17β administered to anoestrous ewes caused the release of prolactin as well as LH. However, prolactin appears to be more readily secreted than LH under conditions of psychological disturbances. Evidence that stress causes prolactin release has been provided by Bryant, Linzell and Greenwood (1970).

Several reports concerning the influence of pituitary gonadotrophins on the maintenance and secretary activity of the corpora lutea of sheep concluded that LH plays a major luteotrophic role in the sheep (Denamur 1968; Kaltenbach, Garber, Niswender & Nalbandov 1969; Hixon & Clegg 1969; McCracken et al 1971). The luteotrophic influence of LH was mainly demonstrated by hypophysectomy during the luteal phase of the cycle and replacement therapy. The luteotrophic properties of prolactin have also been demonstrated by injection of prolactin into hypophysectomized ewes (Denamur 1974). Prolactin treatment increased the weight of the corpora lutea and caused an increase in progesterone concentration in the ovarian venous blood.

Moreover, prolactin was shownto retain its luteotrophic activity after heating to 100^{0}C at pH 7 for several minutes, a treatment which would destroy any contaminating LH (Denamur & Short 1973). These results indicated that prolactin and LH are both involved in the maintenance of corpora lutea, and together form the luteotrophic complex. $PGF_{2\alpha}$ must overcome this luteotrophic effect in order to cause luteolysis.

Having clarified the hormonal events during the cycle let us consider the control (hormonal or otherwise) of prostaglandin production during the different stages of the oestrous cycle. Much work has been carried out to try to determine the nature of the stimulus for the increased production of $PGF_{2\alpha}$ by the uterus. Oestrogen treatment was found to have paradoxical results, being both luteolytic and luteotrophic according to circumstance. Injection of oestradiol from day 9 to 12 in the sheep induced premature regression of the corpora lutea (Howland, Kirkpatrick, Woody, Pope & Casida 1968; Stormshak, Kelley & Hawk 1969; Hawk & Bolt 1970; Piper & Foote 1970; Warren et al 1973). However, the injection of oestradiol on day 11 and 12 failed to cause luteal regression in the hysterectomized ewe (Stormshak et al 1969; Akbar, Rowe & Stormshak 1970; Bolt & Hawk 1972; Denamur & Khan 1973; Bolt & Hawk 1975). The weights of the corpora lutea in both ovaries were reduced by oestradiol administration to unilaterally hysterectomized ewes,

but the corpora lutea in the ovary adjacent to the intact uterine horn were significantly smaller in size than those in the opposite ovary (Akbar et al 1971). Chakraborty and Stormshak (1976) also investigated the role of the uterus in oestradiol induced luteal regression in the ewe, and found that the uterus needs to be present for more than 24 hours after injection of oestradiol for the hormone to bring about premature luteal regression. This suggests that part, at least, of the effect of oestradiol on the corpora lutea was mediated via the uterus. Furthermore, Denamur and Kann (1973) found that the luteolytic effect of oestrogen persists after pituitary stalk section, suggesting that the hormone is not acting centrally on the hypothalamus, but acting directly on the uterus. French and Casida (1973) showed that intra-uterine administration of actinomycin D (a substance that inhibits DNA-dependent RNA synthesis) on days 10 or 11 of the cycle prevented corpus luteum regression. When ewes bearing one corpus luteum on each ovary were unilaterally treated with actinomycin D, the corpus luteum adjacent to the treated horn was maintained when the horn was ligated and severed to prevent transport of actinomycin D from the treated side to the control horn. These findings indicate that DNA-dependent RNA synthesis, which is normally stimulated by oestradiol is necessary for the luteolytic influence of the uterus to occur. Oestradiol has also been found to stimulate increased $PGF_{2\alpha}$

synthetase in the uterus of intact and ovariectomized guinea-pigs (Naylor & Poyser 1975). Barcikowski et al (1974) infused oestradiol into the arterial supply of the ovine uterus during the late luteal phase of the oestrous cycle, and found a large increase in $PGF_{2\alpha}$ output by the uterus some 60 to 90 minutes after the start of infusion. This interval after infusion is probably too short for extensive enzyme reactions to occur, suggesting that the oestradiol may simply release bound arachidonic acid for conversion into $PGF_{2\alpha}$. However this reaction is usually a much more rapid process when induced by other stimuli (Poyser 1981). Hixon, Gengenbach and Hansel (1975) suggested that oestradiol may also potentiate the luteolytic effect of $PGF_{2\alpha}$ in sheep. Their conclusion was based on the observation that, treatment with a dose of $PGF_{2\alpha}$ that was luteolytic in intact ewes, was not effective after destruction of the ovarian follicles by X-irradiation. This suggests that oestradiol is necessary for $PGF_{2\alpha}$ to have a luteolytic effect. Furthermore, the study of Gengenbach, Hixon and Hansel (1977) in which hysterectomized and hysterectomized X-irradiated ewes were treated with oestradiol and/or $PGF_{2\alpha}$ suggested that the luteolytic interaction of oestradiol and $PGF_{2\alpha}$ is independent of the uterus. Although the design of the experiment did not indicate the site of action, it can be assumed that it is the corpus luteum. In view of this and from the evidence reported earlier (e.g. Caldwell,

Tillson, Brook & Speroff 1972; Ford, Pexton, Wilson, Butcher & Inskeep 1973; Barcikowski et al 1974; Ford, Weems, Pitts, Pexton, Butcher & Inskeep 1975) it would appear that oestradiol probably has the dual effect of releasing $PGF_{2\alpha}$ from the uterus and acting with $PGF_{2\alpha}$ to cause luteolysis.

The results of the above investigations using exogenous administration of oestradiol are partialy reinforced by studies of endogenous levels of oestradiol in ovarian and peripheral veins. Cox, Thorburn, Currie and Restall (1974) measured oestradiol and $PGF_{2\alpha}$ in the plasma collected from ewes prepared with indwelling catheters in both utero-ovarian veins. They found that oestradiol concentrations rose about day 11 to 12, just before the first peak of $PGF_{2\alpha}$ on day 13. This peak of oestrogen coincides with the end of the first wave of follicular growth in the ovine cycle (Brand & De Jong 1973). The next major rise of oestradiol occurred during prooestrus on day 15 and 16 and coincided with a further and more sustained output of $PGF_{2\alpha}$ by the uterus. Barcikowski et al (1974) also reported peaks of oestradiol in the uterine venous blood of ewes and found that after day 13 the peaks were associated with peaks of $PGF_{2\alpha}$. Furthermore, Joshi, Watson and Labhsetwar (1973) showed that levels of oestradiol in the ovarian vein of guinea-pigs are low during the early part of the oestrous cycle, but increase around day 11 and again around day 15. The concentration of $PGF_{2\alpha}$ in the utero-

ovarian vein of guinea-pigs (Blatchley & Donovan 1972; Earthy, Bishop & Flack 1975) also follows a similar pattern with an increase recorded on day 11 and a further increase on day 15. When uterine venous samples have been collected at few-hourly intervals, several authors have reported a rise in oestrogen coinciding with the initial rise in $PGF_{2\alpha}$ in gilts, guinea-pigs, hamster and cow (Shaikh and Akbar 1974; Nancarrow et al 1973; Cox, Thorburn, Currie & Restall 1974; Horton & Poyser 1976). The results of these studies show that the ovary starts secreting oestradiol just before the increase in $PGF_{2\alpha}$ output from the uterus. During the oestrous cycle in the sheep, the corpus luteum does not secrete oestradiol, and secretion of this hormone is exclusively from the follicles, and is an index of follicular activity (Baird, Baker, McNatty & Neal 1975).

Progesterone has also been shown to have the ability to alter the length of the oestrous cycle. Exogenous progesterone during the early part of the oestrous cycle in the ewe causes a shortening of the cycle due to premature luteal regression (Ginther 1968; Smith & Robinson 1969) while treatment at a later stage causes a lengthening of the cycle. Thus Woody and coworkers (Woody, Ginther & Pope 1968; Woody 1968) found that hysterectomized ewes treated with progesterone from day 1 to 10 failed to return to oestrus by day 60. Also Ginther (1968) showed that injection of progesterone from days 2 to 5 into unilaterally hysterectomized sheep

with one corpus luteum in each ovary caused the average weight of the corpus luteum on the side of the retained horn to be significantly less than that on the opposite side of the retained horn. This result also indicates the local nature of the luteolytic effect of the uterus on the corpus luteum. However, in guinea-pigs injection of progesterone early in the oestrous cycle does not cause premature luteolysis (Choud y & Greenwald 1968; Bland & Donovan 1970). Although progesterone treatment did not cause luteal regression it did potentiate luteolysis initiated by other mechanisms (in sheep, Warren et al 1973; in guinea-pig, Bland & Donovan 1970). As progesterone concentrations are high between days 5 and 13, but the initiation of the uterine luteolytic mechanism does not occur until about day 12, it has been assumed that this initial period of progesterone exposure is required to initiate the luteolytic function of the uterus. However, these studies did not take into consideration the levels of other endogenous hormones. It has been shown that the combined action of oestradiol and progesterone is more effective and also necessary for the secretion of the uterine luteolytic hormone. Several authors have suggested that a period of progestational influence facilitates the luteolytic effect of oestradiol (Warren et al 1973; Ford et al 1975; Louis et al 1977). Scaramuzzi, Boyle, Wheeler, Land and Baird (1974), collected blood samples from the uterine vein of ovariectomized ewes with the uterus

transplanted to the neck. They found that infusion of progesterone alone had a small influence on $PGF_{2\alpha}$ secretion; infusion of oestradiol alone produced no effect, while oestradiol infusion after pre-treatment with progesterone significantly stimulated the secretion of $PGF_{2\alpha}$. Similarly Warren et al (1973), observed that oestrogen treatment of ewes early in the oestrous cycle caused luteal regression if given after a sequence of progesterone injections. Thus a period of progestational priming appears necessary for oestrogen to cause regression of the corpus luteum. In this context, Baird, Land and Scaramuzzi (1976) suggested a period of 7-10 days of progesterone priming being necessary for the uterus to release prostaglandin F. Barcikowski et al (1974) also confirmed the combined effect of oestrogen and progesterone and also showed that the response of oestradiol after progesterone priming was depressed by indomethacin, an inhibitor of prostaglandin synthesis. This effect of indomethacin on $PGF_{2\alpha}$ release was also subsequently shown by Lewis and Warren (1975). When indomethacin was infused into the uterine horn adjacent to the corpus luteum, it prevented oestradiol-17β induced luteolysis.

The mechanism by which luteolysis is brought about by $PGF_{2\alpha}$ is not yet fully understood and there appears to be variation between different species. Initial studies appeared to support the hypothesis that luteolysis was caused by constriction of the utero-

ovarian vein which resulted in a reduction of the blood flow to the corpus luteum. This was initially proposed by Pharris, Cornette and Gutknecht (1970) who suggested that $PGF_{2\alpha}$ significantly reduced blood flow in the utero-ovarian vein and thus decreased blood flow to the ovaries. This might lead to a build up of metabolites in the ovary which could have a harmful effect on the corpus luteum (Pharris, Tillson & Erickson 1972). In keeping with this, Thorburn and Hales (1972), showed that the infusion of $PGF_{2\alpha}$ into a uterine vein on day 8-9 of the ovine oestrous cycle caused a drop in blood flow through the corpus luteum and ovarian tissue. Gutknecht, Duncan and Wyngarden (1970) also reported a 50% reduction in ovarian blood flow in rabbits receiving an intravenous infusion of $PGF_{2\alpha}$. Moreover, intrauterine or intramuscular injection of $PGF_{2\alpha}$ into sheep resulted in premature regression of the corpus luteum (Douglas & Ginther 1973; Nett, McClellan & Niswender 1976) with a reduction in ovarian blood flow similar to that observed during normal luteal regression (Mattner, Hales & Brown 1972; Niswender, Diekman, Nett & Akbar 1976; Bruce & Moore 1976; Niswender, Reimers, Diekman & Nett 1976). Likewise, infusion of $PGF_{2\alpha}$ via the ovarian artery in ewes with autotransplanted ovaries caused the mean ovarian blood flow to fall by 10-15% during the infusion. This was followed by a dramatic decline in the secretion of progesterone. Thus it initially appeared that luteolysis by $PGF_{2\alpha}$ was simply caused by

venoconstriction in the intact animal, although it had been suggested (McCracken 1971; Thorburn & Hales 1972) that there could perhaps be a redistribution in blood flow through the ovary at the time of luteal regression. However, the subsequent findings in the sheep (Einerjensen & McCracken 1976) and rabbits (Bruce & Hiller 1974) showed that progesterone output from the ovary begins to fall before blood flow through the corpus luteum is reduced, thus indicating that the initiation of luteolysis is not primarily due to a reduction of luteal blood flow. Thus, Horton and Poyser (1976) suggested that the reduction in the luteal blood flow may be a result but not the cause of luteal regression.

The structural and functional changes of the corpus luteum have been studied by many workers. In the sheep the structural changes have been demonstrated using light and electron microscopy by several groups (Bjersing, Hay, Moore, Short & Deane 1970; Thwaites & Edey 1970; Gemmell, Stacy & Thorburn 1975,1976; Umo 1975; Stacy & Gemmell 1976 a,b). Subsequently Niswender et al (1976) found a decrease in the relative volume of the capillary network within the corpus luteum during regression, while Chamley and O`Shea found cellular debris within the capillaries following the injection of $PGF_{2\alpha}$ into the corpus luteum. O`Shea, Nightingale and Chamley (1977) studied the changes in the small blood vessels in the corpus luteum during cyclical regression and concluded that many luteal capillary changes are

caused as a result of obstruction by cellular debris. Similar morphological and biochemical events associated with luteolysis were shown by McClellan, Abel and Niswender (1977). Other studies have suggested a direct effect of $PGF_{2\alpha}$ on the corpus luteum. It has been hypothesized that the biochemical event that initiates luteolysis is the decline in the concentration of luteal receptors. $PGF_{2\alpha}$ combines with its luteal cell membrane receptor in order to induce luteolysis. This binding of $PGF_{2\alpha}$ increases through the oestrous cycle and reaches a maximum when the corpora lutea are actually regressing (Kimball & Wyngarden 1977). Accordingly changes in number and/or affinity of $PGF_{2\alpha}$ receptors during the oestrous cycle may account for the resistance of the corpus luteum to the luteolytic action of $PGF_{2\alpha}$ during the early part of the cycle. On the other hand, Henderson and McNatty (1977) postulated from their studies on ovine tissue that since the steriodogenic potential of the granulosa-luteal cell is related to the amount of LH bound to the cell, this bound LH may protect the newly formed corpus luteum from the lytic action of $PGF_{2\alpha}$. Furthermore Diekman, O'Callaghan, Nett and Niswender (1978) demonstrated that administration of $PGF_{2\alpha}$ to the sheep on day 10 of the cycle reduced LH binding and the number of LH receptors in the corpus luteum. This effect was apparent after 7.5 hours and became more significant by 22.5 hours. However, progesterone secretion decreased before there was a

reduction in LH binding or the LH receptor population. Similarly Behrman, Grinwich, Hickens and MacDonald (1978) proposed that the luteolytic activity of $PGF_{2\alpha}$ initially causes a rapid loss in the ability of the corpus luteum to accumulate LH, even though LH receptors of high affinity are still present; subsequently an actual loss of LH receptors makes luteolysis irreversible. Other evidence is available which suggests that $PGF_{2\alpha}$ causes luteal regression by blocking the effects of luteotrophic hormones on the corpus luteum. Thus, in pseudopregnant rat, Chatterjee (1972) has found that $PGF_{2\alpha}$-induced luteolysis on day 5 can be prevented with prolactin, while in guinea-pigs FSH can partially reverse the luteolytic effects of oestradiol (Choudary & Greenwald 1969). Thus in these species, the action of $PGF_{2\alpha}$ to cause luteolysis may be to reduce the binding of gonadotrophins and reduce cyclic AMP. Henderson and McNatty (1975) suggested that this would result in a decrease in steroidogenesis or a reduction in the progesterone/20α -hydroxy progesterone ratio. However, Sasser, Niswender and Nett (1977) found that $PGF_{2\alpha}$ - induced luteolysis in the ewe is not prevented by administering LH and/or prolactin. Similarly, $PGF_{2\alpha}$ induced luteolysis in the cow is not prevented by LH (Gonzalez-Mencio, Murphy & Manns 1977). More recently, Wakeling and Green (1981) concluded from their investigation that the luteolytic effect of $PGF_{2\alpha}$ is mediated by the binding of $PGF_{2\alpha}$ to plasma membrane

receptors, disruption of the LH-stimulation of adenylate cyclase and the consequent reduction in progesterone sythesis. They further suggested that the resistence of the newly formed corpus luteum to $PGF_{2\alpha}$ may be mediated by a mutually antagonistic relationship between the trophic support fo luteal function and the luteolytic action of $PGF_{2\alpha}$.

The luteolytic mechanism of $PGF_{2\alpha}$ on the corpus luteum has been investigated extensively in the rat; and the biochemical changes following functional luteolysis have been reviewed recently by Poyser (1981). One of the earliest actions of $PGF_{2\alpha}$ on the corpus luteum occurring within 15 mins, is to prevent the LH-induced increase in c-AMP levels. This reduction in c-AMP production causes a reduction in cholesterol esterase activity in the rat ovary.

Before $PGF_{2\alpha}$ was known to be the luteolysin produced by the uterus, several procedures (mainly insertion of foreign bodies) were shown to induce luteal regression (Moore & Nalbandov 1953; Nalbandov, Moore & Norton 1955; Inskeep et al 1961,1962; Ginther, Pope & Casida 1966; Stormshak et al 1966,1967). Subsequently introduction of intra-uterine devices (IUDs) has been shown to cause premature release of $PGF_{2\alpha}$ and luteolysis. This release of $PGF_{2\alpha}$ was shown by the studies of Wilson and coworkers (Wilson et al 1972; Pexton, Ford, Wilson, Butcher & Inskeep 1973). These authors found that insertion of IUDs into each uterine

horn on day 2 of the oestrous cycle in sheep caused an increase in endometrial levels of $PGF_{2\alpha}$. Similarly, Spilman and Duby (1972) showed that IUD insertion into one uterine horn of a ewe on day 2,3 or 4 of the oestrous cycle caused an increase in $PGF_{2\alpha}$ content of the endometrium and the uterine venous plasma together with an inhibition of luteal development and maintenance. Also the shortening effect of IUD on the the oestrous cycle has been shown to be overcome by treatment with indomethacin, an inhibitor of prostaglandin synthesis (Spilman & Duby 1972). These findings demonstrate that IU treatments cause premature luteal regression by a premature increase in $PGF_{2\alpha}$ production and release by the uterus.

Prostaglandin has also been found to be released from the uterus in response to bacterial infection (Skarnes & Harper 1972; Greenwood & Kelly 1975). Prostaglandins have been detected in pyometrial fluid in the cow, bitch and ferret (Heap & Poyser 1975). Inflammatory fluid obtained from the uterus contains mainly $PGF_{2\alpha}$. Uterine fluid in pregnancy also contained high concentrations of $PGF_{2\alpha}$ (up to 1500 ng ml^{-1}) (Harrison, Heap & Poyser 1976). Treatment of non pregnant ewes with progesterone results in accumulation of uterine fluid which is similar to that in the pregnant ewehaving a high concentration of $PGF_{2\alpha}$ (Amoroso, Harrison, Heap & Poyser 1973; Harrison, Heap, Poyser 1976; Alwachi et al 1980). Earlier investigations

by Yamauchi and coworkers (Yamauchi 1963; Yamauchi, Nakahara, Kaneda & Inui 1967) demonstrated that injection of a mass of viscous, gel-like substance into the uterus in cows caused shortening of the oestrous cycle. However extended cycles were obtained by these investigators when it was injected between days 12 and 21. More recently Kindahl, Lindell and Edquist (1981) found that intrauterine infusion lengthened the oestrous cycle in the cow depending on the stage of the cycle. If iodine is perfused in the early or in the middle of the cycle, the cycle is shortened, but if infusion is later (after about day 15) the cycle is prolonged. This has been suggested to cause endometritis, resulting in $PGF_{2\alpha}$ release. It is apparent from the preceding data that, the physiological stimulus factors that control the production and release of $PGF_{2\alpha}$ are by no means settled, although it appears that an interaction between progesterone, oestradiol and oxytocin form the physiological stimulus for $PGF_{2\alpha}$ synthesis and release from the uterus as proposed by Poyser (1981).

Bearing in mind the preceding data on the effect of hormonal and non-hormonal factors on the production and release of $PGF_{2\alpha}$ it is now relevant to examine the endogenous changes of the hormones with respect to prostaglandin secretion. As shown earlier several authors have reported that a rise in oestrogen concentration occurs at the time of the initial rise in

$PGF_{2\alpha}$ in the ewe, , guinea-pig, hamster and cow (Shaikh & Klaiber 1973; Nancarrow et all 1973; Cox et al 1974; Horton & Poyser 1976). Cox et al (1973) collected blood samples every 3 hours from two sheep prepared with indwelling catheters in their utero-ovarian veins. A peak of oestradiol-17β was found on days 10 to 11 of the oestrous cycle i.e. the day before the first observed peak in $PGF_{2\alpha}$. Barcikowski et al (1974) investigated the association between endogenous oestradiol and prostaglandin in sheep via indwelling cannulae from the uterus in situ. Daily sampling of utero-ovarian blood in 4 ewes between days 12 to 16 indicated that the mean level of oestrogen and prostaglandin were found to be significantly higher on day 15 than at other times sampled. To obtain more detailed relationships, the same authors collected samples from three other sheep with utero-ovarian transplants. Two sheep were sampled every 8 hours from day 13 of one cycle to day 1 of the following cycle, while in a third samples were collected every 2 hours from day 11 of the cycle until day 6 of the following cycle. A complex series of $PGF_{2\alpha}$ peaks were observed between day 13 and 16. The prostaglandin peaks were associated with oestrogen peaks only during luteolysis. McCracken et al (1981) showed that the number of $PGF_{2\alpha}$ peaks occuring is important for successful luteal regression in the sheep. When 5 pulses of prostaglandin were infused in 24 hours complete luteal regression occurred. Four peaks in 18 hours were

not sufficient. Even when the dose level was lowered, complete luteal regression occurred provided that 5 pulses were given in 24 hours. Many of the foregoing experiments imply a role for oestrogen in luteolysis. However, the temporal relationship of the different hormones in the latter part of the cycle is not well understood. Frequent serial measurements (i.e. less than 3 hours interval) of uterine $PGF_{2\alpha}$ and ovarian steroids have hitherto been restricted to a study of one cycle in a ewe with utero-ovarian indwelling catheters in the utero-ovarian vein in situ (Thorburn et al 1973). Furthermore in this study no attempt was made to assess the stage of the prostaglandin synthetic pathway effected.

In the current experiments the frequency of sampling was increased, and serial measurements of uterine $PGF_{2\alpha}$ content and synthesizing abililty was also made. The simultaneous measurement of plasma oestradiol-17β , progesterone and $PGF_{2\alpha}$ and the serial measurement of uterine content and synthetic ability for $PGF_{2\alpha}$ and PGE_2 should clarify the temporal relationships between the different factors at the end of the oestrous cycle.

Housing and Husbandry

(i) Animals:

The ewes chosen for these experiments were Dorset Horn X Finnish Landrace crosses. The Dorset Horn has a prolonged reproductive season showing heat periods during the major part of the year (Newton & Belts 1967). The "Dorset-Finn" combines the incidence of multiple births of the "Finn" with this prolonged breeding season of the Dorset Horn (Land 1970), and has been developed for intensive sheep husbandry systems. On account of this, this breed of sheep is well suited to indoor housing.

(ii) Housing and Animal Care:

The ewes were kept at the University of Edinburgh Centre for Laboratory Animals on the Bush Estate. The Centre is situated appoximately 7 miles south of Edinburgh, about 200 metres above sea level at latitude 55^{o} 52' N and longitude 3^{o} 13' W. While under experiment , ewes were kept in individual pens (9.5' x 10') on straw bedding. The sheep house was unheated, well ventilated and illuminated by natural day light. They were allowed free access to water and fed about 1500 g of hay daily. The ewes were dosed with an antihelminthic immediately before housing and the sheep's feet were examined and trimmed regularly.

(iii) Detection of Oestrus

Spontaneous oestrus was checked by teasing daily with vasectomized rams before feeding, between 9.00-

10.00 hrs. The onset of oestrus was considered to be the time at which the ewe first allowed the ram to mount and to complete service. The first day of oestrus was designated Day 1.

Material and Methods

(i) Animals and General Procedures:

Sixteen mature non-pregnant Dorset Horn X Finnish Landrace ewes were used in this experiment. All the ewes exhibited regular 16-19 day cycles. Food was witheld for 24 hrs before surgery and all surgical operations were carried out under full aseptic conditions.Anastomosis of the uterine vein to the anterior mammary vein was attempted on all ewes. The patency of the anastomosis was checked before fistulation was performed. Fistulation of the uterine horn was only done in those ewes with a patent anastomosis. Blood and uterine tissue samples from sheep with a patent anastomosis and a fistulated uterus were collected during 8 cycles only. The frequency of sampling varied during the 8 cycles.

(ii) Anaesthesia

Anaesthesia was induced with an intravenous injection of about 20 mls alphaloxone and alphadolone acetate mixture (Saffan; Glaxo Labs. Ltd.. Greenford, Middlesex) into the recurrent tarsal vein. It was maintained with Halothane, nitrous oxide and oxygen mixture administered via a tracheal tube.

(iii)Anastomosis of the Utero-ovarian Vein to the Mammary Vein:

Introduction:

Anastamosis of the utero-ovarian vein to the mammary vein was first done by Thorburn and Mattner (1971) in pregnant ewes. The utero-ovarian vein was

anastomosed end-to-end, with the anterior mammary vein (vena epigastrica cranialis superficialis),consequently the uterine venous drainage was diverted through the superficial and thus more accessible mammary vein. The method was adapted by Alwachi et al (1979) to avoid interrupting the pathway whereby $PGF_{2\alpha}$ passed from uterus to ovary. Such interruption occurred in Thorburn and Mattner`s original method. This initial modification involved removal of the ovary ipsilateral to the anastomosis to prevent persistence of the corpora lutea. However, removal of the ipsilateral ovary revealed an apparent local effect of the ovary on the uterus (Alwachi et al 1979). The absence of the ovary altered the pattern of prostaglandin production by the ipsilateral uterine horn. On account of this, the technique was further modified in the present study to leave the ovary in situ , but still avoid disrupting the luteolytic pathway.

Procedure of Anastomosis:

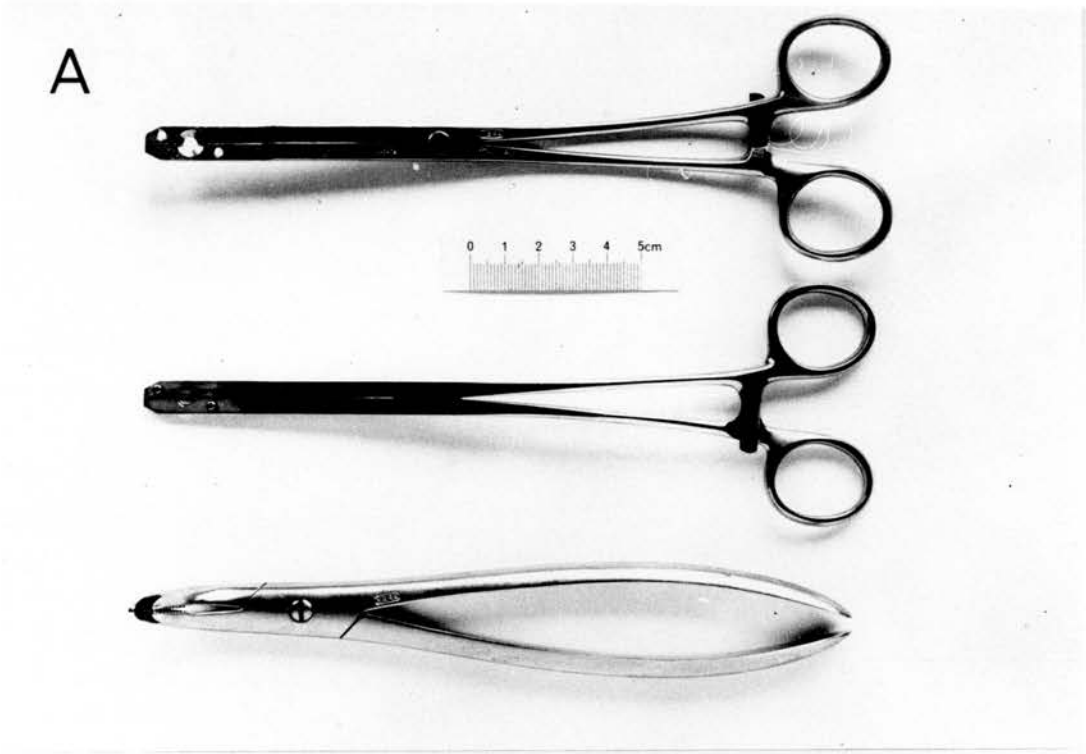
Prior to anastomosis the belly was shaved and the direction of blood flow in the anterior mammary vein was checked. In most virgin sheep the direction of blood flow is towards the udder (Linzell 1960). However, during lactation the valves, which usually ensure that blood flows towards the udder, become incompetent with the result that blood can flow in the opposite direction i.e. away from the udder. For anastomosis only the latter situation is suitable.

Anaesthesia and preparation were followed by a skin incision lateral to and parallel to the mammary vein. Approximately 4cm of the mammary vein was carefully dissected free from the surrounding tissues and placed back under the skin flap to reduce venous spasm. The uterus and ovaries were then exteriorized through a paramedian incision of the abdominal wall and examined. A uterine branch of the utero-ovarian vein cranial to the junction of the uterine and ovarian branches was separated from the surrounding tissue and severed. It was then passed through a small elliptical hole in the linea alba. The free ends of the two veins (Anterior Mammary and a uterine branch of the utero-ovarian vein) were then anastomosed end-to-end using Nakayama`s instrument for small vessel anastomosis (Senko Medical Instrument Manufacturing Co. Ltd., Bunkyoku, Tokyo, Japan)- see Fig. 2 and appendix 1. Following the anastomosis and after ensuring complete haemostasis, the abdominal and skin incisions were closed with mattress sutures of cat gut (chromic 116, Ethicon Ltd. Edinburgh) and nylon (Ethicon 186, Ethicon Ltd. Edinburgh), respectively. Once healing was complete (3-5 weeks) the patency of anastomosis was checked by retrograde injection of the radioopaque material (Urografin, Schering Chemicals Ltd., Burgess Hall, Sussex) into the anastomosed vein and quickly visualizing the vessels by X-radiography (see Fig. 3). If the result of this was inconclusive, the anastomosis was re-checked during

Figure 2: Nakayama's instrument for small vessel anastomosis.

A-Anastomosis equipment consisting of two ring-holding clamps (upper two instruments) and a pair of pliers.

B- Enlargement of the rings used to join the vessels.

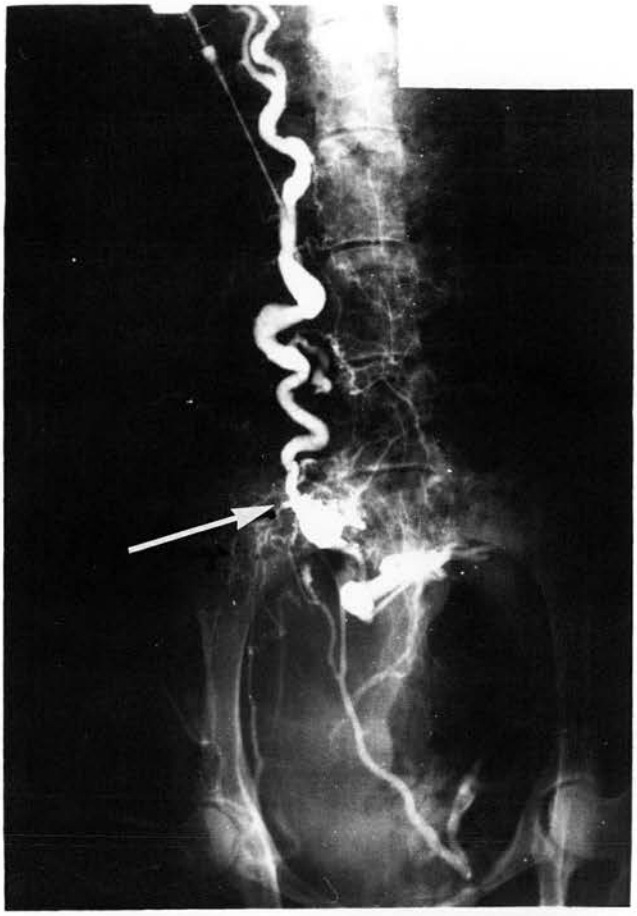

A
0 1 2 3 4 5cm

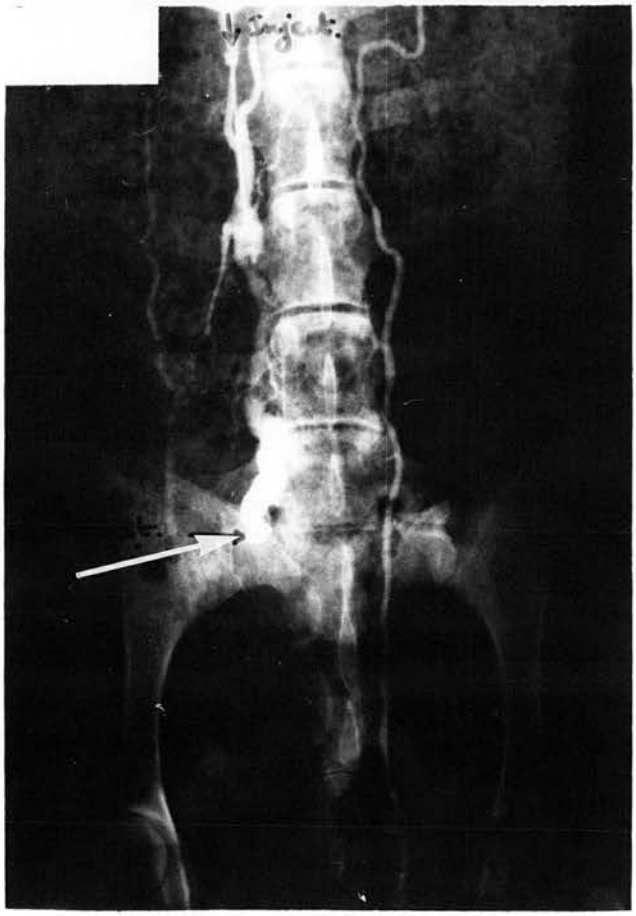

B
1mm

Figure 3: Successful and unsuccessful anastomosis between the uterine vein and the anterior mammary vein in the sheep.

Top- Radiograph showing a patent utero-mammary vein anastomosis in Ewe A10.

Bottom- Radiograph showing the anstomosis in Ewe A16, which was considered not patent. The injected radio-opaque material did not pass through the anastomosis rings (arrowed).





surgery for fistulation. Prior to the actual fistulation radioopaque material was injected into the "anastomosed" branch of the utero-ovarian vein and the vessels again visualized by X-radiography. The persistence of the stainless steel anastomosis rings in the tissue allowed accurate estimation of the patency of the anastomosis, although in some animals the exact superficial course of the mammary vein was further indicated by artery clamps prior to the injection of radioopaque material. The patency of the anastomosis was again checked at autopsy by injection of Indian Ink into the uterine venous branch close to the fistulated uterine horn. If the anastomosis was patent the Indian Ink passes through the anastomosis and into the mammary vein.

(iv)Fistulation of the Uterine Horn:

Intoduction:

To determine the prostaglandin content and synthesizing ability of the uterine endometrium it was necessary to collect uterine tissues regularly. The interlocking folds within the sheep`s cervix are such as to prevent uterine biopsies being taken by the obvious route of per vaginam and cervix. In view of these obstacles it was necessary to devise a surgical technique to make the uterine endometrial tissues accessible in the conscious animal.

Procedure of Uterine Horn Fistulation

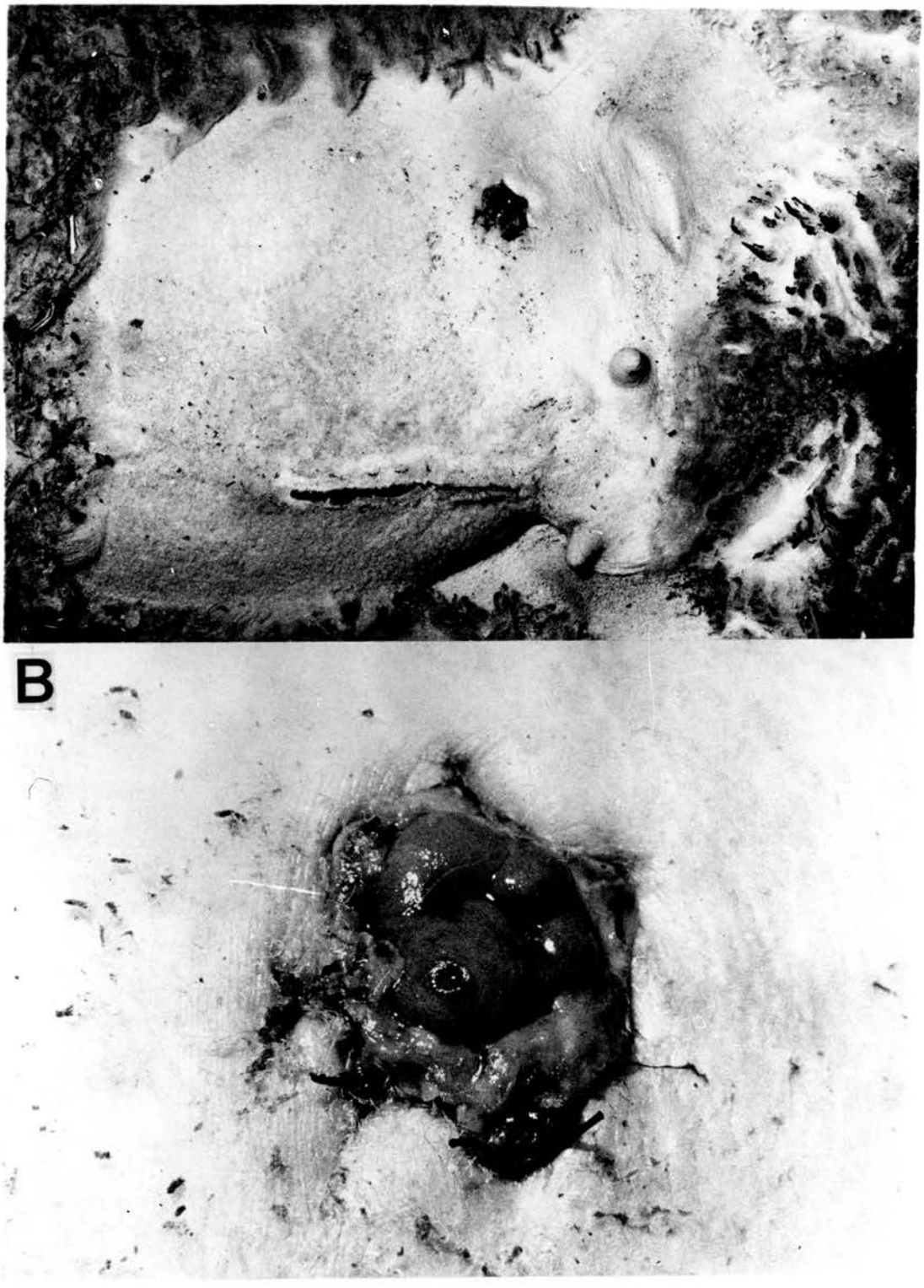
Fistulation of the uterine horn ipsilateral to the anastomosed uterine venous branch was performed only after checking the patency of the anastomosis and following the re-establishment of normal oestrous cycles. Only four animals were thus eligible for this procedure. The uterus and ovaries were first located and exteriorized through a mid-ventral incision. The uterine horn on the anastomosed side was ligated at the base and separated. The cut end was then fistulated to the exterior through a small hole through all layers of the body wall and then sutured to the sides of the hole. The nylon sutures used passed through all the body layers including the skin (see Fig. 4).

Post-Operative Treatment

Following surgery, the ewes were kept under supervision in the clinic overnight, and returned to

Figure 4: A- The ventral abdominal area of Ewe A10 showing the site of fistulation relative to the laparotomy.

B-The exposed uterine tissue of the fistula. Note the healthy appearance of the caruncles. The sutures are sited well clear of the endometrial tissue to prevent abrasion.


B

their own pen the next morning. Consequently sampling was performed in the sheep's normal environment. During sampling the sheep were restrained manually. Due to continuous contact between the sheep and the experimenter the sheep adapted very quickly to the handling procedures and co-operated readily. Consequently the controlling of the animal during the frequent collection of mammary vein blood and uterine tissue could be done easily by one person.

(v) Sampling of Blood and Uterine Tissues:

Blood Sampling from Peripheral and Mammary Vein:

Samples of uterine venous blood were collected from the patent anastomosed utero-mammary vein. At each sampling, 3-5 ml of blood were taken into tubes containing Potassium EDTA (Ethylene diamine tetraacetic acid). Collection of these samples started at 10.00 hr of the selected sampling day in each cycle. Samples were brought back to the laboratory in Edinburgh for processing, but during the half-hour sampling, centrifugation was done immediately on the spot. Previous experiments with Indomethacin treatment of blood and tissue samples had shown that no changes occur in the prostaglandin content of the samples during transportation. Blood samples were then centrifuged and the plasma stored at $-20^{\circ}C$.

Biopsy Procedure for Uterine Tissue:

At each sampling two endometrial samples were taken from the fistulated uterine horn using a home-made

stainless steel biopsy instrument (Fig. 5). Endometrial biopsy samples were either caruncular or non-caruncular or rarely a mixture; no evidence of myometrium was seen in any sample. To determine the inititial prostaglandin content of the endometrial tissue, one sample of tisue was immediately placed in 5 ml absolute alcohol and stored at -20°C. The other tissue sample was first weighed and then homogenized in 15 ml of Tyrode's solution (see below) in a Fison's glass homogenizer and incubated for 90 mins. Methods of extraction and incubation for $PGF_{2\alpha}$ content, and synthesizing ability are as described below.

Composition of Tyrodes's Solution pH 7.5-7.8

Sodium chloride	8.0 gm
Potassium chloride	200 mg
Calcium chloride	200 mg
Magnesium Chloride	100 mg
Sodium acid phosphate	50 mg
Sodium bicarbonate	1 gm
Glucose	1 gm
Distilled water	1,000 cc

(vi)Analysis Of Samples:

Extraction of Prostaglandins in Uterine Tissues

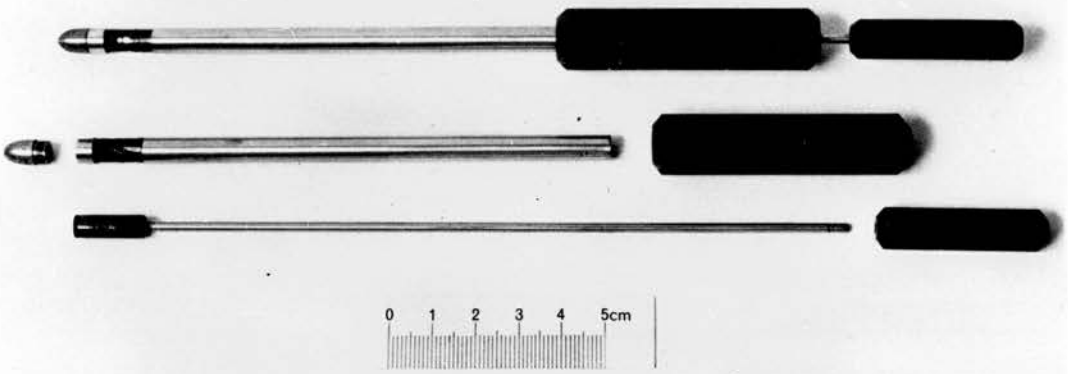
In order to determine the initial $PGF_{2\alpha}$ content of the endometrial tissue a sample of tissue was placed immediately in 5 mls of absolute ethyl alcohol after removal from the animal and then stored at -20°C until extracted.

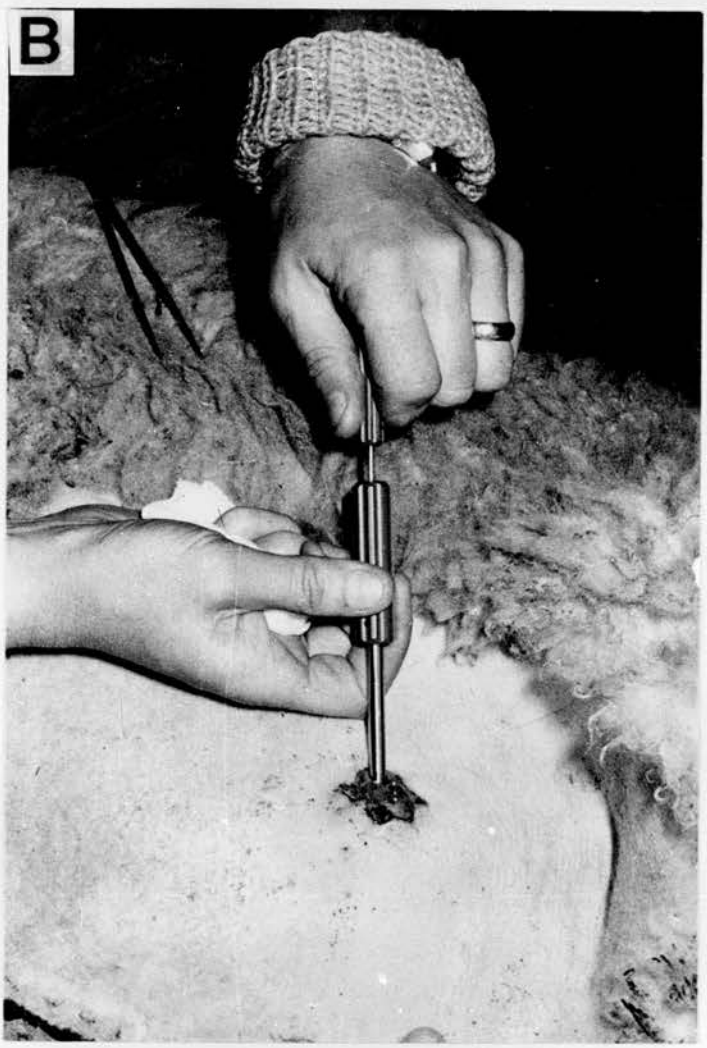
Subsequently this sample was weighed and

Figure 5: A- The home-made stainless steel biopsy instrument used for removing uterine endometrial tissue. The upper part of the picture shows it assembled for use, while in the lower it is stripped down to its component parts.

B- The instrument being used to extract a biopsy.

A





homogenized in the alcohol in which it had been stored and centrifuged. The supernatant was withdrawn and the precipitate washed in 5 mls absolute alcohol and centrifuged at 1300xg in an MSE cool spin centrifuge for 30 mins at 4°C. The supernatant and the washings were combined and evaporated to dryness. The residue was then dissolved in 10 mls of water, the pH of this aqueous extract lowered to 4.5 and the prostaglandins extracted as described below. The extract was then stored in 2 mls of ethyl acetate at -20°C until assayed.

In order to obtain an indication of the ability of uterine tissue to synthesize $PGF_{2\alpha}$ albeit under in vitro conditions, the other tissue sample obtained from the uterine fistula was weighed immediately after removal and then homogenized in 15 mls Tyrode's solution with a Fison's glass homogenizer. The homogenate was then incubated for 90 minutes at 37°C with a constant stream of oxygen bubbling through it. Prostaglandin synthesis was halted after incubation by adjusting the pH to 4.5 with 1M HCl. The sample was then extracted three times with 40 mls of redistilled ethyl acetate and the three portions were combined. After washing with distilled water (pH less than 6.0), the combined fractions were evaporated to dryness and the residue redissolved in 5 mls of ethyl acetate and stored at -20°C until analyzed. $PGF_{2\alpha}$ and PGE_2 concentrations in extracts of uterine tissue were measured by radioimmunoassay based on methods previously described (Blatchley & Poyser 1974; Dighe, Emslie, Henderson, Rutherford & Simon 1975;

Poyser & Horton 1975; Fenwick, Jones, Naylor, Poyser & Wilson 1977). These extraction procedures gave a greater than 90% recovery of $PGF_{2\alpha}$ and PGE_2.

Extraction Procedure of Prostaglandins in Plasma

Blood samples were obtained from the ewes by venepuncture. The collected samples were then immediately centrifuged at 1500 g (3500 rpm) for 10 minutes and the plasma stored at -20°C.

The one step extraction procedure was that of Blatchley and Poyser (1974) and gave >90% recovery of $PGF_{2\alpha}$. For extraction an amount of plasma (e.g. 200 µl) was taken in duplicate from each sample and dispensed into extraction tubes. To each tube was added 100 µl pH 4 citrate buffer and the tubes were mixed using a "Whirlimix". Redistilled ethyl acetate (2 ml) was then added and the samples vortex mixed for 5 mins. The plasma was then frozen using a freezing mixture consisting of dry-ice in acetone. The ethyl acetate fractions from the duplicate extractions of each sample were then pooled and evaporated to dryness in a stream of air at 45°C.

$PGF_{2\alpha}$ Radioimmunoassay

Abbreviations of chemicals and of Solutions for radioimmunoassay that are used in the ensuing text.

Tris = Tris(hydroxymethyl) methylamine

PPO = 2,5-diphenyloxazole

Butyl PBD = 2-(4-tert-H-Butylphenyl)-5-(4-biphenyl)-1,3,4-oxadiazole

DARS = Donkey anti-rabbit serum; Wellcome Reagents Ltd. Stored at 4°C in 25 ml aliquots until use.

NRS = Normal rabbit serum; obtained from non-immunized male New Zealand white rabbits by the method of Dighe, Emslie, Henderson and Simon (1975). Store at -20°C. Prior to use the serum was thawed and there after stored at 4°C.

Solutions used for $PGF_{2\alpha}$ radioimmunoassay:-

Diluent A
- 0.05 M tris buffer pH 8.0
- 0.1 gl^{-1} sodium azide
- 1.0 gl^{-1} gelatin

Scintillant A
- 10.5 g PPO
- 1.5 l toluene
- 0.9 l 2-ethoxyethanol

Sources of Chemicals

Tris (hydroxymethethyl) methylamine -	Analar B.D.H. Chemicals Limited.
Sodium Azide	Hopkin and Williams Ltd.
Gelatin	B.D.H. Chemicals Ltd.
P.P.O.	Fisons
Butyl P.B.D.	Koch Light Laboratories Ltd.

Method of Radioimmunoassay: Following the preperation of the samples the following standards were prepared;

one machine background count using 2 ml diluent A

four "zero standards" consisting of 0.5 diluent A per assay tube.

four "non-specific" binding standards (NSB),

containing an excess of "cold" $PGF_{2\alpha}$ (0.5 ml per tube).

nine triplicate "$PGF_{2\alpha}$" standards of various concentrations in diluent A (0.5 ml per tube).

To each of the above tubes, except the machine background standard, was added 50 µl ^{3}H -$PGF_{2\alpha}$ (160 ci $mmol^{-1}$, 30 pg/ml). Fifty ul of antibody was then added to all the tubes except the counting and machine background standards and the solutions were mixed using a "Whirlimix". All the samples and the standards were then incubated for 1 hr at room temperature, followed by addition of NRS (50 µl 1:140 dilution in diluent A) and 50 ul DARS (50 µl 1:15 dilution in diluent A). The solutions were then mixed and incubated overnight (about 16 hr) at 4°C. Following incubation the tubes were centrifuged at 1300 g for 30 mins at 4°C (Fison's MSE Centrifuge). The supernate was decanted off and the precipitate retained in the tubes and resuspended in 3 mls of scintillant A. A similar volume of scintillant was also added to the "counting standards". The tubes were then put in glass vials and counted for 4 mins in a liquid scintillation counter (Nuclear Chicago). The counts were recorded on a paper tape which was subsequently fed into a PDP 8I computer programmed to calculate the mean % ^{3}H -$PGF_{2\alpha}$ bound by the standards and to apply the logistic curve fit formula of Parker and Ward (1971) to these values. It then supplies the list

of observed values and list of calculated values. The PDP 8I computer is also programmed to calculate from the standards the amount of $PGF_{2\alpha}$ in the samples. For each assay two tubes containing a known quantity of prostaglandin in 0.5 ml diluent were added to check the interassay coefficient of variation.

Standard Curve

Portions of 50 µl standard solutions of $PGF_{2\alpha}$ corresponding to 10,20,40,80,160,320,640,1280 and 2560 pg/ml were added to triplicate disposable plastic tubes. In addition zero standards were set up in quadruplicate. Fifty µl ^{3}H -$PGF_{2\alpha}$ (i.e. 30 pg) was added to each tube followed by 50 µl 1:1600 dilution of antiserum to all tubes except the counting standards. The solutions were mixed and incubated at room temperature for 1 hr. After incubation NRS and DARS were added to all tubes except the counting standards. The standard curve was calculated using the computer programme as described above, and plotted as percentage binding of the ^{3}H -$PGF_{2\alpha}$ against amount of non-radioactive "cold" $PGF_{2\alpha}$.

Specificity

The antibody used was raised in rabbits (Dighe, Emslie, Henderson, Rutherford & Simon 1975). The antibody does not distinguish between $PGF_{2\alpha}$ and PGF-1α but has low cross-reactivity (< 3.4%) with other prostaglandins and their metabolites (Poyser & Scott 1980). In a study by Poyser (1982) GC-MS analysis of prostaglandins formed

by homogenates of sheep uterus during incubation showed that very little $PGF_{1\alpha}$ was synthesized in comparison to $PGF_{2\alpha}$. Consequently in the present study the $PGF_{2\alpha}$ antisera were measuring predominantly the prostaglandin to which it had been raised, (ie $PGF_{2\alpha}$).

Sensitivity and Precision

An example of a standard curve from a prostaglandin $F_{2\alpha}$ assay is shown in Text Fig.6 . When no added unlabelled prostaglandin was present the antibody bound 60-70% of the added radioactivity (3157±40 (±SE) cpm).

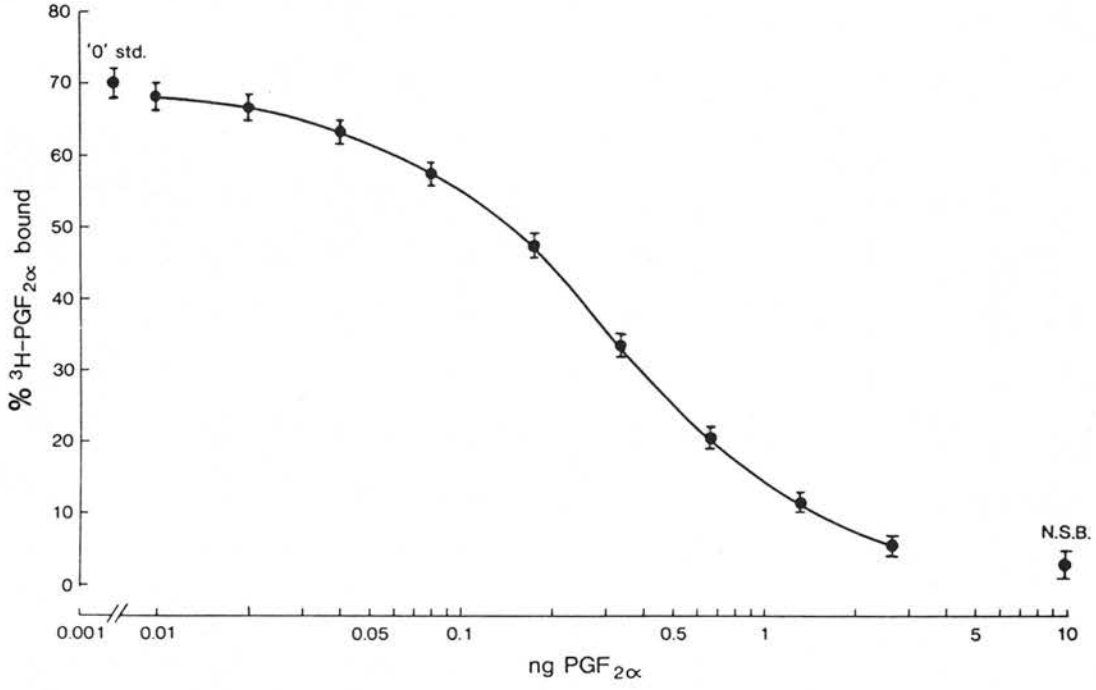
The interassay coefficient of variation calculated from the results obtained by incorporating a known amount of $PGF_{2\alpha}$ (0.64 ng/ml) into each assay was 7.5%. The intra assay coefficient of variation calculated from the variation between the duplicate results obtained was 9.6% (n=65 in 3 assays).

The sensitivity of the assay was 0.025 ng.

Prostaglandin E_2 Radioimmunoassay

Extraction procedure and radioimmunoassay of PGE_2 was the same as for $PGF_{2\alpha}$. PGE_2 was measured using antiserum purchased from the Pasteur Institute, Paris. Other prostaglandins and prostaglandin metabolites had low cross reactivity with the PGE_2 antibodies (Poyser & Scott 1980). The dilution of antibody used was 1:400 in diluent A. After a 2 hr incubation of standards and samples 50 ul of NRS (1:140) and DARS (1:15) were added to each tube and the contents were mixed and incubated for about 16 hr at $4^{\circ}C$. The procedure was then as for

Figure 6: A standard curve used in the $PGF_{2\alpha}$ assay using antiserum at a dilution of 1:1600 and $^{3}H\text{-}PGF_{2\alpha}$. Each point represents the mean of triplicate determinations ±S.D. The standard curve was calculated using the computer programme and plotted as percentage binding of the $^{3}H\text{-}PGF_{2\alpha}$ against amount of non-radioactive "cold" $PGF_{2\alpha}$. The zero standard ("0" std.) and non-specific binding (N.S.B.) are also shown.


80
70
60
50
40
30
20
10
0
'0' std.
% ^{3}H-PGF$_{2\alpha}$ bound
N.S.B.
0.001
0.01
0.05
0.1
0.5
1
5
10
ng PGF$_{2\alpha}$

$PGF_{2\alpha}$ radioimmunoassay, except that the tubes were put in glass vials and counted for 10 mins in the liquid scintillation counter. An example of an acceptable standard curve is shown in Text Fig. 7. Two tubes containing a known amount of prostaglandin E_2 in 0.5 ml diluent were also added to check the interassay coefficient of variation. This was 11.8% while the intraassay coefficient of variation was 8.4%. The limit of sensitivity was defined as the concentration of cold PGE_2 which produced a 10% decrease in binding from the zero standard. The limit of sensitivity of this assay was 40 pg.

Measurement of Progesterone by Radioimmunoassay

Chemicals and solution used for progesterone radioimmunoassay:

Diluent B: 34.5 g disodium hydrogen phosphate

56 ml 0.1 M solution of sodium dihydrogen phosphate

5 l distilled water

0.5 g sodium azide

5 g gelatin

Scintillant B:

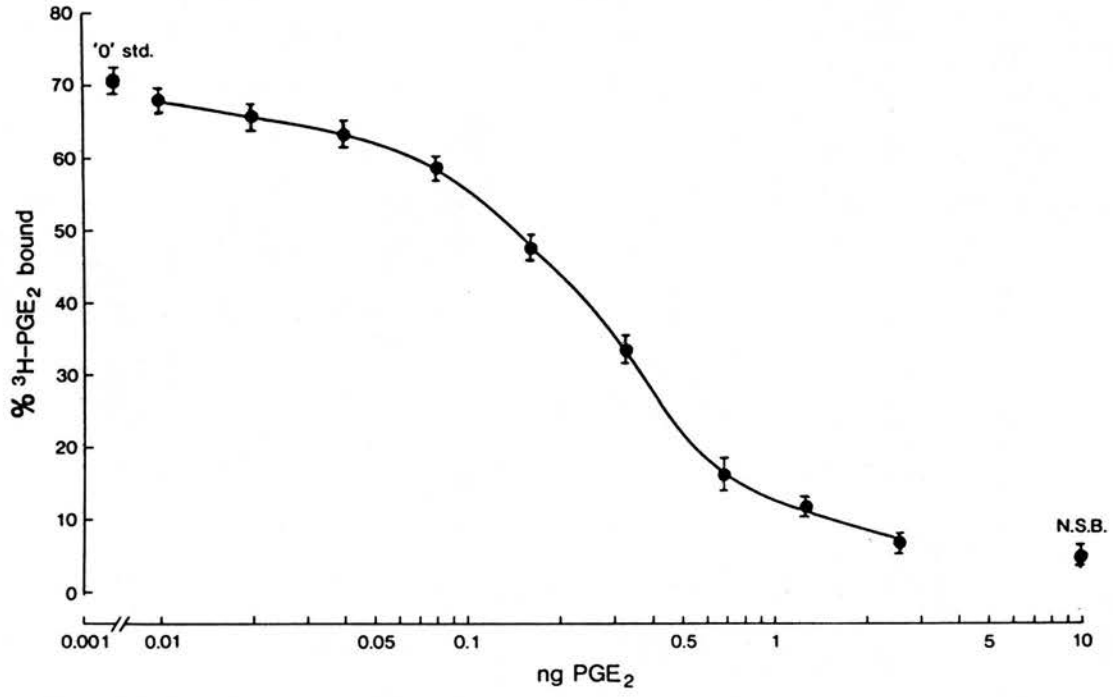
10.0 g butyl PBD

2.5 l toluene

Extraction Procedure for Progesterone from Plasma

Plasma samples for extraction were taken in dupiclates of between 200-500 µl. Half the volume of

Figure 7: A standard curve use in the PGE_2 assay with anitserum at a dilution of 1:400 and ^3H-PGE_2 ($160\ ci\ mmol^{-1}$). The standard curve was calculated using the computer programme and plotted as percentage binding of ^3H-PGE_2 against amount of non-radioactive PGE_2. The zero standard ("0" std.) and non-speicific binding (N.S.B.) are also shown.


80
70
60
50
40
30
20
10
0
'0' std.
N.S.B.
% ^{3}H-PGE$_2$ bound
0.001
0.01
0.05
0.1
0.5
1
5
10
ng PGE$_2$

ethyl alcohol was added to each sample to prevent binding of the proteins in plasma to the antibody. Progesterone was extracted by adding 2.0 ml redistilled petroleum spirit (boiling point 40-60°C) and vortex mixing for 10 mins. The plasma was then frozen using a freezing mixture consisting of dry-ice in acetone and the petroleum spirit fractions were poured off. Extraction was repeated and the two extracts from each sample pooled and evaporated to dryness in a stream of air at 60°C. 1.0 ml of diluent B was then added to each tube.

Radioimmunoassay for Progesterone

Blood samples were extracted in glass tubes with petroleum spirit as above and the progesterone contents were measured by a double antibody technique developed by Dighe and Horton (1975), and described by Poyser and Horton (1975). Following preparation of the standards 30 pg ^{3}H-progesterone (60 $\mathcal{C}$imol^{-1}, in 50 µl of diluent B) and 50 µl of antibody (1:2000) in diluent B were added. The tubes were mixed using "whirlimix" and incubated for 2 hrs at room temperature. Following incubation NRS (50 µl of 1:15 dilution in diluent B) were added. The constituents were then mixed on the "whirlimix" and incubated overnight at 4°C. After incubation 1 ml diluent B was added to each tube before centrifugation. The supernate was then decanted into vials containing 10 ml scintillant fluid B. The vials were shaken for 2 mins before counting for 4 mins on a liquid scintillation

counter.

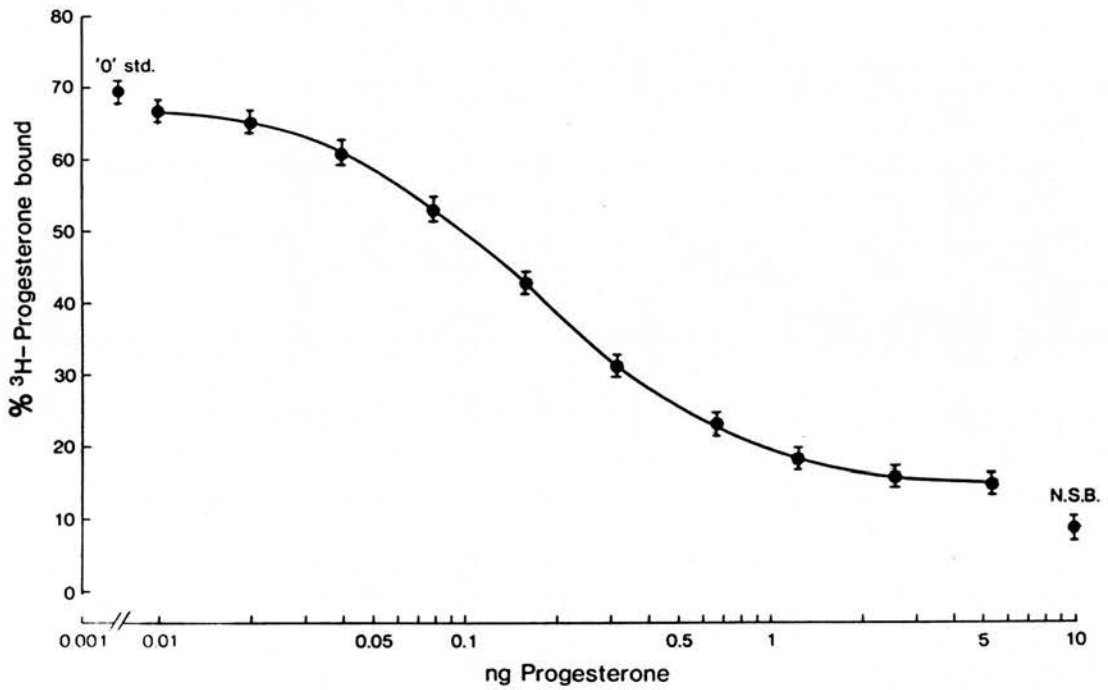
Standard Curve

At the beginning of each assay several standard curves were made to check the accuracy. Ten aliquots of standard solutions of progesterone corresponding to 10,20,40,80,160,320,640,1280,2560 and 5120 pg in a volume of 1.0 ml diluent B were added to triplicate glass tubes. In addition zero standards, non-specific binding and counting standards were set up in quadruplicate. Fifty µl of ^{3}H-progesterone was added to each tube followed by 50 µl 1:2000 dilution of antiserum to all the tubes except the counting standards. The solutions were mixed and incubated for 2 hrs at room temperature. Following incubation NRS (1:140 in diluent B) and DARS (1:10 in diluent B) were added to all tubes except the counting standards. The standard curve was calculated using a computer programme and plotted as percentage of the ^{3}H-progesterone against weight of non-radioactive "cold" progesterone. An example of an acceptable standard curve is shown in Text Fig. 8.

Specificity

At the time of beginning this assay a new batch of progesterone antisera was produced in this laboratory. Specificity studies using this new antibody were made and the cross reactivities with other steroids were determined by the method of Dighe and Hunter (1974), are shown in appendix 2 .

Figure 8: A standard curve used in the progesterone assay with antiserum at a dilution of 1:2000 and ^{3}H-progesterone. The standard curve was calculated using the computer programme and plotted as percentage binding of the ^{3}H-progesterone against the amount of non-radioactive progesterone. The zero standard ("0" std.) and non-specific binding (N.S.B.) are also shown.


80
70
60
50
40
30
20
10
0
'0' std.
N.S.B.
% 3H- Progesterone bound
0.001
0.01
0.05
0.1
0.5
1
5
10
ng Progesterone

Recovery Test for Progesterone

The accuracy of the procedure was evaluated by adding known amounts of progesterone to 2 ml of castrated male sheep plasma and extracting with 2 ml petroleum spirit. The values obtained are shown on table 2.

Table 2

The amount of progesterone (ng/ml) extracted from male sheep plasma.

Amount of progesterone ng/ml		
added	recovered (mean ± S.E.M.)	Number
0.0	0.003±0.60	N=3
0.5	0.059±0.10	N=3
1.0	1.001±0.12	N=3
2.0	2.064±0.40	N=3
4.0	4.032±0.20	N=3

The sensitivity of the assay was 22 pg

The intra-assay coefficient of variation was 9.55% where N=6.

The interassay coefficient of variation was 9.6% where N=6.

Measurement of Oestradiol-17β by Radioimmunoassay

As no current assay procedure for oestradiol-17β was available in this laboratory, method for extraction of oestradiol-17β from plasma and a radioimmumoassay procedure for oestradiol-17β had to be developed as described below.

Antisera

Antisera which had cross-reactivities as shown in appendix 3 , was kindly provided by Dr.D.W. Davidson (Unit of Reproductive Biology, Edinburgh.

The antisera was diluted 1:3200 in diluent B (p.58) and stored at 4°C.

^{3}H-oestradiol-17β (137 μCi mmol^{-1}) was diluted in ethanol and stored at -20°C. An aliquot volume of tritiated oestradiol-17 was evaporated to dryness under a stream of nitrogen and rediluted 1:20 in diluent B before use in the radioimmunoassay. The antibody and tritiated oestradiol-17-β were selected empirically to give 50-60% binding of labelled oestradiol-17β in the control assay tubes containing no unlabelled steroid.

Extraction Of Oestradiol-17β In Plasma Samples

Plasma samples for extraction (100 or 200 μl) were taken in duplicate. Oestradiol-17β was extracted once by adding 3 mls of freshly opened diethyl ether, stabilized with about 1 ppm of pyrogallol, and vortex mixing for 5 mins. The plasma was then frozen in a freezing mixture consisting of dry ice in acetone and the diethyl ether was decanted off. This freezing procedure of the mixture of plasma and diethyl ether was done only once and the extracted oestradiol-17β in the diethyl ether was taken to dryness in a stream of air at 50°C. Half a ml of diluent B was then added to each of the tubes.

Radioimmunoassay for Oestradiol-17β:

Radioimmunoassay of oestradiol-17β was technically the same procedure as for progesterone. After preparation of the extracted experimental samples, eleven triplicate "standards" in a volume of 0.5 mls diluent were prepared. Four tubes containing zero standards and four blanks were added to the assay. In all the experiments the non-specific binding as indicated by the "blanks" was less than 10%. Fifty µl of ^{3}H-oestradiol-17β and 50 µl of antibody were added to all the tubes except the counting standards. The constituents of the tubes were mixed by "whirlimix" and incubated at room temperture for 2 hrs. After incubation NRS (1:140 in diluent B) and DARS (1:10 in diluent B) were added. The solutions were mixed and incubated overnight at 4°C. After incubation 1 ml of diluent B was added to all tubes. The tubes were then centrifuged at 4°C and 1300 g for 30 mins (Fison's MSE centrifuge). The supernatant liquid was decanted into vials containing 10 mls of scintillant B and the vials were shaken for 2 mins before counting for 4 mins in a liquid scintillation counter. The oestradiol-17β content of each experimental sample was determined from the standard curve using a computer programmme as mentioned earlier. The data were expressed as pg oestradiol-17β/ml plasma.

Recovery Estimates

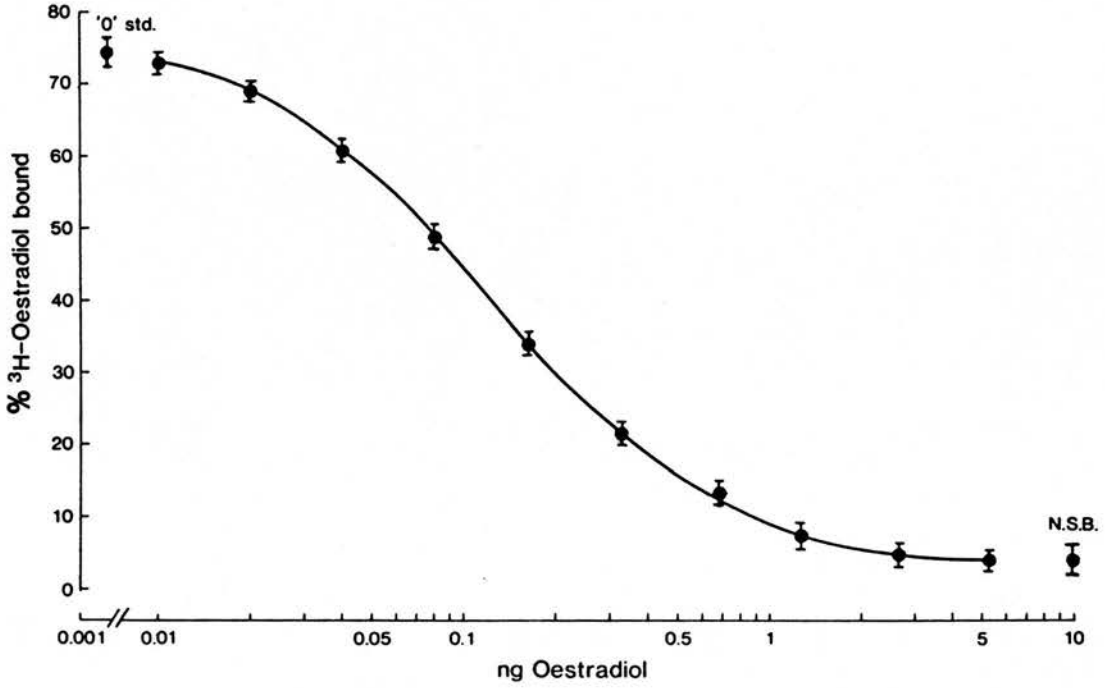
At the beginning of the assay several standard curves were made for accuracy. An example of a standard curve used in the oestradiol assay is shown in Text Fig. 9. When no added labelled oestradiol-17β was present the antibody bound 50-60% of the added radioactive oestradiol-17β.

The percentage recovery of oestradiol-17β was determined by extracting a known amount of radioacative oestradiol-17β added to castrated male sheep plasma. Thus 250 ul castrated male plasma was added to 20 extraction tubes containing 50 µl ^{3}H-oestradiol-17β. Fifty µl ^{3}H-oestradiol was also added to 4 tubes as counting standards. One ml diluent B was then added to all tubes and mixed. Fifty µl of each extracted sample was dispensed into vials containing 10 ml scintillant B (page59), shaken for 2 mins and counted for 4 mins in a liquid scintillation counter. The average percentage tritiated oestradiol recovered was calculated and found to be more than 90%. Accordingly no procedural losses were taken into account during the assay.

Accuracy and Precision

The accuracy and precision were determined by adding known amounts of oestradiol-17β in triplicate to castrated male sheep plasma followed by extraction and assaying. Since the average blank value for plasma was 17 pg, this value was substracted from each value and the results are shown on the table below:

Figure 9: A standard curve used in the oestradiol-17 assay with antiserum used at a concentration of 1:3200 and ^{3}H-oestradiol-17 . The standard curve was calculated using the computer programme and plotted as percentage binding of the ^{3}H-oestradiol-17 against amount of non-radioactive oestradiol-17 . The zero standard ("0" std.) and non-specific binding are also shown.


80
70
60
50
40
30
20
10
0
'0' std.
% 3H-Oestradiol bound
N.S.B.
0.001
0.01
0.05
0.1
0.5
1
5
10
ng Oestradiol

Amount Of Oestradiol-17β (pg/ml)

added	recovered (mean ± S.E.M.)	Number
5	7±3.8	N=3
10	14±1.4	N=3
25	29±4.0	N=3
50	65±5.0	N=3
100	122±8.9	N=3
400	410±3.4	N=3
800	823±22.7	N=3

The intra-assay coefficient of variation was 5.09%. (N=70 from 5 different assays).

Inter assay coefficient of variation was determined by assaying duplicates of standards (640 pg) incorporated into each assay and was 12.2%.

The sensitivity of the assay was 10 pg

(vii)Sampling Frequency During the Experiment

The four animals successfully prepared with a patent utero-mammary vein anastomoses and a uterine fistula were sampled at various frequencies as shown in Table 3 below.

Ewe	Cycle No.	Utero-Mammary Vein		Uterine Tissue	
		frequency	days*	frequency	days*
A76	1	daily	1-16	-	-
A76	2	3 hr	12-17(-1*)	-	-
A76	3	1/2 hr	12-13(-4)	4hr	12-13(-4)
A25	1	3 hr	13-18(1)	3hr	13-16(-2)
A25	2	1/2 hr	15-17(S)	8hr	15-17(S)
A10	1	3 hr	13-19(1)		13-16(-3)
A10	2	1/2 hr	16-18(-1)		16-18(-1)
A19	1	3 hr	12-14(S)		12-14(S)

* Figures in brackets indicate days prior to oestrus while "S" denotes that the oestrus was silent.

Ewe A76 cycle 1 was a preliminary exercise to perfect the technique and monitor the daily changes of $PGF_{2\alpha}$, progesterone and oestradiol-17β in plasma. The initial objective for each animal was to sample during two cycles. In one cycle utero-mammary vein samples would be taken at 3 hr intervals from day 12 or 13 onwards and uterine biopsies at the same frequency. In the other cycle uterine venous samples would be at 1/2 hourly intervals from day 12 or 13 onwards and samples of uterine tissue would be taken as frequently as it was considered feasible. It was found that this idea had to

be modified for a variety of reasons, namely:

1) Half hourly samples for more than 3 days was not feasible.

2) Uterine biopsies in the second oestrous cycle had to be less frequent otherwise all the accessible uterine tissue was used up. Plenty of normal uterine endometrium could still be present in the fistula but the design of the biopsy instrument prevented it being harvested.

3) Ewe A19 failed to return to oestrus at the end of the first cycle.

For the purposes of the results these sampling frequencies are considered as 3 separate studies.

Study 1: Ewe A76 cycle 1-daily samples to assess technique.

Study 2: 4 cycles in which utero-mammary vein blood and uterine tissue was sampled every 3 hrs from day 12 or 13 onwards.

Study 3: 3 cycles in which half hourly samples of utero-mammary vein blood were taken and uterine tissue was sampled at either 4 or 8 hour intervals.

Analysis of Data

Hormonal concentrations for each cycle were constructed into a histogram as shown in fig. 10, from which the modal range* of the hormone was derived. A peak of hormone was defined as one or more values above the modal range. Whereas the fall in progesterone level indicating luteolysis was defined as three consecutive values falling below the modal range. As the data is mainly intended to investigate the events associated with luteolysis, all the time intervals were converted to hours before or after the fall in progesterone concentration below the modal range for the luteal phase of the cycle.

Results

All the ewes in this experiment had a least 2 recorded oestrous cycles before the uterine vein was anastomosed to the mammary vein. Following anastomosis and fistulation also all the ewes had a normal oestrous cycle (16-19 days), except ewe, A19, which did not show behavioural oestrus. In view of this the result from this sheep is discussed separately. Figure 11 shows the plasma concentration for progesterone, oestradiol and $PGF_{2\alpha}$ from daily samples of uteromammary vein blood in ewe A76. These results agree with the now well documented pattern.

The concentration of progesterone, $PGF_{2\alpha}$ and oestradiol in uterine venous plasma, and the endometrial

[*The mode is taken as the most frequently occurring unit value.

The modal range is the three most frequently occurring consecutive unit values that include the mode.]

Figure 10: Hormonal concentrations of ewe A25 constructed into histograms from which the modal range of the hormone was determined.

The upper diagram shows the modal range for progesterone. The fall in progesterone levels was taken as three consecutive values falling below the modal range.

The lower diagram shows the modal range for $PGF_{2\alpha}$. A peak of $PGF_{2\alpha}$ was taken as one or more values above the modal range.

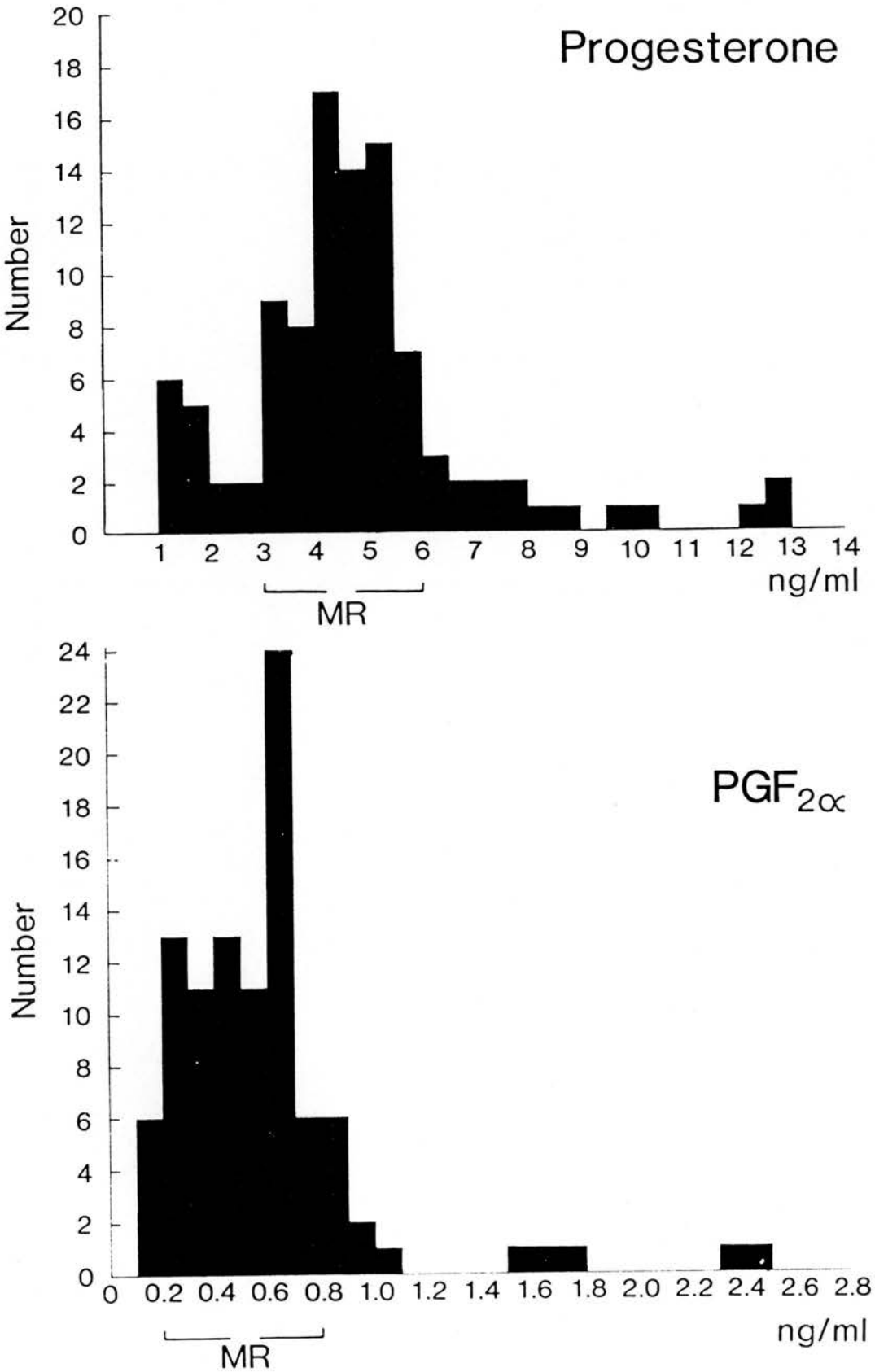
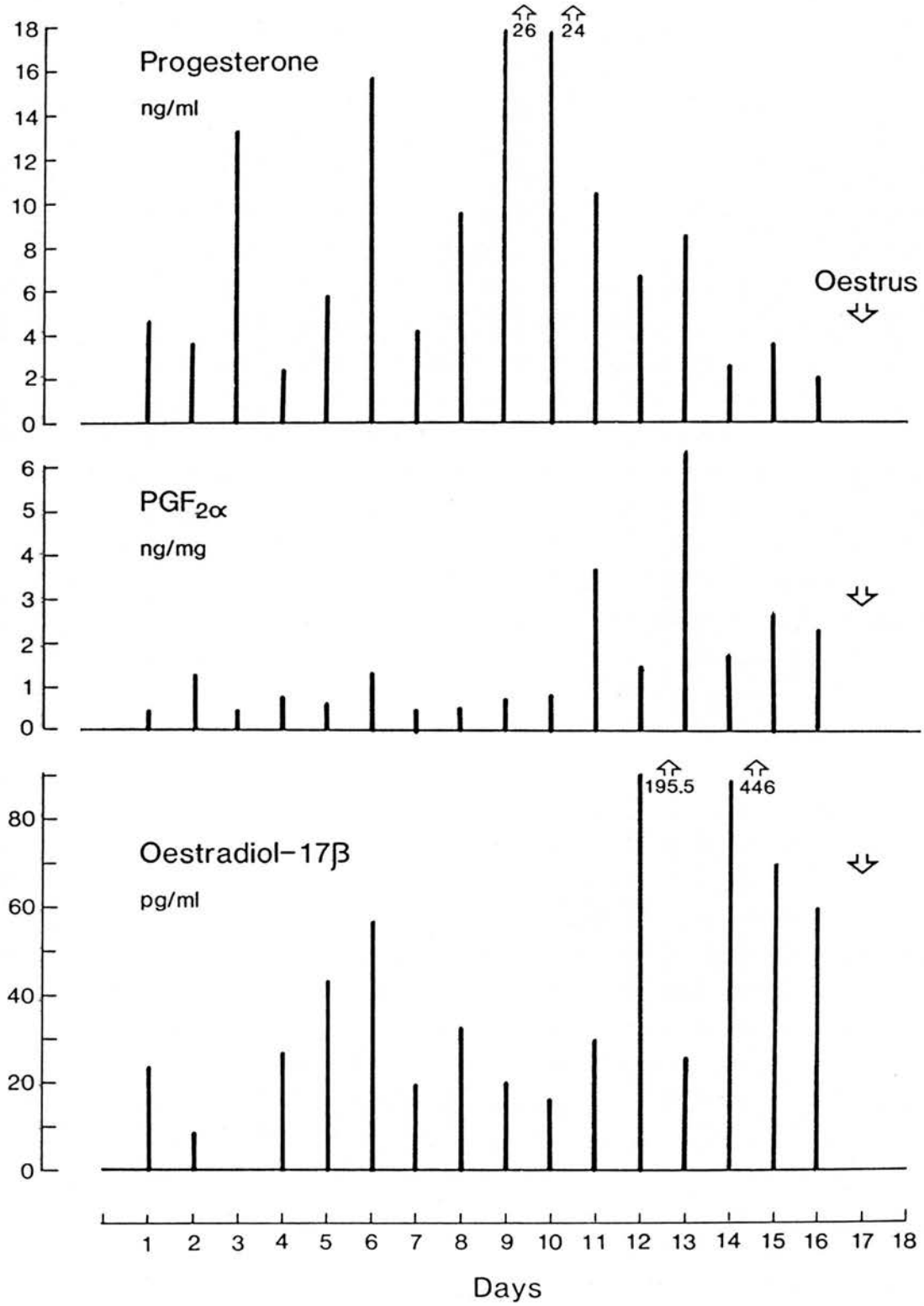

Progesterone
Number
20
18
16
14
12
10
8
6
4
2
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14
ng/ml
MR
PGF2α
Number
24
22
20
18
16
14
12
10
8
6
4
2
0
0 0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8
ng/ml
MR

Figure 11: The plasma concentration of progesterone (ng/ml), $PGF_{2\alpha}$ (ng/ml) and oestradiol-17β (pg/ml) from daily samples of utero-mammary vein blood in ewe A76. Samples were collected once per day during the cycle and the ewe had a normal oestrous cycle of 16 days.


Progesterone
ng/ml
26
24
Oestrus
$PGF_{2\alpha}$
ng/mg
Oestradiol-17β
pg/ml
195.5
446
Days

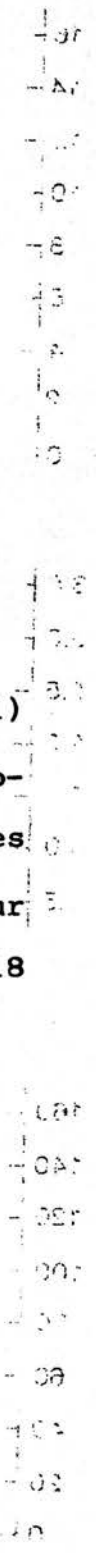


Figure 12:A The concentration of progesterone (ng/ml) $PGF_{2\alpha}$(ng/ml) and oestradiol-17β (pg/ml) in the anastomosed utero-mammary vein, from the second cycle in ewe A76. Samples of utero mammary venous blood were taken every half hour throughout days 12 and 13. The animal had a normal 18 day cycle.

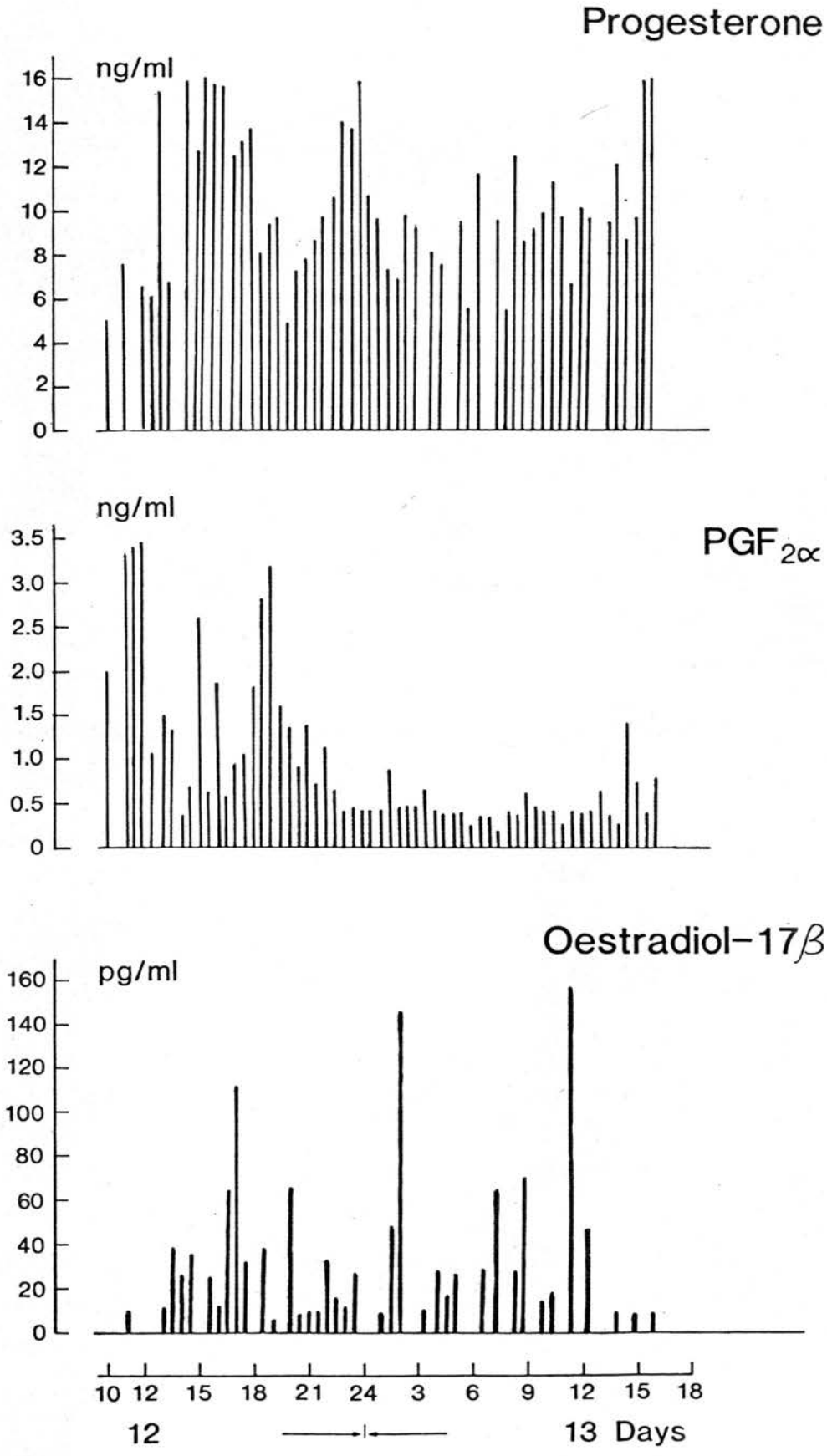

Progesterone
ng/ml
16
14
12
10
8
6
4
2
0
ng/ml
PGF2α
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Oestradiol-17β
pg/ml
160
140
120
100
80
60
40
20
0
10 12 15 18 21 24 3 6 9 12 15 18
12
13 Days

Figure 12:B The concentration of progesterone (ng/ml), prostaglandin $F_{2\alpha}$ (ng/ml) and oestradiol-17β (pg/ml) in the anastomosed utero-mammary vein and the endometrial synthesizing ability (ng/mg/90min) from the second cycle of ewe A25. Throughout day 15,16 and 17 samples of utero-mammary venous blood were taken every half hour and uterine endometrial biopsies were taken every 8 hours.

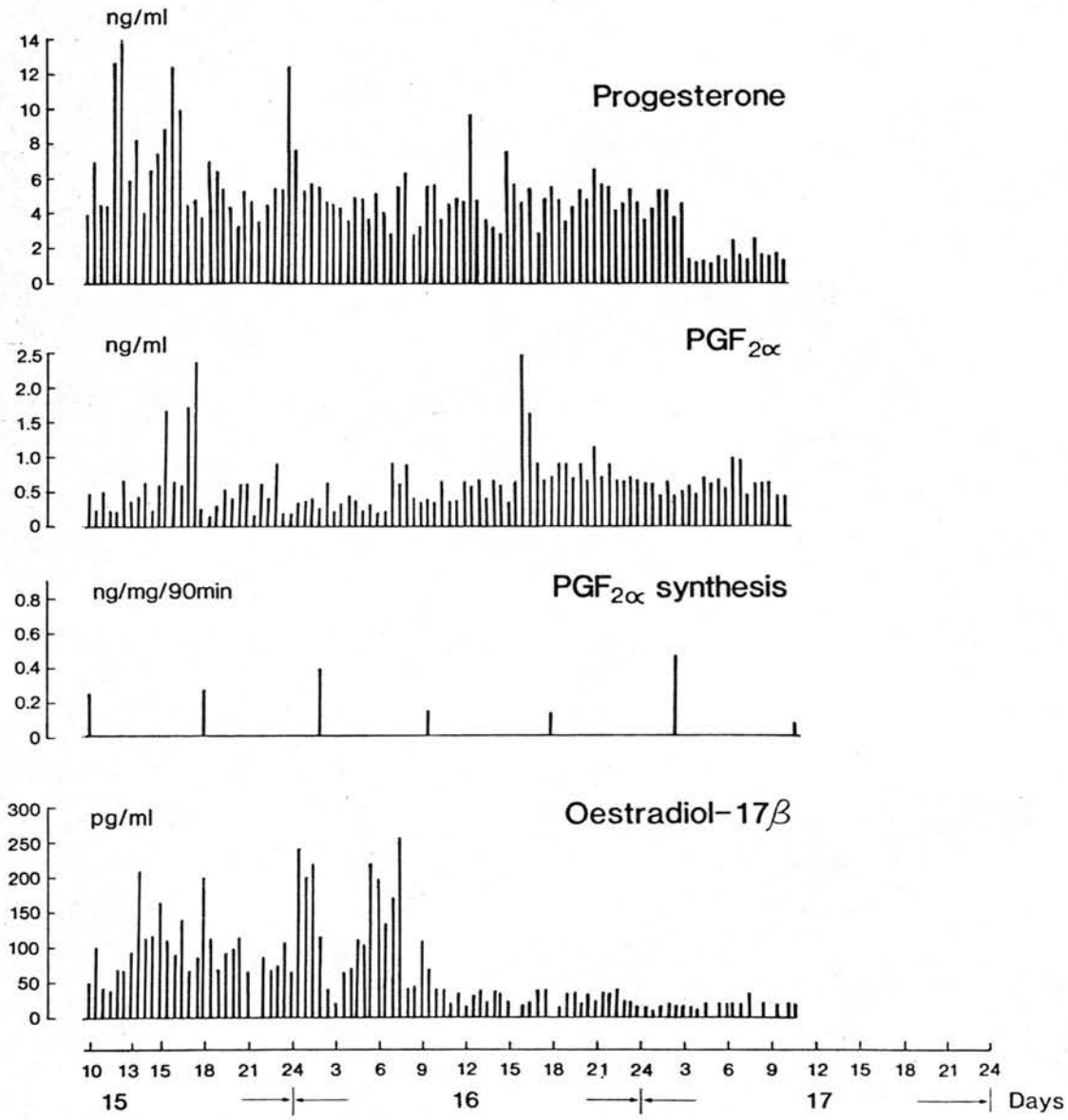
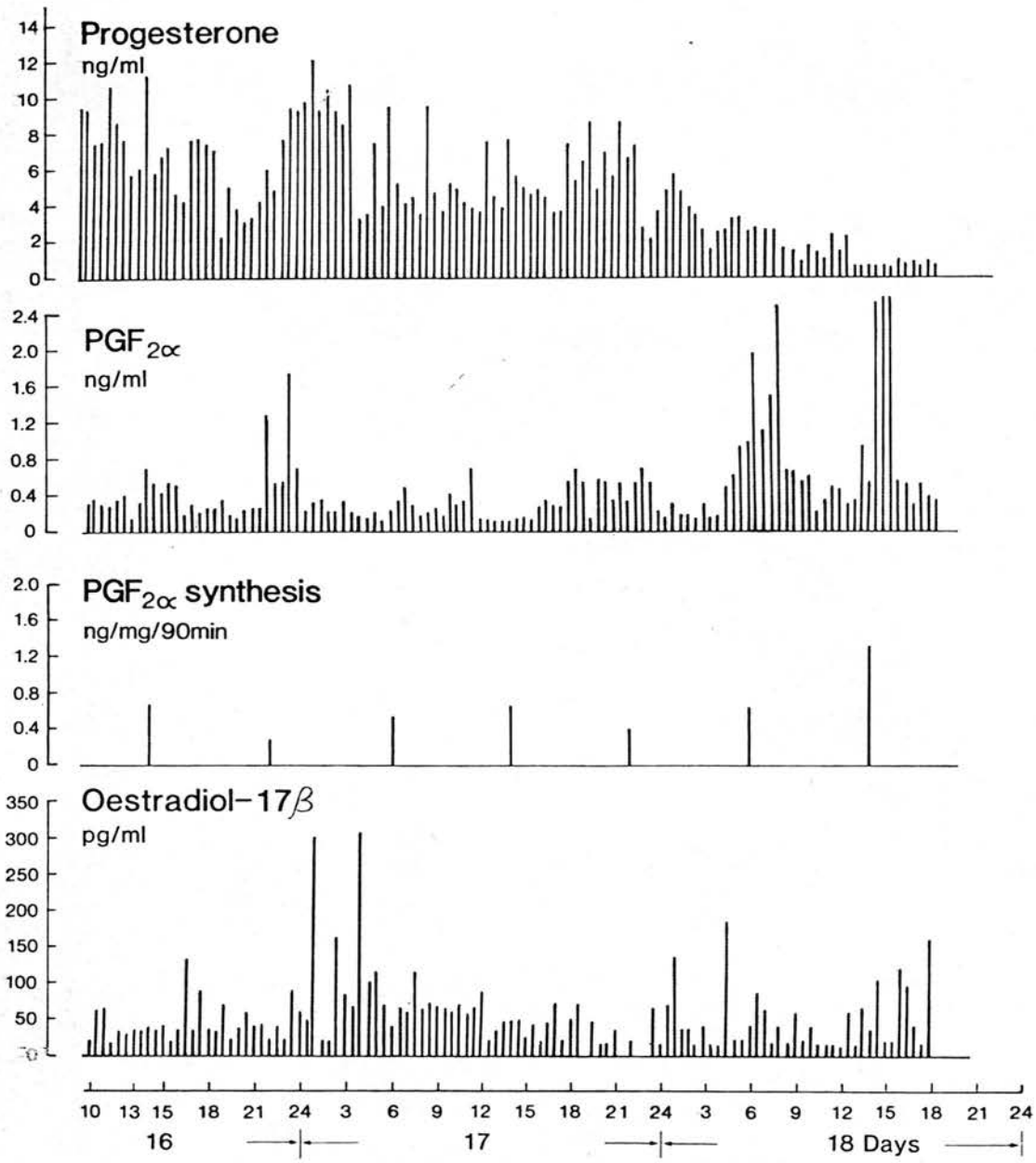

ng/ml
14
12
10
8
6
4
2
0
Progesterone
ng/ml
2.5
2.0
1.5
1.0
0.5
0
PGF2α
ng/mg/90min
0.8
0.6
0.4
0.2
0
PGF2α synthesis
pg/ml
300
250
200
150
100
50
0
Oestradiol-17β
10 13 15 18 21 24 3 6 9 12 15 18 21 24 3 6 9 12 15 18 21 24
15
16
17
Days

Figure 12:C The concentration of progesterone (ng/ml), prostaglandin $F_{2\alpha}$ (ng/ml) and oestradiol-17β (pg/ml) in the anastomosed utero-mammary vein and the endometrial sunthesizing ability (ng/mg/90min) of the fistulated uterine horn from the second cycle of ewe A10. throughout day 16,17 and 18. Samples of utero-mammary venous blood were taken every 1/2 hour and uterine endomtrial biopsies taken every 8 hours. The animal had a normal 18-day cycle.


Progesterone
ng/ml
14
12
10
8
6
4
2
0
PGF2α
ng/ml
2.4
2.0
1.6
1.2
0.8
0.4
0
PGF2α synthesis
ng/mg/90min
2.0
1.6
1.2
0.8
0.4
0
Oestradiol-17β
pg/ml
350
300
250
200
150
100
50
0
10 13 15 18 21 24 3 6 9 12 15 18 21 24 3 6 9 12 15 18 21 24
16
17
18 Days

tissue content and synthesizing ability of $PGF_{2\alpha}$ for 3 cycles that were sampled more frequently are illustrated in figs 12. Progesterone concentration fluctuated episodically before declining at the end of 6 cycles indicating luteal regression occurred in all cases. However, there was variation between the animals in the rate of progesterone decline, and in one instance (ewe A25) decline was very rapid and oestrus failed to reoccur. $PGF_{2\alpha}$ and oestradiol-17β also showed an episodic pattern of release. Table 4 shows the modal values and the modal ranges of the plasma concentrations of progesterone, $PGF_{2\alpha}$ and oestradiol-17β during the 6 oestrous cycles. In order to improve the validity of any conclusions the appropriate timed samples in the 3 cycles which were sampled half-hourly, were selected to standardize all the cycles of apparent 3-hour sampling. The extent of the elevations above the modal range in five cycles are illustrated in Fig. 13 and summarized in fig. 14.

It will be noticed that there were peaks in the release of $PGF_{2\alpha}$ during the period 30-45 hours prior to progesterone decline. There was also one or more peaks during the period 3-9 hours before luteal regression in 4 of the 5 cycles. Further peaks indicating $PGF_{2\alpha}$ release occurred after the initiation of progesterone decline in 4 of the 5 cycles.

Oestradiol-17β showed an increase in level during much of the period 18-42 hours before progesterone

TABLE 4

The Modal values and the Modal Ranges for progesterone, prostaglandin and oestradiol-17β .

EWE	Frequency of sampling	Progesterone (Luteal)(ng/ml)	Prostaglandin $F_{2\alpha}$ (ng/ml)		Oestradiol(pg/ml)	
			Modal Value	Modal Range	Modal Value	Modal Range
A76	half hour	9.0-9.4	0.30-0.39	0.2 -0.69	5- 9	0-24
A76	3 hours	9.5-9.9	0.30-0.39	0.1 -0.69	10-14	0-34
A10	half hour	3.5-3.9	0.2 -0.29	0.0 -0.59	20-24	5-34
A10	3 hours	5.5-5.9	0.40-0.49	0.1 -0.69	40-44	30-50
A25	half hour	4.0-4.4	0.60-0.69	0.3 -0.89	15-19	5-39
A25	3 hours	3.0-3.4	0.50-0.59	0.34-0.59	20-24	10-39
Mean Mode		5.75-6.15	0.38-0.47		13.3-17.3	

Figure 13: (General)

All the next 5 diagrams show the concentration of progesterone, oestradiol-17β and $PGF_{2\alpha}$ in utero-mammary vein blood and $PGF_{2\alpha}$ content and synthesizing ability of uterine biopsies during the latter part of the oestrous cycle. Samples are all at 3 hr intervals and depicted in terms of hours before or after the decline in progesterone (indicated by arrows). Peaks above the white bar indicate levels above the modal range while stippled columns indicate that no sample was available.

Figure 13:A Ewe A10 (cycle 1). Progesterone started to decline at 10.00 hr on day 17. Throughout days 13 to 18 and the day of oestrus, samples of utero-mammary venous blood were taken every three hours; uterine endometrial biopsies were taken every 3 hours up to day 16. The ewe had a normal 18 day cycle.

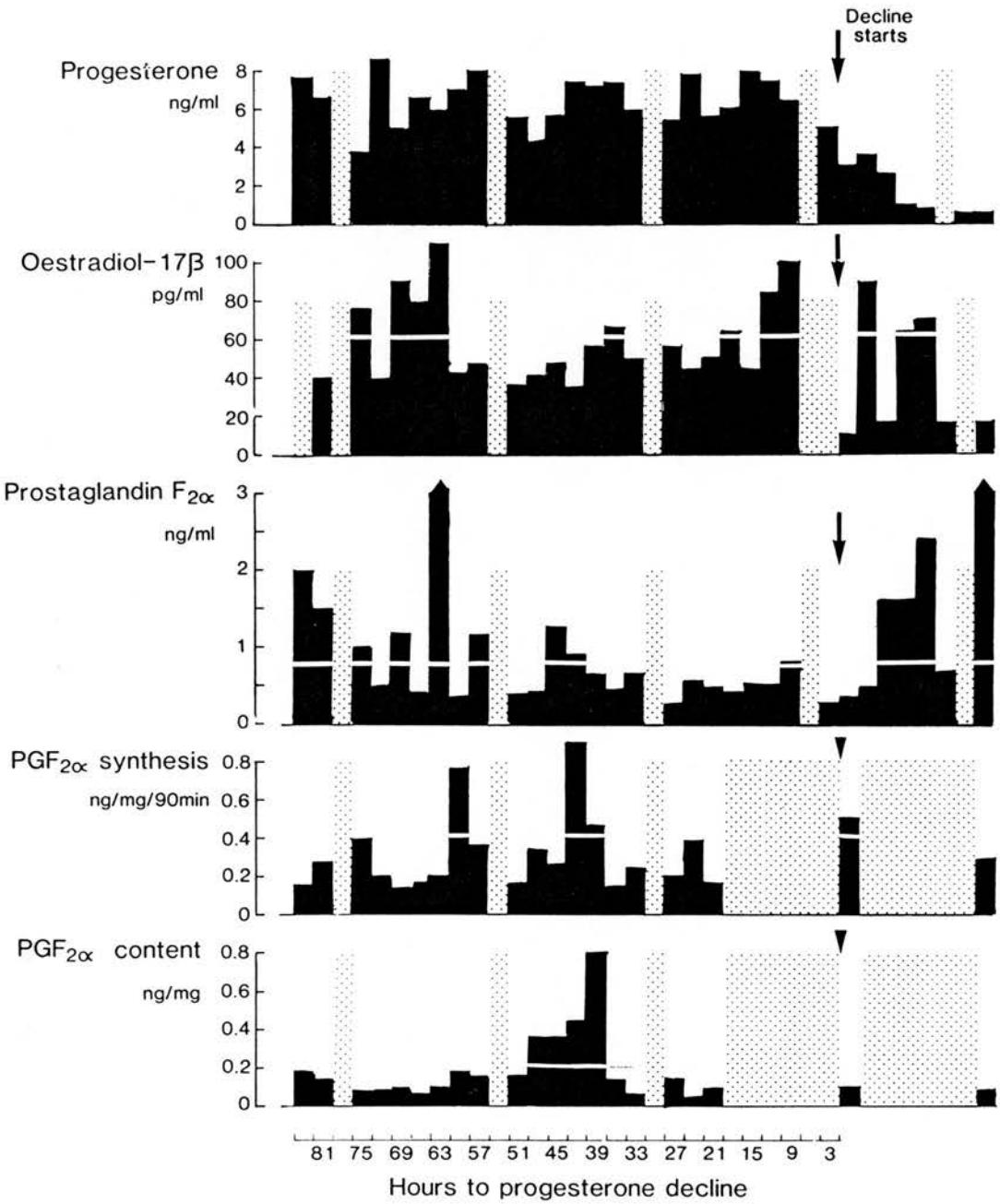

Decline starts
Progesterone
ng/ml
8
6
4
2
0
Oestradiol-17β
pg/ml
100
80
60
40
20
0
Prostaglandin F2α
ng/ml
3
2
1
0
PGF2α synthesis
ng/mg/90min
0.8
0.6
0.4
0.2
0
PGF2α content
ng/mg
0.8
0.6
0.4
0.2
0
81 75 69 63 57 51 45 39 33 27 21 15 9 3
Hours to progesterone decline

Figure 13:B Ewe A10 (cycle 2). Progesterone started to decline at 02.30 hr on day 18. Plasma values are shown at 3 hr intervals from day 16-18. Uterine biopsies were taken at 3 hr intervals throughout the same period. Oestrus occurred on day 19.

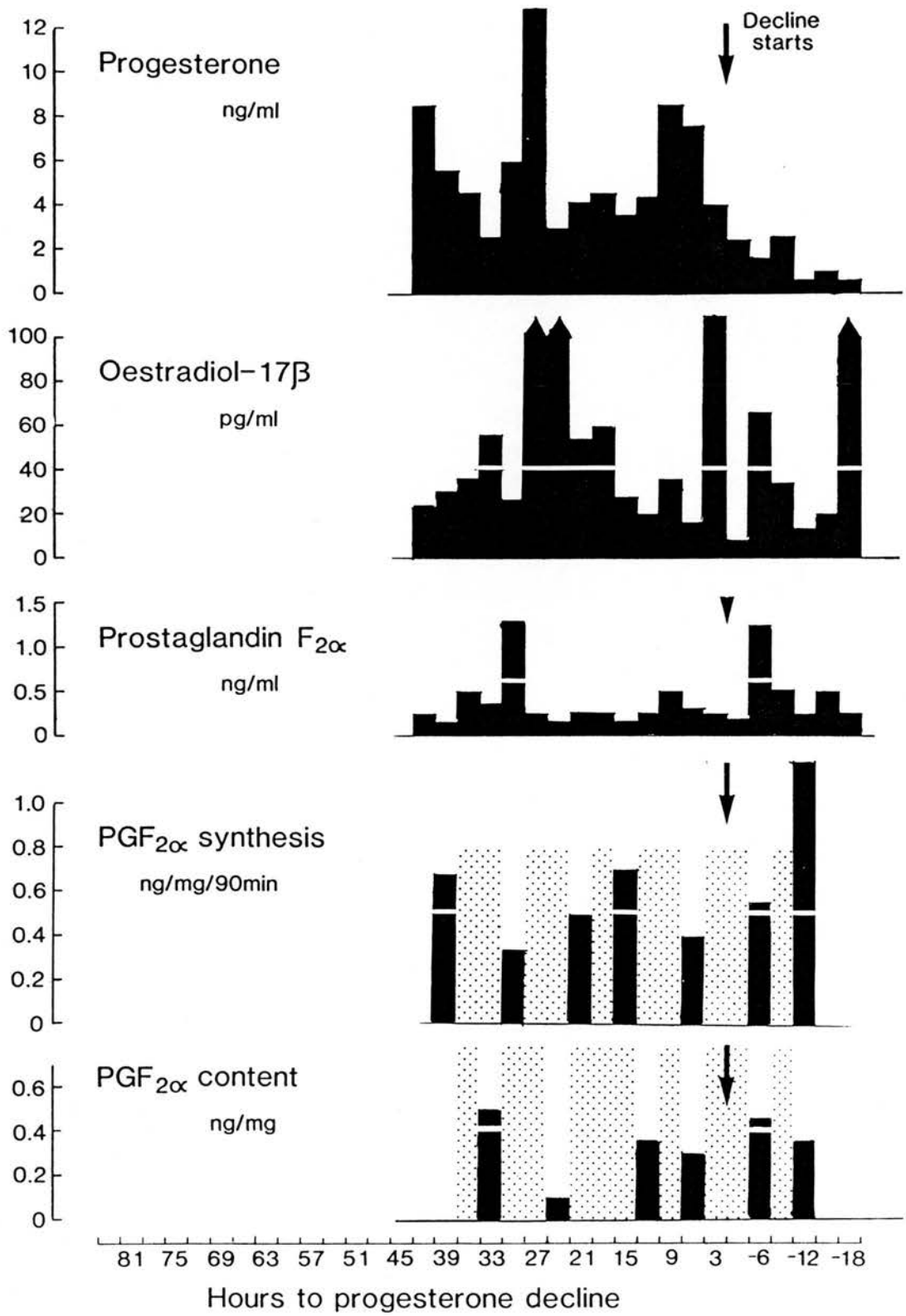

Decline starts
Progesterone
ng/ml
12
10
8
6
4
2
0
Oestradiol-17β
pg/ml
100
80
60
40
20
0
Prostaglandin F2α
ng/ml
1.5
1.0
0.5
0
PGF2α synthesis
ng/mg/90min
1.0
0.8
0.6
0.4
0.2
0
PGF2α content
ng/mg
0.6
0.4
0.2
0
81 75 69 63 57 51 45 39 33 27 21 15 9 3 -6 -12 -18
Hours to progesterone decline

Figure 13:C Ewe A25 (cycle 1). Progesterone started to decline at 13.00 hr on day 15. Throughout days 13 to 17 and the day of oestrus, samples of utero-mammary venous blood and uterine biopsies were taken every 3 hours. The ewe had a normal 17-day cycle.

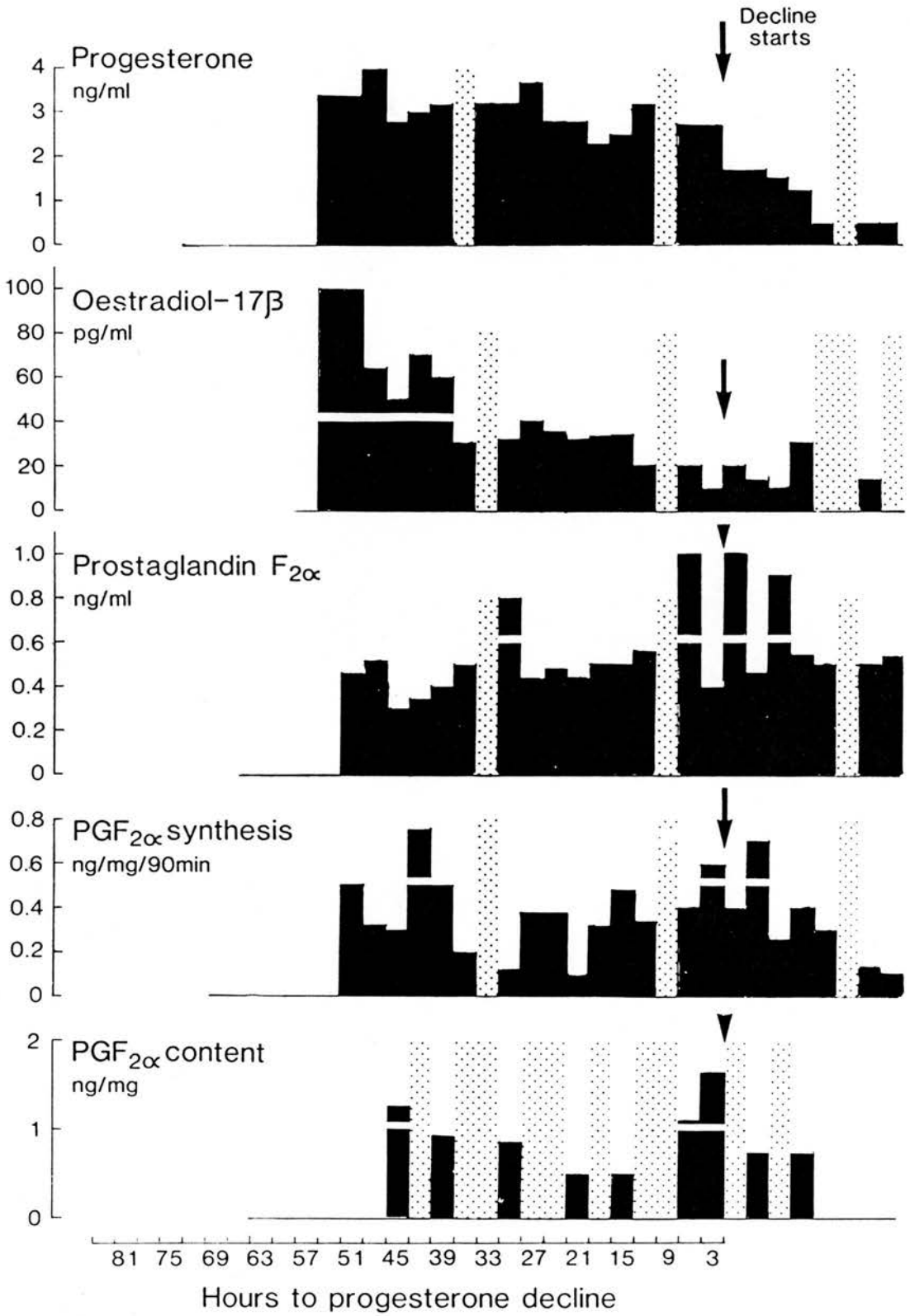

Decline starts
Progesterone
ng/ml
4
3
2
1
0
Oestradiol-17β
pg/ml
100
80
60
40
20
0
Prostaglandin F2α
ng/ml
1.0
0.8
0.6
0.4
0.2
0
PGF2α synthesis
ng/mg/90min
0.8
0.6
0.4
0.2
0
PGF2α content
ng/mg
2
1
0
81 75 69 63 57 51 45 39 33 27 21 15 9 3
Hours to progesterone decline

Figure 13:D Ewe A25 (cyle 2). Progesterone started to decline at 04.00 hr on day 17. Plasma values are shown at 3 hr intervals from day 15-17 while uterine biopsies were taken at 8 hour intervals during the same period. At autopsy on day 21 it was found that this ewe had had a silent heat.

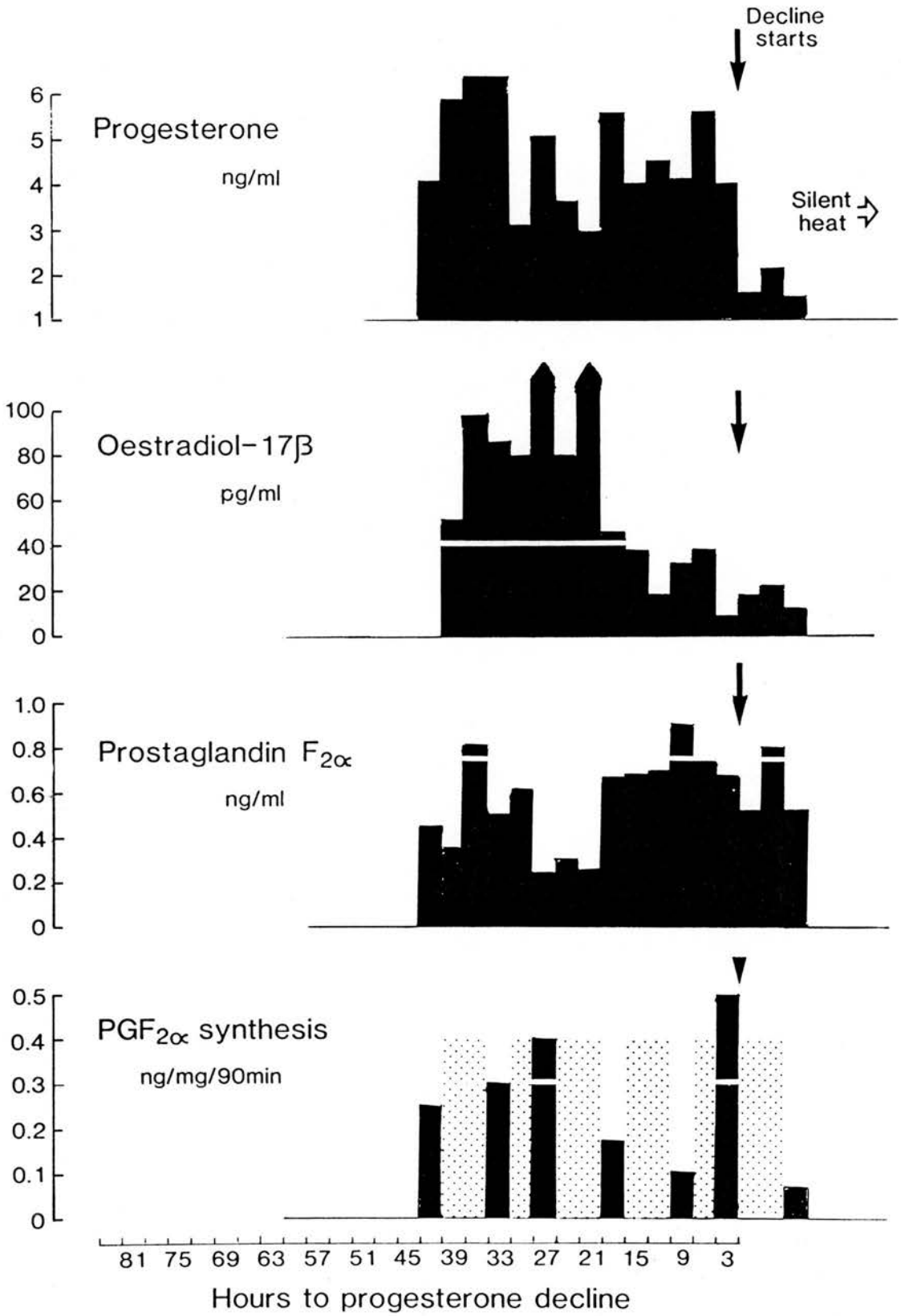

Decline starts
Progesterone
ng/ml
Silent heat
Oestradiol-17β
pg/ml
Prostaglandin F2α
ng/ml
PGF2α synthesis
ng/mg/90min
81 75 69 63 57 51 45 39 33 27 21 15 9 3
Hours to progesterone decline

Figure 13:E Ewe A76 (cyle 1). Progesterone started to decline at 16.00 hr on day 16. Throughout days 12 to 18 and the day of oestrus samples of utero-mammary venous blood were taken every 3 hours. The ewe had a normal 18 day cycle.

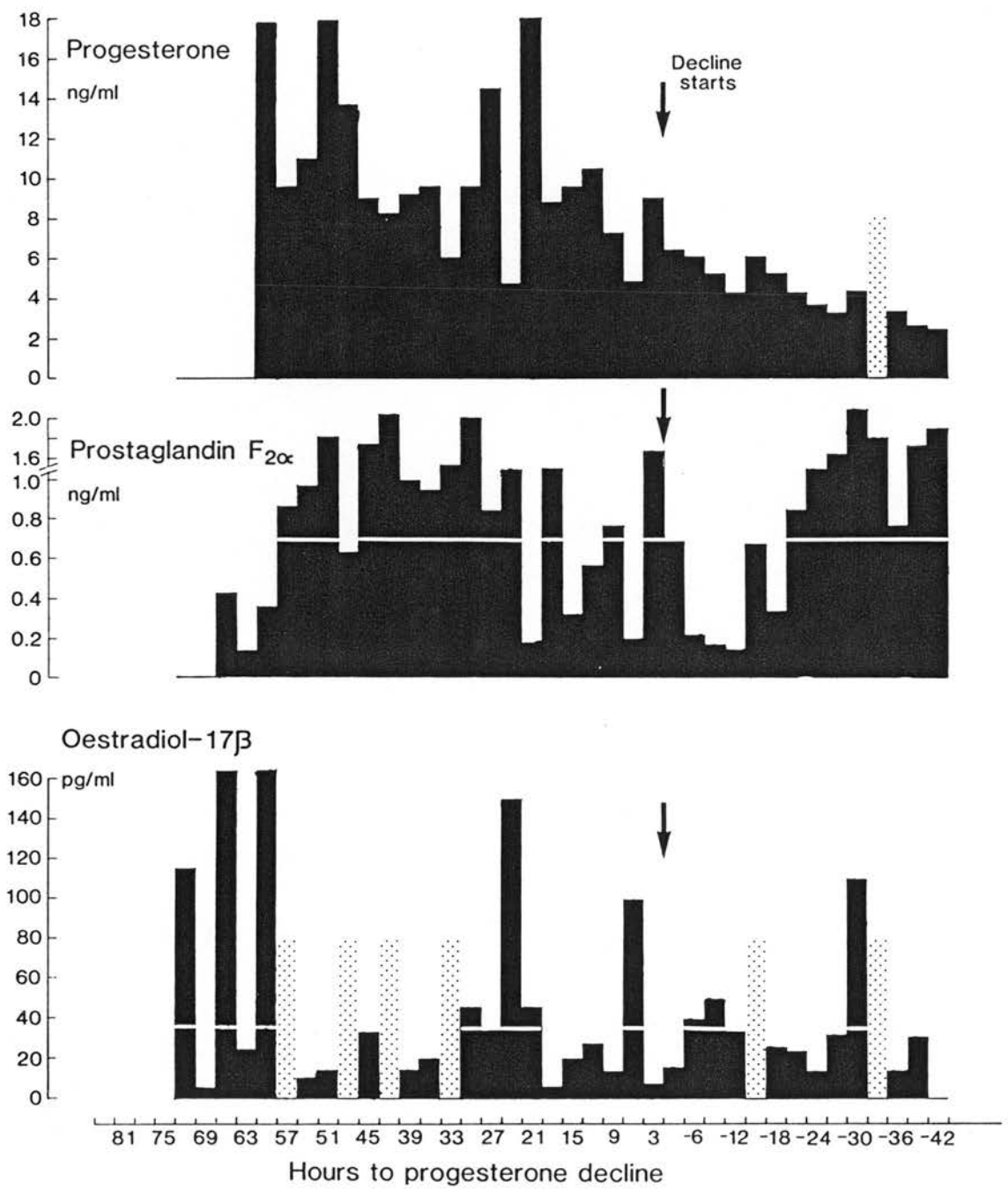
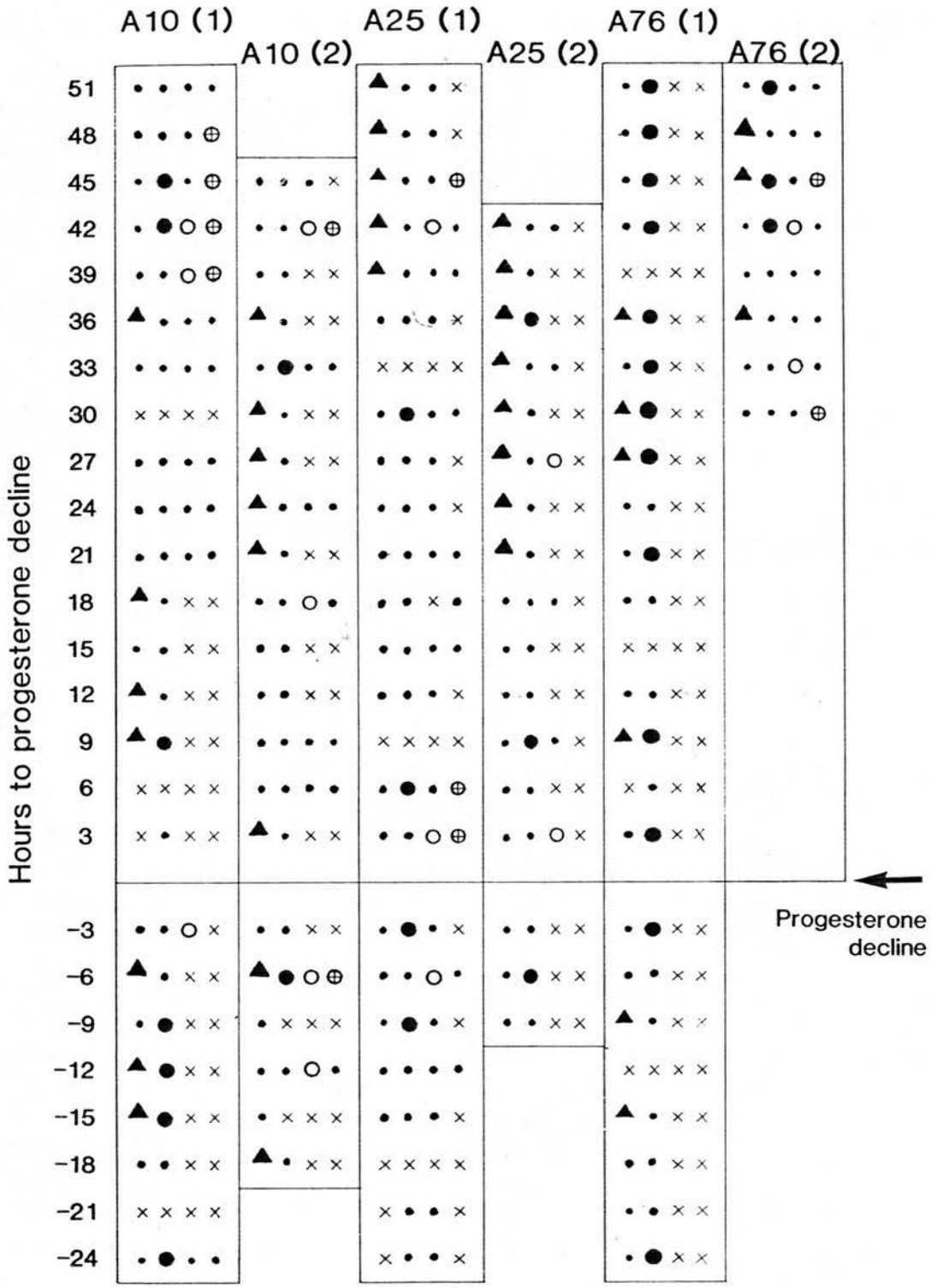

Progesterone
ng/ml
Decline starts
Prostaglandin $F_{2\alpha}$
ng/ml
Oestradiol-17β
pg/ml
Hours to progesterone decline

Figure 14: Summary of the occurrence of peaks (ie above modal range) in $PGF_{2\alpha}$ secretion(●)(ng/ml), $PGF_{2\alpha}$ synthesis(○)(ng/mg/90min), $PGF_{2\alpha}$ content(⊕)(ng/mg) of uterine tissue and the oestradiol-17β (▲) (pg/ml) concentration in the utero-mammary venous blood of all the 6 cycles studied. All values are represented as hrs before or after progesterone decline. Following progesterone decline all the ewes came into heat except ewe A25 (in cycle 2). In the latter sheep there was no increase in oestradiol and $PGF_{2\alpha}$ following luteolysis. The small spots indicate basal levels, while the crosses indicate no samples were availble.


A10 (1)
A10 (2)
A25 (1)
A25 (2)
A76 (1)
A76 (2)
Hours to progesterone decline
51
48
45
42
39
36
33
30
27
24
21
18
15
12
9
6
3
−3
−6
−9
−12
−15
−18
−21
−24
Progesterone decline

decline in all of the 5 cycles. These elevated levels bore a variable relationship to the contemporary $PGF_{2\alpha}$ peaks. A further increase occurred in oestradiol-17β release in the 12 hours prior to progesterone decline in 3 of the 5 cycles. In two of these cycles the oestradiol-17β peaks immediately preceeded a peak of uterine $PGF_{2\alpha}$ release. There were also elevated levels of oestradiol-17β in the uterine venous blood between 6 and 15 hours <u>after</u> the start of the progesterone decline in 3 of the 5 cycles. These coincided with peaks of $PGF_{2\alpha}$ release in all 3 cases.

Elevations of endometrial $PGF_{2\alpha}$ content and synthesizing ability occurred at similar times to $PGF_{2\alpha}$ release, namely 27-48 hours and 3-6 hours before and 3-12 hours after commencement of progesterone decline. However, there was no consistent relationship between either uterine $PGF_{2\alpha}$ content and synthesizing ability or between either of these and $PGF_{2\alpha}$ release. The PGE_2 content and synthesizing ability of uterine tissue (see fig.15A&B) most frequently followed but were invariably lower.

Half hourly samples of utero-mammary vein plasma were also taken in 3 of the above cycles. However, sampling in only 2 of these cycles impinged on the immediate period of luteolysis. These more frequent samples revealed more peaks of $PGF_{2\alpha}$ and oestradiol-17β than is apparent from considering only 3-hourly samples. Thus in cycle A25 (2) frequent peaks of $PGF_{2\alpha}$ occurred

Figure 15:A

Endometrial tissue content (ng/mg) and synthesizing ability (ng/mg/90min) of $PGF_{2\alpha}$ and PGE_2, in ewe A10 (cycle 1). Tissue samples were collected every 3 hours.

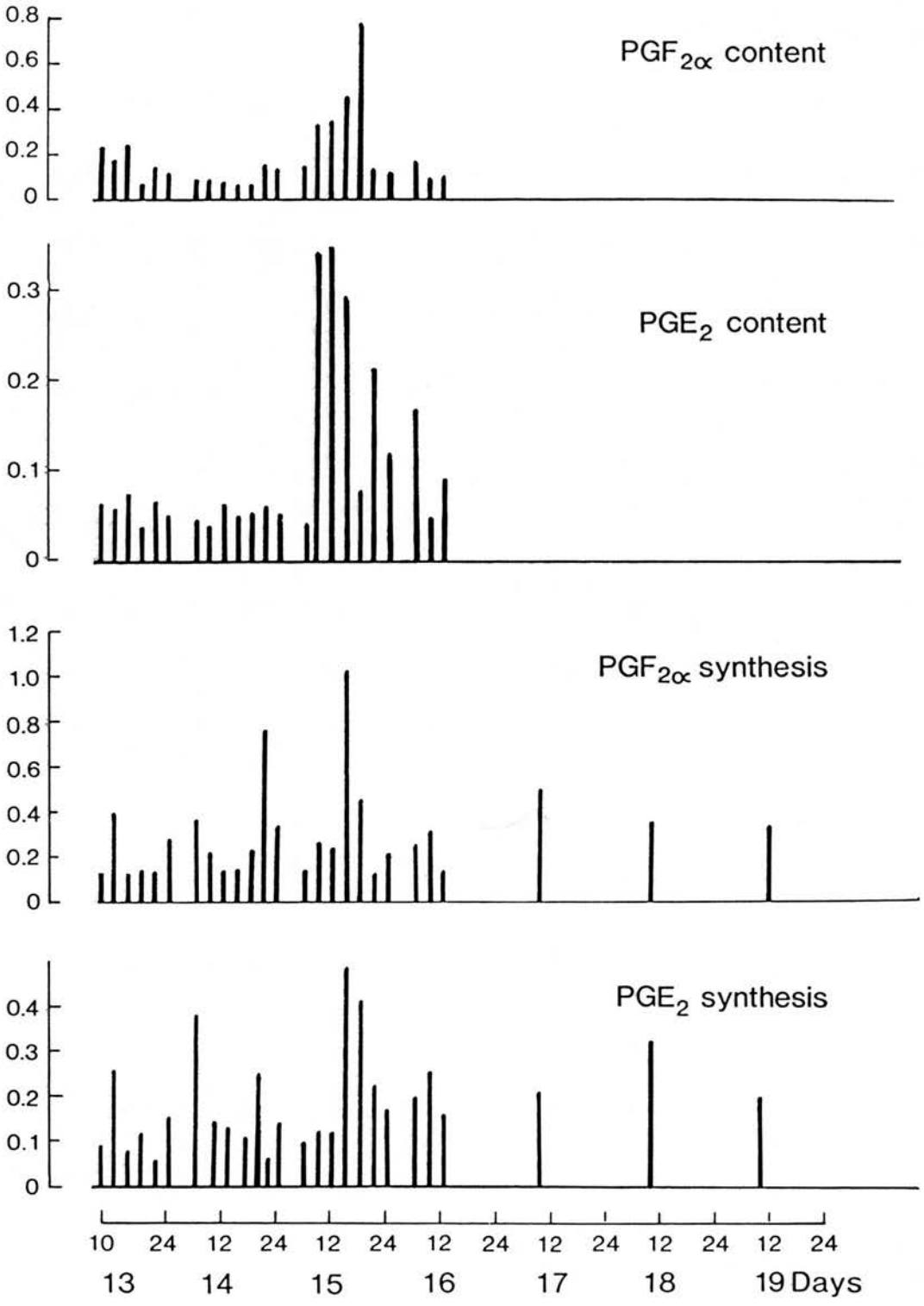
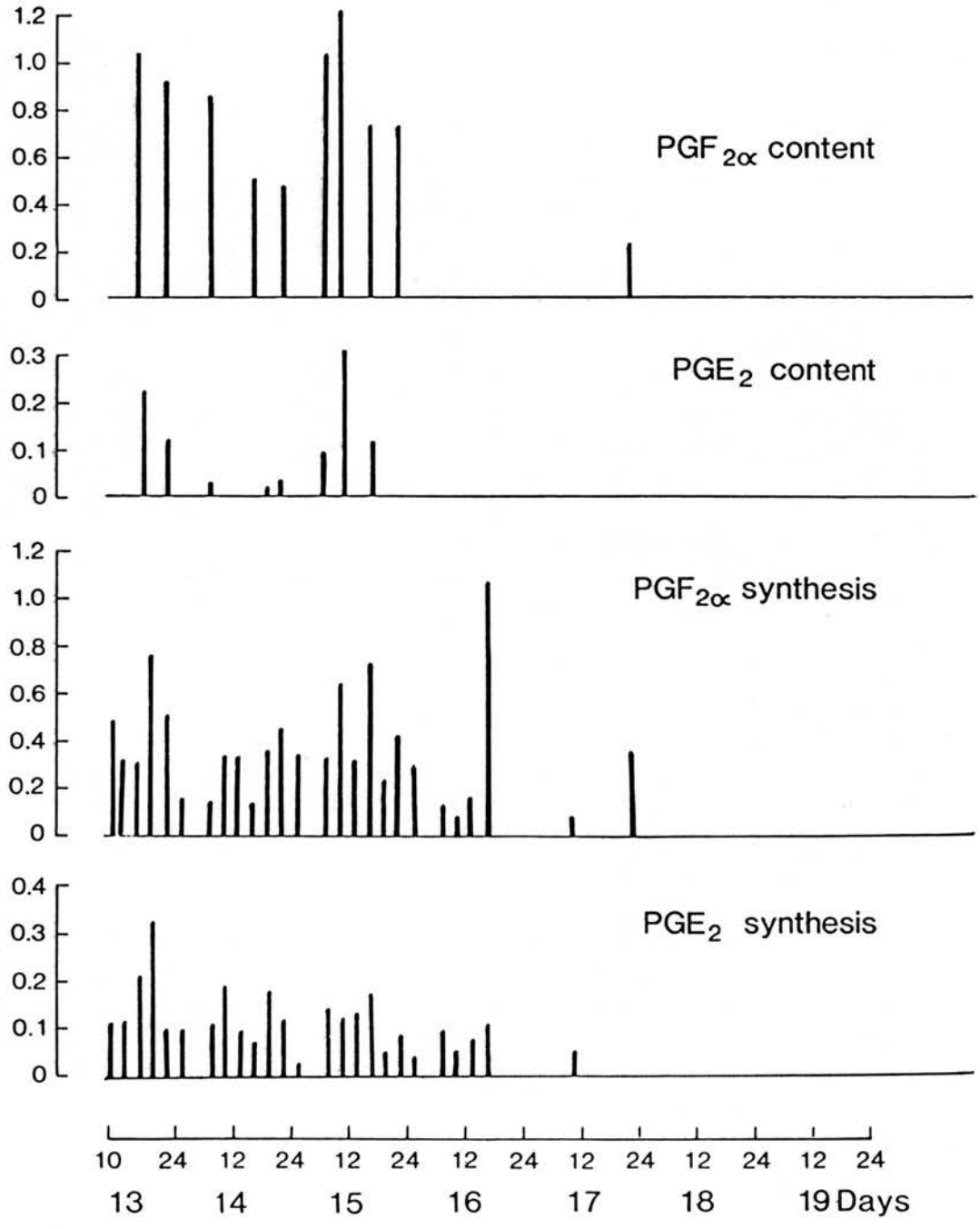

0.8
0.6
0.4
0.2
0
PGF2α content
0.3
0.2
0.1
0
PGE2 content
1.2
1.0
0.8
0.6
0.4
0.2
0
PGF2α synthesis
0.4
0.3
0.2
0.1
0
PGE2 synthesis
10 24 12 24 12 24 12 24 12 24 12 24 12 24
13 14 15 16 17 18 19 Days

Figure 15:B

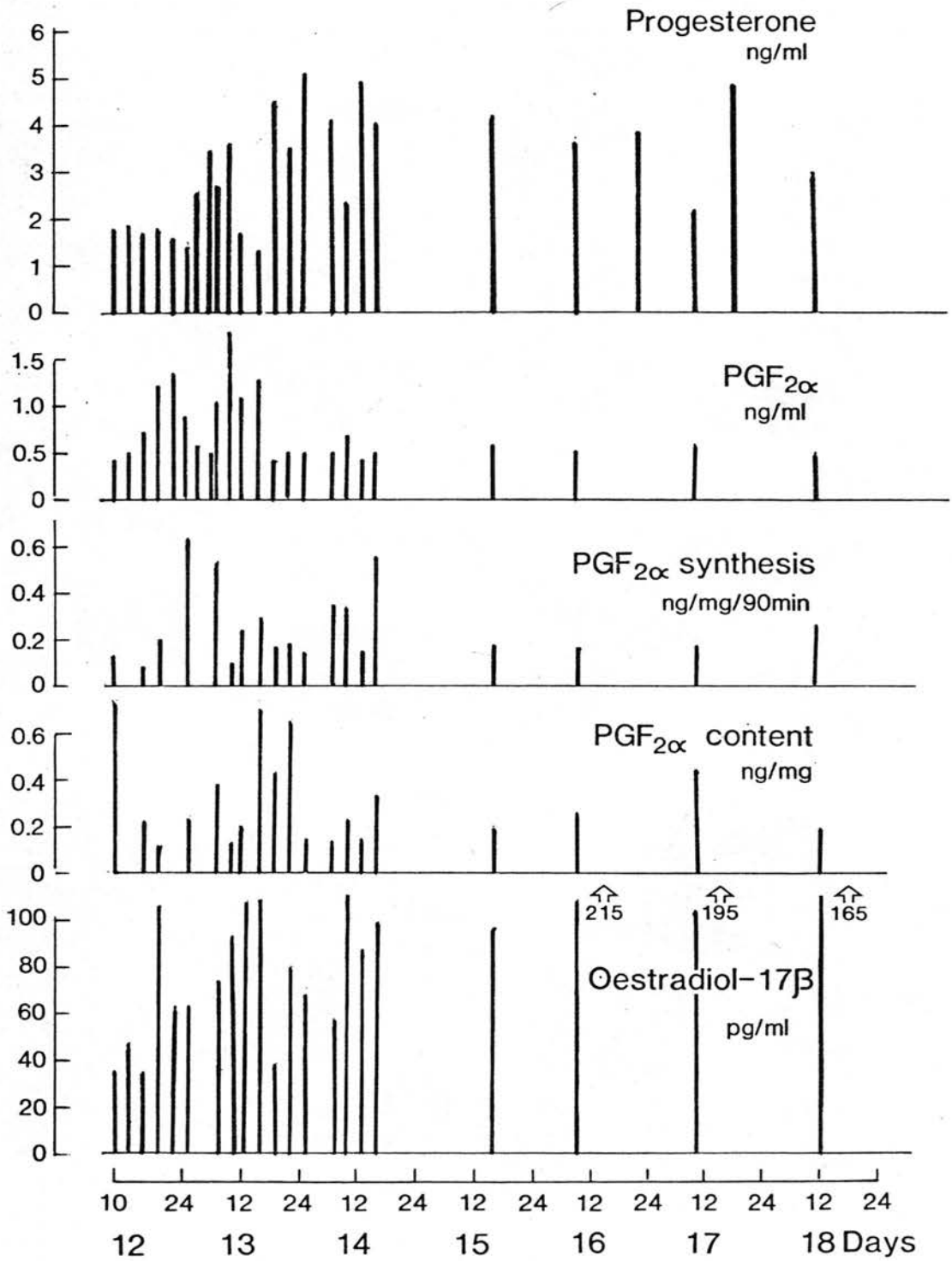
Endometrial tissue content (ng/mg) and synthesizing ability (ng/mg/90min) of $PGF_{2\alpha}$ and PGE_2 in ewe A25 (cycle 1). Tissue samples were collected every 3 hours.


1.2
1.0
0.8
0.6
0.4
0.2
0
PGF 2α content
0.3
0.2
0.1
0
PGE 2 content
1.2
1.0
0.8
0.6
0.4
0.2
0
PGF2α synthesis
0.4
0.3
0.2
0.1
0
PGE2 synthesis
10 24 12 24 12 24 12 24 12 24 12 24 12 24
13 14 15 16 17 18 19 Days

between 5.5 and 14.5 hours before progesterone decline and in cycle A10(2) peaks of $PGF_{2\alpha}$ release occurred at 3 and 7.5 hours before. This means that all 5 cycles showed peaks of $PGF_{2\alpha}$ release between 3-9 hours before progesterone fall. Peaks of oestradiol-17β were associated with these $PGF_{2\alpha}$ peaks, although again the association was not consistent. The post-luteolysis peaks of $PGF_{2\alpha}$ were most apparent in cycle A10(2) where they were accompanied by elevated oestradiol-17β levels. The $PGF_{2\alpha}$ elevations following luteolysis in cycle A25(2) were very much less marked and in this instance were <u>not</u> accompanied by oestradiol-17β peaks. This ewe experienced a silent heat at the end of this cycle.

The hormonal changes of ewe A19 are shown in Fig. 16. It is noticed that progesterone levels at the time of the expected oestrus did not fall to minimum levels. In fact this sheep had not shown oestrous behaviourwhen she was killed. Examination of the ovaries at postmortem revealed that there was no corpus luteum on the ovary opposite to the fistulated side. However, there were two small corpora lutea on the surface of the ovary adjacent to the fistula. The ovaries of this animal were removed and examined histologicaly by staining with haemotoxylin-eosin. The sections revealed 2 small regressing corpora lutea, mainly outside the ovarian fascia but with some luteal tissue within, and 2 large totally regressed corpora lutea. There were also several non-atretic follicles. Examination of the history of

Figure 16: Concentration of progesterone (ng/ml), prostaglandin $F_{2\alpha}$ (ng/ml) and oestradiol-17β in the anastomosed utero-mammary vein, the endometrial tissue content (ng/mg) of and the synthesizing ability (ng/mg/90min) for $PGF_{2\alpha}$ in ewe A19. Throughout days 12 to 14 samples were collected every 3 hours while on days 5 to 25 utero-mammary samples and uterine biopsies were collected daily. In this sheep the fistulated uterine horn has a patent utero-mammary vein anastomosis, but failed to come into oestrus. Progesterone levels did not decline and the sheep had not shown oestrus when she was killed.


Progesterone
ng/ml
6
5
4
3
2
1
0
PGF2α
ng/ml
1.5
1.0
0.5
0
PGF2α synthesis
ng/mg/90min
0.6
0.4
0.2
0
PGF2α content
ng/mg
0.6
0.4
0.2
0
215
195
165
Oestradiol-17β
pg/ml
100
80
60
40
20
0
10 24 12 24 12 24 12 24 12 24 12 24 12 24
12 13 14 15 16 17 18 Days

this animal from the records indicate irregular cycles during the period following anastomosis. Following the anastomosis this animal failed to return to oestrus and it was assumed that she had a persistent corpus luteum. However, it was decided to get the animal cycling again because the season was coming to a close and the animal would soon pass into seasonal anoestrus. Oestrus was induced by injection of 3 mls. of synthetic prostaglandin $F_{2\alpha}$ intramuscularly. The oestrous cycle length following prostaglandin injection was 10 days. The animal exhibited normal behavioural oestrus. Accordingly, it was decided to fistulate and collect blood and tissue samples on day 12 following this oestrus. The data on fig. 16 shows that plasma progesterone levels on day 12 of the cycle were below 2 ng/ml. Progesterone levels increased gradually and were high on the day the animal was killed (day 25). In view of the above findings, it is possible that the sheep had yet another short cycle, but without displaying oestrus, and the progesterone levels on the day she was killed were actually the values for day 13 of the new cycle. However it is not possible to deduce precisely the sequence of events in this animal and so it has been excluded from the assessment of the data.

Discussion

The results of this experiment indicate that it is possible to prepare conscious animals in which uterine venous blood can be collected via an anastomosis of the

uterine vein to the mammary vein. Diversion of part of the uterine venous drainage, leaving the adjacent ovary with a corpus luteum *in situ* did not interfere with the sheep's oestrous cycles. All the ewes (except ewe A19) had normal oestrus cycles postoperatively. The implications of this on the utero-ovarian pathways will be mentioned in a following chapter. However, the difficulty with the anastomosis procedure was the low number of animals in which the anastomosis was patent compared to the number attempted. The poor success rate stemmed primarily from anastomosing only one branch of the uterine venous drainage. On account of this other routes for the uterine venous blood were readily available. A positive drive to maintain the patency of the anastomosis was thus not usually present. Subsequently the uterine horn of the side of the patent anastomosis was also fistulated through the different layers of the body exposing the uterine endometrium to the exterior. This fistulation procedure gave access to the uterine lining in the conscious animal and allowed the collection of tissue samples. The curvature of the fistulated horn also allowed the persistence of an appreciable amount of unsampled endometrium which ensured continuation of the oestrous cycles. Surprisingly, no infections problems were encountered during sampling and removal of tissue biopsies was straight forward. The present results on the concentration of $PGF_{2\alpha}$ and progesterone in uterine venous blood are in agreement

with earlier reports that increase of $PGF_{2\alpha}$ towards the end of the cycle causes luteal regression. Thus peaks of $PGF_{2\alpha}$ release consistently occurred at 30-45 hrs before the decline in progesterone concentrations. Elevation of endometrial $PGF_{2\alpha}$ content and synthesizing ability occurred at similar times to the $PGF_{2\alpha}$ release, but did not consistently either precede, coincide or follow it. This suggests that neither an increase in $PGF_{2\alpha}$ content or an increase in ability of the uterus to synthesize $PGF_{2\alpha}$ are prerequisites for $PGF_{2\alpha}$ release, unless their increase is so transitory to prevent them being detected in these experiments. A similar conclusion can be drawn from the work of Alwachi et al (1979) in the sheep. On the other hand Smith, Husling and Fogwell (1979) found an increase in prostaglandin-forming cyclo-oxygenase during the period of luteal regression, which led them to suggest that an increase in the efficiency with which arachidonate was converted into $PGF_{2\alpha}$ precursor occurred rather than an increase in substrate availability.

Oestradiol-17β showed an increase in levels around the time of both periods of $PGF_{2\alpha}$ increase. These elevated levels of oestradiol-17β often precede and\or coincide with peaks of $PGF_{2\alpha}$, thus confirming a role for oestradiol in $PGF_{2\alpha}$ release. The mechanism by which oestradiol triggers the release of $PGF_{2\alpha}$ is not known, but does not appear to be by either increasing the $PGF_{2\alpha}$ content or synthetic ability of uterine endometrium. The increase in oestradiol-17β probably provides the

stimulus for the increase in endometrial $PGF_{2\alpha}$ synthetase levels which occur at this time

(Smith, Husling & Fogwell, 1979) and that this directly allows $PGF_{2\alpha}$ release. It is likely therefore that, the intermittent increase in oestradiol levels prior to progesterone decline, in conjunction with prior conditioning of the uterus by progesterone (see page 32) causes the luteolytic release of $PGF_{2\alpha}$ from the uterus. The luteolytic interaction between prostaglandin and oestradiol in sheep has previously been suggested by Hixon et al (1975). The studies of these workers demonstrated that treatment of ewes with a dose of PGF_2 that was luteolytic in sham irradiated ewes failed to do so following destruction of the follicles by X-irradiation. Moreover, there was a much more rapid decrease in plasma progesterone in sham and X-irradiated ewes when both $PGF_{2\alpha}$ and oestradiol were given together than when either of these hormones were given alone.

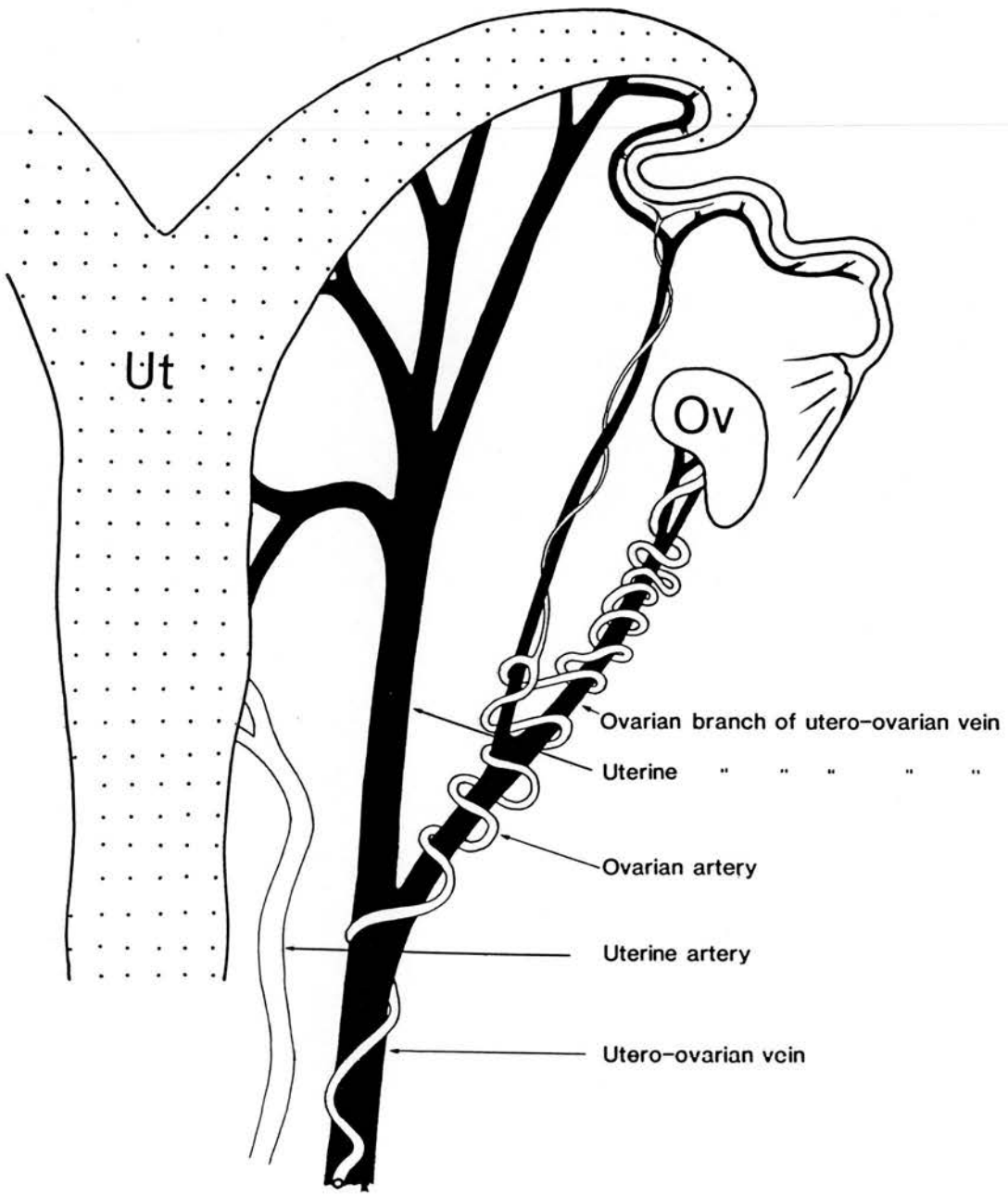
An increase of oestradiol-17β and $PGF_{2\alpha}$ was also observed after the commencement of progesterone decline but before oestrus. This could serve to ensure the irreversible demise of the corpus luteum. The close relationship between oestradiol and $PGF_{2\alpha}$ is well shown in ewe 25 (cycle 1), where oestradiol-17β levels failed to increase at luteolysis. Thus oestrus failed to occur, but the pre-oestrus rise in $PGF_{2\alpha}$ was also absent.

The secretion of $PGF_{2\alpha}$ to cause luteal regression appears to occur in two stages. In response to

progesterone secreted by the corpus luteum, for 7-10 days, the uterus in response to oestradiol starts to secrete $PGF_{2\alpha}$ in an episodic manner. This suppresses progesterone production on about day 13-15. The second event follows the decline in progesterone when $PGF_{2\alpha}$ and oestradiol-17β peaks complete structural luteolysis. Oestradiol not only causes the release of $PGF_{2\alpha}$ from the uterus, but also potentiates the luteolytic action of this hormone at the luteal level (Hixon et al 1975).

The general absence of a relationship between the plasma concentration of $PGF_{2\alpha}$ and its endometrial content and synthesizing ability was surprising and implied that the release of $PGF_{2\alpha}$ is under independent control. The direct relationship between the endometrial content of $PGF_{2\alpha}$ and PGE_2 suggests that either PGE_2 is a by product of $PGF_{2\alpha}$ production or that in both instances it is the availability of a common precursor that controls the tissue content of the two prostaglandins.

Figure 17: Generalized diagram of the vasculature of the uterus and ovary in sheep. The ovary is drained by the ovarian branch of the utero-ovarian vein. The uterine horn is drained by veins which form the uterine branch of the utero-ovarian vein. The main uterine and ovarian branches join together and form the utero-ovarian vein. The ovarian artery is closely associated with the utero-ovarian vein.


Ut
Ov
Ovarian branch of utero-ovarian vein
Uterine " " " " "
Ovarian artery
Uterine artery
Utero-ovarian vein

Studies On $PGF_{2\alpha}$ Transport

General Introduction

The identification of $PGF_{2\alpha}$ as the uterine luteolytic hormone was soon followed by extensive studies to determine the route by which it passed from the uterus to the ovaries. The diagram (Fig. 17) on the facing page shows the utero-ovarian vascular relationship in the ewe.

Previous work has already shown the local nature of the control of the uterus on the corpus luteum. Thus removal of one uterine horn from sheep in which corpora lutea were present in both ovaries resulted in regression of the luteal tissue in the ovary with direct tissue continuity with the residual uterine tissue but not in the contralateral ovary (Caldwell et al 1969; Goding 1974). This local effect is further emphasized by the fact that $PGF_{2\alpha}$ is rapidly metabolized in the lungs (Ferriera & Vane 1967; Piper, Vane & Wyllie 1970; Piper & Vane 1971) thus making transport in the general circulation improbable. Thus various workers (Thorburn & Nicol 1977; Douglas & Ginther 1973; Chamley, Cerini, Cerini, Cumming, Goding & O`Shea 1974) showed that doses of $PGF_{2\alpha}$ equivalent to those found in the uterine venous blood on day 15 when administered, either intramuscularly or via the jugular vein, failed to produce any luteolytic effect. Subsequently extensive studies were made on the anatomical structure of the

vasculature of the uterus and ovaries in different animals. Del Campo and Ginther (1973a) demonstrated that the uterus and ovaries of the sheep are drained by a common vein "the utero-ovarian vein". The venous drainage was found to be considerably complex. In some animals the ovarian vein (i.e. ovarian branch of the utero-ovarian vein) emptied into a tubal branch which in turn drained into the uterine branch while in others it passed directly to the latter (Del Campo & Ginther 1973b). There was also a prominent anastomotic network between the venous systems of the left and right side in the area of the uterine body and cervix. This network was ventral in sheep and swine, and was dorsal in horses. This species difference is due to the manner in which the uterus is suspended. The ovary of the sheep is supplied by the ovarian artery, which originates from the aorta and divides into 2 or 3 branches about 50 mm from the ovary. In the sheep and swine, the ovarian artery from its origin to its division is in close apposition to the utero-ovarian vein. In the sheep the artery is somewhat tortuous on the surface of the vein. A uterine branch of this artery supplies the caudal portion of the oviduct and follows the course of the uterine branch of the utero-ovarain vein and is closely applied to it (Del Campo & Ginther 1973a). The main uterine artery enters the uterus in the region of the uterine body. In the sheep Del Campo and Ginther (1973b) found a close apposition between the ovarian artery and the utero-

ovarian vein over the greater part of the vein's length (approximately 12 cm). Their investigations (Ginther & Del Campo 1973) also suggested that these areas of extensive surface contact may be the site of transfer of the uterine luteolytic substance from the utero-ovarian vein to the ovarian artery. However, histological studies show that there is not a reduction of tissue layers in the area where the two vessels are in apposition. In view of the close apposition between the vessels it has been proposed by Del Campo and Ginther (1974) and Lee and O'Shea (1975) that $PGF_{2\alpha}$ transfer can occur between the utero-ovarian vein and the ovarian artery and that the structure is suitable for a counter-current system.

Other studies on the vascular anatomy of the uterus and ovaries in laboratory and farm animals have also been interpreted as being compatible with the hypothesis that blood vessels can serve as a unilateral pathway for uterine-induced luteolysis in the hamster, rat, swine, cattle and guinea-pig (Hixon & Hansel 1974; Del Campo & Ginther 1972,1973) although in the case of the guinea-pig this has been disputed (Egund & Carter 1974). However, in the horse and the rabbit such a mechanism was found to be unlikely, even though the uterus has a luteolytic effect (Ginther 1974). The pig and rabbit are also unusual since hemi-hysterectomy does not interfere with luteal regression in either ovary. In a pig in which one ovary and its adjacent uterine horn were

removed and the other ovary transplanted to the vacant side, normal oestrous cycles were recorded (Harrison & Heap 1972). There were no common utero-ovarian vessels and the uterine vein and ovarian artery were separated by a distance of 5 cm. However, unlike most species, much of the $PGF_{2\alpha}$ in the blood of the sow passes through the lungs unchanged (Davis, Fleet, Harrison & Maule Walker 1980); consequently $PGF_{2\alpha}$ may pass from the uterus to the ovaries via the sytemic circulation. These data suggest that in the sheep and several other species the uterine luteolytic hormone $PGF_{2\alpha}$ is transferred directly from the utero-ovarian vein to the ovarian artery, possibly by a counter-current mechanism, to cause corpus luteum regression at the end of the oestrous cycle.

However, even though the counter-current transfer of $PGF_{2\alpha}$ from the utero-ovarian vein to the ovarian artery has been widely accepted, several studies have failed to support it as the sole pathway for $PGF_{2\alpha}$ transfer. In the early work of Thorburn & Mattner (1971) separation of the utero-ovarian vein from the ovarian artery failed to interrupt the normal oestrous cycle of the sheep. Similarly, Lamond & Dorset (1973), sectioned the ovarian artery distal to the region where the transfer is believed to take place and did not interrupt the oestrous cycle. Moreover, Coudert and co-workers (Coudert, Phillips, Palmers & Faiman 1972) failed to find consistent rises in $PGF_{2\alpha}$ in the ovarian artery

towards the end of the cycle and then later (Coudert, Phillips, Faiman, Chernecki & Palmer 1974 a,b) were unable to show transfer of radioactive xenon or tritium labelled $PGF_{2\alpha}$ from the uterine vein to the ovarian artery. However, Land, Baird and Scaramuzzi (1976) did find transfer of tritiated $PGF_{2\alpha}$ from the uterine vein to the ovarian artery in sheep with utero-ovarian transplants in the neck. However the precentage transfer was very low. Conversely, they (Baird & Land 1973) found that only 4 out of 10 ewes showed persistent luteal function when the main uterine vein alone was ligated and severed. At about the same time Restall, Hearnshaw, Gleeson and Thorburn (1973) demonstrated that a close apposition of the uterine vein and ovarian artery was not essential for $PGF_{2\alpha}$ to act as a luteolysin. These authors infused $PGF_{2\alpha}$ into the uterine vein following surgical separation of the utero-ovarian vein from the ovarian artery. Peripheral progesterone fell to low levels and the animals returned to oestrus. Recently Alwachi, Bland and Poyser (1981) have shown that in some sheep $PGF_{2\alpha}$ can also reach the ovary via the venous drainage passing alongside the oviduct and suggested that the oviducal vein is an additional pathway. However in this instance any counter-current mechanism would be situated nearer the hilus of the ovary in the ovarian vascular plexus. However, Kotwica (1980) found that injection of $PGF_{2\alpha}$ into the jugular vein of pigs did not cause luteolysis, while infusion into one uterine

vein significantly decreased progesterone levels in the ipsilateral and contralateral utero-ovarian veins. This suggests that yet another pathway may be involved by which $PGF_{2\alpha}$ is transported to both ipsilateral and contralateral ovaries in amounts sufficient to cause luteolysis.

In the light of the above findings further work is definitely needed to conclusively show that a counter-current system between the utero-ovarian vein and ovarian artery is the prime mechanism whereby $PGF_{2\alpha}$ is transferred.

The Effect Of Surgical Separation Of The Uterus And Ovaries And Cannulation Of The Uterine Vein On Oestrous Cycle Length In Sheep

Introduction

An early approach to prove the role of vascular integrity between the uterus and ovary in corpus luteum function was the ligation or sectioning of veins and arteries between the uterine horn and adjacent ovary. Luteal maintenance was then considered as evidence for the role played by those structures. In this respect Kiracofe and coworkers (Kiracofe &Spies 1963; Kiracofe , Menzies, Gier & Spies 1966) showed that bilateral ligation of the major uterine veins and arteries early in the oestrous cycle resulted in maintenance of the corpus luteum in seven of eight ewes, while unilateral ligation resulted in prolonged luteal maintenance in only one of six ewes.Subsequently following the

discovery of a local uterine influence on luteal function (see page 13), several attempts were made to elucidate the pathway involved. Barrett et al (1971) first suggested that $PGF_{2\alpha}$ might be transfered from the utero-ovarian vein to the ovarian artery by a counter-current mechanism. This counter-current hypothesis was subsequently examined by McCracken et al (1973) who found evidence that $PGF_{2\alpha}$ does pass from the utero-ovarian vein to the ovarian artery by such a mechanism. However the efficiency of transfer was low (2 to 10%). Experiments involving surgical interferences were performed by several groups to examine the adequacy of the uterine venous and ovarian arterial blood as the main components of the utero-ovarian pathway for $PGF_{2\alpha}$. For example Ginther's group (Ginther, Del Campo & Rawlings 1974; Mapletoft & Ginther 1975) performed a series of experiments involving surgical anastomosis of uterine veins and ovarian arteries. The results of those experiments suggested that both the utero-ovarian vein and the ovarian artery were involved in the local utero-ovarian pathway. However the interpretation of these surgical interferences only considered the uterine vein and ovarian artery; no other tissues in the region were taken into account. As noted earlier (page 80) other surgical manipulations did not seem to support the counter-current hypothesis. This presence of contradictory evidence suggests that it is necessary to re-examine if blood passing through the uterine vein is

the prime outlet for $PGF_{2\alpha}$ before it is transferred to the ipsilateral ovary. The purpose of the present study was to determine whether the luteolytic hormone passes solely via the blood of the utero-ovarian vein.

MATERIALS AND METHODS

Animals And Experimental Procedure

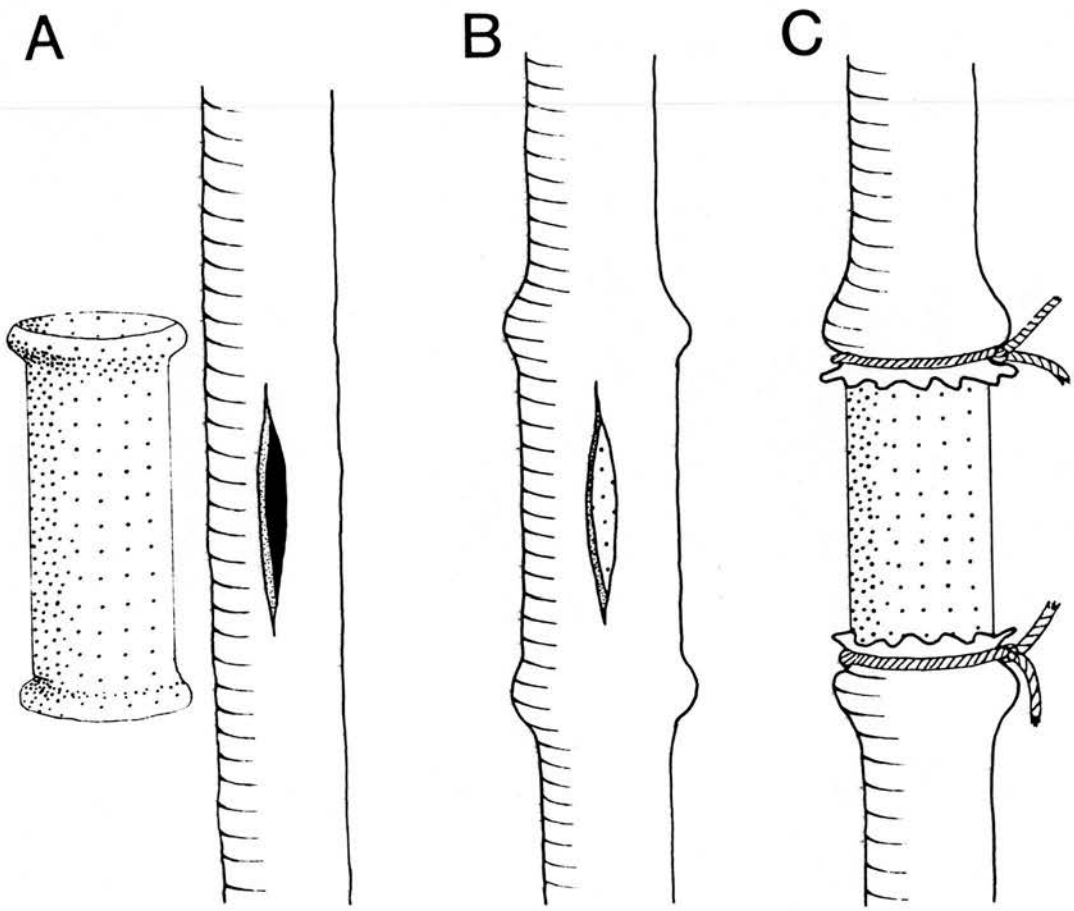
The ewes, 18 Dorset Horn X Finnish Landrace, used is this experiment were allocated to two groups; a cannulated group of 8 animals and a sham operated group of 10. All surgery was performed in the first 7 days of the oestrous cycle. Anaesthesia was induced with Saffan and maintaned under nitrous oxide/halothane. Surgery was performed under aseptic conditions via a mid-ventral laparatomy. In the cannulated group the uterine vein on one side of the uterus was replaced with a glass cannula. The ewes selected for cannulation had all ovulated unilaterally, so that replacement of the uterine vein with a cannula was on the corpus luteum-containing side only. To replace part of the uterine vein with a cannula the main branch of the utero-ovarian vein was first dissected free of connective tissue for 5 or 10 mm, just anterior to the junction with the ovarian branch. Two loose sutures were put around the cleaned vessel. A small cut in the vein was then made and the small siliconized glass cannula (see Fig. 18) inserted into the lumen of the vein and the two ligatures tied securely. The wall of the cannulated part of the vein was then cut away and all the remaining tissue

Figure 18: Diagrams showing the procedure of replacement of part of the uterine branch of the utero-ovarian vein with a glass cannula.

A- Shows the home-made siliconized glass cannula and a small initial cut made in the vein.

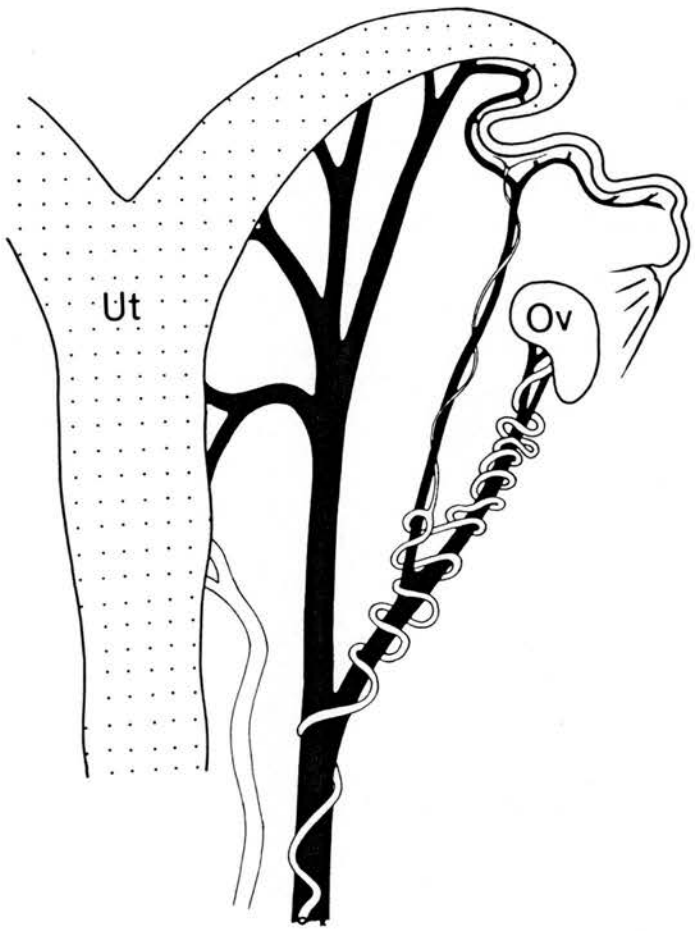
B- Shows the cannula inserted into the lumen of the vein.

C- Shows the cannula ligated in place and the wall of the cannulated part of the vein cut away so that blood passing through the glass cannula was the only connection between the two parts of the vein.


A
B
C

connections between the uterus and ovary were severed and ligated tightly in order to discourage adhesions. In this way all the connections between the uterus and adjacent ovary were either divided or tied securely, with the exception of the venous blood passing through the uterine branch of the vein (see Fig. 19). In one animal (ewe A36), bilateral ovulation had occurred, thus the corpus luteum in the ovary on the side opposite to the cannulation was enucleated. The sham operated ewes were also subjected to laparatomy. The uterine vasculature was manipulated and one of the uterine venous branches severed. In all ewes a sample of blood from the jugular vein was collected daily from the day of surgery until termination. The state of the corpus luteum was monitored by measuring the progesterone concentration in these peripheral blood samples. Plasma progesterone concentrations were measured by radio-immunoassay using a double antibody technique (see page 58). All the ewes were teased daily with a vasectomized ram both before and after surgery. The animals were killed 22-24 days after the pre operative oestrus to examine the patency of the cannula and the state of the corpus luteum in the ovary on the cannulated side. At post-mortem Indian ink was injected into the uterine vein between the uterus and the cannula and the patency of the cannula observed.

Figure 19: Diagrams showing the uterine branch of the utero-ovarian vein intact (upper diagram) and part of the same vein replaced by a glass cannula (lower diagram). Following cannulation of the vein all the remaining connections between the uterine horn and ipsilateral ovary were severed and/or ligated tightly; in this way the only connection left between the uterus and the ovary was the venous blood passing through the cannulated uterine branch of the vein.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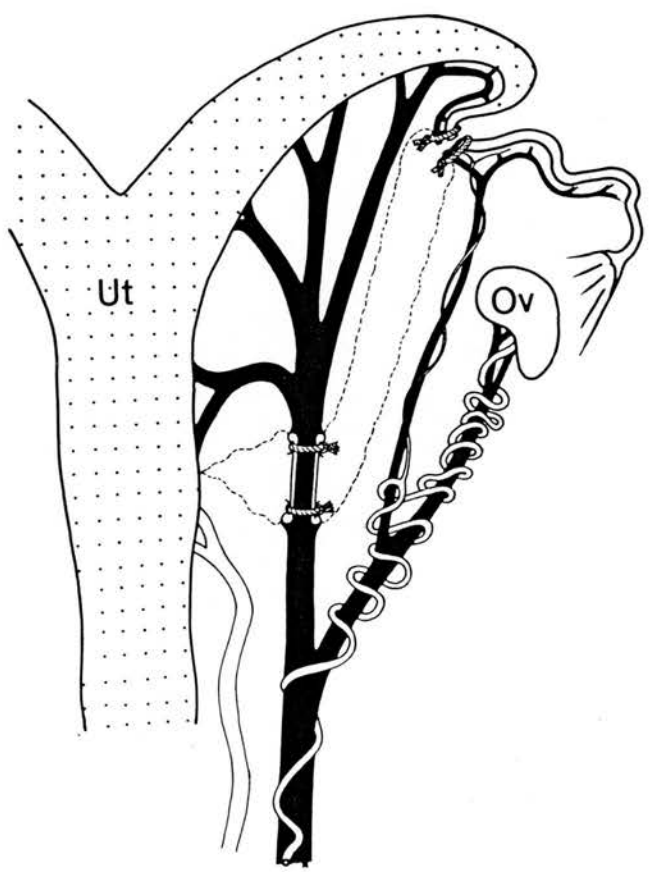
Ov


Ut
Ov

Results

Table 5 shows the outcome of cannulation, day of surgery, days to oestrus following cannulation and the effect of cannulation on the corpus luteum in the cannula operated ewes.

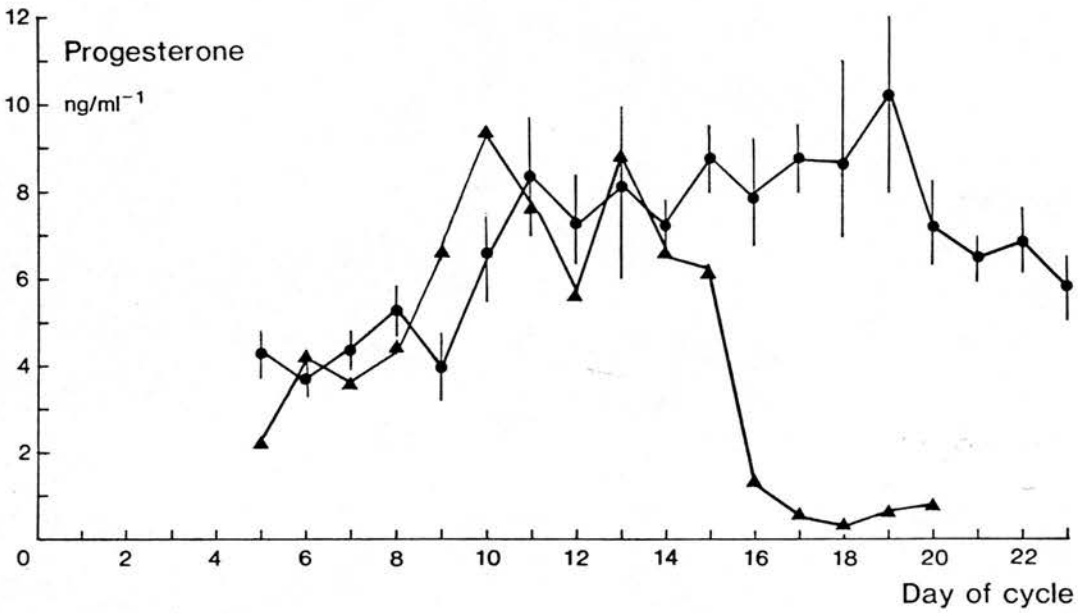
Table 5

Ewe	Day of surgery	Days to oestrus following cannulation	Outcome of cannulation	Day killed	State of corpus luteum
A33	5	19+	patent	24	persistent
A40	5	12	patent	20	fresh cl
A57	5	16+	patent	23	persistent
A30	6	15+	patent	22	persistent
A63	6	18+	patent	24	persistent
A17	7	16+	patent	22	persistent
A36	7	16+	not patent	23	persistent
A44	7	17+	patent	24	persistent

Cannulation of the uterine vein by the present method resulted in persistence of the corpus luteum in the ipsilateral ovary and extended oestrous cycle length. Examination of the area of cannulation indicated a good patency of the cannula in 7 of the 8 ewes. Oestrous cycle length in all the sham operated ewes was normal and within the range of 17-19 days.

In the patent preparations the injection of Indian Ink into the uterine vein between the uterus and the cannula quickly blackened the veins caudal to the

Figure 20: The daily changes of peripheral progesterone concentration (mean ±SE) of the sheep that stopped cycling following cannulation of the uterine vein and of ewe A40(▲)that came into heat following cannulation. Progesterone concentration around the time of expected oestrus was high in the first group, while that in ewe A40 declined to low levels and allowed the occurrence of oestrus.


Progesterone
ng/ml⁻¹
12
10
8
6
4
2
0
2
4
6
8
10
12
14
16
18
20
22
Day of cycle

cannula. However, in one ewe (ewe A36) the cannula was not patent and examination of it revealed that it was blocked by clotted blood and fibrous tissue. In all ewes in the cannulated group (except ewe A40 which came into heat) the corpus luteum on the cannulated side had not regressed. Progesterone concentrations in peripheral plasma indicated that the corpus luteum in the ewes with both patent and blocked cannulae had been maintained and were actively secreting progesterone (fig. 10). The mean concentration of progesterone in animals of the cannulated group in which the corpus luteum was maintained ranged from 3.8 ng/ml to 10.18 ng/ml. However progesterone profiles in the cannulated ewe which returned to oestrus showed the typical decline in progesterone associated with oestrus (fig. 20).

Discussion

The results of the present experiments indicate that the oestrous cycles of the ewes were extended when the uterine vein was cannulated and all other direct connections between the uterine horn and the ipsilateral ovary (oviduct, broad ligament tissue, lymphatics and arteries) were surgically separated. The only connection between the uterus and ipsilateral ovary containing a corpus luteum was the uterine venous blood. Total disconnection between the uterus and ovary has been shown to delay or extend the oestrous cycle (Anderson et al 1961; Inskeep & Butcher 1966); such a delay was found in the present experiment even though uterine venous

blood was still passing down the utero-ovarian vein. The corpus luteum regressed normally in all the sham operated group where a substantial amount of tissue between the uterus and the ovaries was left intact. Baird and Land (1973) found that some ewes, in which all the connecting structures between the uterus and ovaries had been severed except the middle uterine vein, showed normal regression of the corpus luteum, while others showed persistence. Thus former results contrast with those in the present experiment where part of the uterine vein was replaced with a glass cannula so that blood was the only contact between the uterine horn and the ipsilateral ovary. The amount of tissue left around the middle uterine vein in Baird and Land`s experiments (1973), is unknown, but must be the deciding factor in whether the corpus luteum regressed or was maintained.

In ealier studies the corpora lutea were counted and marked with either Indian ink under the tunica albugina (Ciro, Torres & First 1975), or a piece of silk was tied to it then confirmed by a re-examination at laparatomy. In the current experiment the progress of the corpus luteum was monitored by daily progesterone measurements as well as postmortem examination.

In the ewes in which all anatomical structures between the uterus and ovaries were surgically separated the corpora lutea were maintained. This differs from the gilt in which the corpora lutea regressed even though the connections between the uterus and ovary were

severed (Ciro et al 1975). In this context the report by Kotwica (1980) that $PGF_{2\alpha}$ could be transported to the contraleral ovary when infused into one anterior vein is relevant. The results from the present experiment suggest that blood passing through the uterine vein is not sufficient to account for a luteolytic factor passing from the the uterus to the ipsilateral ovary. Possibly, as suggested by Bland and Donovan (1969), Hansel et al (1973), Kotwica (1980) and Staples and Heap (1982), lymph may have a role to play.

ANATOMY OF UTERINE LYMPHATIC DRAINAGE

Introduction

Detailed information on the relationship between the vascular and lymphatic circulation of the uterus and the ovary in sheep is rather sparse. There is detailed information on the gross anatomy on the vascular drainage of the uterus (see page 78) and vascular and lymphatic drainage of the ovary. However, there is very little information in the uterine lymphatic system in cycling sheep. In a comprehensive description of the ovarian lymphatics in the sheep, Morris and Sass (1966) showed that a profuse network of highly permeable lymphatic ducts develop within the corpus luteum during the luteal phase of the oestrous cycle and in early pregnancy. After traversing the ovary these lymphatics join in the region of the hilus (subovarian plexus) to form 4 to 12 main vessels which drain the ovary and are joined by lymphatics from the proximal half of the oviduct. Similarly, Anderson (1926) gave an account of the lymphatics of the ovary of the sow and noticed that the development and regression of the lymphatic vessels coincided with the events of the oestrous cycle.

The lymphatic vessels of the uterus have been studied by Reynolds (1965) and by McClean and Scothorne (1970) in the rabbit. Lymphatic vessels were abundant beneath the uterine peritoneum, between the muscle layers and at the endometrial-myometrial junction. However details of the uterine lymphatic drainage in

other non-human animals is lacking except for the sheep, where the data available on the utero-ovarian lymphatic relationships are few but contradictory. Linder, Sass and Morris (1964), examined for possible connections between the lymphatics of the ovary and the oviduct, but in all ewes examined the lymphatic drainage of the two regions was discrete. Moreover, Meckley and Ginther (1969), in a brief report stated that lymphatic vessels from the caudal part of the uterus leave at interval of 4 to 10 mm and anastomosed 1 to 5 cm from the mesometrial attachement and formed four to 10 major lymphatics which usually accompanied the middle uterine vein. However, neither Meckley and Ginther (1969), nor Morris and Sass (1966) found any direct connection between the uterus and the ipsilateral ovary. This indicates that the lymph cannot pass directly from the uterus to the ovary. In another brief report, Cirmanec (1972) examined 4 ewes on day 4 of the oestrous cycle and 4 more on day 15 and found no evidence for direct lymphatic drainage from the uterus to the ovary, but on day 15 a lymphatic vessel draining the uterine horn was seen passing near the hilus of the ovary in close association with the ovarian artery and vein. An extensive report by Staples, Fleet and Heap (1932) showed evidence for an anastomosis of uterine and ovarian lymphatics in the region of the ovarian pedicle in 4 animals during early pregnancy. Magness and Ford (1931) studied the lymphatic drainage of the uterine

horn in the sow to determine if the lymphatic vessels draining the uterus act as an alternative route for the transport of steroids from the gravid uterus. These authors found that the main lymphatic vessel draining the uterine horn was in close apposition to the ipsilateral uterine and/or ovarian vasculature.

The functional significance of the uterine lymphatic vessels and their contribution to the luteolytic mechanism has not yet been investigated, even though several investigators have suggested that uterine lymphatic vessels could be a possible alternative route for the transport of the uterine luteolytic factor . In view of this it was decided to examine in more detail the anatomy of the uterine lymphatic drainage in the non-pregnant sheep.

Materials and Methods

In order to establish the best method for visualizing the uterine lymphatics, a preliminary study (part I) involving 6 ewes was undertaken.

Part I

Six Dorset Horn X Finnish Landrace ewes were anaesthetized with Halothane/N_2O, opened by a midventral laparatomy to expose the uterus and ovaries and then subjected to one of the following procedures:-

Procedure 1

In 2 ewes 2 ml of Indian Ink was injected into the lumen of one uterine horn after ligating it at the base. The uterus was observed for a period of 30 mins and then the animal was closed and allowed to recover. The animals were killed 2 days later and the uterus examined at post mortem. In both cases there was apparently no drainage of dye into any of the lymphatic ducts and after two days, the Indian Ink was still inside the lumen.

Procedure 2

In this study two ewes were anaesthetized and the uterus and ovaries exposed. Small incisions were made into various parts of the mesometrium to locate any lymphatic vessels. Once the clear vesicle of a lymphatic vessel bulged through, Indian Ink was injected directly into the lymph vessel using a No. 27 syringe needle. The progress of the Indian Ink was studied over the next 20

minutes and the animals were then closed and slaughtered two days later. The injection of Indian Ink directly into a lymph vessel in this study showed a fill right back to the lymph nodes; but revealed none of the side branches of the lymphatics. Surprisingly the Indian Ink injected two days earlier was still clearly visible during post mortem. The dye was also found in the lymph nodes (right and left).

Procedure 3

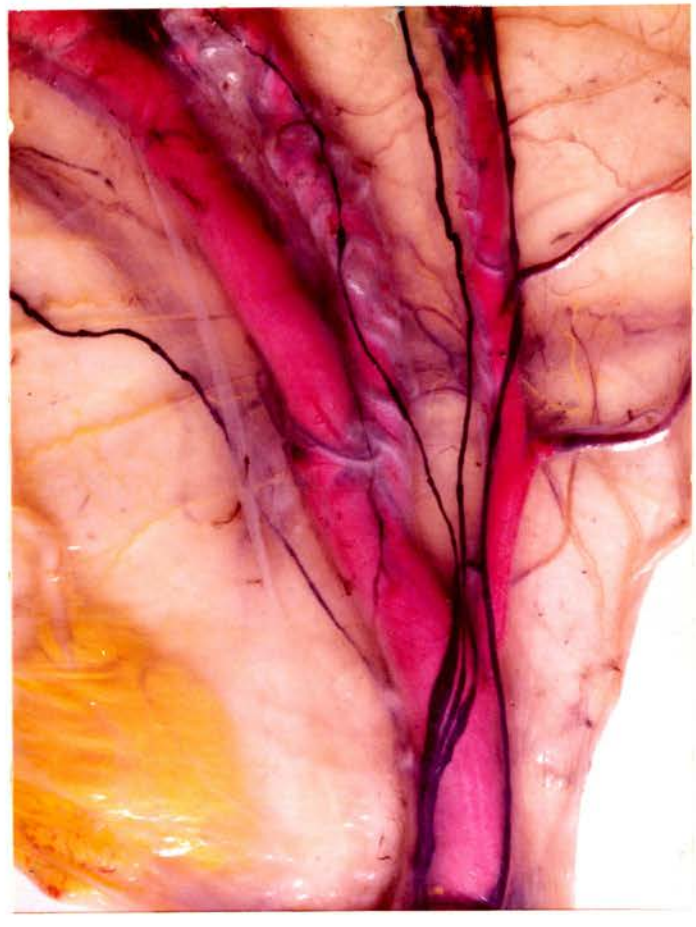
In one sheep Indian Ink was put into a cavity under the serosa covering the uterus. The cavity was made with a probe and a suture and used to keep the dye within the cavity. Some Indian Ink was already leaving in the lymphatic vessels by the time the abdomen was closed. Following recovery this animal was taken for autopsy the next day and the lymphatic drainage examined. At postmortem no lymphatic vessels were apparent but the lymph nodes associated with the lymphatic vessels were very dark, indicating that the dye had already passed into the nodes. This study indicated that the dye should be applied subserosally for identification of the lymphatic drainage.

Procedure 4

In one sheep the uterus was exposed as previously described and Indian Ink was injected subserosally into different parts of the mesometrium. The drainage of the lymphatic vessels were observed and recorded over the subsequent 30 minutes. Injection of Indian Ink revealed

several lymphatic ducts that emerge and join together to form at least two main vessels. After removing the genital tract from the animal the arteries and veins were injected with coloured latex (see Fig. 21). After removing the uterus, colouring latex ("Chromopaque" Damancy & Co Ltd.; Ware; Herts; England) was heated, mixed with starch and gealtin, and injected while still warm. The utero-ovarian vein was injectd with red latex and the ovarian artery with yellow latex. Following injection of the latex the vessels were clamped to stop the coloured latex draining out.

Figure 21: Photograph showing lymphatic drainage of the uterus and ovary in ewe A2. After removing the genital tract from the animal, the utero-ovarian vein was injected with red latex and the uterine artery with yellow latex. The lymphatic vessels are seen black due to the injection of Indian Ink.



Part II

Following these preliminary tests a full study was made on 8 ewes to establish the anatomical arrangement of the uterine lymphatics. The results of the previous studies had indicated the site and method of dye injection for identification of uterine lymphatic drainage. The exact day of the oestrous cycle in these ewes was not known. The ewes were anaesthetized and following laparatomy the rumen and intestines were moved out of the abdomen for better exposure of the uterus. Indian Ink was injected subserosally at intervals along one or both uterine horns avoiding all visible blood vessels. Once the lymphatic drainage was clearly defined the uterus, broad ligament and all the blood vessels were clamped off in order to prevent further drainage. The ewes were then terminated by an overdose of Pentobarbitone solution (Lethobarb, 20% w/v B.P, Loveridge Ltd. Southampton) intravenously. The whole uterus was removed and spread on a flat piece of polystyrene to allow full visualization of the vessels. In some of the specimens the arteries and/or the veins were injected with coloured latex (see page 95). The specimens were then fixed in 10% formal-saline and, after some dissection, drawn. Drawings were preferred to photographs as the latter could not differentiate the finer lymph vessels. The main vessels are named according to the International Committee on Veterinary Nomenclature.

RESULTS

There was much variation between the sheep in the arrangement, number and size of uterine lymphatic vessels (Fig. 22). Lymph vessels draining the uterine horn joined together and drained into the iliac and lumbar lymph nodes. However these lymph vessels were not involved in the ovarian pedicle, but pass parallel to it. There was no direct flow between the uterus and ovaries. A common feature in all animals studied was the close association between the lymphatic vessels and the main utero-ovarian vein.

In a few animals a substantial part of the utero-ovarian vasculature, between the junction of the uterine and ovarian branches of the utero-ovarian vein and the posterior vena cava was available for study. Interestingly in these cases a close association between the uterine lymphatics and the ovarian artery was evident. An enlargement of this portion of the vasculature in ewe A4 is shown in Fig. 23.

Discussion

There was no direct lymph flow between the uterus and ovaries. Similar findings were reported by Cirmanec (1972). The ovarian artery has an intimate relationship with the utero-ovarian vein as it proceeds from its origin in the abdominal aorta. This close relationship is presumed to be important because infusion of $PGF_{2\alpha}$ into the uterine vein results in regression of the corpora lutea in the adjacent ovary (Thorburn & Nicol

Figure 22: A2 to A7

The following drawings show actual examples of the arrangement of the lymphatic drainage of the genital tract. The notations used are consistent throughout, namely:

Ut = Uterus

OV = Ovary

OA = Ovarian artery

UA = Uterine artery

UOV = Utero-ovarian vein

OUOV = Ovarian branch of utero-ovarian vein

UUOV = Uterine branch of utero-ovarian vein

The cross-hatched areas show the site of the original injections. Lymphatics are shown filled in with black, arteries are stippled and veins left unshaded.

Figure 22-A2: This is the same preparation from ewe A2 as shown in the photograph in Figure 21. Indian Ink was injected into various parts of the mesometrium and all the lymphatics revealed converged into common trunks associated with the utero-ovarian vein.

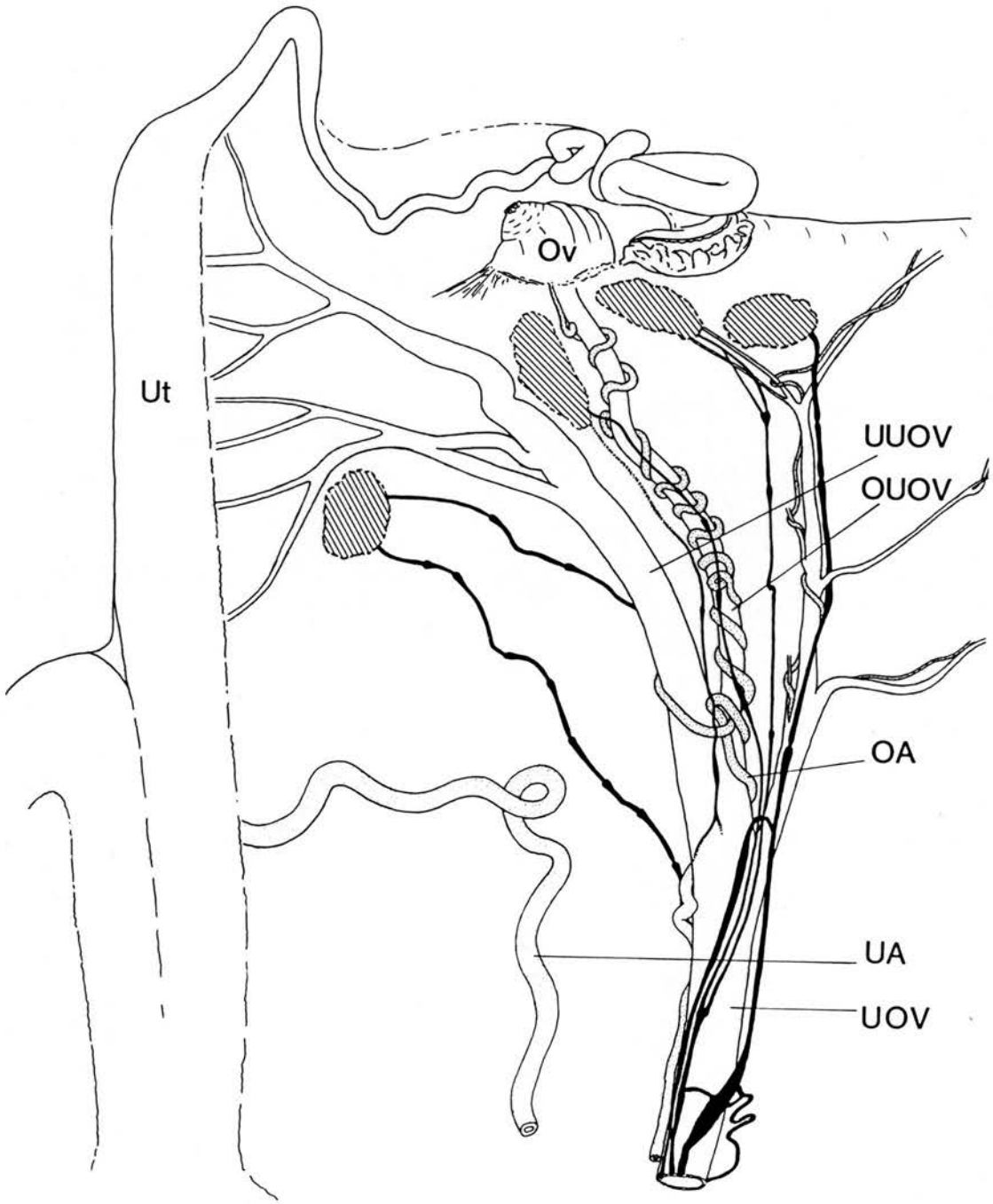

Ov
Ut
UUOV
OUOV
OA
UA
UOV

Figure 22-A3:

In ewe A3 the main uterine lymphatic vessel is parallel to the ovarian pedicle and in close association with the uterine branch of the utero-ovarian vein. This lymphatic vessel joins another lymphatic at the level of utero-ovarian junctions; this latter vessel appears to have been partly filled by back flow. There is no direct connection between the uterus and ovary.

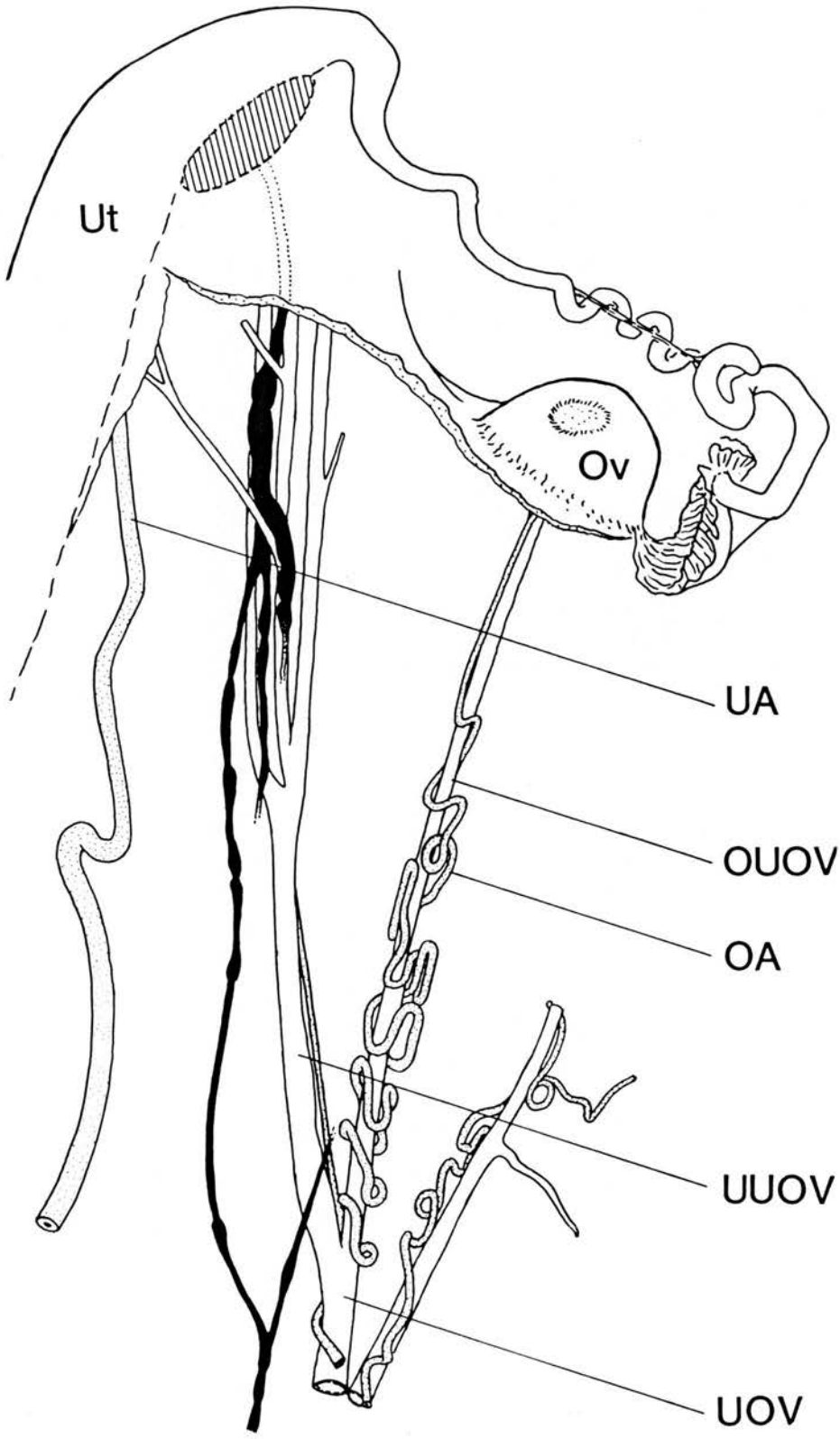

Ut
Ov
UA
OUOV
OA
UUOV
UOV

Figure 22-A4:

In ewe A4 Indian Ink was injected into the ovarian end of the uterus. The several lymphatic ducts filled finally join together to form one main lymphatic vessel that passes along the surface of the utero-ovarian vein in close association with the ovarian artery. The uterine artery in this animal enters the uterus more cranially than usual.

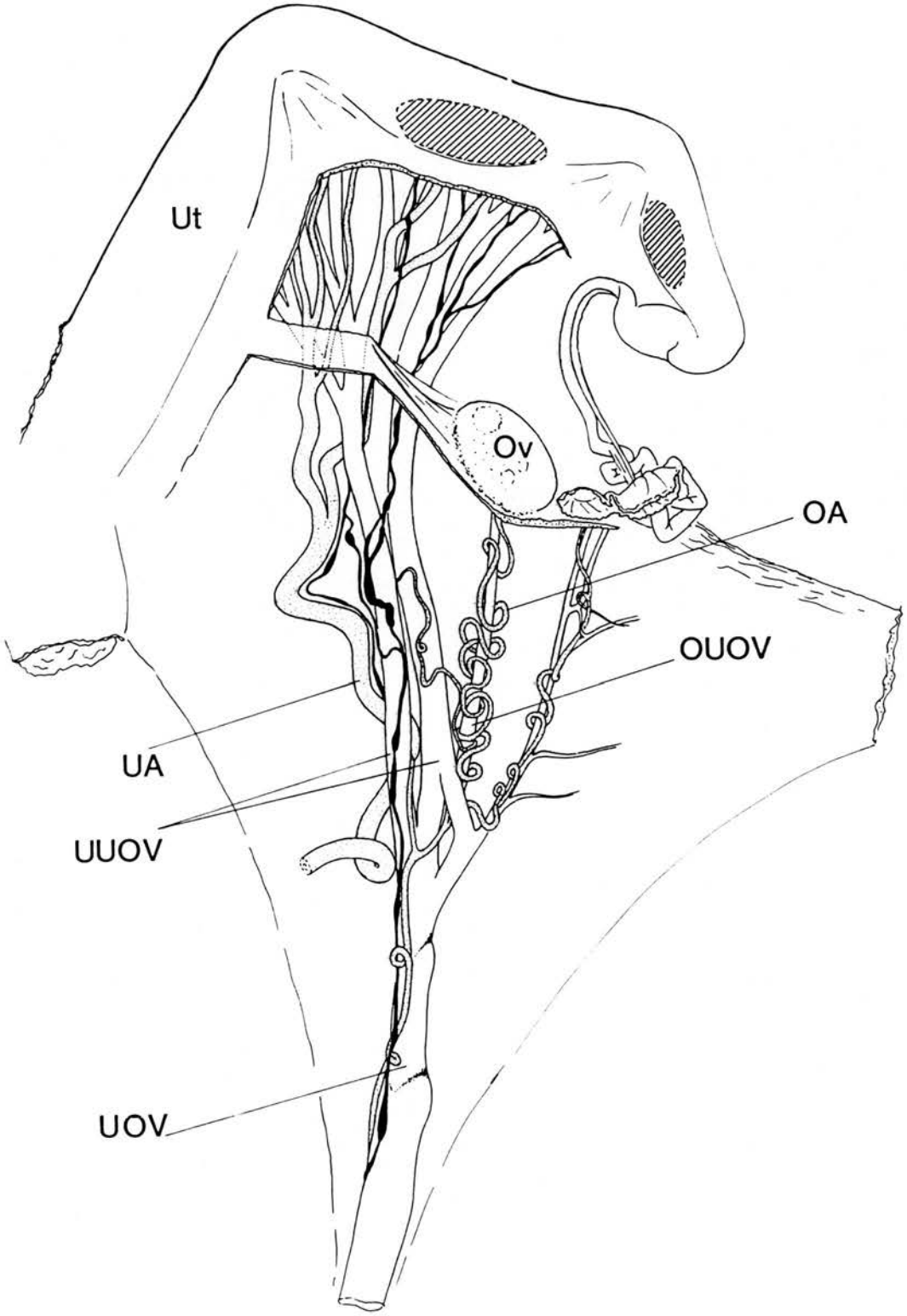

Ut
Ov
OA
OUOV
UA
UUOV
UOV

Figure 22-A5:

In ewe A5 almost the whole length of the uterus was injected showing an extensive pattern of lymphatics. Most of the lymph vessels join together forming two main lymphatic vessels that are in close association with the utero-ovarian vein. Although some of the lymph vessels run close to the ovary, there is no lymphatic connection between the uterus and ovary. One lymphatic is also seen passing down the side of the uterine body.

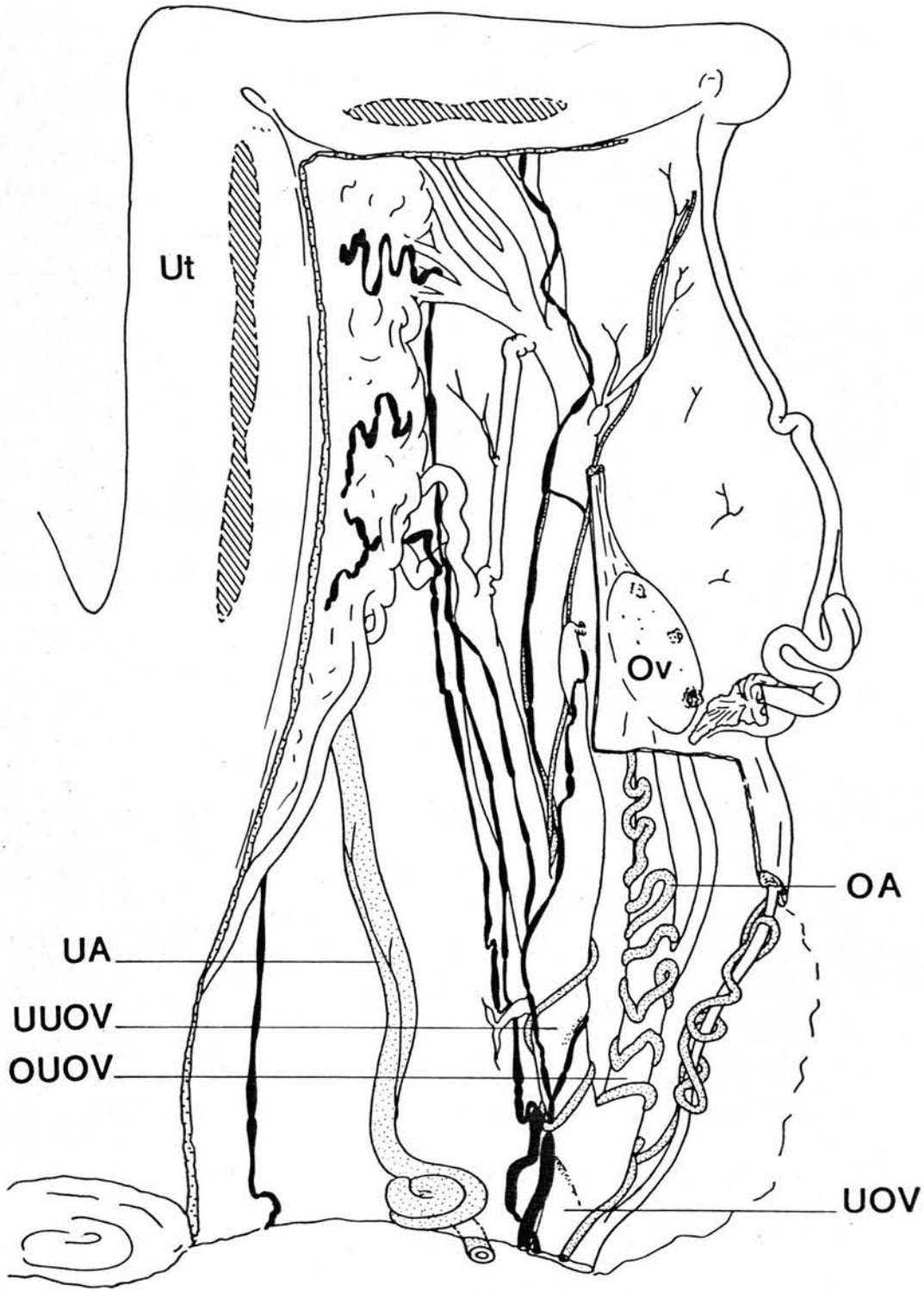

Ut
Ov
OA
UA
UUOV
OUOV
UOV

Figure 22-A6:

A single localized injection into the uterus of ewe A6 revealed several uterine lymphatic vessels running parallel to each other. Two course alongside the uterine branch of the utero-ovarian vein while another appears to be associated with the uterine artery.

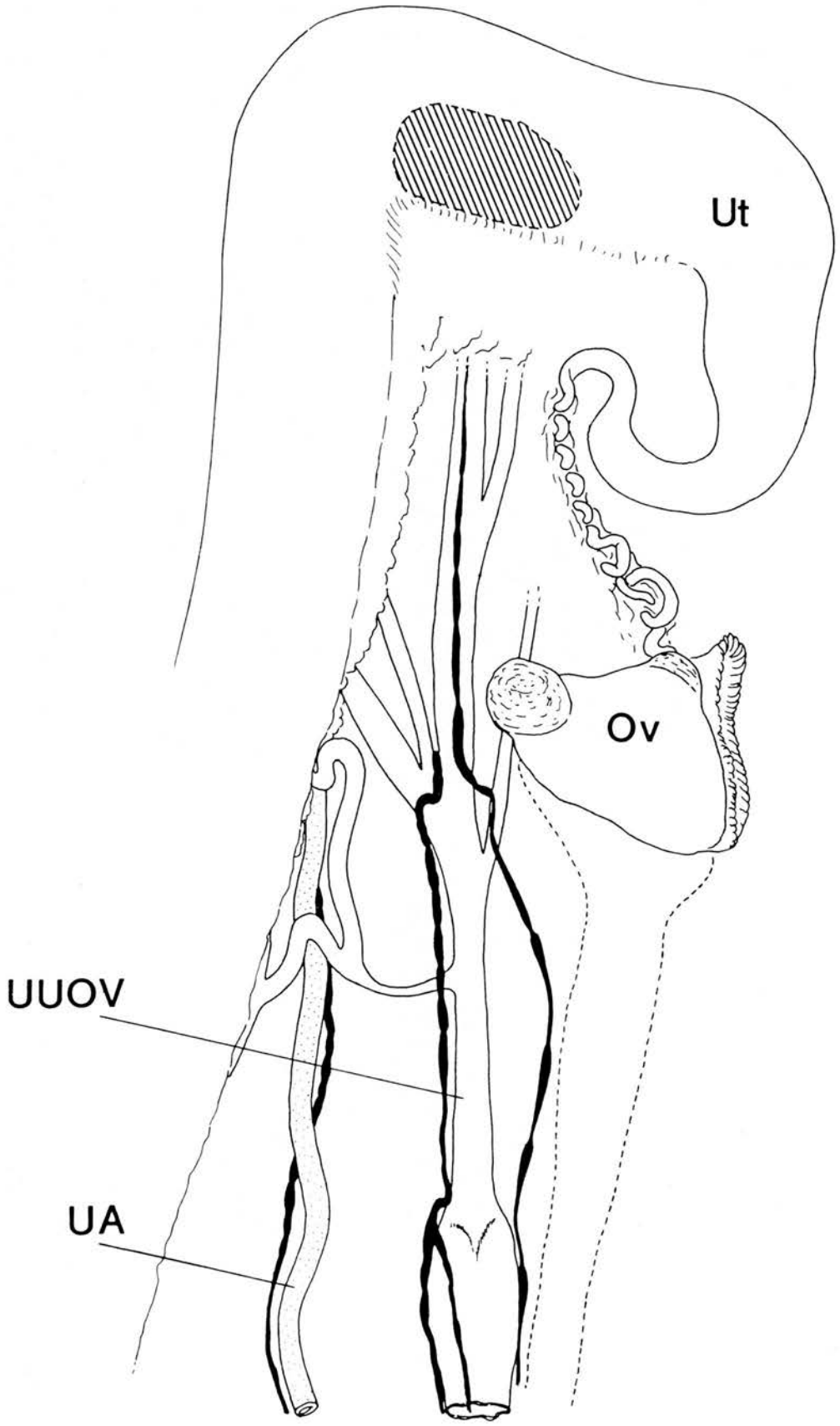

Ut
Ov
UUOV
UA

Figure 22-A7:

In ewe A7 extensive injections along the length of the uterine horn revealed a complex network of lymphatics. These lymph vessels joined together at different levels but eventually encircle the utero-ovarian vein and are also in close contact with the ovarian artery as is shown in the enlargement in the next diagram.

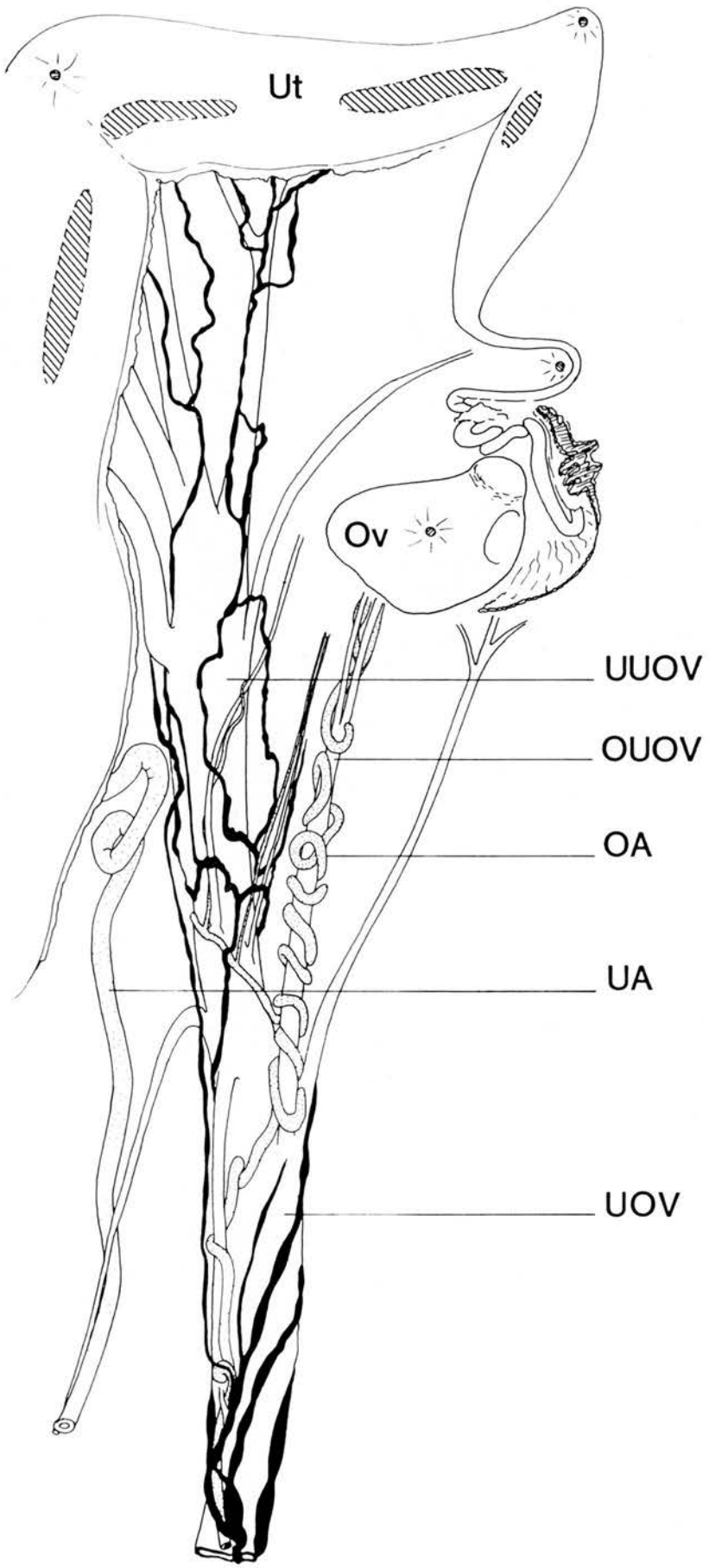
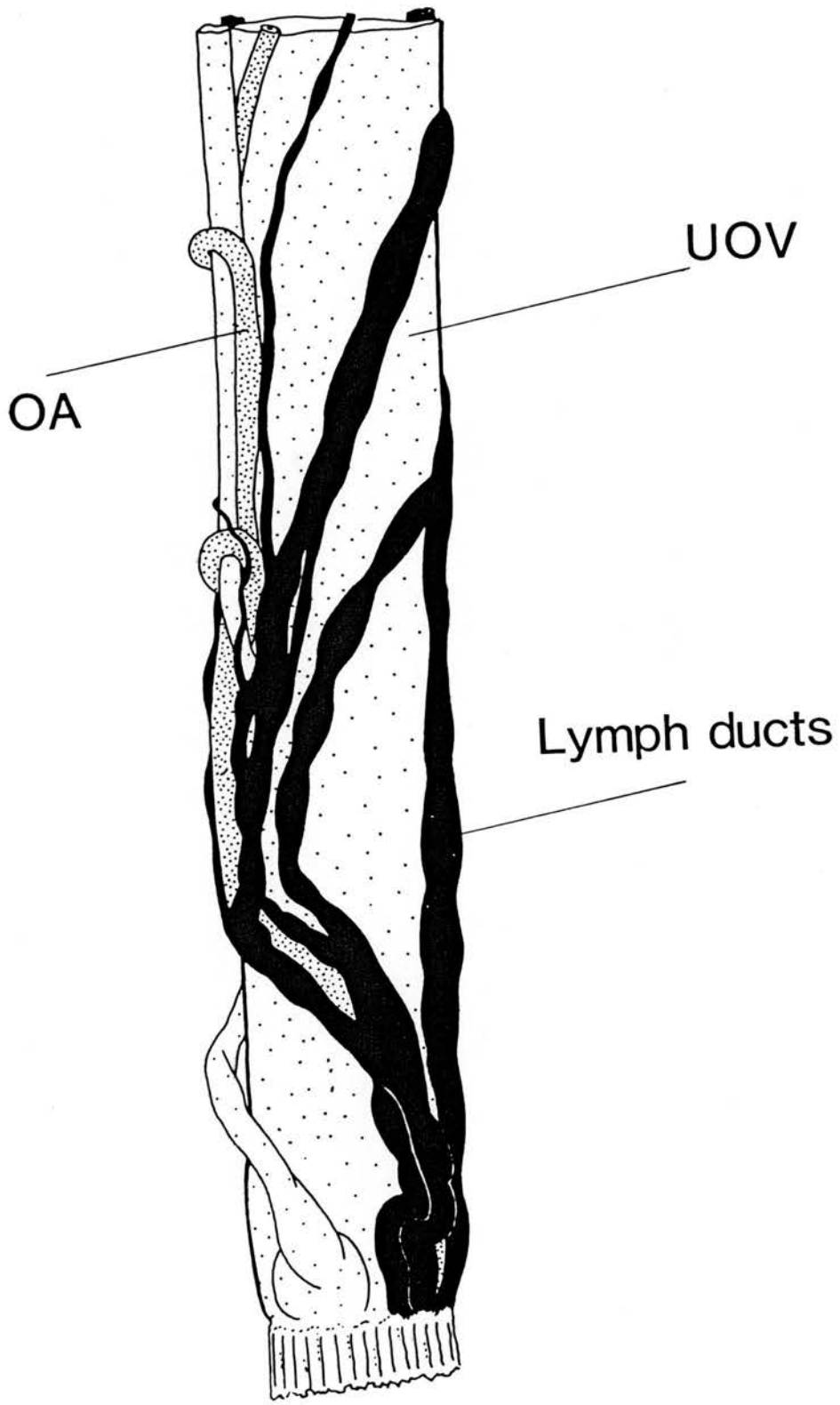

Ut
Ov
UUOV
OUOV
OA
UA
UOV

Figure 23:

An enlarged drawing of part of the utero-ovarian vasculature between the junction of the uterine and ovarian branches of utero-ovarian vein and the posterior vena cava in ewe A7. Main lymphatic vessels converge over the surface of the utero-ovarian vein to a point where they come into close contact with the ovarian artery and encircle it. This point of contact between the vein and artery is the place where $PGF_{2\alpha}$ has been proposed to transfer by a counter-current mechansim and cause luteal regression.


UOV
OA
Lymph ducts

1971; Goding et al 1971). The route of transfer of $PGF_{2\alpha}$ from the utero-ovarian vein to ovarian artery had been investigated by the infusion of tritiated $PGF_{2\alpha}$ which appears in higher concentrations in the ovarian than in the intestinal iliac arterial blood. Thus McCracken et al (1971) suggested that a conter-current system similar to that which operates in the kidney and testes (Waites & Moule 1961) may exist between the utero-ovarian vein and the ovarian artery. However, it was calculated that about 2-10% of the infused radioactivity was tranferred. In contrast Coudert et al (1974 a,b) infused tritium labelled $PGF_{2\alpha}$ into the uterine vein at a similar rate and for the same duration as described by McCracken et al, but no transfer of $PGF_{2\alpha}$ was detected. This finding and others (see page 80) therefore suggest that the proposed counter-current mechanism between the uterine vein and ovarian artery might not be the only route by which $PGF_{2\alpha}$ is transferred locally from the uterus to the ovary.

The results of the present anatomical study show that the main uterine lymphatic system trunks are also present at the proposed site of the counter-current exchange system. In fact, there was a very close association between the uterine lymphatics and the ovarian artery. Thus lymph may be an alternative pathway for $PGF_{2\alpha}$. However, the presence of $PGF_{2\alpha}$ in the lymph in the normal cycling animal has not yet been demonstrated.

Cannulation Of The Uterine Lymph Vessels And Collection Of Samples For Prostaglandin Analysis

Anderson (1926) observed that the lymphatics of the sow's ovary were most conspicuous when the corpora lutea were fully developed. This led her to suggest that the lymphatics maybe an important pathway by which the secretory products of the corpora lutea are removed from the ovary. Daniel, Muriel, Gale and Pratt (1963) were among the first to show that lymphatic vessels may take part in the transport of hormones to and from endocrine glands. They found that the prominent lymphatic vessels in the testes of normal rats, goats and monkeys became very small and insignificant after hypophysectomy or transection of the pituitary stalk. As a result of these observations they planned further experiments to determine whether it might be a general principle that a proportion of the hormone secreted by an endocrine organ is carried by the lymphatics draining that organ. They studied the testes, ovary, adrenal and thyroid of baboons, cats and rats. Lymph vessels draining the ovary of the baboons were found to have a large flow of lymph which contained half the amount of oestradiol found in the ovarian vein plasma (the equivalent of 100-200 µg/100ml). Linder (1963) also obtained direct evidence for the participation of lymphatic drainage in the transport of gonadal hormones by identifying testosterone and androstenedione in the testicular lymph of the ram. Subsequently, Linder et al (1964) showed

that the rate of lymph flow from ovaries containing an active corpus luteum was much higher per unit weight of tissue, than from any other organ studied in sheep. They also found that out of all the progesterone synthesized by the corpus luteum only about 10% appeared in the lymphatic drainage. However, in recent studies,on the progesterone concentration in the utero-ovarian lymphatics, Staples et al (1982), found that progesterone levels were one to three orders of magnitude higher than those in peripheral plasma when the ipsilateral ovary contained a functional corpus luteum. These results show that lymph vessels are capable of carrying hormones produced in the ovary. $PGF_{2\alpha}$ is believed to be transferred from the uterine venous blood to the ipsilateral ovarian artery by a counter-current mechanism (see page 80). It is also possible that a part of the luteolytic activity leaves the uterus by way of the lymph as suggested by Hansel, Concannon and Lukaszewska (1973). Direct lymphatic connections between the uterus and ovaries have not been observed (see page 97) although lymphatic vessels draining the uterus pass close to the ovarian arterial supply. However, the amount of the uterine luteolytic hormone that might be transported in the lymph in the non-pregnant animal is not known.

The purpose of this study is to examine the availability of prostaglandins in the lymph draining from the uterus at different stages of the oestrous

cycle.

MATERIAL AND METHODS

Cannulation And Collection Of Lymph Samples

Thirteen Dorset Horn X Finnish Landrace ewes used in this experiment were kept under natural day light and checked once daily for oestrus with a vasectomized ram. The ewes were anaesthetized with an intravenous injection of 20 ml alphaxalone and alphadolone acetate mixture (Saffan; Glaxo Laboratories, Green, Middlesex) and maintained with halothane/nitrous oxide mixture. The uterus and ovaries were exposed via a midventral laparotomy and the side of the ovary containing a corpus luteum was selected for cannulation. About 1 ml of 5% Evan`s blue in 0.9% saline solution was injected under the serosa of the uterus, carefully avoiding all visible blood vessels. The dye was taken up into the lymph and the coloured lymph vessels were quickly and easily visible. One of the lymph vessels draining the portion of the uterus proximal to the ovary was selected for cannulation. A polyvinyl cannula (Polyvinyl Chloride, Dural Plastics, Dural, New South Wales, Australia, I.D. 0.28-1.00 mm) filled with heparin/saline mixture (1000 iu/ml), was used; the diameter of the cannula was selected according to the size of the lymph vessel chosen for cannulation. The slightly tapered free end of the cannula was inserted through a small longitudinal incision in the lymph vessel and passed up the vessel as far as possible but at least past the first set of

valves. The cannula was considered patent if coloured lymph was seen passing down its lumen. The tip of the cannula inside the lymph vessel was then ligatured in place and anchored. The uterus and ovaries were handled as little a possible during the cannulation. The free end of the cannula was passed into a sterilized container in which the lymph was collected by free-flow. Lymph sample collection did not commence until all the heparin/saline mixture had been displaced from the cannula and lasted between 30 minutes and 1 hour. Immediately after collection, the lymph samples were centrifuged and stored at -20°C. Twenty one samples of uterine lymph were collected in this way.

MEASUREMENT OF PROSTAGLANDINS IN LYMPH:

Prostaglandin $F_{2\alpha}$ and PGE_2 were extracted from the lymph by the method of Blatchley and Poyser (1974). For extraction duplicate aliquots of lymph (e.g. 200 µl) were taken from each sample and dispensed into extraction tubes. To each tube was added 100 µl pH4 citrate buffer and mixed in a "Whirlimix". Redistilled ethyl acetate (2 ml) was then added and all the samples vortex mixed for 5 mins. The lymph was then frozen using a freezing mixture consisting of dry-ice in acetone and the ethyl acetate fractions were taken off. This process of extraction was repeated and the ethyl acetate fractions were pooled and evaporated to dryness in a stream of air. This one-step extraction procedure gave >90% recovery of $PGF_{2\alpha}$ and PGE_2.

a) $PGF_{2\alpha}$ Assay

The procedure of radioimmunoassay of $PGF_{2\alpha}$ in lymph was the same procedure as in plasma using the same antibody (see page 54). However the dilution of antibody used was 1:1600. NRS was 1:140 while DARS was 1:10.

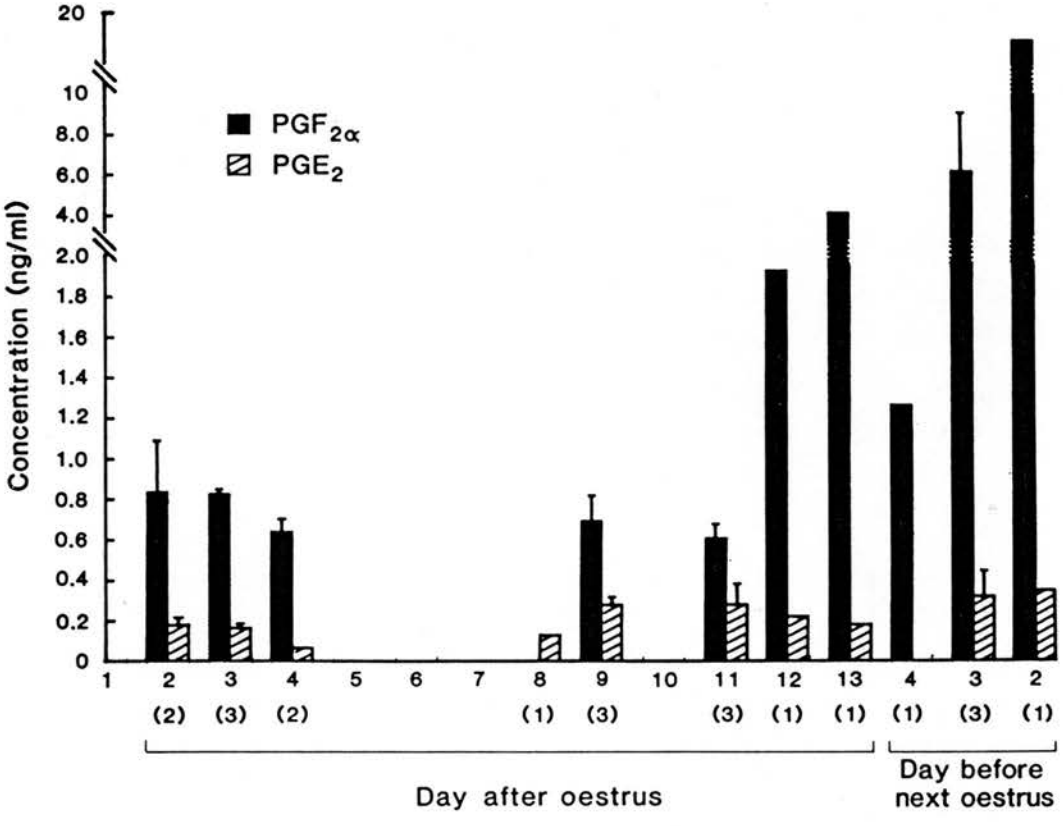
b) PGE_2 Assay

PGE_2 in lymph was measured by radioimmunoassay using antiserum purchased from the Pasteur Institute, Paris. Other prostaglandins and prostaglandin metabolites have only low cross-reactivity with this PGE_2 antibody (Poyser & Scott 1980 and appendix 2). The dilution of antibody used was 1:400 in diluent A (page 54) After a 2 hr incubation of standards and samples 50 ul of NRS (1:140) and DARS (1:15) were added to each tube and the contents of each tube were mixed and incubated for 16 hrs at $4^{\circ}C$. The procedure was then as for $PGF_{2\alpha}$ (see page 55) except that the tubes were put in glass vials and counted for 10 mins in the liquid scintillation counter. Two tubes containing a known quantity of prostaglandin in 0.1 ml diluent were also added to check the intra-assay coefficient of variation. All the PGE_2 samples were measured in a single assay . The intra-assay coefficient of variation was 11.8% in a single assay.

RESULTS

The mean concentration (ng/ml) of $PGF_{2\alpha}$ and PGE_2 in lymph samples from different days of the oestrous cycle of ewes are shown in Fig. 24. All the animals had normal

Figure 24: The mean (±SE) concentration of $PGF_{2\alpha}$ (ng/ml) and PGE_2 (ng/ml) in lymph samples collected from 13 ewes during different days of the oestrous cycle. All the ewes have normal length oestrous cycle (16-19 days). The number of samples is given in brackets under the base line.


Concentration (ng/ml)
20
10
8.0
6.0
4.0
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
PGF2α
PGE2
1 2 3 4 5 6 7 8 9 10 11 12 13 4 3 2
(2) (3) (2) (1) (3) (3) (1) (1) (1) (3) (1)
Day after oestrus
Day before next oestrus

17-19 day cycles during the period of sampling. The mean concentration of $PGF_{2\alpha}$ remained low (<1 ng/ml) until day 12 of the oestrous cycle. Increased amounts of $PGF_{2\alpha}$ ($p< 0.001$) were present in uterine lymph from day 12 onwards. The highest concentration recorded was 16.9 ng/ml in a sheep two days before it returned to oestrus. These levels are comparable to those in uterine venous blood (see page 68). On the other hand, there was no significant variation in the concentration of PGE_2 in the lymph during the oestrous cycle. PGE_2 was in the range 0.07-0.43 ng/ml. The flow rate of lymph varied from 0.25 to 2.0 ml/hr and was not related to the stage of the oestrous cycle.

DISCUSSION

The results of this experiment demonstrated that $PGF_{2\alpha}$ is present in uterine lymph and increases about day 12. This increase in $PGF_{2\alpha}$ on day 12 coincides with the beginning of luteal regression and the decline in progesterone secretion (Baird et al 1976). Thus at the time of luteal regression in the normal oestrous cycle $PGF_{2\alpha}$ is present in high concentrations in both the venous blood (McCracken et al 1971) and in the lymph draining the uterus. The present results also indicate that lymphatic PGE_2 concentrations are low throughout the cycle. The absence of any significant changes in PGE_2 levels during the time of regression of the corpus luteum suggests that this compound is not involved in luteolysis and that the elevated levels of

$PGF_{2\alpha}$ did not result from excessive trauma during sampling.

Prostaglandin $F_{2\alpha}$ is known to be secreted into the uterine venous blood in a pulsatile manner (Thorburn et al 1973; Nett et al 1976; and page 69). It is thus difficult to draw extensive conclusions about the role of $PGF_{2\alpha}$ concentrations in uterine lymph vessels when only single samples were taken from each ewe. Obviously, it is important to clarify the pattern of prostaglandin $F_{2\alpha}$ secretion in lymph and hence the role of uterine lymphatic circulation in the contr_ol of the ovine oestrous cycle. To this end a technique for continuous sampling using chronic cannulation was devised.

$PGF_{2\alpha}$, PGE_2 AND STEROID CONCENTRATIONS IN SEQUENTIAL SAMPLES OF UTERINE LYMPH

Introduction

The results of the preceding experiment have established that lymph vessels draining the uterus carry $PGF_{2\alpha}$ in concentrations comparable to uterine venous plasma levels. The increased concentration of $PGF_{2\alpha}$ in uterine lymph from day 12 onwards coincides with the stage of the oestrous cycle during which regression of the corpus luteum occurs . However, the uterine lymph samples were collected as one-off sample to establish the presence of $PGF_{2\alpha}$. The development of a technique of continuous sampling of uterine lymph to establish whether the episodic pattern of uterine $PGF_{2\alpha}$ secretion into venous blood also applies to the lymph was thus

necessary. Progesterone and oestradiol-17β concentrations were measured simultaneously to establish the temporal relationship between their concentrations and those of $PGF_{2\alpha}$ in lymph.

Methods

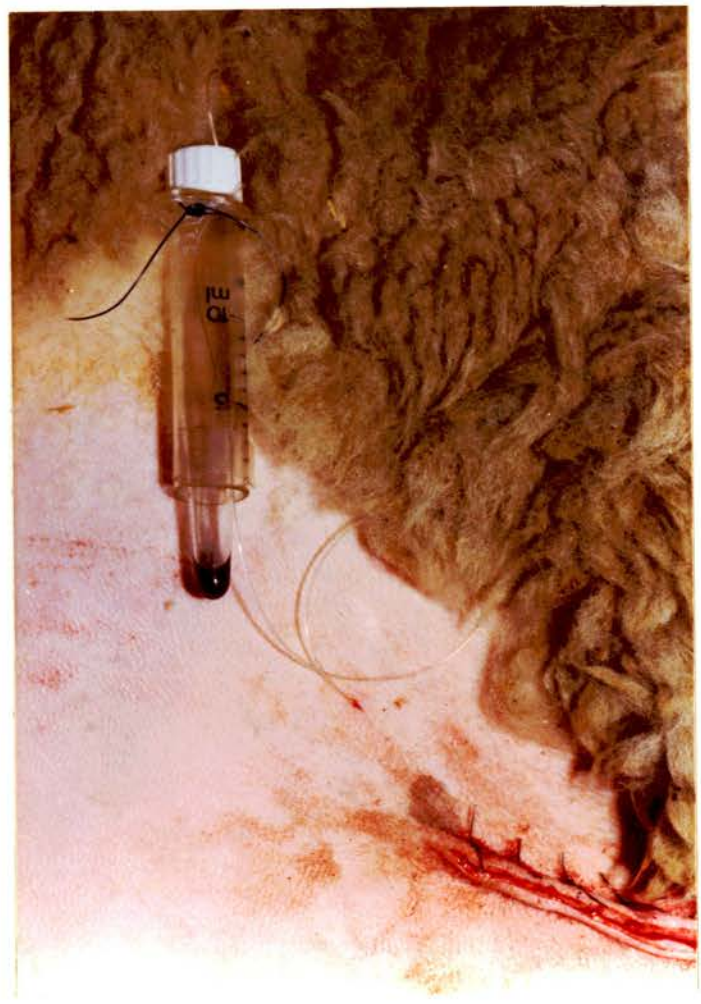
Dorset Horn X Finnish Landrace ewes were checked daily for oestrous activity and only those animals exhibiting at least two consecutive oestrous cycles of normal duration were assigned to this experiment. Thirteen such animals were used. The ewes were anaesthetized with Halothane/nitrous oxide on various days of the oestrous cycle and subjected to laparotomy. Lapraotomy in these animals was made in the axil region of the left leg, giving a good exposure of the uterus right down to the fat surrounding the origin of the utero-ovarian vein. Following exposure of the uterus, 5% Evan's blue was injected subserosally into the uterine horn ipsilateral to the ovary containing a CL. One of the large uterine lymph vessels either close to the main uterine artery or at a point prior to the joining of the uterine and ovarian branches of the utero-ovarian vein was selected for cannulation - this ensured that only lymph of uterine origin was collected. This lymphatic vessel was dissected free from surrounding tissues for a short distance and two ligatures of nylon (Ethicon 186; Ethicon Ltd, Edinburgh) were placed, one above and one below the proposed site of incision. Moderate tension on the ligature below the site of incision was applied to

stop lymph flow in the vessel and increase its size. A small longitudinal slit was then made in the lymph vessel wall with a pointed scalpel. A cannula with as large a bore as possible (ID 0.28-1.00 mm) was then inserted into the vessel lumen and passed up the vessel towards the uterus at least past the first set of valves. Once the lymph flow through the cannula had started the second ligature was tied over the lymph vessel and cannula. The cannula was then anchored to the mesometrium and uterus by 3 or more sutures and exteriorized through an opening in the skin and directed into a sterilized collecting tube containing heparin/saline mixture (0.2 ml) hanging on the left flank (see Fig. 25). During cannulation as much of the uterus as possible was kept within the animal, in order to avoid oedema. The abdomen was then closed and the animal allowed to recover. Postoperatively, the animals received an intra-muscular injection of 900 000 iu Penicillin (3 ml Duphapen, Duphan Veterinary Ltd., Southampton, U.K). Following surgery the animal was penned alone and kept under supervision overnight, and then returned to its own pen the following morning. Sample collection started immediately after surgery and continued at 4-5 hourly intervals (throughout the 24 hrs) until lymph flow ceased. At each sampling the whole contents of the collecting tube were removed through a siphon tube down one side of the collecting tube and measured to monitor the flow rate. Samples were

Figure 25:

A- Photograph of the left rear flank region of ewe A51 showing the external appearance of a lymphatic cannulated preparation.

B- Photograph showing the change in the colour of the lymph during sampling. Introduction of Evan`s blue at surgery caused the dye to be lost gradually from the tissue in the lymph over many hours. The lymph appeared dark blue immediately following cannulation, became progressively paler as lymph flow progressed and finally became pale brownish when all the dye had drained away.



immediately taken back to the laboratory where they were centrifuged and stored at -20°C. If the lymph stopped flowing the cannula was carefully and gently flushed with heparin/saline.

Hormone Assays

$PGF_{2\alpha}$ and PGE_2 were extracted from the lymph and measured by radioimmunoassay using the double antibody technique as shown on page 53 .

Progesterone and oestradiol-17β were extracted from the lymph samples the same way as for blood and measured by the double antibody technique shown on page (59) and (63) respectively.

Results

Of the 13 ewes in which lymph vessel cannulation was attempted, it was possible to collect regular lymph samples from 9 only. In these chronic preparations flow continued for a period of up to 5 days. Lymph flow was usually highest in the immediate post-operative period and became reduced shortly before catheter occlusion. In the intervening period flow rates were fairly stable and showed no consistent diurnal varation. The mean flow rate was 0.79 ± 0.4 ml/hr (mean $\pm$ S.E.M.). Due to the introduction of Evan's blue at surgery there was a steady change in lymph colour during sampling (see Fig. 25). The lymph appeared dark blue immediately following cannulation, became progressively paler as lymph flow progressed and finally became pale brownish when all the dye had drained away. This colour change was useful in

verifying that it was definitely uterine lymph that was being collected.

The mean concentrations (± S.E.) of $PGF_{2\alpha}$ and PGE_2, progesterone and oestradiol-17β in the lymph are shown in Fig. 26 . The mean concentration of $PGF_{2\alpha}$ was low until the first half of day 10 when the amounts increased significantly ($p < 0.001$) until the day before oestrus. PGE_2 levels during the cycle were lower than those of $PGF_{2\alpha}$ and more stable, and there was no significant changes during the time of corpus luteum regression. The secretion of progesterone started to decline from day 12 onwards. However, progesterone levels in lymph did not decline to the same very low levels as seen in venous blood even at oestrus. Oestradiol-17β concentrations in uterine lymph did not show any regular pattern, and the conentrations were higher than in either peripheral or uterine venous blood.

The frequent sampling of $PGF_{2\alpha}$ in the uterine lymph showed that the concentration of this hormone fluctuated considerably. Complex series of peaks were apparent especially around the time of luteal regression. Sequential samples from 3 animals illustrating this point are shown in fig. 27.

In two sheep, where the lymph flow stopped immediately following surgery, the cannula was reexamined by laparotomy. Examination of the cannula revealed that lymph had flowed to about 3/4 of the

Figure 26: Pooled mean (±SE) concentrations of $PGF_{2\alpha}$ (ng/ml), PGE_2 and oestradiol-17β (pg/ml) in the uterine lymph throughout the oestrous cycle from sequential samples in 9 ewes. Samples of uterine lymph were taken every 4 or 5 hours following cannulation of a uterine lymph vessel. All the ewes had normal oestrous cycles (16-19) days.

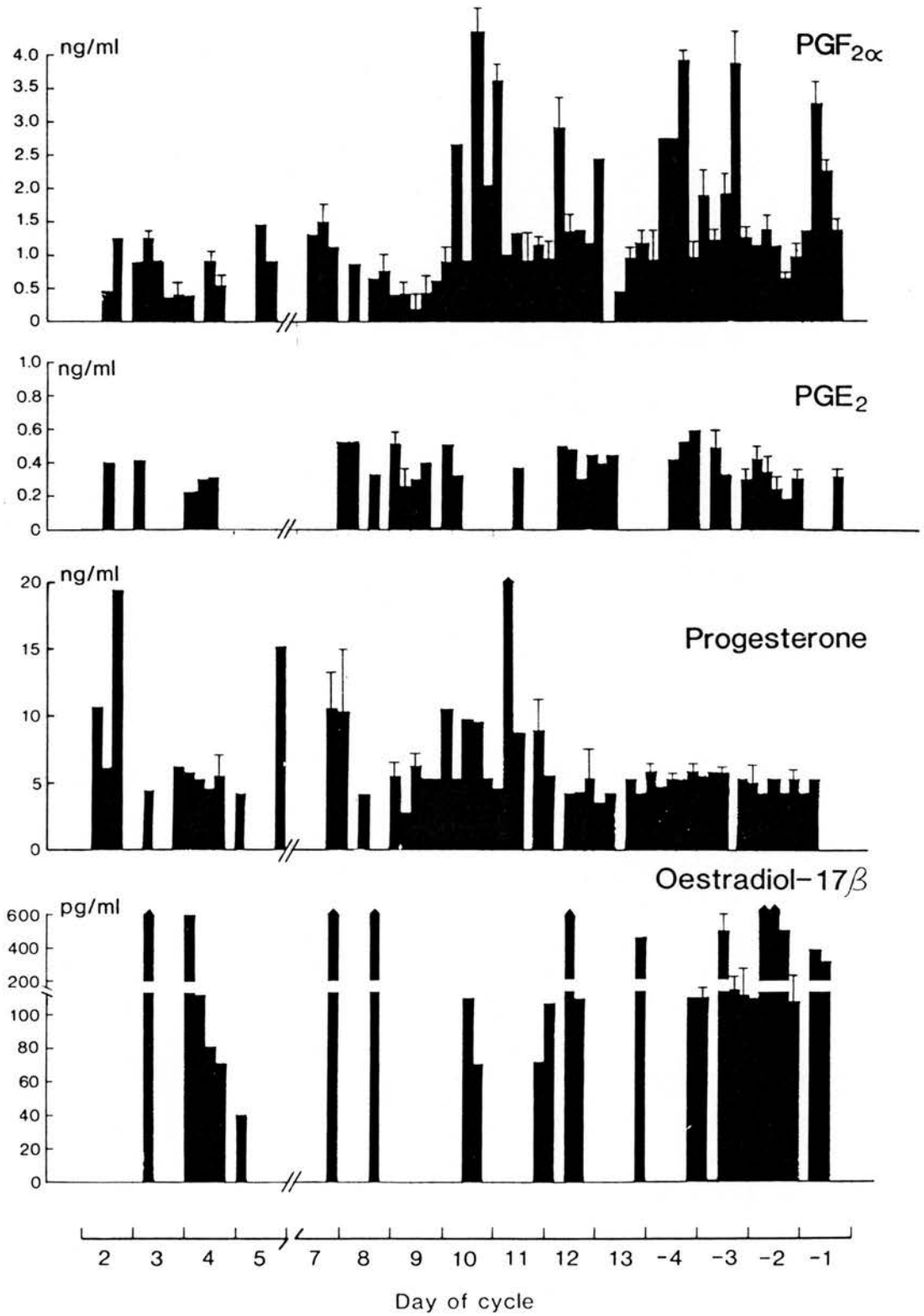
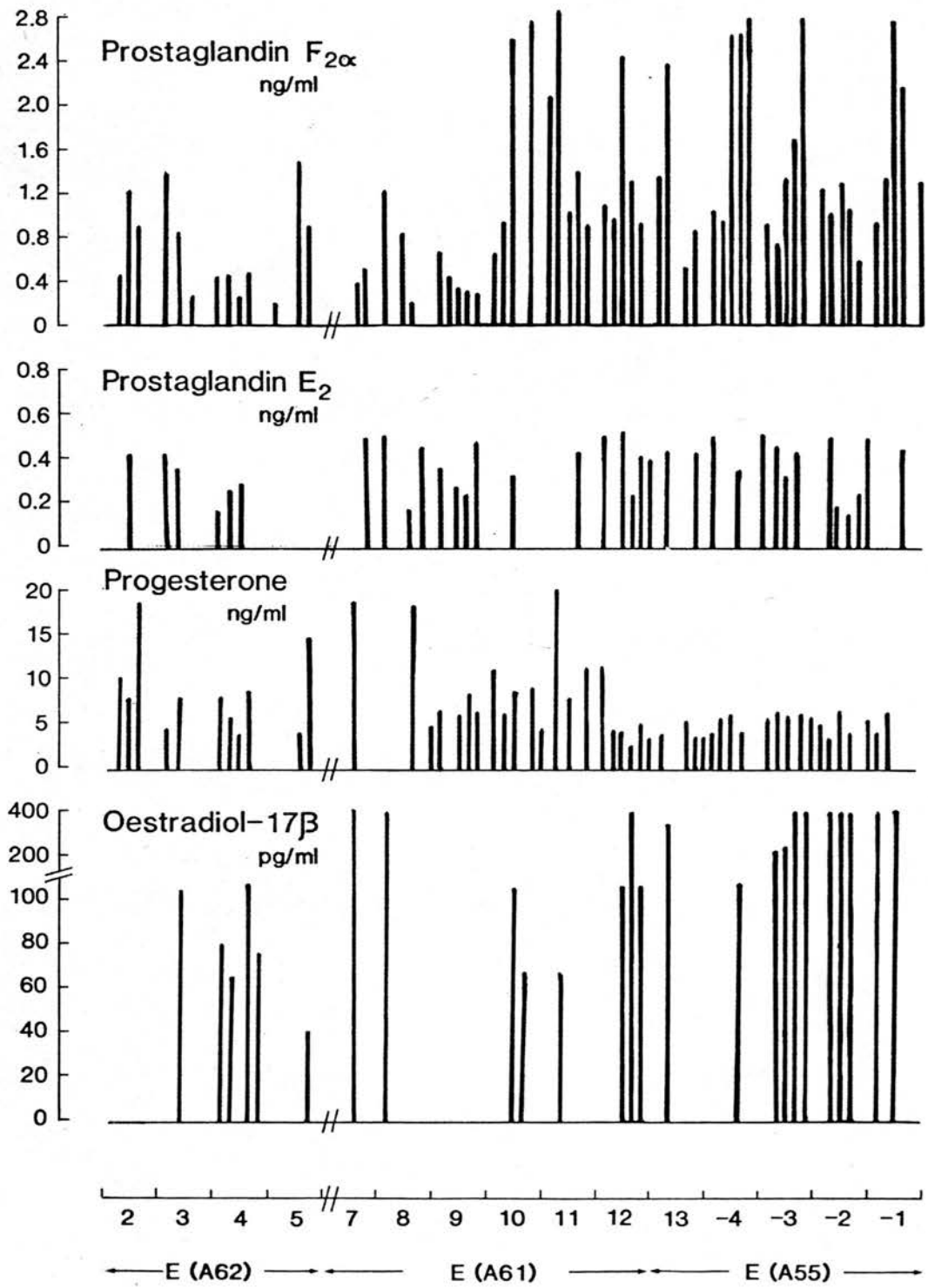

ng/ml
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
PGF2α
ng/ml
1.0
0.8
0.6
0.4
0.2
0
PGE2
ng/ml
20
15
10
5
0
Progesterone
Oestradiol-17β
pg/ml
600
400
200
100
80
60
40
20
0
2 3 4 5 7 8 9 10 11 12 13 -4 -3 -2 -1
Day of cycle

Figure 27:

Concentrations of $PGF_{2\alpha}$, PGE_2, progesterone and oestradiol-17β in uterine lymph in three ewes plotted on the same axis. Ewe A62 was sampled every 5 hrs from day 2 to 5 while ewe A61 and A62 were similarly sampled from day 7 to 12 and day 13 to -1 respectively.


Prostaglandin $F_{2\alpha}$
ng/ml
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0
Prostaglandin E_2
ng/ml
0.8
0.6
0.4
0.2
0
Progesterone
ng/ml
20
15
10
5
0
Oestradiol-17β
pg/ml
400
200
100
80
60
40
20
0
2 3 4 5 7 8 9 10 11 12 13 -4 -3 -2 -1
E (A62)
E (A61)
E (A55)

cannula and stopped. However, it was still in fluid form which indicates that the lymph had stopped flowing rather than clotted. However it was noticed that at the end of a normal flow of several days , a thread like "clot" forms at the tip of the cannula that with time extended back down the tube. When this was pulled out the flow started up again. The "clot" seemed to be composed of a loose mesh of filamentous material. Attempts to flush the cannula with heparin/saline mixture to restore of lymph flow were succesful in two sheep, and the lymph flow was restarted.

Discussion

The results of this experiment confirm those of the previous experiments, namely that $PGF_{2\alpha}$ is present in lymph vessels draining the uterus. The concentration of $PGF_{2\alpha}$ in lymph vessels was comparable to those reported for uterine venous blood. Moreover, there is a significant increase in $PGF_{2\alpha}$ concentrations towards the end of the oestrous cycle at the time when luteal regression takes place. However, the fact that PGE_2 concentrations did not show significant changes during the oestrous cycle confirms that the changes in $PGF_{2\alpha}$ levels were not caused by inflammatory changes in the uterus. The great variation in $PGF_{2\alpha}$ levels found in uterine lymph suggests that the release of this hormone into the lymph may also be episodic in nature.

In sheep as in several other species, the uterus causes regression of the corpus luteum by production of

a luteolytic substance which passes from one uterine horn to the adjacent ovary through a local unilateral pathway. It has been proposed that the local pathway involves discharge of the uterine substance, $PGF_{2\alpha}$, into the uterine venous effluent (see page 80). Prostaglandin $F_{2\alpha}$ then passes from the utero-ovarian vein into the closely adherent ovarian artery by a counter-current mechanism. The present study however, raises the possibility that a similar counter-current transfer system may occur between the uterine lymph and the ovarian arterial blood and so form an additional or even alternative method of tranfer of $PGF_{2\alpha}$ from the uterus to the ovary.

There is no immediate explanation for the high levels of oestradiol noticed in the uterine lymph, but Magness and Ford (1982) in a recent report in pigs suggested that the concentrations of oestradiol in uterine lymph reflected tissue levels of this steroid, since oestradiol concentrations were found to be greater in uterine tissue than in the systemic circulation.

General Discussion

The control of the oestrous cycle in many mammals has been found to be dependent on the release of $PGF_{2\alpha}$ from the uterus. This release of prostaglandin terminates the life-span of the corpus luteum. No single factor or mechanism has been identified as yet to fully explain what causes the release, and regulates the synthesis of $PGF_{2\alpha}$. Several hormones, mainly steroids and peptide hormones have been suggested to have major roles. However, the endogenous pattern of release and relationships of these hormones in the normal cycling animal itself is incompletely understood. From earlier studies it is known that administration of oestrogen in the presence of progesterone causes an increase in uterine $PGF_{2\alpha}$ production towards the end of the oestrous cycle (see page 33). It was apparent from these studies that ovarian hormones can have various effects on the release of $PGF_{2\alpha}$ from the uterus depending on the method and time of application. The luteolytic effects of oestradiol have been found to be mediated through the uterus, as injections of oestradiol on days 11 & 12 failed to cause luteal regression in hysterectomized ewes (Bolt & Hawk 1972, Denamur & Kan 1973, Bolt & Hawk 1975). Similar studies indicate that progesterone has a similar luteolytic effect, mediated through the uterus (Woody, Ginther & Pope 1968, Woody & Ginther 1968). Many studies also suggested that a period of progestational influence facilitates the luteolytic effect of

oestradiol. Thus oestradiol acts on the progesterone primed uterus to cause the production of the luteolytic hormone at the end of the oestrous cycle (Warren et al 1973, Ford et al 1975, Louis et al 1977). Consequently oestradiol released from the follicle is believed to be the physiological stimulus for $PGF_{2\alpha}$ release from the uterus. In view of this, the endogenous level of oestrogen should increase at the time of $PGF_{2\alpha}$ release. Some workers have investigated the endogenous association between the concentrations of these steroids and prostaglandin. Blood samples were either collected by indwelling catheters in the utero-ovarian vein, or with utero-ovarian transplants to the neck (see page 41). The results of these investigations strongly implied a role for oestrogen in luteolysis. However, the temporal relationship of the different hormones during the period of luteolysis is not well documented. A more direct approach to study the concentration of these hormones in the venous effluent of the uterus would be to measure these hormones by frequent sampling of blood. This was the achieved in the present study by performing an end-to-end anastomosis of the utero-ovarian vein to the anterior mammary vein with the adjacent ovary in situ. Thus the uterine venous blood was diverted through the ventral abdominal wall along the anterior mammary vein superificially in the mid-line. The uterine horn on the side of the patent anastomosis was then fistulated to the exterior. Thus collection of simultaneous uterine

venous blood and tissue in the conscious animal was made possible. Surprisingly, such surgical interferences did not interfere with the normal cycling of the animal. Sheep with a patent anastomosis of utero-mammary vein and fistulated uterine horn showed normal oestrous cycles. The effect of the absence or presence of the ovary was examined by Alwachi et al (1979) who found that the presence of an ovary adjacent to the uterine horn was necessary for the normal manifestation of $PGF_{2\alpha}$ synthesizing ability by the endometrium in such a fistulated uterine horn. They suggested that the ovary exerted a local influence over endometrial $PGF_{2\alpha}$ synthesizing ability in the adjacent uterine tissue. In view of this in the present study the uterine branch of the utero-ovarian vein was severed and anastomosed to the mammary vein, and the adjacent ovary was left in situ. Animals with such surgical modifications cycled normally, indicating that $PGF_{2\alpha}$ is being successfully transfered to the adjacent ovary.

The results of the first experiment support earlier reports that an increase of $PGF_{2\alpha}$ towards the end of the oestrous cycle causes luteal regression and decline in progesterone concentration. There were elevations in endometrial $PGF_{2\alpha}$ content, synthesizing ability and release during the period of 30-45 and 3-9 hrs before luteal regression. Oestradiol-17β showed an increase in concentration which was in close association with this increase in $PGF_{2\alpha}$ synthesis and release. A close

relationship between oestradiol and $PGF_{2\alpha}$ release confirms that oestradiol-17β causes the release of $PGF_{2\alpha}$ from the uterus at a time when the uterus is responsive through prior conditioning with progesterone. However, the lack of a consistent increase in either $PGF_{2\alpha}$ content or synthesizing ability of the uterus prior to the increase in $PGF_{2\alpha}$ release indicates that neither of these two parameters are necessary intermediaries between oestradiol-17β increase and $PGF_{2\alpha}$ release.

Another oestradiol-initiated increase in $PGF_{2\alpha}$ release occurs between the decline of progesterone and the occurrence of oestrus. This peak seems to have the role of ensuring the non-recovery of $PGF_{2\alpha}$ synthesis by the corpus luteum. The elevated oestradiol-17β at this time seems to have the dual role of elevating $PGF_{2\alpha}$ release and causing behavioural oestrus of which the latters seems the more important. One sheep failed to exhibit oestrus behaviour even though progesterone concentration declined to a low level. In this animal neither the elevation of oestradiol-17β or $PGF_{2\alpha}$ was present following progesterone decline. An increase in oestradiol release thus appears necessary as the stimulus for an increase in $PGF_{2\alpha}$ release. Evidence that the amount of synthetase enzyme is critical to the amount of prostaglandin produced was reported by Land, Tellier, Rome and Vanderhoek (1973), who found that when studying a partially purified prostaglandin synthetase enzyme complex from sheep vesicular glands, synthesis of

the product stopped before all the precursor was used up. However, the reaction restarted when more enzyme was added, leading them to suggest that the enzyme is destroyed or deactivated during the course of the reaction. This is consistent with the work of Barcikowski et al (1974) who examined the effect of exogenous oestradiol-17β on $PGF_{2\alpha}$ secretion by infusing small amounts of the hormone into the arterial supply of autotransplanted uterus of sheep at different times of the oestrous cycle. It was observed by these authors that oestradiol-17β has no effect on $PGF_{2\alpha}$ secretion until day 14, when there was a 50-100% fold increase in $PGF_{2\alpha}$ in response to oestradiol-17β. This effect could be decreased by indomethacin, which suggests that oestradiol-17β promotes de novo synthesis of $PGF_{2\alpha}$ rather than simply releasing the stored material, an observation which is in keeping with the current results. More recently, Smith et al (1979) have suggested from their investigations that increased $PGF_{2\alpha}$ release depends on an increase in the efficiency with which arachidonate is converted to $PGF_{2\alpha}$ precursor rather than on the availability of the substrate. Other than these works, the pathway by which endogenous steroids control the release of $PGF_{2\alpha}$ in sheep has not been investigated but is probably similar to that in guinea-pig. In the guinea-pig recent studies by Poyser and coworkers (Poyser & Maule Walker 1979, Leaver & Poyser 1981, Poyser 1983a,b, Downing &

Poyser 1983, Poyser & Bryden 1933) indicate that oestradiol acting on a progesterone primed uterus is the optimum stimulus for increasing $PGF_{2\alpha}$ secretion. This hormone combination causes an increase in uterine $PGF_{2\alpha}$ secretion probably by increasing the intracellular Ca^{2+} concentration. Microsomal phospholipase A_2 (PLA_2) in the endometrium is maximally active at 7 mM Ca^{2+}, a concentration which is higher than the normal intracellular Ca^{2+} concentration.

The increase in oestradiol from the ovary on day 11 has been suggested as the cause of this increase in intracellular free Ca^{2+} by acting on the progesterone primed uterus, thereby "switching on" endometrial $PGF_{2\alpha}$ synthesis. Subsequent investigations suggest that intracellular Ca^{2+} concentrations may be more important in controlling the activity of PLA_2 than the absolute activity of the enzyme in controlling the supply of arachidonic acid for endometrial $PGF_{2\alpha}$ synthesis (Downing & Poyser 1933). It has also been found that the endometrial prostaglandin synthetase and PLA_2 activities are higher towards the end of the cycle so that once PLA_2 has been activated a greater quantity of arachidonic acid is released, a greater percentage of which is converted to $PGF_{2\alpha}$. Oestradiol has been found responsible for the increase in prostaglandin synthetase activity, with the majority of the PGH_2 synthesized being directed into the $PGF_{2\alpha}$ forming pathway.

An alternative hypothesis by which exogenous and

endogenous steroids could participate in $PGF_{2\alpha}$ release is perhaps through tissue accumulation. It has been found by Louis et al (1977), that the injection of oestradiol intravenously increased the concentration of this hormone in uterine tissue to 50-100 times greater than the mean plasma concentration. Similar treatment of progesterone also increased the progesterone content in both caruncular and intercaruncular areas. Furthermore Pope, Maurer and Stormshak (1932), found that reproductive tissues (uterine horn, broad ligament and uterine artery) adjacent to the ovary bearing the corpus luteum contained significantly greater quantities of progesterone than more distal tissues. However it is difficult to understand how side differences in tissue concentrations of steroids can exist when exposure of tissues on both sides is via the circulatory system. However, Walsh, Yutrzenka and Davies (1979) suggested that a local counter-current exchange of these steroids between the utero-ovarian vein and the ipsilateral ovarian artery (and vice versa) might provide for these side differences. Increased local concentrations of these steroids may also involve the lymphatic system, as the concentration of these steroids in the lymph may greatly exceed those detected in ovarian venous blood as shown by Linder et al (1964). Consequently exchange of steroids from the lymph to the ovarian artery could increase the quantity of steroids reaching the tissues of the reproductive tract proximal to the ovary. In this

context, it was also clear from the findings of Alwachi et al (1979) that removal of an ovary influenced the physiological status of the ipsilateral uterine horn. Accumulation of steroids in the uterine tissues would also explain the earlier findings of Fairclough et al (1976) who immunized ewes with oestradiol-17β antiplasma from days 13-17 of the cycle and failed to stop luteal regression and fall in progesterone levels. It is possible that oestradiol produced earlier in the cycle might therefore have accumulated in uterine tissues, in significant quantities, that synthesis of endometrial $PGF_{2\alpha}$ was "switched on" normally resulting in luteal regression. Obviously, it would be more interesting to conduct further investigations of the changes in uterine tissue steroids during the oestrous cycle, and its role in $PGF_{2\alpha}$ synthesis and release. Further work is also required to clarify whether oestradiol-17β (with progesterone) is the only stimulus involved in $PGF_{2\alpha}$ release mechanisms, especially in view of the recent work suggesting that uterine $PGF_{2\alpha}$ release may also be controlled by oxytocin. It has been suggested that oestradiol potentiates oxytocin-induced $PGF_{2\alpha}$ release by inducing an increase in the number of endometrial oxytocin receptors (see page 20). It has been demonstrated by McCracken et al (1978) that, infusion of oestradiol into the arterial supply of the ovariectomized sheep with autotransplanted uterus results in an increase in uterine sensitvity to oxytocin within 6 hrs.

Progesterone treatment on the other hand resulted in the uterus becoming refractory to oxytocin treatment and blocked the oestradiol-17β increase in uterine sensitivity to oxytocin . However, after 10 days of progesterone treatment, oestradiol treatment regains its ability to stimulate oxytocin receptor formation and the uterus releases 50-100 fold more $PGF_{2\alpha}$ than when oestradiol-17β is infused alone. These results suggest that an interaction between progesterone, oestradiol and oxytocin form the physiological stimulus for $PGF_{2\alpha}$ release from the uterus in the sheep. However with the new evidence that oxytocin is being produced by the ovary, it is difficult to see whether oxytocin has a selective effect on the adjacent uterine horn, or through the general circulation where both uterine horns receive equal effects. Thus further investigation involving frequent measurements of endogenous oxytocin concentrations simultaneously with oestradiol, progesterone and $PGF_{2\alpha}$ are required. The sheep is a good model for monitoring the utero-ovarian vascular hormones, and full exploitation of the experimental procedures used in part I, in which utero-ovarian vein blood can be collected from the mammary vein through anastomosis, with the adjacent uterine horn fistulated can be made. Such a preparation can facilitate frequent monitoring of these hormones and thus help in solving this complex problem.

The second part of this thesis consists of

experiments performed by the author which extend our understanding of utero-ovarian pathway for $PGF_{2\alpha}$ and the role of uterine lymphatic drainage in this pathway. As mentioned previously $PGF_{2\alpha}$ is believed to be transferred locally from the uterine vein the ipsilateral artery by a counter-current mechanism (see page 80). However, several investigations have failed to demonstrate that this is the only pathway by which $PGF_{2\alpha}$ passes from the uterus to the ovary. In view of this it was decided to investigate further whether the luteolytic hormone, $PGF_{2\alpha}$, passes solely via the blood of the utero-ovarian vein. Part of the uterine branch of the utero-ovarian vein was replaced with a glass cannula. All the remaining tissue connections between thhe uterine horn and the adjacent ovary were either severed or ligated tightly. Thus all the connections between the uterus and adjacent ovary were removed except for uterine venous blood. This procedure caused the oestrous cycle length of the sheep to be extended, indicating that the uterine vein blood is <u>not</u> the only media whereby a luteolytic factor can pass from the uterus to the ipsilateral ovary. Consequently the existence of a utero-ovarian transfer system that did not involve the blood vascular circulation was apparent.

An obvious alternative candidate that could have a role in the utero-ovarian $PGF_{2\alpha}$ pathway is lymph. In fact the possibility of $PGF_{2\alpha}$ transfer from the uterine lymphatics to the ovarian vasculature has been suggested

by several earlier investigators (see page 88). Examination of the literature indicated that the information available on uterine lymphatic vessels is rather sparse and sometime contradictory. Consequently it was necessary to examine the anatomy of the uterine lymphatic drainage in sheep. The results of preliminary investigations indicated that the dye (Evan`s blue) could be applied subserosally for the identification of lymph drainage in the uterus. Injection of the dye into the upper half of one uterine horn revealed several small lymphatic vessels that emerged to join together to form main ducts. However, there was much variation between sheep in the arrangement, number and size of uterine lymphatic vessels. These lymph vessels were not involved in the ovarian pedicle, but were parallel to it, Moreover there was no direct flow of lymph between the uterus and ovaries. Similar findings were reported by Cicmanec (1973). However, in some of the animals examined, a substantial part of the utero-ovarian vasculature between the junction of uterine and ovarian branches of the utero-ovarian vein and the posterior vena cava was available for more detailed examination. These revealed a close association between the lymphatic ducts draining the uterus and the utero-ovarian vein and ovarian artery. Previously it has been suggested that, $PGF_{2\alpha}$ is transferred from the utero-ovarian vein to the ovarian artery by a counter-current system (see page 83). The present results suggest that the main uterine

lymphatic trunks are also in close association with the ovarian artery at the proposed site of the counter current system. Thus lymph could be an alternative pathway for $PGF_{2\alpha}$. However, in the absence of quantitative information on prostaglandin levels in the uterine lymph in the cycling sheep this conclusion would be viewed with scepticism.

The availability of prostaglandins in the lymph draining from the uterus at different stages of the oestrous cycle was thus studied. Consequently, single lymph samples were collected from sheep on different days of the oestrous cycle. It was found that $PGF_{2\alpha}$ in uterine lymph was available in amounts and at times comparable to those in uterine venous plasma. There was an increase in $PGF_{2\alpha}$ on day 12, coinciding with the beginning of luteal regression and decline in progesterone secretion. Thus at the time of luteal regression in the normal oestrous cycle $PGF_{2\alpha}$ is present in high concentrations both in the venous blood and the lymph vessels draining the uterus. Since $PGF_{2\alpha}$ is known to be secreted from the uterus in a pulsatile manner (Thorburn et al 1972; Baird et al 1976; Barcikowski et al 1974), it was important to examine the pattern of $PGF_{2\alpha}$ release into uterine lymph. Conscious sheep were necessary for more frequent sampling of uterine lymph and indwelling polyvinyl cannulae were placed in the lymph vessels draining the uterus. This procedure allowed regular sampling of uterine lymph in the

conscious animal and permitted assessment of the amount of $PGF_{2\alpha}$ and steroids in the uterine lymph. $PGF_{2\alpha}$ appears to be released in an episodic pattern into lymph by the uterus. This is consistent with the nature of release of $PGF_{2\alpha}$ reported in uterine venous blood (see page 41).

An interesting outcome of the present experiments was the finding of substantial amounts of oestradiol-17β and progesterone in the uterine lymph. Concentrations of these steroids were higher than those reported for peripheral blood. Similar high lymphatic steroid concentrations have been reported earlier by Lindner, Sass and Morris (1964) and Peck, Burgner and Clark (1973). Furthermore, Kotwica et al (1981) collected ovarian steroids from the tissue fluid from the pedicle close to the ovary and from lymph and found high steroid concentrations in both lymph and tissue. The significance of these high levels of steroids in lymph is not clear. It may be a function of the slow rate of circulation in the lymph allowing more time for steroid uptake, or they may reflect an equilibration with high tissue levels of steroids as suggested by Louis et al (1977). The physiological importance of these lymphatic vessels requires further study.

The conclusion from the studies on uterine lymphatic drainage and measurements of $PGF_{2\alpha}$ in lymph is that, uterine lymph <u>is</u> an additional pathway of transfer of uterine $PGF_{2\alpha}$ to the ovary in sheep, besides

the possibility of utero-ovarian venous transfer. As our knowledge on the mechanism of these pathways stands, both pathways are possible routes whereby $PGF_{2\alpha}$ can get from the uterus to the ovaries.

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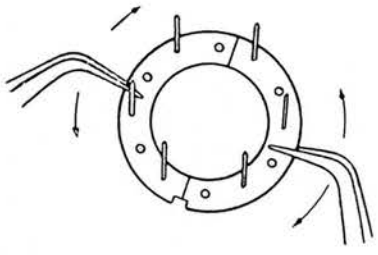
APPENDIX I

Instruction on Nakayama's Instrument for Small Vessel Anastomosis

1) Rings slightly larger than the external diameter of the vessels to be anastomosed are recommended.

2) Before splitting the rings, each ring should be tested to determine if it is properly set in the indentation of the vascular anastomosis clamp (ring-holding clamp) of the corresponding size. (A shallow incision line is made on each ring for easy bisection on use.)

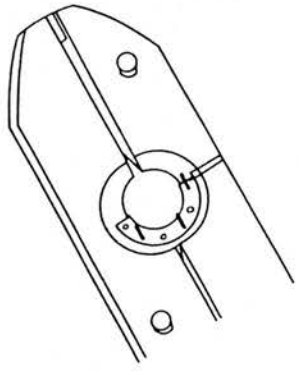
3) Then, the rings are split by a few bending motions with the specially designed forecp (fig. 1.).



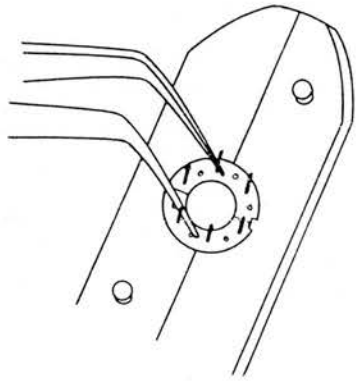
(fig. 1)

4) Separated pieces of each ring should be used as one pair throughout the setting process. Open slightly the vascular anastomosis clamps. One ring half is placed irst in the proximal half of the indentation of the clamp, and the other is placed in the distal half (fig. 2).

It is recommended that the indentation be sustained evenly (horizontally) while setting the halves. The ring is easily cracked away while being manipulated. It is advisable that the setting process be done most gently with our special forcepses on a large aseptic table. After satisfactorily setting the ring, gently close the tips of the vascular anastomosis clamp with applying a forcep over the ring in the indentation to prevent it from falling off (fig. 3.).



(fig. 2)



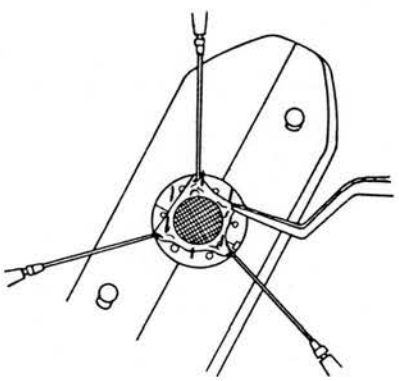
(fig. 3)

If the ring is not properly set, and the two pieces are not on the same exact plane, the setting should be repeated. If after a few attempts the ring is not properly set, gentle filing of the contacting edges of each half usually helps.

5) On completion of the setting, make sure that all the pins are exactly perpendicular to the anastomosis ring plane.

These pins might have been bent during the setting process. If so, these should be carefully straighted out with the special forceps.

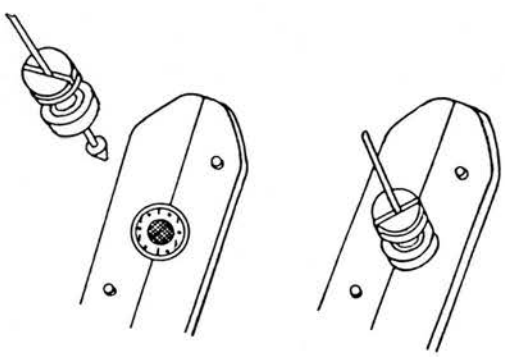
6) The intima of the vessels end is carefully everted and fixed onto the pins with the forcepses and fine hooks. Human vessels are often markedly sclerotic. The following technique is recommended in such circumstances. The vascular end is evenly pulled in outward direction with the three fine hooks evenly spaced on the intima at the vascular end, and the tissue is brought over the anastomosis ring to be hooked to three pins in an equilateral triangle fashion on every other pin. While two of the three points thus hooked are held down with the forcep, the intima between two points is hooked to the unased pin between them. This procedure is carried out on the two remaining unused pins (fig. 4.).



(fig. 4)

Complete eversion of the intima is thus attained with little injury to the intima.

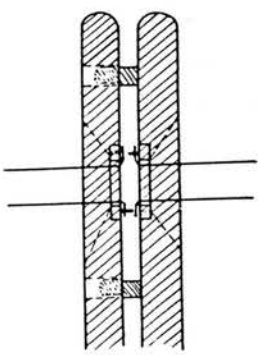
Then the thruster is applied on the vascular end that is everted on the vascular anastomosis ring in order to secure neat and deep fixation of the intima by the pins (fig. 5. a—b)



(fig. 5) a b

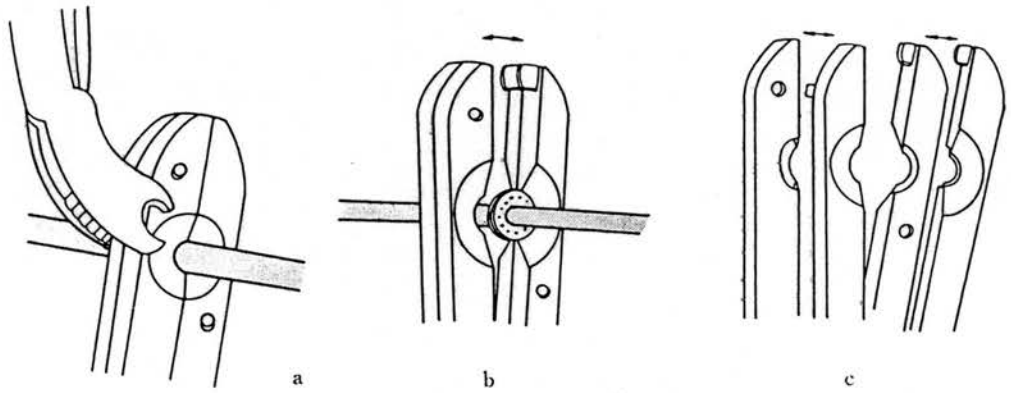
7) After both vascular ends are set on the two anastomosis ring, the anastomosis clamps are joined together by sliding the pins or process of one clamp into the corresponding holes on the other clamp (fig. 6).

8) The two clamps are then pressed on together with a pair of pliers. Pressing should be applied a few times around the ring-set portion to secure perfect cooptation.



(fig. 6)

9) As shown in figure 7, the two clamps should be opened while the upper side of both clamps is being held with the pliers. Then with the release of the clamps the anastomosis is completed (The rings become free from the vessel in a few weeks)

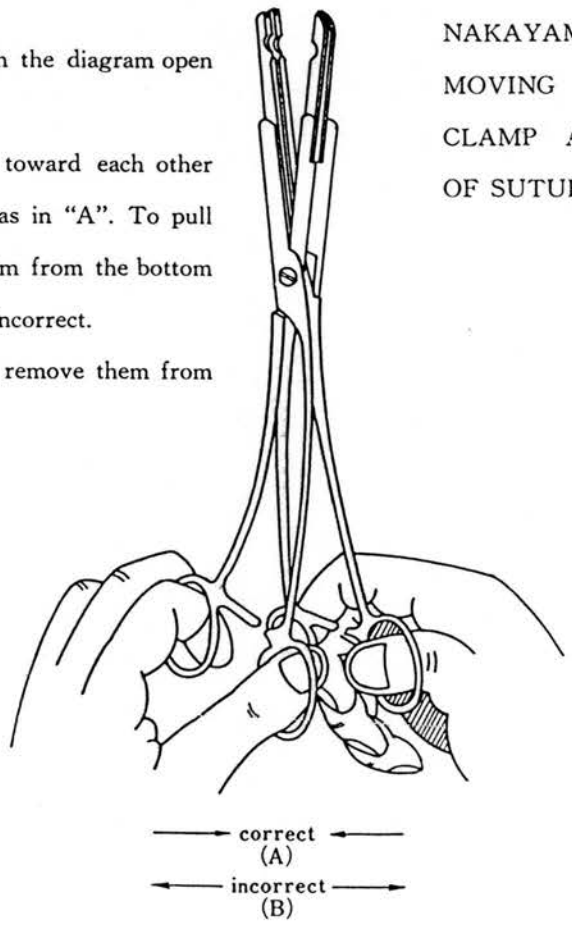


(fig. 7)

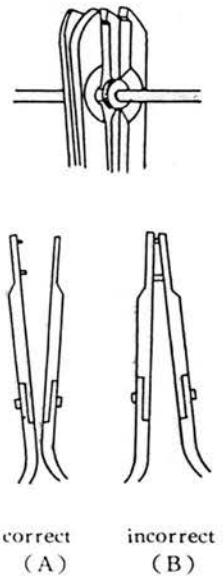
Holding the clamps as in the diagram open the scissors.

Then push the handles toward each other separating the top first as in "A". To pull the clamps separates them from the bottom first as in B. This is incorrect.

After separation simply remove them from the vessel.



NAKAYAMA'S METHOD OF REMOVING THE ANASTOMOSIS CLAMP AFTER COMPLETION OF SUTURE



Appendix II

Cross reactivities of progesterone antibody for radioimmunassay as determined by the method of Dighe and Hunter (1974).

Compound	% cross reactivity
Progesterone	100
Cortisone	0.12
Hydrocortisone	0.21
Estrone	0.03
Testosterone	0.12
Cholesterol	0.02
Estriol	0.11
17α-Hydroprogesterone	0.13
11α-Hydroprogesterone	----
β-Estradiol	0.012
Δ^4-Androstene 3,17-Dione	0.01
11β-Hydroxyprogesterone	0.66
20α-Hydroxy-4-pregnen-3-One	0.20
20β-Hydroxy-4-pregnene-3-One	0.066
Δ^5-Pregnene-3β-ol-20-One	0.054

Appendix III

Cross reactivities of Oestradiol-17β antibody as determined by D.W. Davidson, Unit of Reproductive Biology (1982)

Compound	% cross reactivity
Estradiol-17β 1,3,5(10)-Estratriene-3,17β-diol	100
Estradiol-17α 1,3,5(10)-Estratriene-3,17α-diol	1.0
Estrone 1,3,5(10)-Estratriene-3-ol-17-one	2.1
Estriol 1,3,5(10)-Estratriene-3,16α,17β-triol	0.001
16 Epiestriol 1,3,5(10)-Estratriene-3,16β,17β-triol	15
Androstenedione Δ^4-Adrostene-3,17,-dione	0.6
Testosterone 17β-Hydroxy-3-oxo-4-Androstene	0.9
P_4, Progesterone 4-Pregnene-3,20-dione	0.7
Cortisol 11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	0.001

APPENDIX IV

Prostaglandins Leukotrienes and Medicine 10: 157-161, 1983

PROSTAGLANDIN $F_{2\alpha}$ AND PGE_2 IN UTERINE LYMPH DURING THE OESTROUS CYCLE IN SHEEP

S.E.A. Abdel Rahim, K.P. Bland and *N.L. Poyser

Department of Veterinary Physiology,
Royal (Dick) School of Veterinary Studies,
Summerhall, Edinburgh EH9 1QH (reprint requests to KPB)

and

*Department of Pharmacology,
University of Edinburgh, 1 George Square,
Edinburgh EH8 9JZ

ABSTRACT

The concentration of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in uterine lymph remained low (<1 ng/ml) during the first two-thirds of the oestrous cycle in the non-pregnant sheep. However increased amounts of $PGF_{2\alpha}$ were present from day 12 onwards. The concentration of prostaglandin E_2 in uterine lymph remained low (< 0.43 ng/ml) throughout the cycle.

INTRODUCTION

The uterus of the sheep is important in the control of ovarian function. A uterine luteolytic hormone (prostaglandin $F_{2\alpha}$) is believed to be transferred from the uterine venous blood to the ipsilateral ovarian artery by a counter-current mechanism (1). It is also possible that a part of the luteolytic activity leaves the uterus by way of the lymph (2). Direct lymphatic connections between the uterus and ovaries have not been described, although several workers have described lymph vessels draining the uterus that pass close to the ovarian arterial supply (3, 4, 5). However the amount of the uterine luteolytic hormone that might be transported in this way in a normal animal is unknown. The present study examines for the first time the availability of prostaglandins in the lymph draining from the uterus at different stages of the oestrous cycle.

METHODS

Dorset Horn X Finnish Landrace ewes were used, kept under natural daylight and fed about 1500 g of hay daily. The ewes were checked once daily for oestrus with a vasectomized ram. The first day of oestrus was taken as day 1 of the cycle. Anaesthesia was induced with an intravenous injection of 20 ml alphaxalone and alphadolone acetate mixture (Saffan; Glaxo Laboratories Ltd., Greenford, Middlesex) and maintained with halothane, nitrous oxide and oxygen mixture. Through a lower flank incision about 1 ml 5% Evan's Blue in 0.9% saline solution was injected under the serosa of the uterus, carefully avoiding all visible blood vessels. The dye was taken up into the lymph and the coloured lymph vessels were easily visible. One of the lymph vessels draining the portion of the uterus proximal to the ovary was cannulated with a poly-vinyl cannula filled with heparin/saline mixture. The free end of the cannula was passed into a sterilized container in which the lymph was collected by free-flow. The flow rate of the lymph varied from 0.25 ml/hr to 2.0 ml/hr and was not related to the stage of the oestrous cycle. Lymph sample collection did not commence until all the heparin/saline mixture had been displaced from the cannula. Most of the lymph samples were collected from anaesthetized animals. In a few instances the animal was allowed to recover from anaesthesia before the sample was taken. Such samples from conscious animals showed no significant differences from those of anaesthetized animals and so have not been kept separate. Immediately after collection, the lymph samples were centrifuged and stored at -20°C. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and prostaglandin E_2 (PGE_2) were extracted from the lymph by the method of Blatchley and Poyser (6) and the one-step extraction procedure gave >90% recovery of $PGF_{2\alpha}$ and PGE_2. $PGF_{2\alpha}$ was measured by antibodies raised in rabbits in this laboratory, and the double antibody method of separating bound from unbound labelled ligand was used (7). PGE_2 was measured in a similar manner using antiserum purchased from the Pasteur Institute, Paris. $PGF_{1\alpha}$ cross-reacts significently (100%) with the $PGF_{2\alpha}$ antibodies; other prostaglandins and prostaglandin metabolites have low cross-reactivity with the $PGF_{2\alpha}$ and PGE_2 antibodies (8). Intra-assay coefficient of variation was 8.19% for $PGF_{2\alpha}$, calculated from duplicate results obtained from each sample assayed, and the inter-assay coefficient of variation was 10.01% using the results obtained for 640 pg $PGF_{2\alpha}$ which was incorporated into each assay. PGE_2 samples measured in a single assay and the intra-assay coefficient of variation was 11.8%.

RESULTS

The mean concentrations (ng/ml) of $PGF_{2\alpha}$ and PGE_2 in lymph samples from different days of the oestrous cycle of ewes are shown in Figure 1. All the animals had normal 17-19 day cycles during the period of sampling. The mean concentrations of $PGF_{2\alpha}$ remained low (<1 ng/ml) until day 12 of the oestrous cycle. Increased amounts of $PGF_{2\alpha}$ ($P<0.001$) were present in uterine lymph from day 12 onwards. The highest concentration recorded was 16.9 ng/ml in a

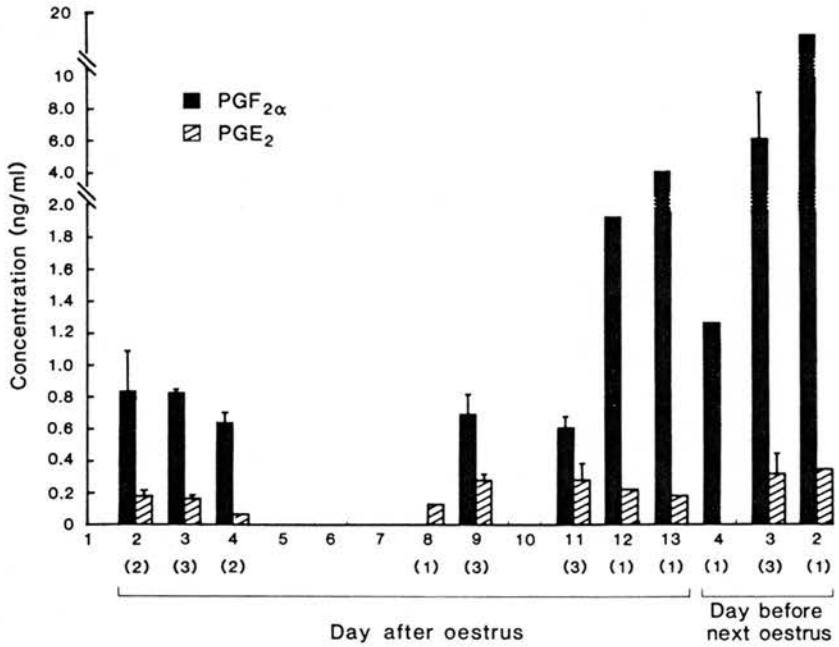


Figure 1. The mean (± S.E.M.) concentration (ng/ml) of $PGF_{2\alpha}$ (Solid bars) and PGE_2 (hatched bars) in lymph samples from different days of the oestrous cycle. Figures in brackets beneath the day of the cycle indicate the number of animals sampled.

sheep two days before it returned to oestrus. On the other hand there was no significant variation in concentration of PGE_2 during the oestrous cycle. PGE_2 values were in the range of 0.07-0.43 ng/ml.

DISCUSSION

The increase in $PGF_{2\alpha}$ on day 12 coincides with the beginning of luteal regression and decline in progesterone secretion (9). Thus at the time of luteal regression in the normal oestrous cycle, $PGF_{2\alpha}$ is present in high concentrations both in the venous blood (1, 9, 10) and the lymph draining the uterus. The possibility of transfer of $PGF_{2\alpha}$ from uterine lymph vessels to the ovarian arterial supply has recently been suggested for both the sheep (5) and the pig (11). The present findings demonstrate that luteal regression coincides with increased concentrations of $PGF_{2\alpha}$ in the uterine lymph, and so such a transfer could be involved in luteolysis. The present results also indicate that lymphatic PGE_2 concentrations are low throughout the cycle. The absence of any significant changes in PGE_2 levels during the time of regression of the corpus luteum suggests both that this compound is not involved in luteolysis, and that the elevated

levels of $PGF_{2\alpha}$ did not result from excessive trauma during sampling. Techniques have now been developed for continuous sampling of uterine lymph and it is hoped that frequent sampling will clarify the role of the uterine lymphatic circulation in the control of the ovine oestrous cycle.

ACKNOWLEDGEMENTS

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