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Estimation And Regulation Of Uterine Prostanoids During Ovine Pregnancy And Delivery

Charles Alexander Evans

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ESTIMATION AND REGULATION OF UTERINE PROSTANOIDS DURING OVINE PREGNANCY AND DELIVERY

by

Charles Alexander Evans

Department of Physiology

Submitted in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

Faculty of Graduate Studies

The University of Western Ontario

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ABSTRACT

The objectives of the work described in this thesis were to delineate major sites of prostanoid (PG) production in pregnant sheep by examining the concentrations of PG in intra-uterine tissues taken at different stages of pregnancy and correlating these with isolated cells prepared from these tissues and with concentration changes in amniotic and allantoic fluids. I examined patterns of output of different PG's *in vitro*, and the variation in PG concentrations within different areas of uterine tissues. The effects of infusing ACTH to the fetal lamb and indomethacin to the mother on patterns of uterine activities and steroid changes were correlated with tissue steroid and PG concentrations during labour and PG outputs *in vitro*. Effects of steroids and protein hormones on PG output *in vitro* were examined in isolated cells. Finally, the sequelae of fetal death, PG concentration and uterine activity changes were examined. The principal findings were:

1) Regional differences in PG concentrations within some intra-uterine tissues in some animals.

2) During normal pregnancy, PG concentrations and PG output *in vitro* from isolated cells were lower on Days 50 and 100 for cotyledons, and on Day 50 for chorioallantois and amnion, than in later stages of pregnancy. In amniotic and allantoic fluids, 6-oxo-$PGF_{1\alpha}$ was the major PG found and concentrations of all PG's were significantly higher on Day 145 than at earlier stages of pregnancy. PG concentrations

in these fluids were significantly correlated with PG concentrations in cotyledons.

3) Labour was initiated after intra-fetal ACTH treatment as indicated by changes in maternal and fetal steroid concentrations, and by increased uterine activity. The tissues with highest PG concentrations were cotyledons and chorioallantois and these high concentrations were correlated with estrone concentrations in these tissues. Concentrations of 6-oxo-$PGF_{1\alpha}$ were elevated in myometrium adjacent to the cervix during ACTH-induced labour and PGF and PGE concentrations were higher at the tubal and cervical ends of the myometrium in all animals. Concentrations of PGE in endometrium and of PGF and 6-oxo-$PGF_{1\alpha}$ in cotyledons and chorioallantois were elevated during ACTH-induced labour. Indomethacin, given to ACTH-treated animals, reduced PG concentrations, the degree of cervical dilation and the frequency of uterine contractions but did not affect the maximum amplitude of contraction, myometrial cyclic AMP or plasma steroid concentrations.

4) The output of PG's *in vitro* from caruncle cells in response to *in vitro* hormone treatments were examined in ovariectomized, non-pregnant ewes, treated *in vivo* with corn oil or estradiol-17β and/or progesterone for 9 days. The *in vitro* output of PG's was not altered by *in vitro* estradiol-17β, progesterone or relaxin treatments. However, the output of PGE from caruncle cells was increased in cells from ewes treated with estradiol-17β *in vivo* and the output of all PG's examined were decreased following *in vitro* ovine placental lactogen treatment.

5) The concentrations of all PG's were elevated in the myometrium in sheep bearing fetuses which had died 12-26 h previously. The proportions of animals showing uterine activity, however, were elevated only in sheep bearing fetuses which had died 34-72 h previously, and the frequency and maximum amplitudes of contractions in these animals were not different from those observed during ACTH-induced labour.

The results of this study suggest an association between elevated PG concentrations and uterine activity during ACTH-induced labour and labour associated with the presence of a dead fetus. An increase in tissue PG concentrations and in PG output in vitro occurs during the last third of pregnancy. Furthermore, these results suggest that estrogen and ovine placental lactogen may play a role in the regulation of uterine PG production.

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LIST OF ABBREVIATIONS

ACTH	adrenocorticotrophic hormone
BSA	bovine serum albumen
°C	degrees Celsius
C.C.	correlation co-efficient
cm	centimetre(s)
CO_2	carbon dioxide
cyclic AMP	cyclic adenosine 3',5'-monophosphate
d.p.m.	disintegrations per minute
E_1	estrone
E_2	estradiol-17β
g	gravity
h	hour(s)
^3H	tritium
I	iodine
i.m.	intramuscular
IU	international unit(s)
kg	kilogram(s)
l	liter(s)
M	molar
min	minute(s)
mg	milligram(s)
ml	milliliter(s)
mm Hg	millimeters of mercury

mOsm	milliosmole(s)
ng	nanogram(s)
N.S.	not significant ($P > 0.05$)
O_2	oxygen
OPL	ovine placental lactogen (somatomammotrophin)
P	progesterone
P.B.	phosphate buffer
PG	prostaglandin (prostanoid)
pg	picogram(s)
RIA	radioimmunoassay
SEM	standard error of the mean
Tx	thromboxane
µg	microgram(s)
µl	microliter(s)
v	volume
wt	weight

INTRODUCTION

Luukkainen and Csapo (1963) showed that the intravenous infusion of a lipid emulsion into pregnant rats resulted in increased responsiveness to oxytocin, suggesting a role for lipids in the initiation of parturition. The active component in these emulsions was later found to be phosphatidylcholine enriched with linoleic acid. In rats, systemic infusions of arachidonic acid induces parturition (Luukkainen and Csapo, 1963). Subsequent studies have suggested a role for arachidonic acid metabolites (prostanoids) in the final train of events leading to parturition in a variety of species. Although evidence indicates a role for prostanoids (PG's) primarily in the regulation of myometrial activity during parturition, the mechanism of action, site of production and the effects and interactions of different PG's on the uterine events associated with parturition remain uncertain. In addition, the control of PG production throughout pregnancy and during parturition is in doubt. Although a variety of hormones has been shown to influence the production of PG's by intra-uterine tissues, it is not known whether production is inhibited throughout pregnancy, and parturition results from the release from inhibition of PG production, or whether PG production increases during pregnancy and is further stimulated during parturition. Until this enigma is resolved, the precise mechanism by which the fetus and/or mother

controls PG production in intra-uterine tissues during pregnancy and parturition remains purely speculative.

Although this thesis is primarily concerned with the role of PG's during pregnancy and parturition in the ewe, it is important to point out that the events occurring from the activation of the fetal adrenal function (initiation of parturition) to the delivery of the fetus involve the interaction of a number of complex events, of which the involvement of PG's represents but one stepping stone. In addition, it is not known exactly how or where these events start, or how they exert their ultimate action.

The primary goals of this study were: 1) to examine the ability of various intra-uterine tissues to produce PG's during different stages of gestation; 2) to test the hypothesis that PG's are mediators in the initiation of ACTH-induced labour; 3) to investigate the association between PG's and labour following fetal death; and 4) to examine the hormonal control of PG production *in vivo* and *in vitro* in pregnant and non-pregnant uterine tissues.

The literature review is divided into the following headings:

1. Role of the fetus in the control of parturition;
2. Effects of glucocorticoids on placental steroidogenesis;
3. Hormonal changes during pregnancy;
4. Uterine activity; possible role of gap junctions;
5. Factors which may regulate uterine PG production during pregnancy and parturition;
6. Possible roles of PG's during pregnancy; and

7. The possible role of PG's during abortive labour and the delivery of dead fetuses.

Where it seemed relevant, information from species other than the sheep is also included.

SECTION I

LITERATURE REVIEW AND RATIONALE

CHAPTER 1

LITERATURE REVIEW

1.1 Role of the Fetus in the Control of Parturition

It is generally accepted that in sheep, parturition which occurs spontaneously on Day 145-150 of pregnancy, is initiated by the activation of the fetal pituitary-adrenal axis. The observations that pregnancy is prolonged following either hypophysectomy or bilateral adrenalectomy of the fetal lamb *in utero*, and that the intra-fetal infusion of ACTH or glucocorticoids results in premature parturition (Drost and Holm, 1968; Liggins *et al.*, 1973) lend strong support for a fetal role in the initiation of parturition. Evidence indicates that there is maturation of the sensitivity of fetal adrenal glands to trophic hormonal stimulation during late pregnancy in sheep. The fetal adrenal secretes little corticosteroid in response to a 60 min intra-fetal infusion of $ACTH_{1-24}$ before Days 130-135, after which there is an increase in plasma cortisol in response to both exogenous and endogenous ACTH (Liggins *et al.*, 1977). *In vitro* studies indicate that there is an increase in 11β- and 17α-hydroxylase activity in adrenal tissue near term and that these enzymes could be stimulated by ACTH (Anderson *et al.*, 1972). The capacity of the fetal adrenal gland to secrete cortisol is high during early pregnancy, is suppressed during mid-pregnancy and increases again after Day 130 (Glickman and

Challis, 1979; Glickman et al., 1979; Wintour et al., 1975). At present, the mechanism by which fetal adrenal function is reactivated around the time of parturition is uncertain.

1.2 Effects of Glucocorticoids on Placental Steroidogenesis

Evidence suggests that the increase in fetal cortisol influences the onset of labour in sheep through its effects on placental steroidogenesis (Flint et al., 1975a,b). During late pregnancy, the ovine placenta is the major source of progesterone (Edgar and Ronaldson, 1958; Bassett et al., 1969; Heap et al., 1973) and under the influence of the increase in fetal cortisol at term, becomes a complete endocrine gland capable of metabolizing pregnenolone to estrogen. Placental tissue previously exposed to cortisol had increased 17α-hydroxylase activity (Anderson et al., 1975) which may be associated with the prepartum decrease in progesterone production (Flint et al., 1975a,b) and for the provision of precursors for aromatization. Flint et al. (1975a) have demonstrated the formation of estrone following the addition of 17α-hydroxyprogesterone in vitro. It has been shown that the activity of placental C_{17-20} lyase was increased by cortisol, possibly explaining the increased preterm estrogen production. Evidence suggests that direct effects of cortisol on placental aromatase (Anderson et al., 1978) and steroid sulfatase (Ash et al., 1973) may also occur in the ovine placenta.

1.3 Hormonal Changes during Pregnancy

A detailed account of hormonal changes during ovine pregnancy and their possible physiological role during pregnancy and parturition has been described previously (Thorburn et al., 1977; Thorburn and Challis, 1979; Challis, 1980). Since these changes contribute to the mechanism of parturition, but are not the focus of this thesis, they will receive only brief consideration here.

1.3.1 Progesterone

Evidence suggests that after Day 50 of pregnancy in sheep, progesterone production occurs primarily in the placenta (Thorburn et al., 1977). In sheep, progesterone concentrations in maternal plasma decrease during the last 5-15 days of pregnancy, following the increase in fetal plasma corticosteroids (Bassett et al., 1969; Fylling, 1970; Bedford et al., 1972a,b). Maternal plasma progesterone concentrations also fall after the administration of glucocorticoids to the fetus (Liggins et al., 1972; Currie et al., 1973; Flint et al., 1974).

A similar pattern in fetal plasma progesterone levels has been reported, although concentrations were lower than maternal plasma (Nancarrow and Seamark, 1968). Myometrial progesterone concentrations increase progressively from Day 45 to Day 140 (Rawlings and Ward, 1976). Similarly, there is a progressive increase in progesterone concentrations in amniotic fluid between Days 50 and term (Power et al., 1981), concentrations increasing markedly during the last 10 days of gestation (Challis et al., 1981). The source of this progesterone is

uncertain, however recent evidence suggests that fetal membranes have some steroidogenic activity (Swartz et al., 1977; Gibb et al., 1978, 1980).

In the rabbit, the effect of progesterone in maintaining uterine quiescence is well established (Csapo, 1969, 1973). In the sheep, however, progesterone withdrawal does not appear to be a prerequisite for parturition. Daily doses of 80 mg (Bengtsson and Schofield, 1963) or 100 mg (Liggins et al., 1972) of progesterone do not prevent the onset of labour in sheep. At larger doses, however (150-200 mg/day), cervical dilatation and uterine activity were blocked (Bengtsson and Schofield, 1963; Liggins et al., 1972). In contrast, Stys et al. (1978) has shown that the administration of progesterone, in amounts which suppressed uterine contractions did not inhibit the increase in cervical compliance accompanying dexamethasone-induced parturition in sheep.

1.3.2 Estrogens

In sheep urinary estrogens first appear at about Day 70 of gestation (Fevre and Rombauts, 1966) and their excretion increases markedly between Day 90 and term. In contrast, the maternal plasma concentrations of estrone and estradiol-17β are low throughout pregnancy, increasing only during the last 24 h before parturition (Challis, 1971; Thorburn et al., 1972) when estrogen production rate increases (Challis et al., 1973). There is also a massive increase in estrogen sulfate concentrations in fetal (Currie et al., 1973) and maternal (Tsang, 1974) plasma at this time. Myometrial estrogen

concentrations correlate well with estrogens in maternal plasma (Rawlings and Ward, 1976).

In sheep, the onset of parturient uterine activity is preceded by increased concentrations of unconjugated estrogen and $PGF_{2\alpha}$ in maternal plasma (Rawlings and Ward, 1976, 1978). A marked increase in the sensitivity to oxytocin and spontaneous contractions were observed 12-24 h after a single injection of 20 mg of stilbesterol to pregnant ewes (Hindson et al., 1967; Liggins et al., 1973). Although induction of parturition with estrogens has been reported (Hindson et al., 1967), this treatment was associated with impaired cervical ripening.

1.3.3 Placental Lactogen

A placental hormone with somatotrophic and lactogenic activities has been recently purified and characterized for the sheep (Handwerger et al., 1974; Martal and Djiane, 1975; Martal et al., 1975, 1976; Chan et al., 1976). Concentrations of this hormone, ovine placental lactogen (OPL), have been measured in blood and uterine tissue by radioreceptor assay and radioimmunoassay (Kelly et al., 1974; Djiane and Kann, 1975; Martal and Djiane, 1977; Chan et al., 1978). Concentrations of maternal plasma OPL generally became detectable between Days 48-60 and increased as pregnancy advanced, to peak levels on Days 131-141, followed by a decline in OPL concentrations about 5 days before parturition. Using a specific radioimmunoassay, Chan et al. (1978) found that OPL concentrations were detectable as early as 40 days of gestation in uterine vein

blood and amniotic fluid. In contrast to concentrations found in maternal peripheral sera, OPL was detected as early as Day 18 in allantoic fluid, reaching peak levels between Days 35-50. In addition, OPL concentrations in fetal sera were found to be 9 times higher than maternal sera from Days 46-70, but after Day 110, OPL concentrations in maternal sera were 5 times higher than fetal sera (Chan et al., 1978). Before Day 40, OPL concentrations in fetal membranes were higher than in maternal caruncles (Chan et al., 1978).

The role of OPL during ovine pregnancy is uncertain. For humans, Kaplan (1974) proposed the placental lactogen is the "growth hormone" of pregnancy that induces a constant tonic effect on maternal metabolism and ensures for the fetus a steady source of various fuels. In the sheep, OPL may exert direct growth-promoting effects during early gestation (Handwerger et al., 1974; Chan et al., 1976, 1978; Martal and Djiane, 1977) and influence the rate of mammogenesis and lactation during late pregnancy (Djiane and Kann, 1975; Martal et al., 1976; Martal and Djiane, 1977).

1.3.4 Relaxin

In general, data from both bioassays and radioimmunoassays (RIA's) show that blood levels of relaxin tend to rise as pregnancy progresses, reaching a maximum shortly before birth and falling at parturition (rat - O'Byrne and Steinetz, 1976; Anderson et al., 1973; Sherwood and Crnekovic, 1979; guinea pig - O'Byrne and Steinetz, 1976; rabbit - Marder and Money, 1944; hamster - O'Byrne et al., 1976; mouse - O'Byrne and Steinetz, 1976; and sow - Sherwood et al., 1975).

Using the assay of Bryant-Greenwood (Bryant, 1972), immunoreactive relaxin has been reported in the blood of sheep during the estrous cycle (Chamley et al., 1975), pregnancy and parturition (Bryant and Chamley, 1976). However, it appears that this radioimmunoassay was not measuring "immunoreactive relaxin" due to the impurity of the relaxin preparation and the iodination procedures used (O'Byrne et al., 1978). Some care must therefore be taken when evaluating these results.

Evidence for a physiological role of relaxin during pregnancy and parturition has been presented in reviews by Hall (1960) and Schwabe et al. (1978).

An overwhelming body of evidence suggests that relaxin inhibits spontaneous uterine activity in a variety of species (see Hall; 1960; Schwabe et al., 1978), including the sheep (Lye, 1981). This response appears to be immediate. However, the uterus remains responsive to stimulants such as oxytocin and prostaglandins (Sawyer et al., 1953; Steinetz et al., 1957; Porter, 1972; Chamley et al., 1977; Porter et al., 1979). The mechanism of this inhibition action is unknown. The involvement of catecholamines has been suggested (Miller and Murray, 1959) and discounted in vitro (Paterson, 1965) and in vivo (Porter et al., 1979). However, relaxin increases myometrial cyclic AMP concentrations (Sanborn et al., 1980; Judson et al., 1980), possibly accounting for the inhibition of uterine contractions.

Porter (1974) has suggested that in species in which progesterone inhibits myometrial activity, relaxin may provide a mechanism under which progesterone can be withdrawn without the development of

premature spontaneous activity. Since responsiveness to oxytocin and $PGF_{2\alpha}$ is retained during relaxin inhibition, maximal myometrial activity can be developed rapidly when these substances are released at parturition. In the absence of reliable data concerning the plasma concentrations of relaxin in the ewe during pregnancy and parturition, it is premature to speculate on its role in this species.

1.3.5 Oxytocin

Plasma oxytocin levels are very low or undetectable in late pregnancy or early labour in the pig (Forsling et al., 1979) and goat (Chard et al., 1970) and show a great increase in second-stage labour. These observations have led to suggestions that rather than initiating parturition, oxytocin acts to increase further uterine activity during fetal expulsion in these species (Chard, 1973; Forsling et al., 1979). This suggestion is supported by observations by Denamur and Martinet (1961) and Bosc (1972) showing normal parturition at term in sheep after maternal hypophysectomy at Days 50-134. It seems likely, therefore, that oxytocin secreted during second-stage labour plays a supportive role in labour.

Vaginal distension is known to provoke the reflex release of oxytocin (Ferguson, 1941; Debackere et al., 1961; Roberts and Share, 1968), accounting for the increasing amounts of oxytocin in maternal blood at the time of parturition (Forsling et al., 1979). Evidence suggests that the release and action of oxytocin may also be influenced by changes in steroid hormones at this time. In the sheep, oxytocin release in response to vaginal distension is augmented in

estrogen-dominated animals and reduced in progesterone-dominated animals (Roberts and Share, 1969). In addition, Alexandrova and Soloff (1980a,b) have shown in the rat that exogenous estrogen increased myometrial oxytocin receptor concentrations and that during parturition there is a parallel rise in cytosol estrogen receptor and oxytocin receptor concentrations coinciding with progesterone withdrawal. They suggested that the increase in estrogen receptors resulting from progesterone withdrawal allowed the circulating estrogen to effect an increase in oxytocin receptors. It remains to be established whether myometrial oxytocin receptors are controlled by a similar mechanism in sheep.

1.4 Uterine Activity; Possible Role of Gap Junctions

Pertinent to the above studies are the findings of Garfield and co-workers (Garfield et al., 1977, 1978, 1979, 1980, 1981) who noted the presence of gap junctions between the uterine muscle cells of the rat, guinea pig, sheep and human only immediately before, during and after delivery.

These junctions were not found, or were present with only a low frequency in non-pregnant animals or at any other time during pregnancy. There were fewer gap junctions in the non-gravid than in the gravid horns in unilaterally pregnant rats at term (Garfield et al., 1978). In the rat, progesterone in the presence of estrogen inhibits, whereas estrogen stimulates the formation of gap junctions

(Garfield et al., 1979, 1980). Evidence suggests that products of arachidonic acid metabolism may also be involved in gap junction formation, possibly as mediators of the steroid effects (Garfield et al., 1980).

Garfield et al. (1979) proposed that gap junctions are essential for normal labour and delivery and in the synchronization of uterine contractions. The electrical and contractile activity of the uterus in situ are irregular and localized until just before and during parturition when they become synchronized and spread throughout the muscle (Fuchs, 1969; Csapo, 1973; Kao, 1977). However, it is not clear how or if gap junctions are related to these physiological changes. Moreover, there are few, if any, gap junctions in the non-pregnant uterus, yet the cells in this muscle appear to be electrically coupled (Abe, 1970; Kuriyama and Suzuki, 1976). Gap junctions, therefore, may not be the only route for current spread between cells. Membranous interdigitations and projections frequently come in close contact (10-15 nm) with neighbouring cells becoming especially prominent in uteri from pregnant or estrogen-treated rats (Garfield and Daniels, 1974). Sperelakis and Mann (1977) have postulated that electrical current might spread between cells by way of the extracellular space in these regions of close contact.

Gap junctions, therefore, appear in the myometrium when co-ordination of contractions is essential. Whether their formation is related to the spread of electrical activity within the uterus remains to be established.

1.5 Prostaglandins

In 1930, human seminal fluid was shown to contain a factor resulting in contraction and relaxation of uterine strips in vitro (Kurzrok and Lieb, 1930). A few years later, Goldblatt (1935) in England and Von Euler (1936) in Sweden independently reported smooth muscle contracting and vasopressor activity in seminal fluid, and Von Euler identified the active material as a lipid-soluble acid which he named "prostaglandin". It is now known that there is not one but a whole family of prostaglandins which are all C_{20} acids formed from polyunsaturated fatty acids by oxygenation and cyclization. Prostaglandins are synthesized from three essential unsaturated fatty acids: 8,11,14 eicosatrienoic acid, 5,8,11,14 eicosatetraenoic acid (arachidonic acid) and 5,8,11,14,17 eicosapanaenoic acid, which lead to prostaglandins of the 1, 2 and 3 series respectively (example PGE_1, PGE_2 and PGE_3; for review, see Bygdeman and Green, 1980). Quantitatively, the most important precursor acid is arachidonic acid and therefore the physiological role of PG's of the 2 series has received much attention.

Arachidonic acid is released from membrane glycerophospholipids by the action of phospholipases. In most tissues, the concentrations of free arachidonic acid is low (Leaver and Poyser, 1981). For this reason, it is generally believed that endogenous biosynthesis of PG's and related compounds depend on the phospholipid stores. Flower (1978) and Marcus (1978) have hypothesized that phospholipase A_2 is the common rate-limiting step in the enhanced biosynthesis that occurs in response to widely divergent physical,

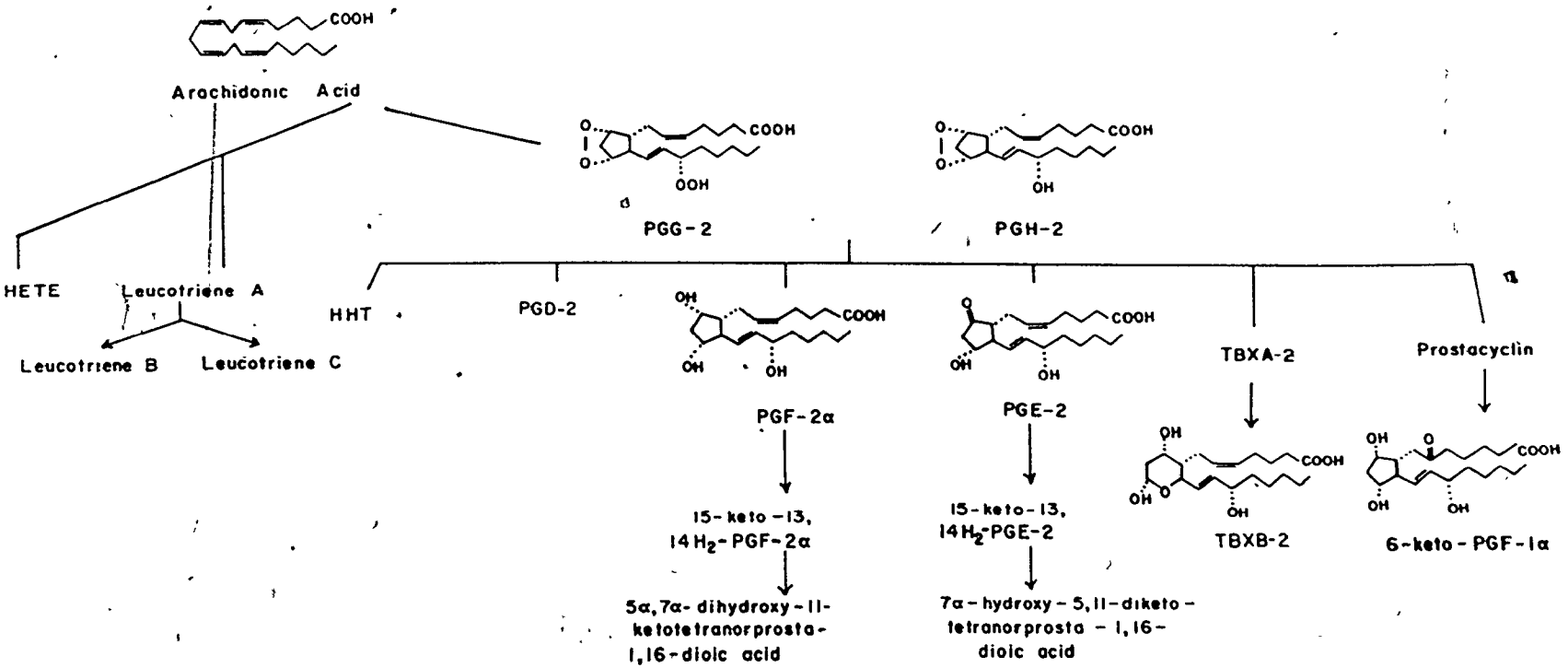
chemical, hormonal and neurohumoral influences.

Once released, arachidonic acid is rapidly metabolized to oxygenated products by two distinct enzymatic mechanisms, a cyclo-oxygenase and a lipoxygenase. A summary of the known routes of bioconversions of arachidonic acid is shown in Figure 1. Through the action of lipoxygenases, arachidonic acid is oxidized to form hydroperoxy acids which are reduced to hydroxy-eicosatetraenoic acids (HETE). Although these compounds are formed by a number of tissues, the physiological significance of hydroperoxy acids is unknown (Samuelsson et al., 1975). The leukotrienes are formed in muscle cells and leukocytes and although nothing is known about their possible role of reproduction, they appear to be the constituents of slow-reacting substance and thus may play a role in conditions such as allergen-provoked asthma (Murphy et al., 1979; Parker et al., 1979).

The enzyme, prostaglandin endoperoxide synthetase (prostaglandin synthetase, cyclo-oxygenase), catalyses the transformation of arachidonic acid to the cyclic endoperoxides (PGG_2 and PGH_2), which are key intermediates in the biosynthesis of PGE_2, $PGF_{2\alpha}$, thromboxane A_2 (TXA_2) and PGI_2 (prostacyclin). Some of the enzymes involved in the biosynthesis of these compounds from the endoperoxides have been partially purified and studied (see Samuelsson et al., 1978 for references). The enzymes PG endoperoxide E isomerase and PG endoperoxide $F_{2\alpha}$ isomerase catalyse the conversions of endoperoxide to PGE_2 and $PGF_{2\alpha}$, respectively. Several proteins catalyse the formation of PGD_2. Thromboxane A isomerase catalyses the formation of TXA_2 and prostaglandin endoperoxide I isomerase catalyses the formation of PGI_2.

FIGURE 1

Summary of some of the presently known bioconversions of arachidonic acid (from Gréen et al., 1981).


COOH
Arachidonic Acid
HETE
Leucotriene A
Leucotriene B
Leucotriene C
PGG-2
PGH-2
HHT
PGD-2
PGF-2α
PGE-2
TBXA-2
Prostacyclin
15-keto-13, 14H2-PGF-2α
15-keto-13, 14H2-PGE-2
TBXB-2
6-keto-PGF-1α
5α,7α-dihydroxy-11-ketotetranorprosta-1,16-dioic acid
7α-hydroxy-5,11-diketo-tetranorprosta-1,16-dioic acid

Thromboxane B_2 and 6-oxo-$PGF_{1\alpha}$ are formed by non-enzymatic hydrolysis of TXA_2 and PGI_2, respectively. Prostacyclin may also be metabolized enzymatically in the lung to form 6,15-dioxo-$PGF_{1\alpha}$ (Sun and Taylor, 1978) and in platelets to form 6-oxo-PGE_1 (Wong et al., 1980). The latter compound has potent PGI_2-like activity.

It is generally agreed that the production of arachidonic acid metabolites represents, at least in part, the final common pathway by which the different hormonal events associated with parturition exert their action on the myometrium. Prostaglandins have been shown to cause stimulation of uterine activity in a variety of species, both in vivo and in vitro (Ramwell and Shaw, 1970). The emphasis in this section will be placed on the role of PG's in non-pregnant animals and during parturition (see Challis, 1980 and Thorburn and Challis, 1979 for recent reviews).

1.5.1 Sites of PG Production and Metabolism

In the non-pregnant sheep with a 16-day estrous cycle, evidence suggests that $PGF_{2\alpha}$ is produced in the uterus and exerts a local effect on the adjacent ovary, resulting in regression of the corpora lutea (see Horton and Poyser, 1976). During the estrous cycle, PGF concentrations in the utero-ovarian vein increase slowly and progressively between Days 6 and 12 (Thorburn et al., 1973; Robinson et al., 1976). A complex series of peaks in the concentrations of PGF was observed between Days 13 and 17 of the cycle, normally lasting less than 6 hours. The first major peak was observed on Day 13, followed by another peak on Day 14 and a series of peaks

of high concentrations on Days 15 and 16 (Thorburn et al., 1972, 1973). The high PGF concentrations in the utero-ovarian vein on Days 15 and 16 are associated with regression of the corpora lutea and the subsequent decrease in plasma progesterone concentrations (Thorburn et al., 1973). In vitro studies suggest that the caruncles, specialized foldings of endometrium which give rise to the maternal cotyledons (placentomes) during pregnancy, may be the major site of PGF biosynthesis and metabolism during the luteal phase of the estrous cycle (Louis et al., 1976). Using an immunocytofluorescence procedure, Huslig et al. (1979) measured the activity of PG forming cyclo-oxygenase during the estrous cycle in sheep. The specific activity in uterine microsomes was higher on Days 13 to 15 than earlier in the cycle, correlating with a three-fold increase in cyclo-oxygenase activity in caruncular endometrium but not in other tissues. Incubation in vitro of sheep uterine tissue homogenates revealed that although both caruncular and non-caruncular endometrium can synthesize $PGF_{2\alpha}$, PGE_2 and 6-oxo-$PGF_{1\alpha}$ (Alwachi et al., 1980; Marcus, 1981), the myometrium synthesizes predominantly 6-oxo-$PGF_{1\alpha}$ (Alwachi et al., 1980). Endometrial cells and intact embryos on Day 15 of pregnancy, and chorionic membrane on Day 20 of pregnancy have been shown to produce PGI_2, $PGF_{2\alpha}$ and PGE_2 from labelled arachidonic acid in vitro (Marcus, 1981). Although PGF concentrations in the utero-ovarian vein increase slightly between Days 12 and 14 in pregnant sheep, PGF peaks on Days 15 and 16 were not observed, PGF levels fell to relatively low concentrations by Day 20, and there was no overall change in plasma progesterone concentrations (Thorburn et al., 1973; Roberts et al., 1975).

The reduced levels of PGF appear to persist throughout pregnancy since low concentrations of both PGE and PGF in the utero-ovarian vein plasma were also found from Day 125 to just before parturition when concentrations increased sharply (Challis et al., 1976). Since PG concentrations have not been measured between Days 20 and 125 of pregnancy, it is not certain whether the reduced levels observed on Days 20 and 125 are indicative of decreased synthesis of all PG's throughout pregnancy. Clearly, more studies need to be carried out before statements can be made on the roles of PG's during this period.

During late pregnancy in sheep, the concentrations of PGF and 6-oxo-$PGF_{1\alpha}$ in maternal plasma increased during spontaneous delivery (Thorburn et al., 1972; Mitchell et al., 1979), and at delivery induced by intrafetal infusions of ACTH or of glucocorticoids (Currie et al., 1973; Kendall et al., 1977; Mitchell et al., 1979). Similarly, the concentrations of PGE in fetal plasma (Challis et al., 1976) and of PGE, PGF and 6-oxo-$PGF_{1\alpha}$ in amniotic fluid (Mitchell et al., 1977b, 1978; Challis et al., 1978), increase prior to delivery. In contrast, however, concentrations of TXB_2 in maternal and fetal plasma did not increase during spontaneous delivery (Mitchell et al., 1979). Concentrations of TXB_2 in amniotic fluid and of all arachidonic acid metabolites in allantoic fluid have not been measured in sheep.

Concentrations of PGE and PGF have been measured in myometrium and placental tissue (cotyledons) in sheep (Liggins and Grieves, 1971; Mitchell and Flint, 1977). In this species, the maternal and fetal placentomes can be separated during late pregnancy

(Liggins et al., 1973). Commencing 24 h after an intrafetal infusion of dexamethasone, before the onset of uterine contractions, concentrations of PGF, but not PGE, increased in maternal cotyledons followed by elevated concentrations in fetal cotyledons and myometrium after the establishment of labour (Liggins and Grieves, 1971). In contrast, other investigators (Mitchell and Flint, 1977) reported high levels of PGE in fetal cotyledons, but not in myometrium or maternal cotyledons after spontaneous or dexamethasone-induced delivery. These seemingly paradoxical results may be due, in part, to the considerable variation among single observations taken from the few animals used in these studies. Furthermore, PG determination in separated fetal and maternal cotyledons may be influenced by incomplete separation of the placental components and by the artifactual effects of trauma associated with manual separation. Since PG concentrations have not been measured in whole (unseparated) cotyledons, the possible contribution of these artifactual sources of PG's is undetermined.

Using a superfusion system, Mitchell and Flint (1976) observed higher PGE "output" by fetal and maternal cotyledons from 3 sheep, than of PGF, possibly explaining the high PGE concentrations found in fetal plasma (Challis et al., 1976). In addition to PG's of the E and F series, human placental tissue has been shown to generate an unstable substance with properties resembling those of PGI_2 (Myatt and Elder, 1977). The production of PGI_2 has since been demonstrated in rat (Fenwick et al., 1977; Jones et al., 1977; Williams et al., 1978), sheep (Jones et al., 1977) and human (Abel and Kelly, 1979) uterine tissues. During parturition, myometrial PGI_2 synthesis

in vitro, increased 18.5-fold in the rat (Williams *et al.*, 1978) and 6-oxo-$PGF_{1\alpha}$ output *in vitro* from minced human myometrial tissue increased 4-fold during labour (Bamford *et al.*, 1980). In contrast, however, Satoh *et al.* (1981) reports a 50% decrease in 6-oxo-$PGF_{1\alpha}$ output from minced human myometrial tissue using essentially the same procedure. The changes in myometrial PGI_2 synthesis during labour in the human therefore remain uncertain.

In humans, PGF output *in vitro* increased during labour in the myometrium and decidua but not in the amnion (Satoh *et al.*, 1981). Other investigators have also reported elevated PGF output by human decidua during labour (Mitchell, 1980; Okazaki *et al.*, 1981). Both human chorion and amnion make predominantly PGE (Mitchell *et al.*, 1978a; Okazaki *et al.*, 1981; Satoh *et al.*, 1981). Kinoshita and Green (1980) demonstrated that ^{14}C-arachidonic acid is mainly converted into PGE_2 in whole homogenates of human amnion following vaginal delivery. However, confusion exists as to whether PGE production in amnion is stimulated during labour. Using similar techniques, increased PGE output *in vitro* by amnion obtained from primates has been observed by some investigators (Okazaki *et al.*, 1981) but not by others (Mitchell *et al.*, 1978a; Satoh *et al.*, 1981). Results from studies on PG output *in vitro* by minced or homogenates of human tissues therefore are inconclusive. The paradoxical results obtained from human tissues, possibly as a consequence of different incubation conditions, suggest that serious reservations must be considered when interpreting such studies, especially when tissue concentrations or other supportive data are not included. For this reason, it is difficult to determine

which uterine tissues in sheep may be the major sites of PG production during labour. The possibility that fetal membranes and endometrium are also sites of PG production at this time in sheep, and the identity of PG's produced in these tissues have not been investigated.

1.5.2 Factors Regulating Uterine PG Production during Pregnancy and Parturition

The apparent low plasma PG concentrations during most of pregnancy, compared to the luteal phase of the estrous cycle in non-pregnant ewes, and during parturition suggests that parturition is initiated by the "release of a suppressive influence" on arachidonic acid metabolism and/or by the "emergence of a stimulatory influence" on uterine PG synthesis.

PG's produced by homogenates of bovine seminal vesicles is inhibited by a factor present in human plasma (Saeed et al., 1977). Although an endogenous circulating inhibitor of PG synthesis has also been found in the plasma of pregnant women (Brennecke et al., 1981a), inhibition of PG synthesis in bovine seminal vesicles by plasma of pregnant and non-pregnant women does not differ and parturition does not appear to be preceded by a withdrawal of this inhibitor (Brennecke et al., 1981b). However, if PG production during pregnancy is further suppressed by a factor of fetal, placental or ovarian origin, receptors for this factor may not be present in bovine seminal vesicles and hence inhibition and/or withdrawal of inhibition may not have been detected. Since a factor which inhibits PG synthesis specifically during pregnancy has not been identified, most authors

believe that the increase in PG production during labour is a consequence of the emergence of stimulatory factors.

1.5.2.1 Steroids

The possibility that steroids regulate uterine PG production was suggested by the observation that concentrations of estrogen and PGF in the utero-ovarian vein rise in parallel during the last 24 h of pregnancy (Challis et al., 1972; Currie et al., 1973; Liggins, 1973), corresponding with the evolution of uterine activity.

The role of estrogen in the regulation of uterine PG production during late pregnancy was supported by the observation that diethylstilbestrol, administered to pregnant ewes (130 days post coitum), resulted in elevated PGF concentrations in maternal cotyledons, myometrium and utero-ovarian vein plasma. This effect could be blocked by high doses (200 mg per day) of progesterone (Liggins et al., 1973). Although the administration of diethylstilbestrol resulted in the stimulation of uterine activity and PGF release at earlier stages of pregnancy (Day 125), cervical ripening and delivery were not observed (Hindson et al., 1967; Liggins et al., 1973). In the experiments of Liggins et al. (1973), uterine activity and utero-ovarian vein PGF levels during the induction of premature parturition by the intra-fetal infusion of dexamethasone were suppressed by high doses of progesterone, although the increased PGF concentrations in myometrium and maternal cotyledons were not affected by progesterone treatment. Unlike larger doses, however, sheep treated with progesterone (150 mg/24 h) did not prevent the spontaneous onset of labour at term and uterine activity in these animals was indistinguishable from that of normal labour.

The results of these in vivo experiments in pregnant ewes suggest that estrogen stimulates uterine PG production and release while the main effect of progesterone is inhibition of PG release. In an attempt to control the effects of endogenous steroids on uterine PG production, a number of investigators have examined the effects of steroids on uterine PG production in non-pregnant sheep. In the non-pregnant, ovariectomized ewe, injection of estradiol is followed by an increase in the concentration of PGF in the caruncles and utero-ovarian vein plasma, provided that the animals were primed with progesterone (Caldwell et al., 1972; Ford et al., 1975; Louis et al., 1977; Scaramuzzi et al., 1977). The effects of estrogen or progesterone alone on uterine PG production are not fully understood. Although progesterone alone increases PGF production and release from uterine caruncles (Louis et al., 1977), a decline in its plasma concentrations in pregnant or non-pregnant ewes lead to further PG release (Challis et al., 1976). In contrast, concentrations of PGF in uterine tissues and utero-ovarian vein plasma were not affected by estradiol treatment alone in non-pregnant ewes (Ford et al., 1975; Louis et al., 1977; Scaramuzzi et al., 1977; Rexroad, 1978). Thus in vivo studies in non-pregnant sheep suggest that progesterone "priming" is essential for uterine PG synthetase activity and that after the uterus is sufficiently primed, progesterone withdrawal and/or increase in estrogen will enhance the release of PGF. The existence of alternate mechanisms for the stimulation of uterine PG production during parturition is supported by the observation that passive immunization against total unconjugated estrogens did not affect the normal increase in maternal venous plasma PGF concentrations during parturition in ewes (Rawlings

et al., 1978). The potential role of progesterone withdrawal in stimulating uterine PG production is further suggested by the work of Mitchell and Flint (1977a). In one animal, the intra-amniotic administration of cyanoketone (an inhibitor of progesterone synthesis) on Day 120 of pregnancy, resulted in a decrease in the concentration of progesterone, and a corresponding increase in 14,15-dioxo-$PGF_{2\alpha}$ concentrations with no significant effect on total unconjugated estrogens in the utero-ovarian vein plasma.

In cycling and ovariectomized, estrogen-treated rats, the in vitro determination of PG synthetase activity in uterine microsomal fractions suggested that the primary action of estrogen was not to increase the rate of PG production but rather to alter the direction of PG's produced, resulting in an increased ratio of PGF:PGE (Ham et al., 1975). The possibility that treatments with progesterone and/or estrogen results in a similar alteration in the nature of PG's produced in sheep uterine tissue remains uncertain. The output of PGF but not PGE from non-pregnant human endometrium obtained during the secretory but not the proliferative phase of the cycle, maintained in organ culture, was increased by estradiol. Progesterone suppressed the spontaneous output of PGE and PGF by endometrium obtained at all stages of the cycle and also prevented the estrogen-induced stimulation (Abel and Baird, 1980). More recently, marked and dose-dependent stimulation was observed in PGF and PGE production by rat uterine homogenates following the addition of catecholestrogen, while suppressing the production of 6-oxo-$PGF_{1\alpha}$ (Kelly and Abel, 1980). It is possible, therefore, that the increasing estrogen concentrations

during parturition in sheep is converted to catecholestrogens which then stimulate the production of specific PG's, primarily PGF.

It has been proposed that factors regulating lysosomal stability, and hence the availability of phospholipase A_2, may also control PG biosynthesis during parturition (MacDonald et al., 1978; Challis, 1980). Lipid droplets accumulate under the influence of progesterone in the epithelial cells of the endometrium of ovariectomized sheep, while estrogen results in their depletion from progesterone-treated ewes. The possibility that these droplets contain fatty acid precursors for PG synthesis has been suggested (Louis et al., 1977).

In humans, decidual and amnion lysosomes are particularly susceptible to fracture at the time of labour (Gustavii and Brunk, 1974; Schwarz et al., 1980). Grieves and Liggins (1976) demonstrated high phospholipase A_2 activity in human amnion, and ovine amnion and chorioallantois and suggested that these tissues may participate in PG biosynthesis by releasing stored arachidonic acid. In women, the fetal membranes are enriched in phosphatidylethanolamine which contains approximately 60% of the total esterified arachidonic acid (MacDonald et al., 1978). In human fetal membranes, phospholipase A_2 has substrate specificity for phosphatidylethanolamine, and during labour there is a reduction in the arachidonic acid content of phosphatidylethanolamine in amnion (Ókita et al., 1980). This finding is consistent with the hypothesis (MacDonald et al., 1978) that the liberation of arachidonate as a result of decreased lysosomal stability and subsequent increased phospholipase A_2 activity may be a major regulatory

step in PG biosynthesis during parturition: The recent observation that arachidonic acid is selectively lost from the phosphatidylinositol fraction of human amnion during labour (Okita et al., 1980) and the presence of phosphatidylinositol-specific phospholipase C activity in human fetal membranes and decidua may provide an additional mechanism for arachidonic acid release (DiRenzo et al., 1981). The observation that the specific activities of phospholipase C and A_2 increase in amnion tissue with advancing gestation (Okazaki et al., 1981), is consistent with the finding that unesterified arachidonic acid concentrations increase disproportionately to other free fatty acids in the amniotic fluid of women in labour (MacDonald et al., 1974, 1978).

Recently, attention has focussed on the physiological regulation of lysosomal phospholipase activities in the fetal membranes. According to the hypothesis of MacDonald and co-workers (1978) lysosomal stability during pregnancy is maintained, in part, by progesterone and the appearance of a high-affinity progesterone-binding protein in the fetal membranes a few weeks prior to parturition (Schwarz et al., 1976) affects local progesterone withdrawal, and subsequently augments phospholipase A_2, and possibly phospholipase C, activity. Although there is no direct evidence for a role of progesterone in promoting uterine lysosomal stability, low concentrations of progesterone have been shown to stabilize erythrocyte membranes against hypotonic hemolysis (Seeman, 1966). The progesterone-binding protein is found in significant quantities only after the thirty-seventh week of human pregnancy and binds progesterone, 5α-dihydroprogesterone and cortisol (MacDonald et al., 1978). Moreover, these investigators have shown

that there is a decrease in progesterone metabolism by human fetal membranes in late pregnancy (Milewich et al., 1979), possibly due to effective progesterone withdrawal as a result of the appearance of this binding protein.

There is also substantial evidence that glucocorticoids inhibit the release of arachidonic acid from phospholipids, possibly by increasing lysosomal stability and therefore decrease the formation of PG's and related compounds (Gryglewski et al., 1975; Hong and Levine, 1976; Blackwell et al., 1978). Although a progesterone-binding protein in association with fetal membranes has not been identified in sub-primate species, such a protein may also bind cortisol, and result in decreased fetal membrane lysosomal stability and the liberation of free arachidonic acid. Szego (1974) has suggested that estrogen may have the opposite effect of progesterone and cortisol on lysosomes, and labilize their membranes. The hypothesis that estrogen, present in the amniotic fluid, results in increased phospholipase A_2 and PG biosynthesis has also been advanced (Challis, 1980). In addition, once PG's are produced and released by the fetal membranes, they may also stimulate PG synthesis in other uterine tissues by labilizing lysosomes (Gustavii, 1977).

It should be emphasized that most of the studies implicating phospholipase A_2 and C activities as the rate-limiting step in the synthesis of PG's and related compounds during pregnancy and parturition have been carried out on human tissues. The roles of phospholipase A_2 and C and the fetal membranes in the control of PG biosynthesis during pregnancy in sheep remain uncertain.

1.5.2.2 Oxytocin

In addition to progesterone and estrogen, oxytocin also appears to cause the release of PGF into utero-ovarian vein blood. Elevations of PGF concentrations occur in plasma of late pregnant sheep (Currie, 1974; Mitchell et al., 1975) and of estrogen-treated anestrous ewes (Sharma and Fitzpatrick 1974) following the intravenous infusion of oxytocin. In addition, PG's are released in association with suckling (Currie, 1974) and a marked increase in PGF release occurs immediately before delivery during second-stage labour (Currie et al., 1973). The release of oxytocin in response to vaginal distension was followed 1-4 min later by an increase in both utero-ovarian venous PGF concentrations (Flint et al., 1975c) and uterine activity (Mitchell et al., 1975), an effect which was augmented towards the time of parturition. Oxytocin also enhanced the in vitro release of PGF from ovine endometrial but not myometrial tissue during the estrous cycle (Roberts et al., 1976).

The progressive increase in the responsiveness to oxytocin during late pregnancy in sheep is likely due, in part, to an increase in the number of oxytocin receptors. The changes in circulating levels of estrogen and progesterone at the time of parturition may cause the appearance of more oxytocin receptors (see Section 1.3.5) which, in the presence of oxytocin, could result in increased PGF release from the uterus. It should be pointed out, however, that the increased uterine activity following oxytocin injection is not inhibited in the ewe when PG production is suppressed by indomethacin (Roberts and McCracken, 1976). Furthermore, the stimulatory effect of oxytocin on uterine activity in vitro is not suppressed by indomethacin

in the rat (Chan, 1977; Dubin et al., 1979). It appears, therefore, that an increase in PG synthesis is not essential for the stimulatory effects of oxytocin; however, oxytocin and PGF may act synergistically to increase myometrial activity.

In addition to an effect of oxytocin on uterine PG production, there is also evidence suggesting that high circulating levels of PG's augment oxytocin release. The injection of PGF in humans (Gillespie et al., 1972) and pigs (Forsling et al., 1979) resulted in a sharp increase in plasma oxytocin concentrations. During second-stage labour, therefore, a positive feedback relationship between oxytocin release and uterine PG production may play an important role in the generation of strong contractions and subsequently placental delivery.

1.5.2.3 Placental lactogen and relaxin

Thorburn and Challis (1979) have suggested that parturition in the rat may result from the decrease in circulating placental lactogen prior to delivery which then results in decreased estrogen receptor concentrations in luteal cells and subsequently decrease progesterone production. These authors further suggest that the increased uterine PG released as a result of changes in circulating steroid levels results in luteolysis and parturition. Possible effects of placental lactogen on uterine PG synthesis and metabolism as well as on relaxin production in the corpora lutea have also been suggested (Thorburn and Challis, 1979); however at present there is little direct evidence which supports this hypothesis.

Ovarian autotransplantation studies in rats indicate that a factor causing the release of relaxin from the corpora lutea prior to parturition travels sytemically (Sherwood et al., 1977) and evidence suggests that this factor may be $PGF_{2\alpha}$ (Sherwood et al., 1976, 1979). In rats, immunoreactive relaxin levels increase gradually from Day 10 until 12 to 24 h before parturition; levels then fall progressively until the surge occurring during delivery (Sherwood et al., 1980). It is possible that this relaxin surge results from the elevated utero-ovarian vein PGF levels at this time. Consistent with this suggestion is the observation that although parturition can be delayed by progesterone (Sherwood et al., 1978) and indomethacin (Sherwood et al., 1979), only the latter will delay the relaxin surge occurring at the time of parturition (Sherwood et al., 1979).

The direct effect of relaxin and/or placental lactogen on uterine PG production has not been investigated. During early pregnancy in sheep, ovine placental lactogen production coincides with a period of uterine quiestence, possibly due to inhibition of uterine PG production. In addition, the inhibitory effects of relaxin on uterine activity may be mediated by a similar mechanism.

1.5.2.4 Other factors

In guinea pigs, mechanical distention of the uterus in vitro has been shown to increase PG release (Horton et al., 1971). "Physiologically", uterine distention occurs during pregnancy as a result of fetal growth. As pointed out earlier, low PG concentrations in the utero-ovarian venous plasma appear to persist until the time of delivery. These results suggest that PG output is not

increased during "physiological" uterine stretch, possibly the result of adaptive mechanisms operating during this period. PG output in response to uterine distention in vitro may result in increased muscle tension, thus preventing excessive stretch and subsequent muscle injury. It should be stressed, however, that PG concentrations in myometrial tissues have not been measured at different gestational ages. Since the cotyledons are thought to be the major source of PG's in the utero-ovarian venous plasma (Challis, 1980), small changes in PG content in the myometrium may go undetected. Hence, until these measurements are made, the effects of uterine stretch on PG output from the myometrium remain uncertain.

1.6 Possible Roles of PG's during Pregnancy

1.6.1 Uterine Activity

It is generally accepted that PG's play an important role in the regulation of uterine contractility during parturition (Liggins et al., 1973; Flower, 1977; Thorburn and Challis, 1979; Challis, 1980). Prostaglandins cause stimulation of uterine contractile activity in a variety of species both in vivo and in vitro (Ramwell and Shaw, 1970). When infused during estrus or following estrogen treatment in ovariectomized ewes, intravenous $PGF_{2\alpha}$ causes an increase in the frequency and amplitude of uterine contractions (Rexroad and Barb, 1975; Roberts and McCracken, 1976). During the luteal phase of the estrous cycle,

however, the uterus is unresponsive to exogenous $PGF_{2\alpha}$ (Roberts and McCracken, 1976). Moreover, elevated myometrial $PGF_{2\alpha}$ synthesis is associated with the high levels of uterine activity and responsiveness to oxytocin during estrus (Roberts et al., 1976). These results are consistent with the observation that the concentrations of high affinity PG receptors in the myometrium of monkey, human, rat and hamster vary with the cycle (Goldberg and Ramwell, 1976). It appears, therefore, that PG receptors are regulated by ovarian steroids and the concentrations of PG receptors is proportional to the rate of PG production and myometrial activity.

In human subjects, as well as in rats, mice and monkeys, exogenous PG's have been shown to induce parturition and abortion (Weeks, 1972). In pregnant sheep, however, the effects of exogenous PG's on uterine contractile activity is uncertain. The injection or infusion of high doses (up to 10 μg/kg) of $PGF_{2\alpha}$ or PGE_2 into near-term sheep failed to cause an immediate increase in spontaneous uterine activity (Liggins et al., 1972; Oakes et al., 1973). Since PG infusions were not associated with concomitant changes in maternal or fetal cardiovascular functions, Oakes et al. (1973) concluded that sheep were insensitive to the effects of PG's. Although Mitchell et al. (1976a) were able to elicit spontaneous uterine activity using high $PGF_{2\alpha}$ infusion rates (94 μg/min), the data were not analyzed and control data were not presented. Similarly, chronic infusions of $PGF_{2\alpha}$ into the maternal aorta have been reported to increase spontaneous uterine activity in pregnant ewes after a time lag of about 24 h (Liggins et al., 1973). These results suggest that in pregnant sheep, myometrial PG receptors are present in low concentrations. In humans,

however, $PGF_{2\alpha}$ receptors were found in high concentrations in myometrium during pregnancy (Bauknecht et al., 1980).

The contribution and interactions of other arachidonic acid metabolities in the regulation of uterine activity in sheep is unknown. The bolus administration of PGI_2 (20 µg/kg) had no effect on uterine activity in a near-term sheep, although PGE_2 (10 µg/kg) was stimulatory (Rankin et al., 1979). Some investigators have suggested that in primates, PGI_2 inhibits spontaneous myometrial and tubal contractions in vitro in a dose-dependent manner (Omini et al., 1979), as well as inhibit those contractions induced by PGF (Bennett and Sanger, 1979). The effects of PGI_2 on the human Fallopian tube was studied in greater detail by Lindblom et al. (1979). These investigators found that although PGI_2 caused relaxation of the circular muscle layer in vitro, it contracted the longitudinal muscle layer. Thorburn and Challis (1979) reasoned that since PGI_2 is produced in large quantities by the human myometrium (Omini et al., 1979; Satchet et al., 1981), it may function as an endogenous inhibitor of uterine activity during pregnancy. However, in vitro studies on uterine strips from pregnant rats show that PGI_2, though less effective than $PGF_{2\alpha}$, stimulated uterine contractility and potentiated the stimulatory effects of oxytocin (Williams et al., 1979). In view of these paradoxical results, the role of PGI_2 in the regulation of uterine activity during pregnancy is difficult to reconcile. In a recent study, Lye and Challis (1982) demonstrated a dose-dependent inhibition of uterine contractility following the administration of 50 µg and 200 µg of PGI_2 into ovariectomized, non-pregnant sheep. These results support the hypothesis that PGI_2 may act as an endogenous inhibitor of uterine

activity during pregnancy in this species. In contrast, TXA_2 contracts vascular smooth muscle strips (Dusting et al., 1978; Samuelsson et al., 1978) and TXA_2 was found to stimulate contractions in non-pregnant human uterine smooth muscle in vitro (Wilhelmsson et al., 1981). However, the effects of thromboxanes on uterine activity in sheep have not been investigated. Challis (1980) suggests that in the human and possibly the sheep, changes in myometrial activity during pregnancy may be regulated by alterations of the relative concentrations of different arachidonic acid metabolites. Since these metabolites have not been measured in uterine tissues and fluids at different times during pregnancy and parturition, their relative importance in the regulation of uterine activity during pregnancy is uncertain.

The importance of PG's during parturition has been confirmed in a number of species by the use of drugs that inhibit the biosynthesis of PG's and related compounds. The delivery of fetuses, induced by the intrafetal administration of dexamethasone is delayed in sheep (Mitchell and Flint, 1978) and pigs (Nara and First, 1981), and the duration of gestation is prolonged in the human (Lewis and Schulman, 1973) and rhesus monkey (Novy et al., 1974a) by inhibitors of PG synthesis. However, these investigators also reported high fetal mortality rates in indomethacin (Novy et al., 1974a; Nara and First, 1981) and meclofenamic acid (Mitchell and Flint, 1978)-treated animals. In addition, some inhibition by indomethacin of myometrial phosphodiesterase activity has been reported (Beaty et al., 1976) and in sheep, Kendall et al. (1977) have shown that estrogen concentrations

rise to unphysiological levels at the amounts of dexamethasone used by Mitchell and Flint (1978). Therefore, the possibility that the effects of inhibitors of PG synthesis on the length of gestation were due to an elevation of myometrial cyclic AMP or fetal distress, cannot be excluded. In humans, Schwartz et al. (1978) demonstrated a decrease in uterine PG output following the administration of flufenamic acid which preceded the inhibition of uterine contractility during pre-term labour, suggesting that PG's may play a role in the initiation of uterine contractions at this time. At present, the role of PG's in the onset of uterine contractions in sheep and the mechanism(s) by which PG's act on the myometrium remain uncertain.

Garfield et al. (1978) have suggested that PG's may be involved in the control of gap junction formation, which may be important in the generation of strong synchronized uterine contractions. The observation that indomethacin prevents gap junction formation when myometrial tissues from pregnant rats are incubated in vitro (Garfield et al., 1978), is consistent with this hypothesis. However, the possibility that these results were due to the accumulation of cyclic AMP as a result of the non-specific actions of the drug were not considered. The increase in size and frequency of gap junctions towards parturition in sheep has been correlated with the increases in fetal and maternal estrogen to progesterone ratios (Garfield et al., 1979). Since an increase in this ratio is thought to stimulate PG synthesis in the sheep uterus (see Section 1.5.2.1), it is possible that PG's play a role in gap junction formation in this species.

1.6.2 Cervical Dilatation

Dilatation of the uterine cervix is an essential part of the process of birth. The ovine cervix is a long (about 10 cm) and firm structure that is tightly closed by rings of cartilaginous-like connective tissue. The cervix shows little change in its physical properties throughout pregnancy until 12 h before the onset of labour, when the cervix rapidly softens (Liggins, 1978). Cervical ripening is associated with increased secretion of glycosaminoglycans, altered composition of glycosaminoglycans and perhaps reduced secretion of collagen (see Liggins, 1978). The mechanism of cervical ripening therefore appears to depend on altered fibroblast activity. However, the stimulus that promotes these changes is unknown.

Stys et al. (1978) have demonstrated in the sheep that dissociation between uterine activity and changes in cervical compliance is possible after mechanical isolation of the cervix from the uterus. These authors recorded intra-amniotic pressure and cervical compliance and found that compliance increased 10-fold within the 12 h preceding uterine activity. These results suggest that cervical ripening is not directly related to uterine activity. The observation (Liggins et al., 1973) that the cervix does not dilate during spontaneous labour in sheep receiving low doses of progesterone (150 mg/24 h) is consistent with this hypothesis.

The role for relaxin, estrogen and PG's has been implicated in the control of cervical ripening (see Liggins, 1978). Injections of PGE_2 cause cervical softening in the pregnant rat (Zarrow and Yochum, 1961) and mouse (O'Byrne and Steinetz, 1976). In

addition, the local application of estradiol to the unripe cervix at term in humans appears to hasten ripening (Gordon and Calder, 1977). These results suggest that changes in circulating estrogen levels at the time of parturition may play a role in the process of cervical dilatation.

Evidence suggests that PG's may play a role in cervical ripening in sheep. Liggins and co-workers (1977) showed cervical softening, shortening and dilatation, accompanied by little or no change in uterine activity after the intra-arterial infusion of $PGF_{2\alpha}$ and PGE_2 (5 to 10 μg/min) in sheep at 125 days of pregnancy. In addition, cervical softening and dilatation in the absence of uterine activity have been demonstrated after the intraluminal infusion of $PGF_{2\alpha}$ and PGE_2 into the cervix (Fitzpatrick, 1977; Liggins et al., 1977). The effects of PG's on cervical ripening appear to be augmented when circulating progesterone levels are low (Liggins et al., 1977), suggesting that progesterone blocks the action of prostaglandin on the cervix, possibly by reducing the population of PG receptors.

Cervical tissue obtained from late pregnant sheep has been shown to produce PG's during in vitro superfusion (Ellwood et al., 1979a; Ellwood and Mitchell, 1980). In addition, PGE and 6-oxo-$PGF_{1\alpha}$ production, but not PGF or TXB_2, were shown to be elevated in cervical tissues obtained from sheep after delivery. Moreover, PGE and 6-oxo-$PGF_{1\alpha}$ concentrations are elevated in the cervical venous drainage during parturition in this species, suggesting that these PG's may be related to the connective tissue changes which take place in the

cervix at parturition (Ellwood et al., 1979b).

MacKenzie and Embrey (1979) have shown that the efficacy of PGE is greater than that of PGF in promoting cervical softening in women. There are also reports that both in vivo and in vitro administration of PGE reduces the stretch modulus of human cervical tissue strips (Conrad and Ueland, 1976, 1978). The effect of prostacyclin on the cervix is unknown.

The importance of PG synthesis in the process of cervical ripening in sheep is suggested from the studies by Mitchell and Flint (1978). These authors report that both uterine activity and cervical softening were inhibited by meclofenamic acid, an inhibitor of PG synthesis, during the induction of labour by the intrafetal infusion of dexamethasone in sheep, despite the occurrence of increasing estrogen and decreasing progesterone concentrations. These observations suggest that progesterone and estrogen are not directly involved in cervical ripening, and they support a direct action of PG's on the cervix. Further work is needed to establish the exact roles of PG's in the cellular mechanism involved in cervical softening.

1.6.3 Uterine Blood Flow

Von Euler (1938) observed that a crude PG preparation increased the resistance of isolated human placental vessels to perfusion. More recently, Novy et al. (1974b) observed an increased fetal arterial pressure and umbilical blood flow following the intrafetal administration of $PGF_{2\alpha}$ (100-300 µg/kg) in anesthetized sheep on 115 to 135 days of pregnancy. Prostaglandin E_2 (20-100 µg/kg)

exerted a profound active vasoconstrictor effect on the fetal placental bed and decreased umbilical blood flow in these animals (Novy et al., 1974b). Similarly, Rankin and Phernetton (1976a) have also shown that in the awake, unanesthetized, chronically catheterized near-term sheep, the injection of PGE_2 into the fetal circulation was followed by profound vasoconstriction in the umbilical vasculature. These findings, combined with the observation that PGE is present in high concentrations in the fetal blood (Challis et al., 1976), suggest that PGE_2 may play a role in the regulation of placental hemodynamics. Rankin (1978) has recently provided evidence suggesting that PGE_2 meets the criteria of the substance which may regulate perfusion ratios in the placenta.

In addition to its vasoconstrictor properties in the umbilical circulation, there are several reports in the literature indicating that PGE_2 may have a vasodilating action on the uterine placental circulation (Ryan et al., 1974; Terragno et al., 1974; Venuto et al., 1975; Rankin and Phernetton, 1976b). Evidence suggests that the pregnant uterus synthesizes PGE and Terrango et al. (1974) have shown that both the rate of PGE_2 synthesis and uterine blood flow decrease after indomethacin treatment.

It is not certain whether PGE_2 crosses the placenta and reaches the umbilical circulation in its vasoactive form. Persaud and Jackson (1977) have shown that tritiated PG's appear to cross the placenta in rats. Similarly, Beazley and co-workers (1972) have shown placental transfer of $PGF_{2\alpha}$ or its radioactive metabolites, during human pregnancy. However, high concentrations of

15-hydroxy-PG dehydrogenase have been reported in the human placenta (Jarabak, 1972). Moreover, this enzyme has been shown to be active in the human (Keirse et al., 1975, 1976) and sheep (Keirse et al., 1975) placenta.

In addition to PGE_2, evidence is emerging which suggests that PGI_2 and possibly TXB_2 may also play a role in the regulation of placental perfusion ratios. Prostaglandin I_2 is synthesized in the pregnant uterus (Myatt and Elder, 1977; Williams et al., 1978) and in human umbilical arteries (Hamberg et al., 1979). Rankin and co-workers (1979) found that PGI_2 (20 µg/kg) given into the maternal circulation caused an increase in the vascular resistance of maternal cotyledons in sheep. In contrast, Hamberg et al. (1979) found that PGI_2 was four times more effective than PGE_1 in relaxing the human umbilical artery in vitro. Prostacyclin, therefore, may play a similar role as PGE in the regulation of placental perfusion ratios.

It appears, therefore, that the substance which best suits the criteria described by Rankin (1976) for the regulation of placental-umbilical blood flows may be arachidonic acid. It is possible that placental and umbilical vessels metabolize arachidonic acid into either contractile or relaxing prostanoids, depending on local conditions, in an attempt to stabilize the perfusion ratios of the placenta.

In the sheep, blood flow to the cotyledons increases markedly with advancing gestation, while the flows to the myometrium and endometrium show little (Rosenfeld et al., 1974) or no (Markowski et al., 1968) changes. In addition to the redistribution of blood flow during pregnancy, there is a large increase in overall uterine

blood flow (Rosenfeld et al., 1974). During spontaneous (Assali et al., 1958) labour, however, uterine contractions are accompanied by a significant decrease in uterine blood flow. In addition, these investigators observed that uterine blood flow decreased with each contraction and that the degree of flow reduction correlated with the intensity of the uterine contractions. The overall decrease in uterine blood flow at this time likely reflects a shunting of blood flow away from the cotyledons in an attempt to minimize blood loss following placental separation. Griess (1965) reasoned that the maternal expulsive efforts (bearing down) stimulated uterine sympathetic nerve discharge with secondary vasoconstriction. It is also possible that catecholamines released from the maternal and possibly fetal adrenal medulla influence uterine hemodynamics (Rankin et al., 1979).

Recent evidence suggests that the change in uterine blood flow during parturition may be related to changes in placental steroid production. The uterine vasculature of non-pregnant, oophorectomized ewes is extremely sensitive to the vasodilatory effect of physiological doses of estradiol. Rosenfeld et al. (1973, 1976) and Anderson and Huckshaw (1974) observed a 15-fold increase in uterine blood flow following the systemic infusion of estradiol (1 mg/kg). This increase in blood flow seemed to be evenly distributed between myometrium, endometrium and caruncles. In addition, Rosenfeld et al. (1976) noted that the responses of the vasodilatory effects of estrogen in these tissues changed during the course of gestation. In the last third of gestation, the systemic infusion of

estradiol resulted in a 3-fold increase in blood flow to the myometrium and a 4-fold decrease in placental cotyledons. In non-pregnant, oophorectomized ewes, concurrent estradiol and progesterone treatment reduced the elevated blood flow observed with estradiol alone (Anderson et al., 1977). These results are consistent with those of Resnik (1976), who reported a significant decrease in the magnitude of the uterine blood flow response to estradiol when given simultaneous infusions of progesterone. These authors also noted that the intrauterine distribution of blood flow to the myometrium is favoured by estradiol and that caruncular flow is favoured by progesterone. It is possible, therefore, that the decreased blood flow occurring during parturition in sheep results from the increase in the estrogen:progesterone ratio in the circulating blood and the subsequent redistribution of blood flow. Since an increase in this ratio may also stimulate PG biosynthesis, it seems reasonable that these changes in blood flow might be PG-mediated. The ability of estrogens to increase the uterine blood flow may be mediated by the secondary production of specific PG's (Ryan et al., 1974). In the rat uterus, estrogen stimulates the production of PGF preferentially over PGE (Kuehl et al., 1976; Ham et al., 1975). If estrogen exerts a similar action on the pregnant sheep uterus, then a decreased blood flow to the placental cotyledon would be predicted. In addition, prostacyclin has recently been shown to cause placental vasoconstriction and myometrial vasodilation (Rankin et al., 1979). During parturition in sheep, PGF concentrations increase in the placental cotyledon and myometrium (Mitchell and Flint, 1977; Liggins and Grieves, 1971); however, concentrations of PGI_2 or its stable hydrolytic product

(6-oxo-$PGF_{1\alpha}$) in these tissues are unknown.

1.7 Possible Role of PG's during Abortive Labour and the Delivery of Dead Fetuses

Spontaneous parturition in sheep is thought to be triggered by fetal adrenal activation which results in changes in the pattern of placental steroidogenesis and subsequent PG biosynthesis. However, labour ensues in the absence of fetal adrenal activity following fetal death in sheep. Evidence suggests that in these circumstances, increased intra-uterine PG production may represent the major signal for the initiation of labour.

After fetal death *in utero* in ewes following fetal catheterization (Carson and Challis, 1981) or due to infection (Smith and Hughes, 1974; Carter *et al.*, 1976), abortion was preceded by a decline in the progesterone concentrations in maternal plasma in the absence of a significant rise in estradiol. The mechanisms leading to the rapid decrease in progesterone and subsequent development of labour in the presence of a dead fetus are uncertain. The importance of PG's in the initiation of abortive labour is suggested from the studies of Mitchell and Flint (1978). The authors found that the maternal administration of meclofenamic acid, a PG synthetase inhibitor, delayed delivery after fetal death *in utero* in sheep. In addition, these authors claim that the intra-uterine fetal death is associated with increased plasma PG concentrations and heightened

responses to oxytocin and $PGF_{2\alpha}$; however, the data were not presented.

For the sheep, Carson and Challis (1981) suggested that the delivery of the dead fetus may be initiated by an abrupt drop in endogenous progesterone levels, leading to the complete withdrawal of the block of PG production and myometrial contractility.

In two monkeys bearing dead fetuses, maternal venous plasma concentrations of 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ did not increase until the time of delivery, approximately 25 and 50 days after fetal death (Mitchell et al., 1976). However, in the monkey, fetal death appears to result in decreased peripheral plasma estrogen with no changes in progesterone concentrations (Mitchell et al., 1976; Challis et al., 1977).

These may explain the rapid onset of abortive labour in sheep following fetal death (Carson and Challis, 1981), compared to monkeys (Mitchell et al., 1976). PG's may also be released from necrotic placental tissue after fetal death (Myers et al., 1974) and/or from the dead fetus. It is possible that in the monkey, tissue necrosis following fetal death may represent the major stimulus for PG production. Alternatively, Mitchell and Flint (1978) have suggested that fetal death may be associated with the withdrawal of a fetus-associated factor which has inhibitory effects on PG synthetase. Further insight on the action of PG's during abortive labour awaits studies investigating the identity and intra-uterine sources of the different arachidonic acid metabolites produced in response to fetal death.

Increased concentrations of $PGF_{2\alpha}$ in amniotic fluid have been observed following the extra-amniotic injection of hypertonic

saline during mid-pregnancy (Gustavii and Green, 1972; Llewellyn-Jones et al., 1975). The decidual cells and their lysosomes have been shown to be extremely fragile (Brunk and Gustavii, 1973) and rupture of decidual lysosomes during saline-induced abortion has been reported (Gustavii and Brunk, 1974; Vassilakos et al., 1974). On the basis of these observations, Gustavii (1973) suggested that the saline-induced rupture of decidual lysosomes resulted in the release of phospholipase A_2 and subsequent PG biosynthesis. In addition, the acidosis resulting from the contractile effects of PG's on smooth muscle may lead to the release of additional lysosomal enzymes (Bengmark et al., 1974). In support of this hypothesis, Waltman and co-workers (1973) found that the administration of PG synthesis inhibitors, such as aspirin or indomethacin, resulted in the prolongation of the instillation-abortion interval in saline-induced abortions in the human. In addition, exogenous PG's or their analogues are commonly used to induce abortion and labour in women (Karim and Sharma, 1971). However, recent evidence suggests that the fetal membranes, particularly the amnion in humans are the major sites of arachidonic acid liberation and phospholipase activity (Okazaki et al., 1981; MacDonald et al., 1978).

Labour induced by amniotomy also appears to result from a local stimulation of PG production. During the last 2-3 weeks of pregnancy, Mitchell et al. (1977) demonstrated higher PG concentrations in amniotic fluid collected by amniotomy than by amniocentesis. Whether the increased PG synthesis following amniotomy results from damage and subsequent labilization of the lysosomes in the fetal

membranes and/or from the loss of excessive amounts of amniotic fluid is unknown. Nor is it known how PG's that are produced in the fetal membranes and/or decidua, or how PG's placed exogenously in the vaginal canal or amniotic cavity, reach the myometrium to exert their action.

CHAPTER 2

RATIONALE

The following hypotheses were investigated in sheep: 1) prostanoid concentrations and output *in vitro* from intra-uterine tissues remain at low levels throughout pregnancy and do not increase until shortly before or during parturition; 2) prostanoids are essential mediators of ACTH-induced labour, and labour associated with the presence of a dead fetus; 3) the amniotic and allantoic membranes produce prostanoids throughout pregnancy, and this production is further increased during parturition; and 4) estrogen, progesterone, placental lactogen and relaxin play a role in the regulation of uterine prostanoid production.

This investigation was assisted by the development of a chronic fetal lamb preparation which allowed the simultaneous measurements of fetal breathing movements, heart rate, blood pressure, uterine activity and the concentrations of various hormones in fetal and maternal blood during normal and experimentally manipulated pregnancy. This development is paramount in studies on fetal physiology since anesthesia and postural changes during acute experimentation profoundly influence blood flow to the fetus and placenta, fetal CNS activity and fetal hormone production (Rudolph and Heymann, 1973).

If prostaglandins are essential mediators in the initiation of labour, it was anticipated the prostanoid concentrations

would be elevated during labour and that indomethacin, an inhibitor of PG synthesis, would inhibit the initiation of parturition. Moreover, if the ratio of estrogen:progesterone was important in the regulation of uterine prostanoid production, then this ratio should be elevated in the maternal circulation during parturition and in the intra-uterine tissues showing elevated prostanoid concentrations at this time. Since PGE, PGF, PGI_2 and TxB_2 have been shown to exert effects upon smooth muscle activity (see Chapter 1), concentrations of PGE, PGF, 6-oxo-$PGF_{1\alpha}$ (the stable product of PGI_2) and TxB_2 were measured in uterine tissue by radioimmunoassay.

If estrogen, progesterone, placental lactogen and/or relaxin influences the pattern of arachidonic acid metabolism in intra-uterine tissues, then it was anticipated that the addition of these hormones to incubation media containing dispersed cells from different intra-uterine tissues would alter the output of prostanoids into the incubation media.

In addition, it was anticipated that a description of the changes in prostaglandin concentrations in the various intra-uterine tissues and fluids at different gestational ages and during labour would provide insight regarding the roles of prostanoids at different stages of pregnancy and during parturition.

SECTION II

GENERAL METHODS AND EXPERIMENTAL DATA

CHAPTER 3

GENERAL MATERIALS AND METHODS

3.1 Animals

Pregnant and non-pregnant sheep of mixed breeds were obtained from a local farmer. For pregnant sheep, the day of successful mating was designated as Day zero of pregnancy. The ewes were fed ad libitum and were housed with at least two other animals in a quiet room with 12 h light/dark cycles at about 25°C.

3.1.1 The Chronic Fetal Lamb Preparation

The technique of fetal and maternal catheterization used in these studies has been published previously (Manchester et al., 1979). Before surgery, the ewes received an injection of Pen-di-strep (4 ml, 1,000,000 IU im). Surgery was performed between Days 110 to 115 following mating.

Anesthesia was induced with sodium pentothal, and was maintained by using a 50:50 nitrous oxide:oxygen mixture, with 2-3% halothane delivered at 2-3 l/min. A vertical incision was made in the lower midline of the maternal abdomen after it was clipped and washed with proviodine detergent. The catheters and electrocardiogram electrodes, which had been sterilized by gamma irradiation, were fed into

the abdominal cavity through a wound made with a large bladder trochar in the right flank of the ewe.

The hind limb of the fetal lamb was withdrawn through a small uterine incision and a catheter (Bolab V4; 0.5 mm bore; vinyl tubing) was implanted in the fetal saphenous vein and advanced to the level of the inferior vena cava. An amniotic fluid catheter (Bolab V10; 1.0 mm bore; vinyl tubing) was left free-floating in the amniotic cavity. After exposing the fetal head and neck through a separate incision, catheters (Bolab V4; 0.5 mm bore; vinyl tubing) were implanted into the fetal carotid artery, jugular vein and trachea. At this time, four leads, each having 24 strands of woven stainless steel wire (Cooner Corp., California, U.S.A.) were sutured over the sternum. Through a third uterine incision in the region of the utero-tubal junction, a separate catheter (Bolab V10; 1.0 mm bore; vinyl tubing) was introduced into the allantoic fluid cavity and was left free-floating.

In most animals, catheters (Bolab V10; 1.0 mm bore; vinyl tubing) were also implanted in the maternal femoral artery and vein. The maternal femoral artery and vein catheters were advanced to the descending aorta and inferior vena cava, respectively.

Penicillin (1,000,000 IU) was injected into the amniotic fluid and into a fetal vascular catheter at the time of surgery. In addition, penicillin was delivered to the fetus and amniotic fluid and Pen-di-strep was given to the mother (i.m.) for three days following surgery for prophylactic purposes.

The catheters and wires were secured in plastic bags on the back of the ewes after testing each catheter for its patency and filling them with sterile heparinized saline (1:250). Fetal demise occurred in some animals 8 to 16 days after surgery, possibly due to infection incurred during subsequent sampling. These animals delivered dead fetuses 2 to 5 days after fetal death.

In a separate group of non-pregnant sheep, ovariectomies were performed under general anesthesia, using a single mid-line incision. In these animals, catheters were also implanted in the femoral artery and vein and prophylactic antibiotics were administered before and after surgery.

3.1.2 Estimates of Uterine Activity

The frequency (contractions/hour) and maximum amplitude of uterine contractions were estimated from recordings of amniotic fluid pressure, using the saline-filled amniotic catheter, a Grass polygraph D.C. Driver Amplifier (Model 7DAC) and Statham pressure transducers calibrated from 0-50 mm Hg. Uterine contractions were defined as increases in resting amniotic fluid pressure exceeding 5 mm Hg. The amplitude of contraction was taken as the distance (mm Hg) between the peak and base of each contraction.

3.1.3 Treatments

Indomethacin, an inhibitor of prostaglandin biosynthesis (Ferreira et al., 1975), was added to 0.12 M phosphate buffer (pH 8.0) and dissolved by heating to approximately 50-55°C while stirring.

After titrating to pH 7.4, the solution (308 mOsm) had a final concentration of about 5 mg/ml which did not precipitate after cooling. Indomethacin (25 mg/kg/day) was given to some animals by a continuous infusion into the maternal femoral vein.

In an attempt to induce parturition prematurely at Day 130 of pregnancy, some animals received a 70 h continuous infusion of $ACTH_{1-24}$ (240 µg/day dissolved in saline) into the fetal saphenous vein.

Estradiol (50 µg) and/or progesterone (50 mg) was administered in corn oil by an i.m. injection, once daily for 9 days in a group of ovariectomized, non-pregnant sheep.

3.1.4 Collection of Plasma

Plasma was collected for measurements of steroid concentrations in some animals containing vascular catheters. First, the heparinized saline occupying the dead space in the catheters was collected and discarded. Fetal (2 ml) and maternal (5 ml) blood samples were then collected into chilled, heparinized plastic tubes and the catheters were flushed with heparinized saline. The plasma was separated by centrifugation at approximately 1,500 x g at 4°C for 15 min. The plasma for each sample was then transferred into plastic culture tubes and stored at -20°C until assayed.

3.2 Materials

3.2.1 Chemicals

The following chemicals were used in this investigation:

absolute alcohol	Commercial Alcohols, Ltd. Gatinea, Quebec
acetic acid	Fisher Scientific Co. Fair Lawn, New Jersey, U.S.A.
chloroform	Fisher Scientific Co.
dextran T70	Pharmacia Fine Chemicals HB Uppsala, Sweden
diethyl ether (reagent grade)	Fisher Scientific Co.
gelatin	Fisher Scientific Co.
Norit A (activated charcoal)	Matheson, Coleman and Bell Syracuse, New York, U.S.A.
petroleum ether (re-distilled)	Fisher Scientific Co.
PPO (2,5-diphenyloxazole)	Canadian Scientific Products London, Ontario
proviodine detergent	Rougier Inc, Quebec
saline (0.9% NaCl)	Travenol Laboratories, Inc. Malton, Ontario
sesame oil (laboratory grade)	Fisher Scientific Co.
sodium azide	Fisher Scientific Co.
toluene	Fisher Scientific Co.

3.2.2 Drugs and Hormones

The following drugs and hormones were used in this study:

estradiol	Sigma Chemical Co. St. Louis, Missouri, U.S.A.
estrone	Sigma Chemical Co.

indomethacin	Sigma Chemical Co.
Pen-di-strep (penicillin-streptomycin)	Roger STB London, Ontario
pentothal	Abbott Laboratories Montreal, Quebec
progesterone	Sigma Chemical Co.
prostaglandins E_2, $F_{1\alpha}$, 6-oxo-$PGF_{1\alpha}$, thromboxane B_2	Dr. J. E. Pike The Upjohn Co. Kalamazoo, Michigan, U.S.A.
Synacthen ($ACTH_{1-24}$)	Ciba-Geigy Co.

3.3 Analytical Methods

3.3.1 Collection of Tissues for Prostaglandin Extraction

All tissues were collected under general anesthesia, induced with sodium pentothal. In some animals anesthesia was maintained by using 50:50 nitrous oxide:oxygen mixture with 2-3% halothane delivered at 2-3 l/min. The pregnant uterus was exposed by a vertical abdominal incision. Samples of myometrium, endometrium, cotyledons, chorioallantois and amnion were collected from the antimesometrial side of the uterus. Tissues were not collected from uterine areas adjacent to the incision made at the time of catheterization.

Tissues were placed immediately in ice-cold phosphate buffer (pH 7.4, 0.12 M) containing indomethacin (100 µg/ml). The tissues were blotted and then frozen on dry ice. Edges of frozen tissue, except cotyledons, were trimmed and discarded in an attempt

to minimize the effect of trauma on PG concentrations. The remaining tissue was weighed, homogenized at 4°C in ethanol and centrifuged. The supernatant was stored at -20°C until assayed.

3.3.2 In Vitro Experiments

Isolated amnion, chorioallantois, cotyledons and caruncle cells were prepared using a modification of the method of Glickman et al. (1979). The tissues were collected as outlined above (Section 3.3.1), weighed and cut into small pieces. The fragments were dispersed in 15 ml Krebs-Ringer bicarbonate buffer containing 0.2% glucose and 0.05% collagenase (Type I; Sigma) at 37°C under an atmosphere of 95% O_2 and 5% CO_2. Dispersion was performed in a 50 ml plastic beaker containing 8x3 mm glass beads and a small magnetic stirrer. Harvests of medium (10 ml) were made at 10 min intervals, at which time the enzyme preparation was replenished. A total of 3 collections were made.

The individual harvests were stored at 4°C, pooled and centrifuged at 200 x g for 30 min at 4°C after slow acceleration (40 x g/min). After centrifugation, the cell pellet was resuspended in 10 ml Krebs-Ringer buffer supplemented with glucose (0.2%) and trypsin inhibitor (0.05%, Type 1; Sigma). The cells were recentrifuged at 200 x g for 30 min after slow acceleration and the second pellet was resuspended in 20 ml of the above buffer. After filtration through a wet cotton gauze into a 100 ml beaker, an aliquot of the filtrate was taken for counting using a hemocytometer. Cell viability was also assessed at this time after a 4 h incubation using trypan blue

viability exclusion stain. Incubations were performed in polystyrene culture tubes (12 x 75 mm; Canlab) in a total volume of 1 ml. Each incubation was carried out at 37°C for 4 h under 95% O_2-5% CO_2 in a shaking water bath. Incubations were terminated by centrifuging the tubes for 10 min at 1,500 x g, and the tubes were stored at -20°C until assayed.

3.3.3 Radioimmunoassays

3.3.3.1 Steroids and cyclic AMP

Progesterone, estrone, estradiol and cortisol were measured by radioimmunoassay using methodology described previously (Garfield et al., 1979; Glickman et al., 1979).

Progesterone was extracted from plasma with petroleum ether. In nine random samples of maternal sheep blood, the concentration of progesterone was measured either directly after petroleum ether extraction or after extraction and subsequent thin layer chromatography in the system cyclohexane:ethyl acetate (1:1, v/v). The mean ratio of progesterone concentrations determined by the two methods were 0.97 ± 0.03 (S.E.M.). the progesterone antibody (No. 465/5, donated by Dr. B. J. A. Furr, ICI Limited, Macclesfield, England) had the following cross-reactivities: 11α-hydroxyprogesterone, 35.9%; deoxycorticosterone, 23.8%; 11β-hydroxyprogesterone, 3.4%; corticosterone, 0.9%. Other C_{21}, C_{19} and C_{18} steroids tested had less than 0.2% cross-reactivity. The intra-assay and inter-assay co-efficients of variation were 13.9% and 14.7%, respectively. The sensitivity of the progesterone immunoassay was 8 pg/tube and the 50% binding point was 60 pg.

Total cortisol was measured in ethanolic extracts of plasma using the antibody F21-53 (Endocrine Sciences, California, U.S.A.) prepared against cortisol-21-bovine serum albumin. The sensitivity of the cortisol immunoassay was 5 pg/tube and solvent blank values were always below this value. The principal cross-reacting steroids were cortisol sulphate (155%), corticosterone (48.4%) and deoxycorticosterone (4.3%). The inter-assay and intra-assay co-efficients of variation were 4.0% and 8.9%, respectively.

Estrone and estradiol-17β were measured by radioimmunoassay after extraction from plasma with diethyl ether. The estrone and estradiol antibodies were raised in sheep against 1,3,5(10)-estratrien-3-ol-6,17dione 6-CMO-BSA (Steraloids) and 1,3,5(10)-estratrien-3,17β-diol-6-one 6-CMO BSA (Steraloids), respectively. The sensitivity of estrone and estradiol immunoassays were 3-5 pg/tube and the 50% binding points were 30 pg and 45 pg, respectively. The estrone antibody used at an initial dilution of 1:8,000 cross-reacted with epiestriol (2.6%), 16-oxo-estradiol-17β (1.9%), estradiol-17α (1.5%), estradiol-17β (0.1%) and progesterone, testosterone and cortisol (< 0.1%). The estradiol antibody used at an initial dilution of 1:13,000 cross-reacted with epiestriol (3.0%), estrone (1.1%), estradiol-17α (0.7%), 16-oxo-estradiol-17β (0.4%), progesterone and cortisol (< 0.1%). The intra-assay co-efficients of variation were 7.9% and 9.4% and the inter-assay co-efficients of variation were 14.9% and 9.8% for estrone and estradiol-17β, respectively.

All samples from any one sheep were measured in the same assay. All measurements were corrected for methodological losses,

assessed from the mean recovery of (^{3}H)progesterone, (^{3}H)cortisol, (^{3}H)estrone and (^{3}H)estradiol-17β added to quadruplicate samples prior to extraction.

Measurements of cyclic adenosine 3',5'-monophosphate (cyclic AMP) in ethanolic extracts of myometrium were performed as described previously (Goff and Armstrong, 1977).

3.3.3.2 Prostaglandins

(1) Tissue concentrations: Prostaglandin concentrations were estimated by RIA, as described previously for PGE (Evans, 1978) and for 6-oxo-$PGF_{1\alpha}$ (Kennedy et al., 1980), using PGE_2 and 6-oxo-$PGF_{1\alpha}$ respectively as standards. The RIA for PGF was similar to that for 6-oxo-$PGF_{1\alpha}$ (Kennedy et al., 1980), but used PGF antiserum, $PGF_{2\alpha}$ standard and (^{125}I)-labelled histamine $PGF_{1\alpha}$ complex as tracer. Similarly, the TXB_2 RIA was similar to that for 6-oxo-$PGF_{1\alpha}$ (Kennedy et al., 1980), but used TXB_2 antiserum, TXB_2 standard and (^{125}I)-labelled histamine TXB_2 complex as tracer. Sensitivities of the assays varied between 5 and 15 pg/tube. Specificities, as assessed by inhibition of binding of tracers to antisera by a series of prostaglandins, are presented in Table 1. It should be noted that the PGE antibody cross-reacted with PG's of the A and B series; hence the PGE concentrations that are reported may be overestimated.

To assess further the specificities of the various PG assays, the specific activities for PGE, PGF and 6-oxo-$PGF_{1\alpha}$ were determined in ethanolic extracts of cotyledons, collected on Day 135 of pregnancy, following silicic acid chromatography. Samples, to which had been added approximately 10,000 dpm purified (^{3}H)PGE_2 or

TABLE 1: Cross-Reactivities of the Antisera Used in These Studies

	Percent Cross-Reactions with Antisera Raised Against			
	$PGF_{2\alpha}$	PGE_2	TxB_2	6-oxo-$PGF_{1\alpha}$
$PGF_{2\alpha}$	100	N.D.*	< 1	4.0
$PGF_{1\alpha}$	125	< 1	N.D.*	N.D.*
PGE_1	< 1	45.8	N.D.	N.D.
PGE_2	< 1	100	< 1	2.1
PGA_1	< 1	13.2	N.D.	N.D.
PGA_2	< 1	8.9	N.D.	< 1
PGB_1	< 1	108	N.D.	N.D.
PGB_2	< 1	88.5	N.D.	N.D.
13,14 Dihydro-15-keto-$PGF_{1\alpha}$	1.3	< 1	N.D.	< 1
13,14 Dihydro-15-keto-$PGF_{2\alpha}$	< 1	< 1	N.D.	< 1
13,14 Dihydro-15-keto-PGE_1	< 1	2.7	N.D.	< 1
13,14 Dihydro-15-keto-PGE_2	< 1	N.D.	N.D.	N.D.
15-keto-$PGF_{2\alpha}$	< 1	< 1	N.D.	< 1
15-keto-PGE_2	< 1	< 1	N.D.	N.D.
15-keto-PGE_1	< 1	< 1	N.D.	N.D.
6-oxo-$PGF_{1\alpha}$	< 1	< 1	< 1	100
TxB_2	< 1	< 1	100	< 1
PGD_2	N.D.	N.D.	< 1	< 1

*N.D., not determined

(^{3}H)6-oxo-PGF$_{1\alpha}$, or 15,000 dpm (^{3}H)PGF$_{1\alpha}$ were applied to silicic acid columns, prepared as described previously (Kennedy, 1978) in 2 x 200 ml of 2% methanol in chloroform containing 0.5% acetic acid. Fractions (800 µl) were collected during elution with 6 ml of 2%, 5 ml of 6%, 2 ml of 10% and 6 ml of 20% methanol in chloroform. For each fraction, the amount of radioactivity and the content of PGE, PGF and 6-oxo-PGF$_{1\alpha}$ estimated by RIA and corrected for column blanks were estimated by applying 2 x 200 µl 2% methanol in chloroform containing 0.5% acetic acid in separate columns and eluting as described above.

The specific activities, calculated in fractions containing detectable mass and the co-elution profiles of PGE, PGF and 6-oxo-PGF$_{1\alpha}$ in ethanolic extracts of cotyledons following silicic acid chromatography are shown in Figures 2-4. Recoveries of mass from all fractions estimated by direct assay of a separate aliquot were 87.5%, 102.9% and 82.9% for PGF, 6-oxo-PGF$_{1\alpha}$ and PGE, respectively. The possibility that the PG antisera cross-reacted with other compounds present in extracts of cotyledons seems unlikely in view of the relatively uniform specific activities. For PGE, the initial mass peak determined by RIA may be due to the presence of PG's of the A and B series, since these PG's elute before PGE in this system and cross-react with the PGE antibody.

Accuracy was estimated by determining the content of PG's before and after the addition of standard amounts of PGE, PGF, TXB$_2$ and 6-oxo-PGF$_{1\alpha}$ to separate ethanolic extracts of non-pregnant sheep uteri. The recoveries of known amounts of PGE, PGF, TXB$_1$ and 6-oxo-PGF$_{1\alpha}$ expressed by linear regression analysis were $Y = 2.02 + 0.86\ X$

FIGURE 2

Specific activities calculated from the co-elution of ^{3}H-prostaglandin E_2 and extracts of sheep cotyledons containing detectable mass of prostaglandin E, estimated by radioimmunoassay (RIA).

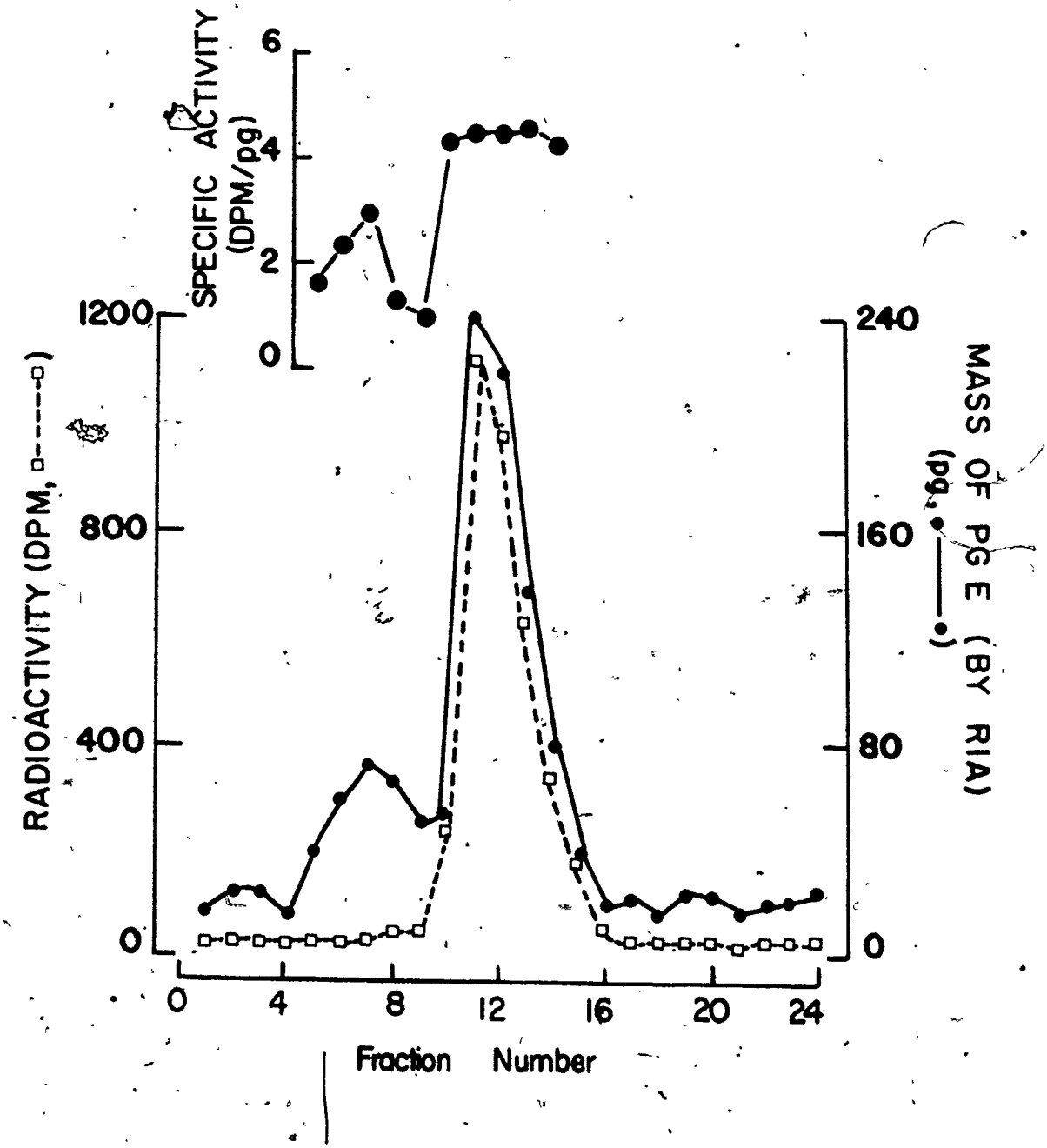

SPECIFIC ACTIVITY (DPM/pg)
6
4
2
0
RADIOACTIVITY (DPM, □----□)
1200
800
400
0
MASS OF PG E (BY RIA) (pg, •——•)
240
160
80
0
0
4
8
12
16
20
24
Fraction Number

FIGURE 3

Specific activities calculated from the co-elution of ^{3}H-prostaglandin $F_{2\alpha}$ and extracts of sheep cotyledons containing detectable mass of prostaglandin $F_{2\alpha}$ estimated by radioimmunoassay (RIA).

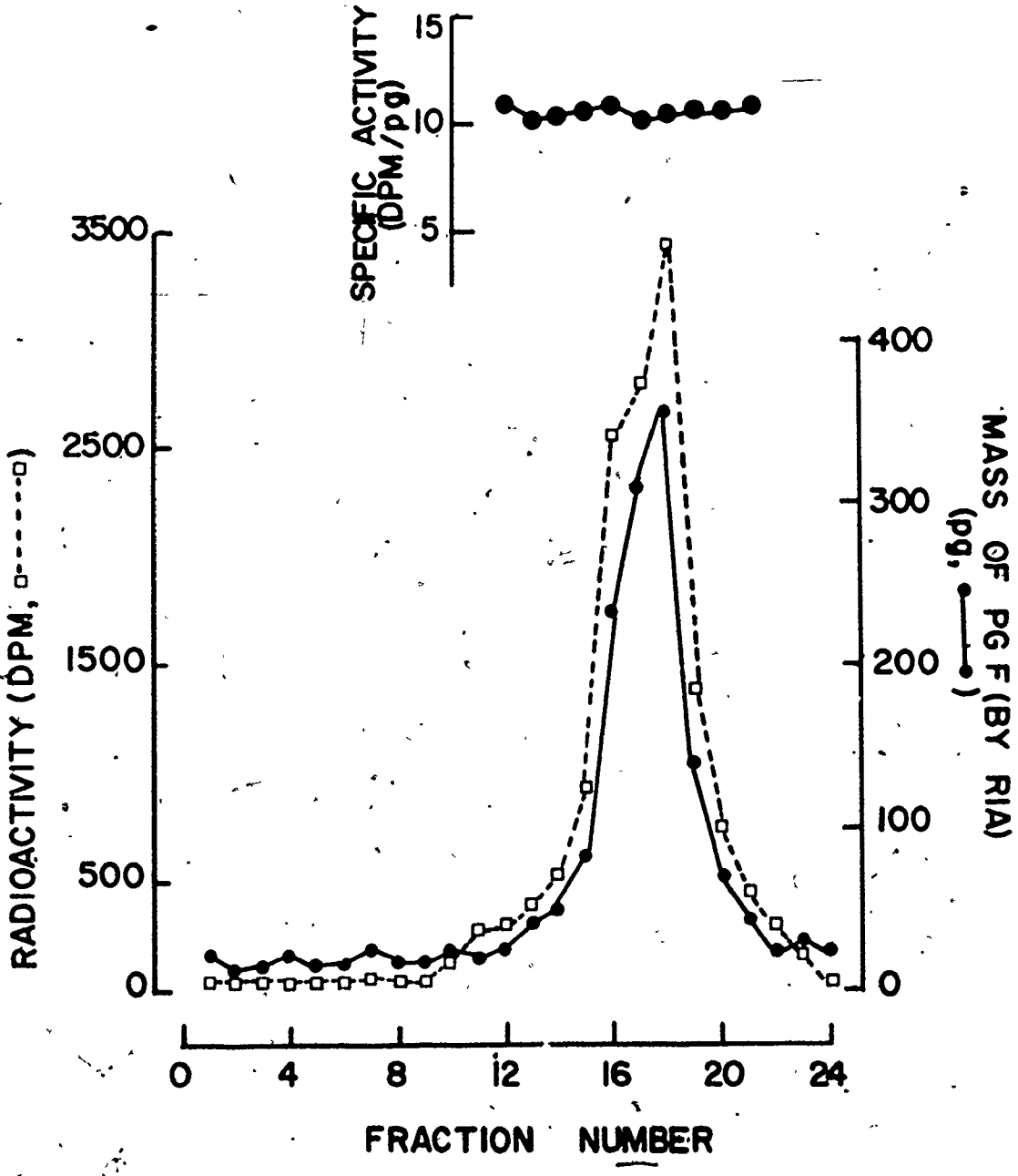
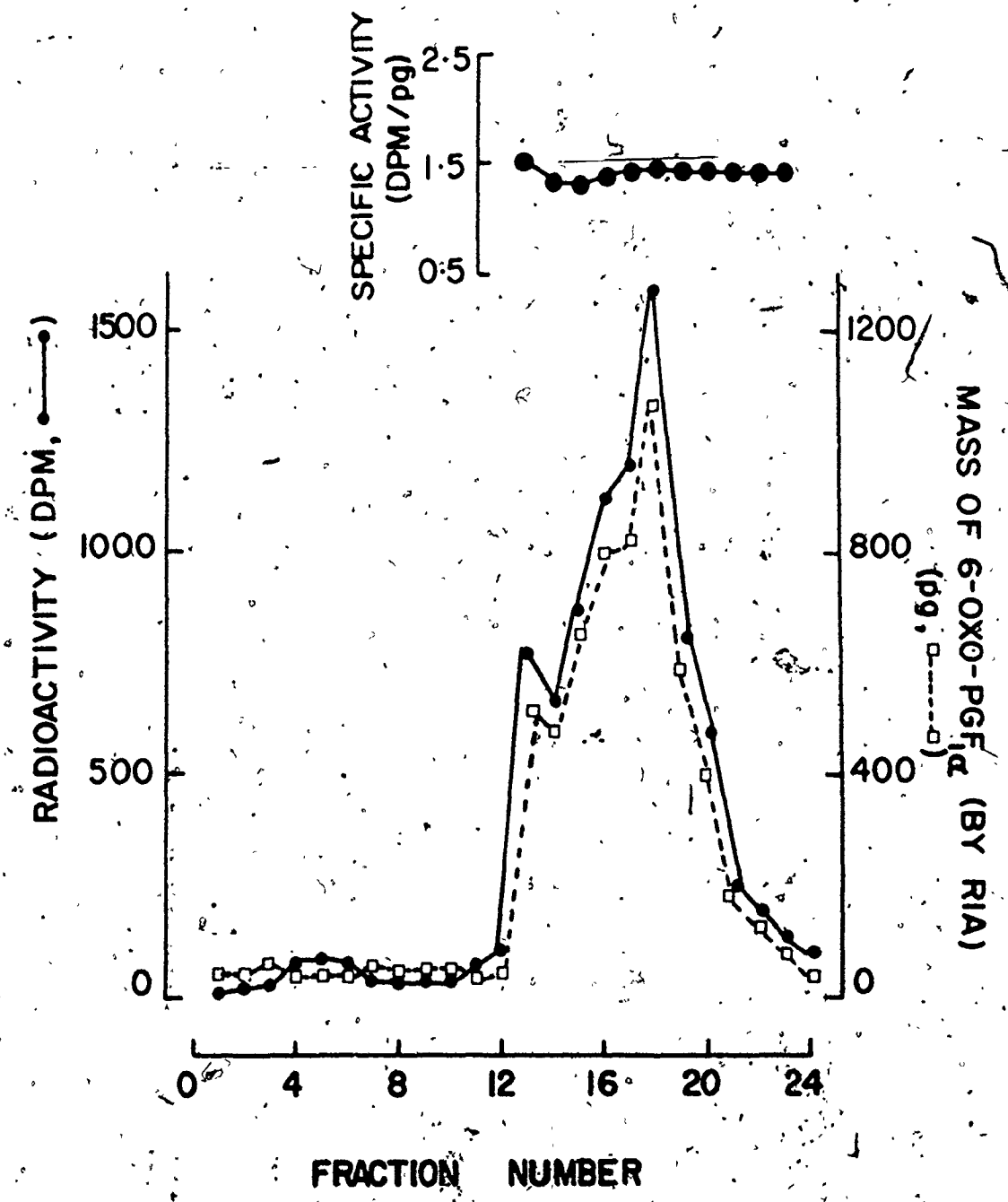

SPECIFIC ACTIVITY (DPM/pg)
15
10
5
3500
2500
1500
500
0
RADIOACTIVITY (DPM, □----□)
400
300
200
100
0
MASS OF PG F (BY RIA) (pg, ●—●)
0
4
8
12
16
20
24
FRACTION NUMBER

FIGURE 4 -

Specific activities calculated from the co-elution of ^{3}H-6-oxo-prostaglandin $F_{1\alpha}$ and extracts of sheep cotyledons containing detectable mass of 6-oxo-prostaglandin $F_{1\alpha}$, estimated by radioimmunoassay (RIA).


SPECIFIC ACTIVITY (DPM/pg)
2·5
1·5
0·5
RADIOACTIVITY (DPM, ●——●)
1500
1000
500
0
1200
800
400
0
MASS OF 6-OXO-PGF1α (BY RIA) (pg, □------□)
0
4
8
12
16
20
24
FRACTION NUMBER

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Full Name of Author — Nom complet de l'auteur

CHARLES ALEXANDER EVANS

Date of Birth — Date de naissance	Country of Birth — Lieu de naissance
1953	Canada

Permanent Address — Résidence fixe

526 - 37th Street N.W.
Calgary, Alta.
Canada

Title of Thesis — Titre de la thèse

ESTIMATION AND REGULATION OF UTERINE PROSTANOIDS DURING OVINE PREGNANCY AND DELIVERY

University — Université

The University of Western Ontario, London, Ontario

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Doctor of Philosophy

Year this degree conferred — Année d'obtention de ce grade	Name of Supervisor — Nom du directeur de thèse
1982	Doctor J.R.G. Challis

Date	Signature
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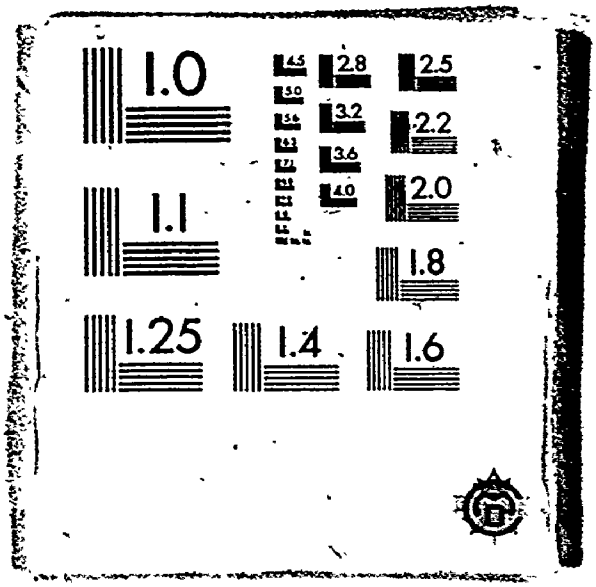

1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
5.0

($r = 0.996$, $n = 10$), $Y = 0.86 + 0.80\ X$ ($r = 0.987$, $n = 10$), $Y = 1.07 + 0.91\ X$ ($r = 0.991$, $n = 10$), $Y = 1.6 + 0.96\ X$ ($r = 0.982$, $n = 10$), respectively, where X = amount added and Y = amount recovered (corrected for endogenous concentrations). The intra-assay co-efficients of variation for PGE, PGF, 6-oxo-$PGF_{1\alpha}$ and TXB_2 were 3.5%, 10.5%, 4.5% and 3.4%, respectively, and the inter-assay co-efficients of variation were 13.5%, 13.35%, 12.8% and 13.5%, respectively. The efficiencies of the extraction procedure were determined for each uterine tissue by estimating the recoveries of (^{3}H)PGE_2, (^{3}H)$PGF_{2\alpha}$, (^{3}H)6-oxo-$PGF_{1\alpha}$ and (^{3}H)TXB_2 added prior to homogenization. Within each tissue, recoveries were consistent (Table 2), and PG concentrations were not corrected for methodological losses. In five random samples of sheep myometrium, the concentrations of PGE, PGF, TXB_2 and 6-oxo-$PGF_{1\alpha}$ were estimated either directly after ethanolic extraction or after extraction and subsequent silicic acid column chromatography, prepared as described above and eluted with 6 ml of 2% and 8 ml of 20% methanol in chloroform. The mass of PGE, PGF, TXB_2 and 6-oxo-$PGF_{1\alpha}$ determined by direct assay, compared to that determined by column chromatography expressed by linear regression analysis was $Y = 1.25\ X - 2.22$ ($r = 0.928$, $n = 5$), $Y = 1.15\ X - 1.28$ ($r = 0.978$, $n = 5$), $Y = 1.09 + 0.89\ X$ ($r = 0.982$, $n = 5$), $Y = 5.49 + 0.82\ X$ ($r = 0.998$, $n = 5$), respectively, where X = mass determined after column chromatography and Y = mass determined by direct RIA of ethanolic extracts.

(2) Amniotic and allantoic fluids: Prostaglandin concentrations in amniotic and allantoic fluids were determined by RIA

TABLE 2: Percent recovery of prostaglandins during ethanolic extraction from sheep uterine tissues (n=5)

Uterine Compartment	^{3}H-PGE$_2$		^{3}H-PGF$_2$		^{3}H-6-keto-PGF$_{1\alpha}$		^{3}H-Thromboxane B$_2$	
	Mean±SEM	% C.V.*	Mean±SEM	% C.V.	Mean±SEM	% C.V.	Mean±SEM	% C.V.
Myometrium	104.3±1.9	4.1	91.6±0.8	1.7	88.2±1.7	4.4	100.0±1.8	4.0
Endometrium	104.3±1.2	2.5	92.3±1.0	2.3	86.6±1.8	4.7	100.7±2.3	5.1
Whole cotyledons	86.3±1.3	3.4	74.6±2.4	7.0	71.7±4.1	12.7	85.8±2.9	7.6
Chorioallantois	100.2±1.8	3.6	92.5±1.4	3.5	87.7±6.9	15.7	97.9±1.6	3.6
Amnion	101.3±0.9	1.9	94.8±2.2	5.2	82.2±6.3	15.4	98.0±2.4	5.4

*co-efficient of variation

TABLE 3: Recoveries of [^{3}H]-labelled and cold PGF, PGE, 6-oxo-$PGF_{1\alpha}$ and TxB_2 from amniotic and allantoic fluids (mean ± SEM, n = 6)

	Recovery of radioactivity (%)				Recovery of mass[a] (%)			
	PGF	PGE	6-oxo-$PGF_{1\alpha}$	TxB_2	PGF	PGE	6-oxo-$PGF_{1\alpha}$	TxB_2
Amniotic fluid	74.8±1.8	84.3±1,0	68.9±1.9	73.0±1.8	76.9±2.4	85.6±2.1	68.9±1.6	76.4±2.4
Allantoic fluid	88.7±1.4	87.1±2.3	77.8±2.1	80.2±1.3	90.1±1.0	88.2±1.4	82.4±2.3	81.9±2.9

[a] 2 ng added prior to extraction

after acidification to pH 3 and subsequent extraction with 20% methanol in chloroform. The efficiency of the extraction procedure was estimated by determining the recoveries of 2 ng of PGF, PGE, 6-oxo-$PGF_{1\alpha}$ and TXB_2 and of (^{3}H)PGF, (^{3}H)PGE, (^{3}H)6-oxo-$PGF_{1\alpha}$ and (^{3}H)TXB_2 (Table 3). All measurements were corrected for methodological losses, assessed from the mean recovery of (^{3}H)$PGF_{2\alpha}$, (^{3}H)PGE_2, (^{3}H)6-oxo-$PGF_{1\alpha}$ and (^{3}H)TXB_2 added to quadruplicate samples prior to extraction.

In seven random samples of amniotic and allantoic fluids, the concentrations of PGF, PGE, 6-oxo-$PGF_{1\alpha}$ and TXB_2 were estimated either directly after ethanolic extraction or after extraction and subsequent silicic acid column chromatography. Columns were prepared as described above and prostanoids were eluted with 6 ml of 2% and 8 ml of 20% methanol in chloroform. For amniotic fluid the concentrations of PGF, PGE, TXB_2 and 6-oxo-$PGF_{1\alpha}$ determined by direct assay, compared to those determined by column chromatography, expressed by linear regression analysis were $Y = 1.16 + 0.87 X$ ($r = 0.956$), $Y = 1.78 + 0.94 X$ ($r = 0.981$), $Y = 1.04 X - 2.61$ ($r = 0.961$), and $Y = 3.27 + 0.98 X$ ($r = 0.989$), respectively. Similarly, regression analysis for PGF, PGE, TXB_2 and 6-oxo-$PGF_{1\alpha}$ concentrations in allantoic fluids were $Y = 2.1 + 0.82 X$ ($r = 0.97$), $Y = 2.71 + 1.14 X$ ($r = 0.972$), $Y = 0.31 + 1.21 X$ ($r = 0.961$) and $Y = 1.01 + 0.97 X$ ($r = 0.986$), respectively, where X = the concentration determined after column chromatography and Y = concentration determined by direct RIA following extraction with 20% methanol in chloroform.

(3) Prostaglandin output *in vitro*: Prostaglandin output *in vitro* was estimated by RIA in aliquots of Krebs-Ringer buffer following

centrifugation. To determine net output, the PG content at the start of the incubation period (T_0 concentration) was subtracted from that measured at the end of the incubation. Evaluation of the PG assays was performed by adding known amounts (0-4 ng) of PG to Krebs-Ringer buffer. Regression analyses for PGE, PGF and 6-oxo-$PGF_{1\alpha}$ concentrations were $Y = 0.071 + 1.002\ X$ ($r = 0.996$, $n = 6$), $Y = 0.187 + 0.802\ X$ ($r = 0.987$, $n = 6$) and $Y = 0.142 + 0.974\ X$ ($r = 0.976$, $n = 6$), respectively, where Y = amount of PG added and X = amount of PG recovered. The intra-assay co-efficients of variation for PGF, PGE and 6-oxo-$PGF_{1\alpha}$ were 10.5%, 3.5% and 4.5%, respectively. The inter-assay co-efficients of variation for PGF, PGE and 6-oxo-$PGF_{1\alpha}$ were 13.3%, 13.5% and 12.8%, respectively.

CHAPTER 4

EVALUATION OF TISSUE COLLECTION PROCEDURE FOR PG DETERMINATIONS; EFFECTS OF UTERINE AREA AND OF TRAUMA

4.1 Introduction

A number of investigators have reported PG concentrations in intra-uterine sheep tissues during late pregnancy and parturition (Liggins and Grieves, 1971; Mitchell and Flint, 1977). However, interpretation of the results from these studies have been confounded by the high variability in PG concentrations between the single samples taken from each animal.

Mechanical distention of the guinea pig uterus *in vitro* has been shown to increase PG release (Horton *et al.*, 1971). During pregnancy, physiological distention of perifetal uterine tissues, as a result of fetal growth, may result in increased PG release in these areas. Since the degree of distention is not uniform throughout the uterus, intra-uterine PG concentrations may vary. Similarly, Thorbert and his colleagues (1977, 1979) have recently shown that during pregnancy in the guinea pig, there is a reduction in norepinephrine content, number of neurons and (^{3}H)norepinephrine uptake in perifetal tissue and that there are qualitative differences in different areas of the uterus. These changes may also influence intra-uterine PG production (Hedquist, 1973, 1976). In addition, the effects of trauma,

such as that occurring during the collection of intra-uterine tissues, on the production of different arachidonic acid metabolites has not been investigated in sheep.

In the present study, therefore, the effects of uterine area and of crushing intra-uterine tissues on PG concentrations were investigated by measuring the levels of PGE, PGF and 6-oxo-$PGF_{1\alpha}$ (the hydrolytic product of PGI_2) in tissue samples collected from myometrium, endometrium, cotyledons, chorioallantois and amnion taken from five different areas of the uterus.

4.2 Materials and Methods

Eight sheep of mixed breeds between 120 and 145 days of pregnancy were used. Gestational ages were estimated from fetal crown-rump lengths and fetal weights (Barcroft, 1946). In one ewe, a vascular catheter was implanted in the femoral vein on Day 115 of pregnancy. Beginning on Day 127 of pregnancy, indomethacin (25 mg/kg/day) was infused in phosphate buffer (pH 7.4, 0.12 M) at a continuous rate until tissues were collected on Day 130. All tissues were collected under general anesthesia as described previously (see Chapter 3).

The effects of incubating freshly dissected myometrium for 1-4 min in ice-cold phosphate buffer, with or without indomethacin (100 µg/ml) prior to freezing on dry ice, on PG concentrations were investigated in five animals. A sample of myometrium was collected

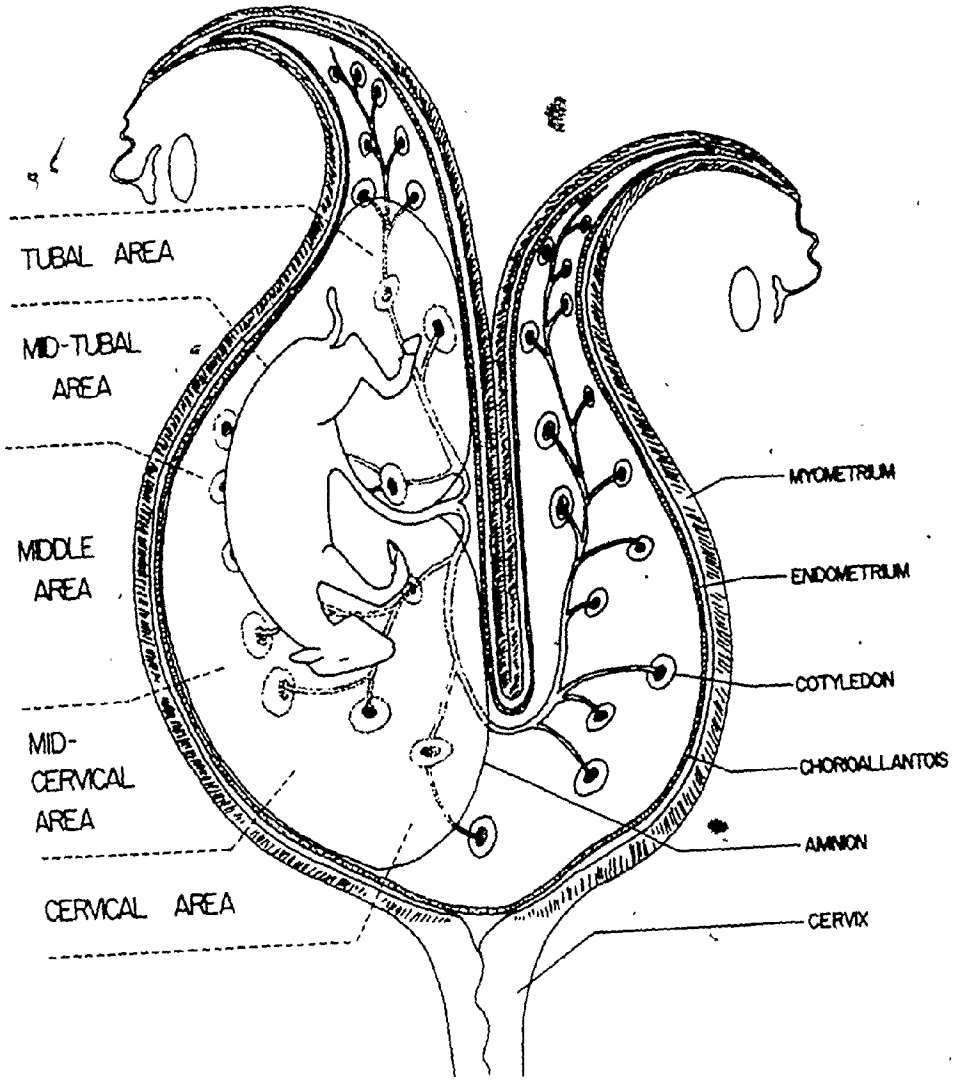
from the middle of the uterus in each animal. Each sample was cut into three pieces: one section was placed in phosphate buffer containing indomethacin; a second section was placed in phosphate buffer alone; and the third section was frozen immediately.

To determine the effects of uterine area on tissue PG concentrations within animals, samples were collected from tubal, middle and cervical uterine areas for myometrium, endometrium, cotyledons, chorioallantois and amnion. Samples were also obtained from regions intermediate between tubal and middle, and between middle and cervical, designated mid-tubal and mid-cervical, respectively. A diagrammatic representation of a pregnant sheep uterus showing these different areas of the uterus is shown in Figure 5. Sampling proceeded from middle, mid-cervical, cervical, mid-tubal to tubal uterine areas and from myometrium, endometrium, chorioallantois, amnion to cotyledon tissues. For each tissue the sampling protocol was repeated four times in each sheep. Samples were placed immediately in phosphate buffer containing indomethacin (100 μg/ml) for 1-4 min, blotted and frozen on dry ice.

The effects of crushing on intra-uterine PG concentrations were investigated in five animals. Ten samples of myometrium, endometrium, chorioallantois and amnion were collected randomly from the different uterine areas in each animal. After dissection, each of the samples was cut into two pieces: one section was crushed repeatedly using a hemostat while the other served as a control. Samples were incubated for 1 min in phosphate buffer containing indomethacin (100 μg/ml). The surface moisture and blood were then blotted and the samples were frozen on dry ice. The possibility that crushing does

FIGURE 5

A diagrammatic representation of a pregnant sheep uterus showing myometrium, endometrium, cotyledons, chorioallantois, amnion, and the different uterine areas from which these tissues were collected (adapted from D.H. Steven, 1975).


TUBAL AREA
MID-TUBAL AREA
MIDDLE AREA
MID-CERVICAL AREA
CERVICAL AREA
MYOMETRIUM
ENDOMETRIUM
COTYLEDON
CHORIOALLANTOIS
AMNION
CERVIX

not result in large increases in uterine PG concentrations because the "trauma" of dissection induces maximal PG production rates was investigated in a ewe which received a continuous infusion of phosphate buffer containing indomethacin (25 mg/kg/day) for 70 h. Ten samples of myometrium, endometrium, chorioallantois and amnion were collected randomly from the different uterine areas. After dissection, each of the samples was cut into two pieces: one section was crushed using a hemostat while the other served as a control. Samples were then placed in phosphate buffer containing indomethacin (100 µg/ml), blotted and frozen on dry ice.

The possibility that the mechanical "trauma" induced during the manual separation of fetal and maternal cotyledons results in increased PG production in these tissues was also investigated. Eight cotyledons were collected from a ewe 135 days pregnant. "Fetal"[a] and "maternal"[a] components of four placentomes were crudely separated by squeezing the base of each cotyledon. The remaining four cotyledons were left intact and all tissues were placed in phosphate buffer containing indomethacin (100 µg/ml) prior to freezing on dry ice. The concentrations of PG's in both components of separated cotyledons were calculated by adding the content of PG's in the maternal component and dividing by the total mass of the cotyledons.

Prostaglandins E and F and 6-oxo-$PGF_{1\alpha}$ were extracted and assayed as described previously (see Chapter 3).

The results are expressed as either proportions or means ± S.E.M., the latter being based on within or between animal variation

[a]Since these tissues interdigitate "fetal" cotyledons likely contain some maternal tissue also and vice versa.

TABLE 4: The effects of incubating myometrial tissue samples in the presence or absence of indomethacin (a) in phosphate buffer (b) on prostaglandin concentrations (pg/mg; mean ± S.E.M.; n = 5 animals)

	Incubated (1-4 minutes)		
	With Indomethacin	Without Indomethacin	Not Incubated
6-oxo-$PGF_{1\alpha}$	16.8 ± 1.8	26.2 ± 6.7*	20.9 ± 3.6
Prostaglandin F	3.4 ± 1.8	8.8 ± 2.6*	5.2 ± 1.6
Prostaglandin E	27.6 ± 9.8	42.4 ± 10.2	31.2 ± 7.9

*Significantly different from indomethacin incubations ($P < 0.01$, Duncan's New Multiple Range Test)

(a) 100 µg/ml

(b) 0.12 M, pH 7.4

as indicated in Results. The significance of uterine area effects within each tissue was determined by one-way analysis of variance. The significance of the effects of crushing and of separating maternal and fetal placentomes of PG concentrations was determined by paired or unpaired Student's *t* tests, as indicated in Results. When heterogeneity of variance was present, determined by the F_{max} test (Sokal and Rohlf, 1969), the data were transformed logarithmically prior to statistical analysis.

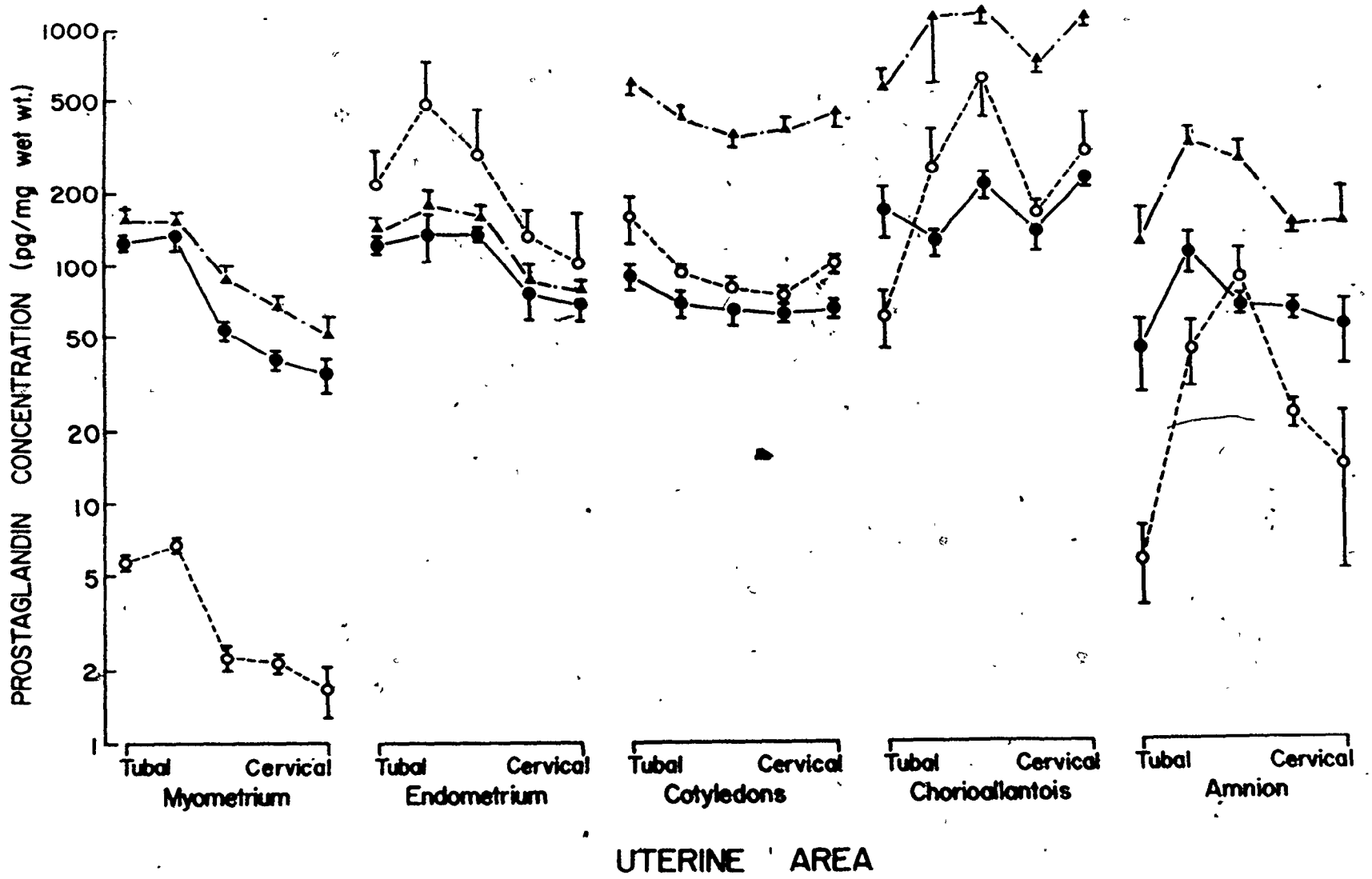
4.3 Results

The possibility that "artifactual" PG production occurs after dissection during the 1-4 min while tissues are incubating in phosphate buffer containing indomethacin was investigated for myometrium collected from five sheep (Table 4). Concentrations of PGF and 6-oxo-$PGF_{1\alpha}$ were significantly higher (Duncan's New Multiple Range Test) in samples which were incubated in ice-cold phosphate buffer compared to those incubated in the presence of indomethacin. However, PG concentrations in samples which were frozen immediately were not significantly different from those incubated in the presence or absence of indomethacin.

In a preliminary study, the effects of uterine area on tissue PG concentrations were examined in a ewe (No. 016), 140 days pregnant (Figure 6). Significant effects of uterine area on PG concentrations were found in some tissues in this animal (see

FIGURE 6

Concentrations of prostaglandin E (PGE) (—— . ——), PGF (— — — —) and 6-oxo-$PGF_{1\alpha}$ (————) in samples taken from different areas of myometrium, endometrium, cotyledons, chorio-allantois and amnion in ewe No. 016.


PROSTAGLANDIN CONCENTRATION (pg/mg wet wt.)
1000
500
200
100
50
20
10
5
2
1
Tubal
Cervical
Myometrium
Endometrium
Cotyledons
Chorioallantois
Amnion
UTERINE AREA

Tables 5-19). To ascertain if these effects of uterine area were consistent between animals, PG concentrations were determined in intra-uterine tissues obtained from a number of animals.

The effects of uterine area on myometrial PGF, 6-oxo-$PGF_{1\alpha}$ and PGE concentrations are summarized in Tables 5-7. For PGF, significant effects (analysis of variance) of uterine area were observed in six out of seven animals (Table 5). The concentrations of PGF were generally higher (paired *t* tests) between the tubal and middle myometrial areas, than between the middle and cervical myometrial areas. Significant effects of uterine area on 6-oxo-$PGF_{1\alpha}$ concentrations were observed in four out of seven animals examined (Table 6). In these sheep, 6-oxo-$PGF_{1\alpha}$ concentrations were generally higher (paired *t* tests) towards the tubal end of the uterus than adjacent to the cervix. Concentrations of PGE in samples taken from different areas of myometrium are summarized in Table 7. Significant effects (analysis of variance) of uterine area were observed in two out of seven sheep examined. In these animals, concentrations of PGE were significantly higher (*t* tests) between the tubal and mid-uterine areas than towards the cervix. The sheep which did not have significant effects of uterine area on PG concentrations were generally at earlier stages of pregnancy than were animals having significant effects of uterine area.

Concentrations of PGF, 6-oxo-$PGF_{1\alpha}$ and PGE in samples taken from different areas of endometrium in six sheep 120 to 140 days pregnant are summarized in Tables 8-10. Significant effects of uterine area (analysis of variance) were observed in two (PGF and PGE) or

TABLE 5: Concentrations[a] of prostaglandin F in samples taken from different areas of myometrium (mean ± S.E.M.; 4 samples/area)

Sheep Number	Approx. Fetal Age (days)	Area of Myometrium					Analysis of Variance	
		Tubal	Mid-tubal	Middle	Mid-Cervical	Cervical	F (4,15)	P Value
016	140	5.7±0.4*	6.7±0.3*	2.3±0.3	2.2±0.2	1.7± 0.4	13.557	< 0.001
L260	120	2.2±0.5	2.4±0.3	2.5±0.8	7.0±4.8	1.7± 0.5	0.759	N.S.
03	145	6.3±0.8*	3.3±1.1	5.3±0.2*	1.8±0.5	3.5± 0.5	6.527	< 0.005
260R	120	1.5±0.6	1.0±0.3	1.1±0.1	2.1±0.3*	9.0± 1.9*	15.177	< 0.001
09	135	10.1±2.9*	5.2±0.7	7.3±0.5	5.0±0.9	5.5± 0.3	3.883	< 0.03
08	140	5.5±0.3	13.4±4.0*	4.7±0.6	3.1±0.8	2.8± 0.4	6.946	< 0.005
907	135	9.5±1.8	15.5±3.6*	15.8±3.5*	4.2±1.2	26.1±11.0*	3.668	< 0.03

[a] pg/mg wet wt.

* Concentrations significantly higher than other areas of the uterus ($P < 0.05$; t tests)

TABLE 6: Concentrations[a] of 6-oxo-$PGF_{1\alpha}$ in samples taken from different areas of the myometrium (mean ± S.E.M.; 4 samples/area).

Sheep Number	Fetal Age (days)	Area of Myometrium					Analysis of Variance	
		Tubal	Mid-Tubal	Middle	Mid-Cervical	Cervical	F (4,15)	P Value
016	140	125.4±0.8*	132.2±14.4*	52.4±4.3	40.7±3.3	35.1±6.0	38.131	< 0.001
L260	120	47.9±5.9	51.0± 7.7	48.8±5.8	37.8±1.2	45.9±3.0	1.001	N.S.
03	145	40.0±3.6	22.9± 3.8	29.3±6.0	21.5±5.2	24.7±3.2	2.777	N.S.
260R	120	52.9±6.1	42.5±11.4	49.3±8.2	44.6±8.8	60.4±4.8	0.755	N.S.
09	135	33.0±2.9	37.0± 3.5	52.0±2.2*	62.9±6.5*	40.5±5.0	8.389	< 0.001
08	140	88.4±7.4*	90.5±25.2*	43.0±5.2	20.1±5.3	17.4±2.6	10.919	< 0.001
907	135	18.2±0.3	27.1± 1.7*	12.5±0.1	13.0±2.4	20.2±2.0	12.190	< 0.001

[a] pg/mg wet wt.

* concentrations significantly higher than other areas of the uterus ($P < 0.05$; t-tests)

TABLE 7: Concentrations[a] of prostaglandin E in samples taken from different areas of the myometrium (mean ± S.E.M.; 4 samples/area)

Sheep Number	Approx. Fetal Age (days)	Area of Myometrium					Analysis of Variance	
		Tubal	Mid-Tubal	Middle	Mid-Cervical	Cervical	F (4,15)	P Value
016	140	152.5± 7.7*	146.8±12.4*	86.0± 9.8	66.1± 4.8	51.4±8.9	26.388	< 0.001
L260	120	59.3± 5.7	65.2±12.1	72.2±13.2	55.4± 1.7	52.6±4.3	0.716	N.S.
03	145	33.0± 2.7	21.6± 5.1	35.8± 8.7	24.3± 6.3	23.0±4.0	1.253	N.S.
260R	120	96.2±21.1	50.5±19.3	79.7±21.3	54.0±18.4	91.3±9.8	1.343	N.S.
09	135	25.2± 1.7	26.3± 3.1	35.4± 2.8	32.2± 2.5	33.7±2.9	2.597	N.S.
08	140	24.8± 1.7*	27.0± 3.0*	16.5± 1.9	14.5± 3.5	17.5±1.9	4.868	< 0.02
907	135	40.8 2.5	42.4 3.3	36.9 3.9	36.0 3.8	35.1 5.0	0.821	N.S.

[a] pg/mg wet wt.

* concentrations significantly higher than other areas of the uterus ($P < 0.05$; t-tests).

TABLE 8: Concentrations[a] of prostaglandin F in samples taken from different areas of endometrium (mean ± S.E.M.; 4 samples/area).

Sheep Number	Approx. Fetal Age (days)	Area of Endometrium					Analysis of Variance	
		Tubal	Mid-Tubal	Middle	Mid-Cervical	Cervical	F (4,15)	P Value
016	140	219.3± 83.7	483.7±235.5	309.3±136.3	135.0± 33.2	102.1± 60.9	1.371	N.S.
L260	120	12.9± 6.3	2.9± 0.6	2.6± 0.6	2.5± 0.2	3.1± 1.2	1.641	N.S.
03	145	421.3±216.0	678.1±205.6	216.1± 48.9	379.0± 67.6	318.1± 68.9	1.121	N.S.
260R	120	3.8± 0.1	3.5± 0.8	3.6± 1.1	1.3± 0.3	2.1± 0.5	2.914	N.S.
09	135	429.4±171.7	337.8± 21.2	229.7± 30.9	406.2±107.3	1153.8±440.0*	3.890	< 0.03
907	135	32.6± 3.2*	22.2± 7.9*	6.9± 1.2	10.0± 0.9	24.9± 6.4*	6.760	< 0.005

[a] pg/mg wet wt.

* concentrations significantly higher than other areas of the uterus ($P < 0.05$; t-tests)

TABLE 9: Concentrations[a] of 6-oxo-$PGF_{1\alpha}$ in samples taken from different areas of the endometrium (mean ± S.E.M.; 4 samples/area)

Sheep Number	Approx. Fetal Age (days)	Area of Endometrium					Analysis of Variance	
		Tubal	Mid-Tubal	Middle	Mid-Cervical	Cervical	F (4,15)	P Value
016	140	123.6± 8.8*	134.9±30.9*	133.9± 2.8*	75.8±17.5	68.3±11.0	4.605	< 0.02
L260	120	35.2± 2.2	44.0± 1.0	47.6± 3.6	44.2± 1.8	37.3± 8.9	1.359	N.S.
03	145	181.4±21.6	197.0±49.8	189.4±17.2	152.9±10.3	168.2±28.2	0.791	N.S.
260R	120	69.4± 4.0	82.2± 0.2	84.0± 4.3	66.5± 2.7	78.3±12.6	2.758	N.S.
09	135	103.4± 7.7	78.5± 3.2	59.0± 5.2	105.9±13.3	155.3±25.1*	10.264	< 0.001
907	135	45.3±10.1*	28.5± 7.3*	16.9± 2.0	14.8± 1.0	23.2± 1.9	5.487	< 0.01

[a] pg/mg wet wt.

* concentrations significantly higher than other areas of the uterus ($P < 0.05$; t-tests).

TABLE 10: Concentrations[a] of prostaglandin E in samples taken from different areas of endometrium (mean ± S.E.M.; 4 samples/area).

Sheep Number	Approx. Fetal Age (days)	Area of Endometrium					Analysis of Variance	
		Tubal	Mid-Tubal	Middle	Mid-Cervical	Cervical	F (4,15)	P Value
016	140	136.2±19.5*	171.6±33.2*	151.2±24.4*	78.9±22.2	67.2± 5.5	4.058	< 0.02
L260	120	52.6± 4.2	59.1± 2.3	68.7± 4.6	52.5± 6.8	54.6± 9.9	1.227	N.S.
03	145	72.8±10.1	89.6±15.2	81.3±12.3	69.2± 4.3	75.4± 6.2	0.459	N.S.
260R	120	116.7±17.1	110.9± 7.8	108.8± 4.9	88.1± 6.2	93.7± 4.6	2.231	N.S.
09	135	121.8± 8.8*	37.1± 3.3	30.7± 4.9	418.9±10.2*	79.8±21.9*	10.161	< 0.001
907	135	77.9±10.9	56.6± 9.9	64.2± 5.5	52.2± 5.2	61.8± 6.8	1.515	N.S.

[a] pg/mg wet wt.

* concentrations significantly higher than other areas of the uterus ($P < 0.05$; t-tests).

TABLE 11: Concentrations[a] of prostaglandin F in cotyledons taken from different areas of the uterus (mean ± S.E.M.; 4 cotyledons/area)

Sheep Number	Approx. Fetal Age (days)	Area of Uterus					Analysis of Variance	
		Tubal	Mid-Tubal	Middle	Mid-Cervical	Cervical	F(4,15)	P Value
016	140	1048.5±343.9	1979.9±359.6	2881.8±609.1*	3609.8±628.4*	2275.1±438.7	4.157	< 0.02
L260	120	40.6± 2.8	63.9± 3.2*	59.6± 3.6	47.6± 3.5	38.6± 1.8	13.556	< 0.001
03	145	2800.6±310.6*	1053.1± 47.7	1181.4± 63.2	1826.0±129.4	1047.7±102.8	20.156	< 0.001
260R	120	157.1± 36.0*	92.9± 5.3	79.3± 9.8	75.4± 3.9	100.1± 5.4	10.600	< 0.001
907	135	201.8± 94.0	116.4± 38.7	85.0± 15.7	70.7± 10.5	43.4± 7.9	2.523	N.S.

[a] pg/mg wet wt.

* concentrations significantly higher than other areas of the uterus ($P < 0.05$; t-tests).

three (6-oxo-$PGF_{1\alpha}$) out of six sheep examined. In these animals, concentrations were higher (t-tests) in middle and/or mid-cervical uterine areas compared to the rest of the uterus. The high between animal variation observed in these animals did not appear to be related to the age of the fetus.

The effects of uterine area on concentrations of PGF, PGE and 6-oxo-$PGF_{1\alpha}$ in cotyledons are summarized in Tables 11-13. Significant effects (analysis of variance) of uterine area were observed in one out of five sheep for PGE and 6-oxo-$PGF_{1\alpha}$. In this animal, concentrations were higher (t-tests) in tubal areas than elsewhere in the uterus. For PGF, significant effects of uterine area were observed in four out of five sheep and concentrations were lower (t-tests) in cervical areas than elsewhere in the uterus.

Tables 14-16 summarize the effects of uterine area on PGF, 6-oxo-$PGF_{1\alpha}$ and PGE concentrations in the chorioallantois. Significant effects of uterine area (analysis of variance) were observed in three out of five sheep for PGF and two out of five sheep for 6-oxo-$PGF_{1\alpha}$ and PGE. For PGF, concentrations were higher (t-tests) in the middle areas of the uterus than in tubal areas of sheep showing significant overall effects of uterine areas (analysis of variance).

For amnion, concentrations of PGF, 6-oxo-$PGF_{1\alpha}$ and PGE in different uterine areas are summarized in Tables 17-19. Significant effects (analysis of variance) of uterine area were observed in four out of five animals for PGF and 6-oxo-$PGF_{1\alpha}$ and three out of five animals for PGE. In these sheep, the areas having higher PG concentrations (t-tests) were also found to correspond with areas having little or no apparent vascularity.

TABLE 12: Concentrations[a] of 6-oxo-$PGF_{1\alpha}$ in cotyledons taken from different areas of the uterus (mean ± S.E.M.; 4 cotyledons/area)

Sheep Number	Approx. Fetal Age (days)	Area of Uterus					Analysis of Variance	
		Tubal	Mid-Tubal	Middle	Mid-Cervical	Cervical	F (4,15)	P Value
016	140	449.8±122.3	376.5±54.2	678.9±45.7	578.2± 44.3	448.9±84.8	2.811	N.S.
L260	120	45.4± 2.4	46.7± 1.9	42.5± 2.1	44.1± 3.6	39.9± 1.3	1.848	N.S.
03	145	998.8± 15.3*	427.9±19.4	544.3±59.4	787.1±126.5	436.8±45.8	8.272	< 0.001
260R	120	87.5± 10.5	67.7± 9.7	63.8± 9.9	62.1± 4.3	65.2± 5.2	1.321	N.S.
907	135	195.9± 2.9	127.1±29.9	96.7±28.3	85.5± 7.3	76.3±12.2	0.454	N.S.

[a] pg/mg wet wt.

* concentrations significantly higher than other areas of the uterus ($P < 0.05$; t-tests).

TABLE 13: Concentrations[a] of prostaglandin E in cotyledons taken from different areas of the uterus (mean ± S.E.M.; 4 cotyledons/area)

Sheep Number	Approx. Fetal Age (days)	Area of Uterus					Analysis of Variance	
		Tubal	Mid-Tubal	Middle	Mid-Cervical	Cervical	F (4,15)	P Value
016	140	412.5±152.8	293.6±45.4	567.3±51.1	412.7± 55.9	439.1±123.2	1.114	N.S.
L260	120	361.5± 47.6	315.1±25.3	280.6±24.0	261.9± 47.5	243.8± 26.6	1.690	N.S.
03	145	4384.1±517.1*	1844.1±93.7	1816.7±91.5	3374.4±668.7	1942.9±244.5	10.515	< 0.001
260R	120	665.4± 96.6	410.1±26.0	342.1±41.7	398.2± 48.5	451.2± 43.9	1.307	N.S.
907	135	379.1±125.5	360.3±17.1	227.6±38.7	271.8± 25.1	317.8± 80.3	0.656	N.S.

[a] pg/mg wet wt.

* concentrations significantly higher than other areas of the uterus ($P < 0.05$; t-tests).

TABLE 14: Concentrations[a] of prostaglandin F in samples taken from different areas of chorioallantois (mean ± S.E.M.; 4 samples/area).

Sheep Number	Approx. Fetal Age (days)	Area of Chorioallantois					Analysis of Variance	
		Tubal	Mid-Tubal	Middle	Mid-Cervical	Cervical	F (4,15)	P Value
016	140	59.4±16.8	248.7±106.4	653.1±224.9*	159.4± 22.3	291.4±127.1	5.108	< 0.01
L260	120	30.3± 7.8	196.1± 42.2*	202.4± 85.6*	178.7± 18.5*	112.5± 34.5*	5.911	< 0.005
03	145	70.5±29.8	82.3± 12.6	268.1± 83.4*	84.2± 11.1	78.6± 21.6	4.321	< 0.02
260R	120	154.0± 1.4	161.3± 77.5	69.8± 4.4	313.7±161.9	291.3± 39.1	1.715	N.S.
907	135	101.6±16.4	23.8± 7.2	38.8± 18.4	65.3± 13.7	73.1± 34.0	2.297	N.S.

[a] pg/mg wet wt.

*concentrations significantly higher than other areas of the uterus ($P < 0.05$; t-tests).

TABLE 15: Concentrations[a] of 6-oxo-$PGF_{1\alpha}$ in samples taken from different areas of chorioallantois (mean ± S.E.M.; 4 samples/area)

Sheep Number	Approx. Fetal Age (days)	Area of Chorioallantois					Analysis of Variance	
		Tubal	Mid-Tubal	Middle	Mid-Cervical	Cervical	F (4,15)	P Value
016	140	166.6±39.9	119.1±16.1	210.2±24.0*	131.0±23.4	220.5±17.5*	4.404	< 0.02
L260	120	57.5± 2.5	87.4± 9.7	63.4± 4.5	70.9± 9.9	62.8± 8.4	2.367	N.S.
03	145	68.5±13.5	85.4±23.2	122.2±20.9	66.4± 7.9	76.7±16.1	1.857	N.S.
260R	120	66.2±13.5	76.6±11.2	101.8± 6.9	118.0±24.5	109.1±11.9	2.579	N.S.
907	135	112.1± 2.9*	71.1±10.6	57.8±10.8	75.9± 9.6	92.7±15.3*	3.914	< 0.03

[a] pg/mg wet wt.

* concentrations significantly higher than other areas of the uterus ($P < 0.05$; t-tests).

TABLE 16: Concentrations[a] of prostaglandin E in samples taken from different areas of the chorioallantois (mean ± S.E.M.; 4 samples/area).

Sheep Number	Approx. Fetal Age (days)	Area of Chorioallantois					Analysis of Variance	
		Tubal	Mid-Tubal	Middle	Mid-Cervical	Cervical	F (4,15)	P Value
016	140	527.0±116.1	1106.7±495.1	1138.4±172.3	703.0± 91.4	1114.2±165.2	1.171	N.S.
L260	120	159.1± 21.7	524.2± 64.2*	272.4± 53.3*	390.1± 58.0*	338.4± 92.9*	4.740	< 0.02
03	145	260.[illegible] 70.5	434.5±153.3	362.1± 42.6	272.2± 50.9	263.0± 81.7	0.830	N.S.
260R	120	261.7± 62.3	438.2±114.1	337.4± 88.1	812.1±192.2	1010.0±216.7	2.030	N.S.
907	135	57.7± 5.9	88.8± 6.9	118.4± 6.4*	130.1± 10.2*	119.0± 8.2*	14.783	< 0.001

[a] pg/mg wet wt.

* concentrations significantly higher than other areas of the uterus ($P < 0.05$; t-tests).

TABLE 17: Concentrations[a] of prostaglandin F in samples taken from different areas of amnion (mean ± S.E.M.; 4 samples/area).

Sheep Number	Approx. Fetal Age (days)	Area of Amnion					Analysis of Variance	
		Tubal	Mid-tubal	Middle	Mid-Cervical	Cervical	F (4,15)	P Value
016	140	5.7± 2.1	42.4±12.5*	85.5±26.2*	22.8± 2.4	13.9± 8.9	8.178	< 0.002
L260	120	28.9±12.6	46.3±16.5	54.8±32.3	63.4±21.5	23.9±11.1	0.683	N.S.
03	145	4.9± 0.8	9.0± 1.4	9.9± 4.5	32.8± 3.0*	36.4± 3.6*	24.102	< 0.001
260R	120	3.4± 1.6	58.7±14.1*	44.9±14.4*	6.8± 1.8	5.7± 2.1	10.445	< 0.002
907	135	339.9±84.0*	255.2±49.5*	54.1±10.8	56.1±16.3	174.0±65.6	10.109	< 0.001

[a]pg/mg wet wt.

*concentrations significantly higher than other areas of the uterus ($P < 0.05$; t-tests).

TABLE 18: Concentrations[a] of 6-oxo-$PGF_{1\alpha}$ in samples taken from different areas of amnion (mean ± S.E.M.; 4 samples/area).

Sheep Number	Approx. Fetal Age (days)	Area of Amnion					Analysis of Variance	
		Tubal	Mid-tubal	Middle	Mid-Cervical	Cervical	F (4,15)	P Value
016	140	42.7±14.4	108.7±21.6*	64.5± 6.0	63.2± 6.0	51.7±15.8	4.250	< 0.02
L260	120	39.8± 6.7	56.5±10.5	45.8± 7.2	56.2± 4.4	42.0±18.2	0.555	N.S.
03	145	68.5±13.5	85.4±23.2	122.2±20.9*	66.4± 7.9	76.7±16.1	22.558	< 0.001
260R	120	66.2±13.5	76.6±11.2	101.8± 6.9*	118.0±24.5*	109.8±10.2*	23.756	< 0.001
907	135	142.3±21.0*	117.9±14.8*	49.8±16.3	47.5± 7.5	87.0±22.5	5.828	< 0.005

[a]pg/mg wet wt.

*concentrations significantly higher than other areas of the uterus ($P < 0.05$; t-tests).

TABLE 19: Concentrations[a] of prostaglandin E in samples taken from different areas of amnion (mean ± S.E.M.; 4 samples/area).

Sheep Number	Approx. Fetal Age (days)	Area of Amnion					Analysis of Variance	
		Tubal	Mid-tubal	Middle	Mid-Cervical	Cervical	F (4,15)	P Value
016	140	114.8±48.7	321.4±40.3*	260.5±74.4*	141.6±7.5	142.2±59.6	3.076	< 0.05
L260	120	99.1±24.4	100.6±13.7	125.7±25.0	133.7±8.7	77.0±27.5	1.146	N.S.
03	145	26.1± 3.5	66.6± 3.6	47.4±21.0	73.0±4.4*	115.6± 7.2*	10.366	< 0.001
260R	120	37.2± 8.9	139.6± 7.2*	189.7±21.2*	68.6±4.9	42.6±14.2	7.468	< 0.005
907	135	49.4± 1.8	68.4± 3.1	69.7±10.9	62.3±4.5	65.6± 6.5	1.728	N.S.

[a] pg/mg wet wt.

*concentrations are significantly higher than other areas of the uterus ($P < 0.05$; t-tests).

The proportions of sheep having significant effects of uterine area on PG concentrations in intra-uterine tissues are shown in Table 20. These results indicate that in several animals there were significant effects of uterine area on PG concentrations in each tissue. The greatest variability in PG concentrations within animals was found in the myometrium and amnion.

The possibility that trauma delivered to tissues during sample collection induced the biosynthesis of prostaglandins was investigated. The effects of crushing samples collected from myometrium, endometrium, chorioallantois and amnion on PGE, PGF and 6-oxo-$PGF_{1\alpha}$ concentrations in five ewes are summarized in Table 21. For myometrium, concentrations of PGE and 6-oxo-$PGF_{1\alpha}$ were significantly higher in tissues which had been crushed, compared to controls. For endometrium, PGF concentrations were significantly lower in tissues which had been crushed, compared to controls. For amnion, crushing did not significantly alter PG concentrations, and 6-oxo-$PGF_{1\alpha}$ concentrations were significantly higher in chorioallantois which had been crushed, compared to control tissue.

Concentrations of PGE, PGF and 6-oxo-$PGF_{1\alpha}$ in samples collected from myometrium, endometrium, chorioallantois and amnion of a ewe 130 days pregnant and treated for 70 h with indomethacin are summarized in Table 22. In this animal, concentrations of PGE, PGF and 6-oxo-$PGF_{1\alpha}$ were significantly higher in myometrium which had been crushed, compared to control tissue. For endometrium, PGF concentrations were significantly lower in tissues which had been crushed, compared to controls. Concentrations of 6-oxo-$PGF_{1\alpha}$ in chorioallantois

TABLE 20: The proportions of sheep having significant ($P < 0.05$, analysis of variance) effects of uterine area on prostaglandin (PG) concentrations in intra-uterine tissues.

Tissue	PGF	PGE	6-oxo-PGF$_{1\alpha}$
Myometrium	6/7	2/7*	4/7
Endometrium	2/6	2/6	3/6
Cotyledons	4/5	1/5	1/5
Chorioallantois	3/5	2/5	2/5
Amnion	4/5	3/5	4/5

*Number of animals showing significant effects of uterine area/Number of animals examined.

TABLE 21: Effects of "trauma", induced by crushing, on intra-uterine prostaglandin concentrations (mean ± S.E.M.; n = 10, 5 animals).

Tissues	Treatment	Prostaglandin Concentrations (pg/mg) PGE	PGF	6-oxo-PGF$_{1\alpha}$
Myometrium	control	29.8 ± 4.3	3.6 ± 0.6	24.8 ± 3.2
	crushed	63.7 ± 8.1**	7.9 ± 2.5	43.3 ± 4.7**
Endometrium	control	82.4 ± 8.9	589.5 ± 124.1	187.7 ± 27.9
	crushed	111.2 ± 15.5	188.2 ± 42.3**	127.0 ± 19.3
Chorioallantois	control	286.1 ± 35.7	163.0 ± 42.2	76.6 ± 7.7
	crushed	280.0 ± 37.8	194.2 ± 85.5	170.7 ± 32.2*
Amnion	control	53.8 ± 8.0	9.0 ± 1.7	30.1 ± 1.9
	crushed	56.9 ± 5.7	12.4 ± 2.6	38.8 ± 4.4

Values significantly different compared with respective control *P < 0.02, **P < 0.01 (paired t-tests).

TABLE 22: Effects of "trauma", induced by crushing, on prostaglandin concentrations in intrauterine tissues collected from a ewe treated with indomethacin (25 mg/kg/day) for 70 h previously by continuous infusion (mean ± S.E.M.; n = 10, 1 animal).

Tissues	Treatment	Prostaglandin (PG) Concentrations (pg/mg)		
		PGE	PGF	6-oxo-PGF$_{1\alpha}$
Myometrium	control	17.7 ± 2.6	1.8 ± 0.4	8.8 ± 1.2
	crushed	23.0 ± 3.5**	2.4 ± 0.5*	13.1 ± 1.7**
Endometrium	control	32.5 ± 5.5	2.5 ± 0.5	8.9 ± 1.8
	crushed	37.3 ± 8.5	1.7 ± 0.3**	9.3 ± 2.9
Chorioallantois	control	17.7 ± 4.2	5.8 ± 1.3	6.4 ± 1.4
	crushed	16.5 ± 2.8	7.0 ± 1.8	9.7 ± 1.7**
Amnion	control	12.6 ± 3.6	2.2 ± 0.5	4.0 ± 0.8
	crushed	14.5 ± 4.2*	2.7 ± 0.5	4.4 ± 0.9

Values significantly different compared with respective control *P<0.05, **P<0.01 (paired t-tests).

and of PGE in amnion were significantly higher in crushed tissue, compared to their respective controls.

The possibility that PG production is stimulated following manual separation of fetal and maternal cotyledons was examined in a ewe 135 days pregnant. Concentrations of PGF, PGE, and 6-oxo-$PGF_{1\alpha}$ in fetal, maternal and whole cotyledons are summarized in Table 23. For comparison with whole cotyledons, the calculated overall PG concentrations in separated cotyledons are also presented. In separated cotyledons, concentrations of PGE and 6-oxo-$PGF_{1\alpha}$ were significantly higher in fetal cotyledons, compared to maternal cotyledons. In addition, the overall concentrations of these PG's were significantly higher in separated cotyledons, compared to whole cotyledons which were not separated prior to PG extraction.

4.4 Discussion

The results of this study show that prostaglandin (PG) concentrations are not uniform throughout tissues in some pregnant sheep uteri, suggesting that estimations based on single samples may not be representative of the entire uterus. In non-pregnant sheep, Rexroad (1980) showed higher PG concentrations in myometrium and endometrium collected from anterior areas compared to posterior areas. Whether these differences reflect different rates of production and/or metabolism, or clearance remains to be established. The possibility that variable PG concentrations within uterine tissues was

TABLE 23: The effects of "trauma", induced by separating fetal and maternal cotyledons on PG concentrations in a ewe, 135 days pregnant (mean ± S.E.M.).

	Prostaglandin Concentrations (pg/mg)		
	PGF	PGE	6-oxo-$PGF_{1\alpha}$
Fetal cotyledons	192.8 ± 18.3	546.4 ± 39.8*	345.5 ± 11.3*
Maternal cotyledons	221.7 ± 14.4	230.8 ± 21.4	182.4 ± 10.1
Separated cotyledons			
overall (n = 4)	214.5 ± 11.9	377.2 ± 299.9**	227.9 ± 10.6**
whole (n = 4)	211.9 ± 19.8	271.9 ± 42.3	97.6 ± 8.4

* Values significantly different from maternal cotyledons ($P < 0.05$; paired t-tests)

** Values significantly different from whole cotyledons ($P < 0.05$; t-tests)

an artifact caused during tissue collection cannot be ruled out. Although the experimental protocol was able to control for the absolute effects of time between the dissection of tissues from different areas of the uterus, it is possible that some areas of the uterus may have a higher capacity to produce PG's compared to others.

The significance of regional differences in intra-uterine PG concentrations is unknown. Several investigators have suggested that PG's may regulate uterine blood flow (Novy et al., 1974b; Rankin and Phernetton, 1976b; Rankin, 1978). Uneven blood flow distribution between cotyledons within pregnant sheep (Power et al., 1967), and in myometrium in pregnant women (Jansson, 1969) has been demonstrated. The differences in PG concentrations within uterine tissues, therefore, may be important in the regulation of regional tissue blood flow. Alternatively, local differences in blood flow may alter the clearance of PG's from these sites. The amnion in sheep is relatively avascular throughout most of pregnancy, blood vessels being visible only in small localized areas, usually tubal and/or cervical regions, after Day 100 of pregnancy. That PG concentrations in this tissue are significantly lower in these areas, compared to non-vascularized areas, lends strong support to the possibility that regional differences in PG concentrations result from regional differences in clearance rates. In the present study, regional differences in myometrial PG concentrations appeared to occur during later stages of pregnancy. The possibility that these differences are related to the redistribution of uterine blood flow at this time (Rosenfeld et al., 1974) deserves further investigation. In guinea pigs, uterine distention

has been shown to increase PG release (Horton et al., 1971) and functional adrenergic denervation occurs in association with uterine distention during pregnancy (Thorbert et al., 1977, 1979). The variable PG concentrations in the myometrium and endometrium may therefore result from changes in PG production and/or clearance due to altered norepinephrine availability or distention caused by the presence of the fetus. Alternatively, regional differences in PG concentrations may be important in the regulation of electrical activity in the myometrium. Clearly, more studies must be conducted before the significance of these regional differences in PG concentrations may merit further speculation.

Others have suggested that measurements of tissue PG concentrations reflect the capacity for formation rather than endogenous levels (Green, 1979; Liggins et al., 1980), since PG output is stimulated from a number of tissues in response to trauma (Piper and Vane, 1971). The effects of trauma on uterine PG output is, however, poorly documented. The present results suggest that the capacity of intra-uterine tissues to produce PG's in response to trauma differs between tissues and between different arachidonic acid metabolites. Since crushing uterine tissues after they were dissected resulted in changes in PG concentrations, the capacity for PG production could not have been maximally stimulated during tissue collection. The possibility that PG production was already at a maximal rate in those tissues where PG concentrations did not change after crushing was examined in one ewe which was treated with indomethacin, an inhibitor of PG synthesis. It was reasoned that PG production in response to dissection would be attenuated in this animal and that this reduced PG

production rate may be stimulated by repeated crushing. The observation that in this animal, PG concentrations after crushing were similar to those observed in animals not receiving indomethacin, is consistent with the notion that the capacity for PG production was not maximally stimulated during tissue collection. However, for tissues not showing changes in PG concentrations in this animal, PG concentrations may be maximally stimulated during tissue dissection due to the limited availability of the cyclo-oxygenase enzyme. Therefore, the possibility that trauma, as a result of tissue collection, induces PG synthesis cannot be ruled out.

In the present study, crushing resulted in changes primarily in PGE and/or 6-oxo-$PGF_{1\alpha}$ concentrations. It has been suggested that the release of PGE and PGI_2 may be important in the genesis of the signs and symptoms of the inflammatory process (Ferreira et al., 1974; Vane, 1978). Prostaglandins of the E series increase vascular permeability in inflammatory sites in man and in various animals (Glenn et al., 1972; Willis et al., 1972; Ikeda et al., 1975). Prostaglandins E_2 and I_2 have also been shown to potentiate the pain-producing and the edema-inducing effect of bradykinin and/or histamine (Moncada et al., 1973; Williams and Morley, 1973; Ikeda et al., 1975; Williams and Peck, 1977). It is possible, therefore, that the directed synthesis of these PG's in response to crushing may represent local defense mechanisms responding to cell injury.

Using a superfusion technique, Mitchell and Flint (1978a) also showed that the output of PGE was quantitatively greater than other PG's in fetal and maternal cotyledons as well as in myometrium,

and that the fetal cotyledons were the major source of PGE under the conditions of superfusion employed. These results are consistent with the high levels of PGE in fetal plasma during late pregnancy in sheep (Challis et al., 1976) and with the role that PGE is believed to play in the control of the patency of the ductus arteriosus (Coceani et al., 1976). However, caution should be exercised when interpreting results of PG determinations in studies where fetal and maternal components of cotyledons were manually separated. In the present study, increased concentrations of PGE and 6-oxo-$PGF_{1\alpha}$ were calculated in cotyledons which were separated into fetal and maternal components, compared to whole cotyledons which were not separated. Interpretation of these results are limited in view of the small sample size collected from one animal; however the changes in PG concentrations following the separation of cotyledons may represent a local reaction to cell injury. The observation that PGE concentrations are higher in fetal cotyledons compared to maternal cotyledons is in agreement with the results of Mitchell and Flint (1977, 1978a).

In summary, the salient findings in this chapter include: 1). PG concentrations are not uniform throughout intra-uterine tissues in some pregnant sheep; 2) the capacity of intra-uterine tissues to produce PG's in response to trauma differs between tissues and different arachidonic acid metabolites; and 3) PG concentrations are higher in cotyledons where fetal and maternal components had been separated manually, compared to intact cotyledons.

CHAPTER 5

EVIDENCE FOR A ROLE OF PROSTAGLANDINS (PG'S) IN THE INITIATION OF LABOUR

I. PROSTAGLANDIN AND STEROID CONCENTRATIONS DURING ACTH-INDUCED LABOUR AND THE EFFECTS OF INDOMETHACIN ON UTERINE ACTIVITY

5.1 Introduction

Evidence supporting a role for prostaglandins (PG's) in the control of parturition in sheep has been presented (see Chapter 1). Briefly, PG concentrations are elevated in fetal (Challis et al., 1976) and maternal (Thorburn et al., 1972; Currie et al., 1973; Mitchell et al., 1979) plasma and in amniotic fluid (Mitchell et al., 1977, 1978; Challis et al., 1978) in association with birth. Furthermore, the administration of meclofenamic acid, a PG synthetase inhibitor, delayed the delivery that normally occurs during dexamethasone infusion to the lamb (Mitchell and Flint, 1978). However, in that study, fetal death occurred in one-half of the treated animals and Kendall et al. (1977) have shown that estrogens rise to unphysiological levels at the amounts of dexamethasone used.

Previous measurements of concentrations of PGE and PGF in sheep placenta and myometrium during late pregnancy and labour (Liggins and Grieves, 1971; Mitchell and Flint, 1977) have suggested that these

tissues may be sites of PG production. However, PG concentrations in endometrium and fetal membranes have not been determined and the concentrations of 6-oxo-$PGF_{1\alpha}$ in different uterine tissues during late pregnancy and parturition are unknown.

The increased production of PG's during labour may be triggered by an increase in the estrogen:progesterone ratio (see Chapter 1). However, concentrations of steroid hormones in the different intra-uterine tissues during parturition have not been determined and estimations of circulating steroid hormones may not necessarily reflect the concentrations of steroid hormones in uterine tissues (Rawlings and Ward, 1976).

In the present study, therefore, concentrations of estradiol-17β, progesterone, PGE, PGF and 6-oxo-$PGF_{1\alpha}$ were measured in tissue samples of myometrium, endometrium, cotyledons, chorioallantois and amnion obtained from different areas of the uterus during late pregnancy and labour induced by the intra-fetal administration of ACTH. The infusion rate of ACTH that was used in this study caused delivery after about 75 h and was associated with maternal steroid changes similar to those seen at full term (Kendall et al., 1977). In addition, it was reasoned that if inhibitors of endogenous PG biosynthesis delay parturition, analysis of uterine activity in these animals might provide insight into the mechanism(s) by which PG's act on the myometrium. Therefore, the effects of indomethacin, a PG synthetase inhibitor, on steroid concentrations in maternal and fetal plasma, PG concentrations in intra-uterine tissues and on the frequency and maximum amplitude of uterine contractions during ACTH-induced labour, were also examined.

5.2 Materials and Methods

Twelve sheep of mixed breeds and known gestational ages were used. Fetal and maternal vascular catheters and an amniotic catheter were implanted on Days 113 to 117 of pregnancy as described previously (see Chapter 3). On Day 127 of pregnancy, the sheep were assigned to one of three treatment groups. One group received continuous infusions of saline (10 ml/day) into the fetal saphenous vein and of phosphate buffer (P.B., 0.12 M, pH 7.4, 275 mOsm, 5.0 ml/kg/day) into the maternal femoral vein (n = 4). A second group received continuous infusions of $ACTH_{1-24}$ (Synacthen, Ciba; 240 µg/day in 10 ml of saline) into the fetal saphenous vein and of P.B. into the maternal femoral vein (n = 4). The third group received continuous infusions of $ACTH_{1-24}$ (240 µg in saline/day) into the fetal saphenous vein and indomethacin (25 mg/kg/day in P.B., pH 7.4, 308 mOsm) into the maternal femoral vein (n = 4). Fetal (2 ml) and maternal (5 ml) blood samples were collected at 8 h intervals for 24 h prior to infusion and during the 70 h infusion period for plasma steroid determinations (see Chapter 3).

The frequency (contractions/hour) and maximum amplitude (mm Hg) of uterine contractions were estimated from recordings of amniotic pressure as described in Chapter 3. In all animals uterine activity was not evident prior to 19 h after the beginning of the infusions. After this time, pressure records were analyzed at 8 h intervals for 1 h periods, until 2-3 h before tissues were collected for PG and steroid measurements.

All tissues were collected under general anesthesia as described in Chapter 3. Samples of myometrium, endometrium, chorio-allantois, amnion and cotyledons were collected from tubal, middle and cervical uterine areas for each tissue. Samples were also obtained from regions intermediate between tubal and middle and between middle and cervical designated mid-tubal and mid-cervical, respectively. All tissues were processed as described in Chapter 3, homogenized in ethanol at 4°C and centrifuged. The supernatants were stored at -20°C until assayed. After sample collection was completed, the degree of cervical dilatation was assessed by digital examination.

Measurements of prostaglandins and cyclic AMP in ethanolic extracts of uterine tissues and of plasma steroids were performed as described previously (see Chapter 3). Estradiol-17β and progesterone concentrations were also estimated by RIA in the same tissue ethanolic extracts from which PG and cyclic AMP determinations were made.

The data are expressed as either proportions or as means ± S.E.M. Average daily plasma steroid concentrations were calculated from the three daily estimates during the infusion period. For each intra-uterine tissue, average steroid concentrations were calculated from the five individual estimates from different uterine areas in each animal. When heterogeneity of variance was present, determined by the F_{max} test (Sokal and Rohlf, 1969), the data were transformed logarithmically prior to statistical analysis. The significance of treatment effects within each tissue was determined by analysis of variance. The significance of the effects of indomethacin on the maximum amplitude and frequency of uterine contraction and day of infusion on steroid concentrations and of uterine area was determined

by Student's t tests. The significance of treatment effects on cyclic AMP, steroid and PG concentrations was also determined by Duncan's New Multiple Range Test; for steroids and PG's this test was used only when analysis of variance indicated significant effects which may be partitioned.

5.3 Results

The concentrations of cortisol in fetal plasma collected from animals receiving saline + P.B., ACTH + P.B. and ACTH + indomethacin are summarized in Table 24. In fetuses treated with ACTH, cortisol concentrations increased 3-fold during the infusion period. Cortisol concentrations were not different in ACTH + P.B.- and saline + P.B.-treated animals at the beginning of the infusion ($P > 0.05$; Duncan's New Multiple Range Test); however, they were significantly elevated on Day 1 of the infusion due to elevated concentrations 8 h and 16 h after the start of infusion. Concentrations of cortisol in ACTH + indomethacin-treated animals did not differ from animals receiving ACTH + P.B.

Concentrations of progesterone in maternal arterial plasma are summarized in Table 25. In ACTH-treated animals progesterone concentrations were significantly lower on Day 3 of the infusion compared to Day 3 in saline + P.B.-treated animals and on Day 1 of infusion in ACTH + P.B.- and ACTH + indomethacin-treated animals. Duncan's New Multiple Range Test indicated that concentrations at the

TABLE 24: Effects of continuous infusions of ACTH or saline to the fetus and of indomethacin or phosphate buffer (P.B.) to the mother on cortisol concentrations in fetal arterial plasma (mean ± SEM) after logarithmic transformation of concentrations (ng/ml; n = 4 sheep/group)

Treatment	Day of Infusion			
	Day -1	Day 1	Day 2	Day 3
Saline[a] + P.B.[b]	1.124±0.131 (13.3)[e]	1.177±0.106 (15.0)	1.079±0.142 (12.0)	1.138±0.148 (13.7)
ACTH[c] + P.B.	1.158±0.119 (14.4)	1.616±0.099[f] (41.3)	2.000±0.072[fg] (100.0)	2.125±0.161[fg] (133.4)
ACTH + indomethacin[d]	1.141±0.173 (13.8)	1.612±0.230 (40.9)	1.819±0.200[f] (79.3)	2.126±0.174[fg] (133.7)

[a] 70 h intra-fetal infusion (10 ml/day)

[b] 70 h intra-maternal infusion (0.12 M, pH 7.4; 5.0 ml/kg/day)

[c] 70 h intra-fetal infusion (240 µg/day)

[d] 70 h intra-maternal infusion (25 mg/kg/day)

[e] Geometric mean

[f] Significantly greater than saline + P.B. ($P < 0.05$; nested analysis of variance and Duncan's New Multiple Range Test)

[g] Significantly greater than Day 1 of infusion ($P < 0.05$; nested analysis of variance and paired t-tests)

TABLE 25: Effects of continuous infusions of ACTH or saline to the fetus and of indomethacin or phosphate buffer (P.B.) to the mother on progesterone concentrations in maternal arterial plasma (mean ± SEM) after logarithmic transformation of concentrations (ng/ml; 4 sheep/group)

	Day of Infusion			
Treatment	Day -1	Day 1	Day 2	Day 3
Saline + P.B.[a]	1.062±0.152 (11.5)[d]	1.138±0.251 (13.7)	1.079±0.134 (12.0)	1.172±0.161 (14.9)
ACTH + P.B.[b]	1.110±0.098 (12.9)	0.998±0.025 (10.0)	1.172±0.117 (14.9)	0.810±0.112[ef] (6.5)
ACTH + indomethacin[c]	1.028±0.108 (10.1)	0.910±0.113 (8.1)	0.860±0.105 (7.2)	0.670±0.162[ef] (4.7)

[a] 70 h intra-fetal saline infusion (10 ml/day) and intra-maternal P.B. infusion (0.12 M, pH 7.4; 5.0 ml/kg/day)

[b] 70 h intra-fetal ACTH infusion (240 µg/day) and intra-maternal P.B. infusion (0.12 M, pH 7.4; 5.0 ml/kg/day)

[c] 70 h intra-fetal ACTH infusion (240 µg/day) and intra-maternal indomethacin infusion (25 mg/kg/day)

[d] Geometric mean

[e] Significantly lower than control group ($P < 0.05$; nested analysis of variance and Duncan's New Multiple Range Test)

[f] Significantly lower than Day 1 of infusion ($P < 0.05$; nested analysis of variance and paired t-test)

beginning of the infusion period (Day -1) were not significantly different ($P > 0.05$). In addition, concentrations in ACTH + indomethacin-treated ewes did not differ from those in animals treated with ACTH + P.B.

Table 26 summarizes maternal plasma estrone concentrations in saline + P.B.-, ACTH + P.B.- and ACTH + indomethacin-treated sheep. Analysis of variance and paired *t* tests indicated that concentrations on Day 3 were significantly higher than on Day 1 of the infusion in ACTH-treated sheep, compared to the saline + P.B.-treated animals. However, concentrations in the latter group of animals were significantly higher than those in the ACTH-treated sheep on Day 1 of the infusions ($P < 0.05$, Duncan's New Multiple Range Test), but not during the pre-treatment period.

At the time of sacrifice, the cervix had dilated to the point of admitting 1-3 fingers in sheep treated with ACTH + P.B. and 0-1 fingers in ACTH + indomethacin- and saline + P.B.-treated ewes. Viable fetuses were delivered from all animals at this time.

Steroid concentrations in intra-uterine tissues in animals receiving intra-fetal infusions of saline + P.B. or ACTH + P.B. and intramaternal infusions of phosphate buffer are summarized in Figure 7. Tissue progesterone concentrations were highest in the chorioallantois and lowest in the cotyledons (both $P < 0.05$, analysis of variance and paired *t* tests). In addition, progesterone concentrations in the cotyledons were significantly lower ($P < 0.05$, analysis of variance and unpaired *t* tests) in sheep receiving intra-fetal ACTH + P.B. compared to those receiving saline + P.B. For all animals, tissue

TABLE 26: Effects of continuous infusions of ACTH or saline to the fetus and of indomethacin or phosphate buffer (P.B.) to the mother on estrone concentrations in maternal arterial plasma (mean ± SEM) after logarithmic transformation of concentrations (pg/ml; 4 sheep/group)

Treatment	Day of Infusion			
	Day -1	Day 1	Day 2	Day 3
Saline + P.B.[a]	1.421±0.282 (26.4)[d]	1.540±0.079 (34.7)	1.665±0.119 (46.2)	1.584±0.113 (38.4)
ACTH + P.B.[b]	1.281±0.121 (19.1)	1.220±0.051 (16.6)	1.187±0.077 (15.4)	1.486±0.152[e] (30.6)
ACTH + indomethacin[c]	1.309±0.146 (20.4)	1.283±0.101 (19.2)	1.422±0.141 (26.4)	2.075±0.411[e] (118.6)

[a] 70 h infusion of saline (10 ml/day) into the fetus and of P.B. (0.12 M, pH 7.4; 5.0 ml/kg/day) into the mother (n = 4)

[b] 70 h infusion of ACTH (240 µg/day) and of P.B. (0.12 M, pH 7.4; 5.0 ml/kg/day) into the mother (n = 4)

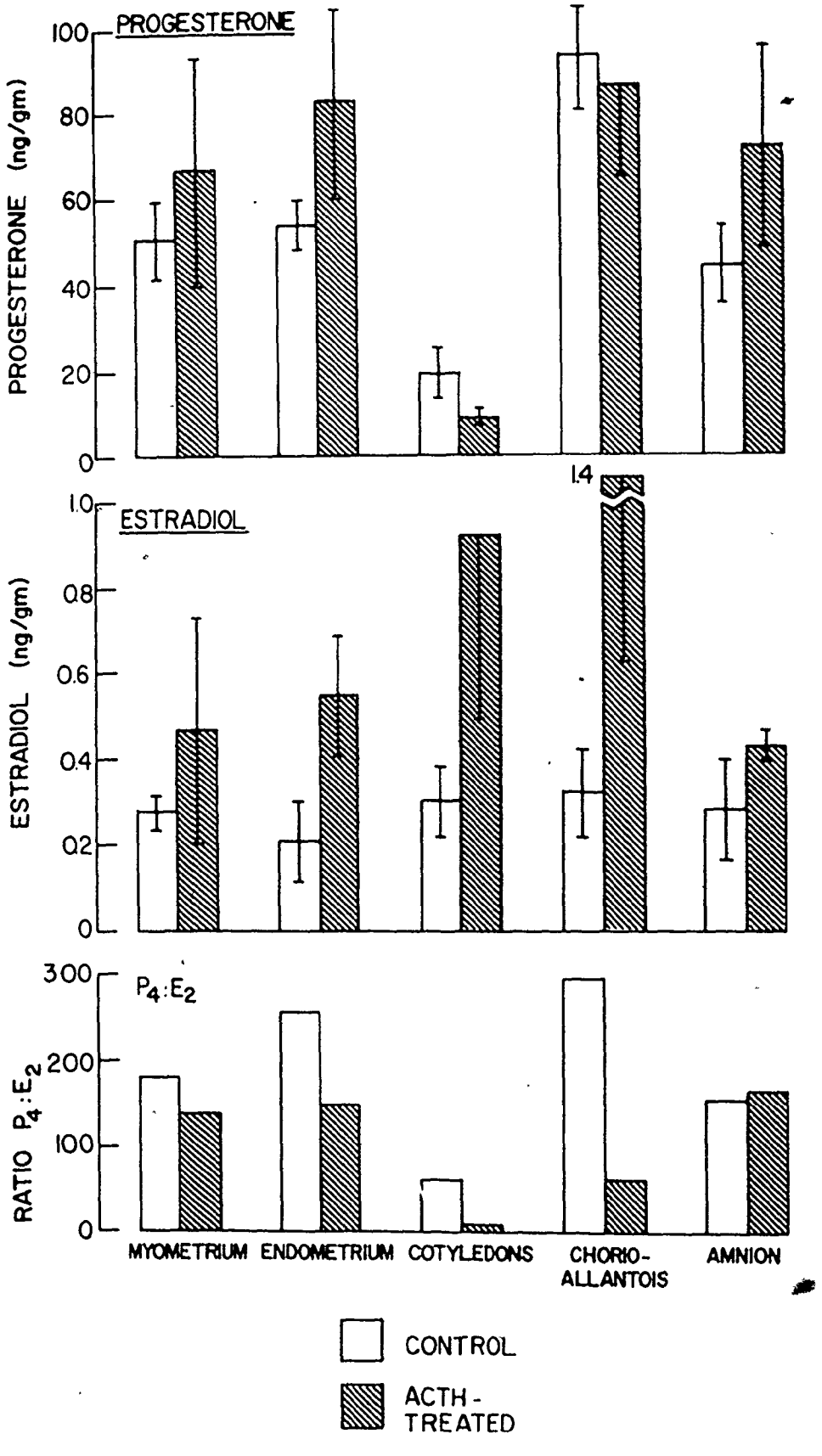
[c] 70 h infusion of ACTH (240 µg/day) and of indomethacin (25 mg/kg/day) into the mother (n = 4)

[d] Geometric mean

[e] Significantly greater than Day 1 of infusion ($P < 0.05$; nested analysis of variance and paired t-tests)

FIGURE 7

Concentrations of progesterone and estrone, and the ratio of progesterone:estrone concentrations in myometrium, endometrium, cotyledons, chorioallantois and amnion, collected from five different uterine areas in ewes 130 days pregnant after the continuous intra-fetal infusion for 70 h of either ACTH (240 µg/day, n = 4), or of saline (n = 4).


PROGESTERONE
PROGESTERONE (ng/gm)
100
80
60
40
20
0
ESTRADIOL
ESTRADIOL (ng/gm)
1.0
0.8
0.6
0.4
0.2
0
1.4
P4:E2
RATIO P4:E2
300
200
100
0
MYOMETRIUM
ENDOMETRIUM
COTYLEDONS
CHORIO-ALLANTOIS
AMNION
CONTROL
ACTH-TREATED

estradiol-17β concentrations were significantly higher in cotyledons and chorioallantois than in other uterine tissues (both $P < 0.05$, analysis of variance and paired *t* tests). Moreover, estradiol-17β concentrations in these tissues and in endometrium were elevated in sheep treated with ACTH + P.B. (all $P < 0.05$, analysis of variance and unpaired *t* tests). However, ratios of progesterone to estradiol-17β concentrations were significantly reduced only in the cotyledons and chorioallantois (both $P < 0.05$, analysis of variance and unpaired *t* tests), decreasing about 3-fold in each tissue. Steroid concentrations were not determined in tissues obtained from ACTH + indomethacin-treated sheep. There was no significant effect of uterine area on tissue steroid concentrations.

Concentrations of PGF, PGE and 6-oxo-$PGF_{1\alpha}$ in myometrium taken from sheep treated with saline + P.B., ACTH + P.B. and ACTH + indomethacin are shown in Figure 8. For PGF, concentrations tended to be higher in sheep treated with ACTH + P.B.; however, they did not attain significance (Table 27). The concentrations of PGF in ewes receiving ACTH + indomethacin were significantly lower than in those receiving ACTH + P.B., but not significantly different from those in saline + P.B.-treated animals. Analysis of variance (Table 27) also indicated a significant effect of uterine area on PGF concentrations in all treatment groups, concentrations being higher in tubal areas in saline + P.B. and in ACTH + indomethacin groups and in tubal and cervical areas in ewes receiving ACTH + P.B. (all $P < 0.05$, paired *t* tests). For PGE, analysis of variance (Table 27) indicated that concentrations were not significantly reduced in ACTH + indomethacin-

FIGURE 8

Concentrations of prostaglandin·E (PGE), PGF and 6-oxo-$PGF_{1\alpha}$ in samples taken from different areas of myometrium in ewes, 130 days pregnant, receiving either saline + phosphate buffer (P.B.) (— ·· — ; n = 4), ACTH + P.B. (——— ; n = 4) or ACTH + indomethacin (— · — ; n = 4) treatments for 70 h.

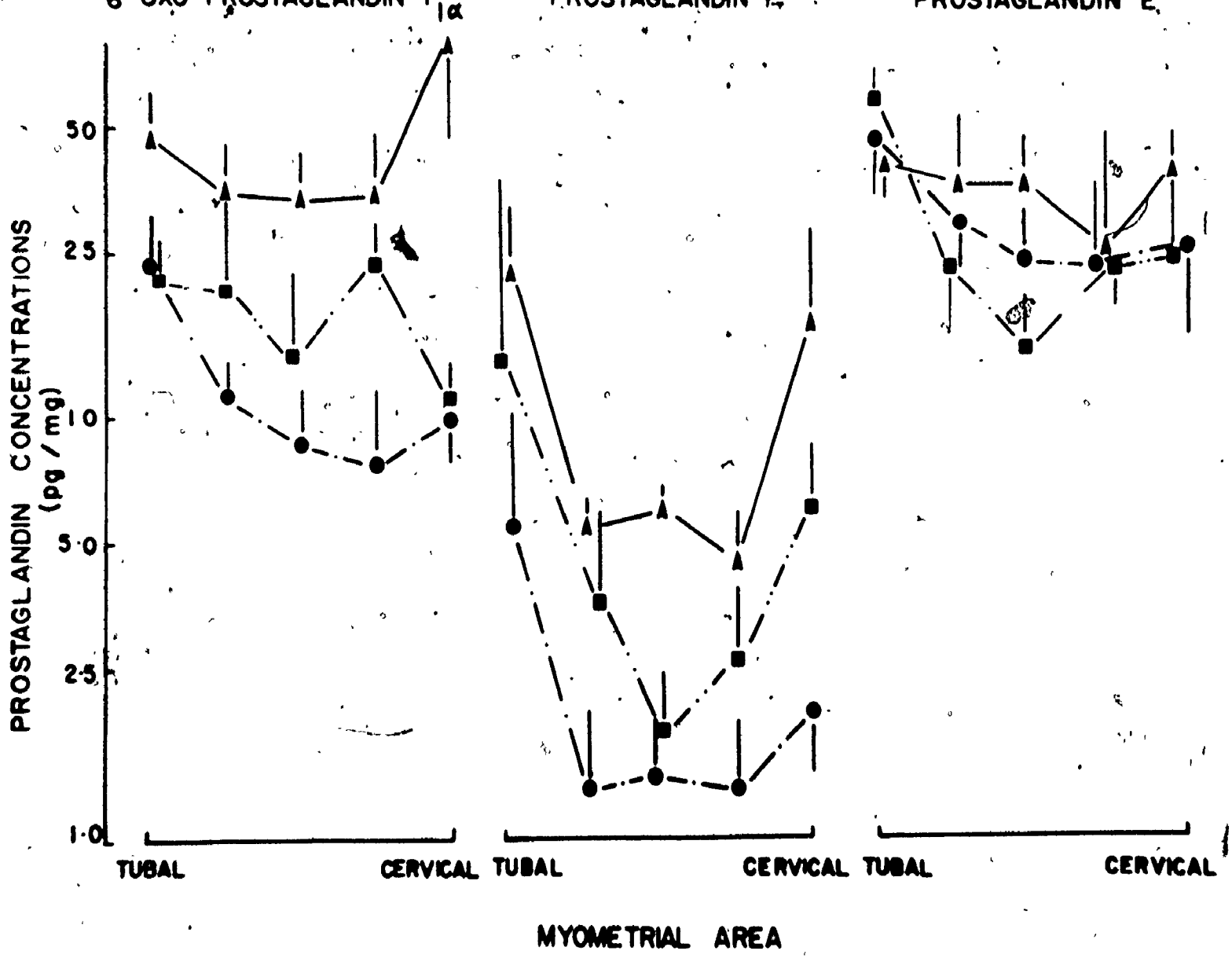

6-OXO-PROSTAGLANDIN F1α
PROSTAGLANDIN F2α
PROSTAGLANDIN E2
PROSTAGLANDIN CONCENTRATIONS (pg / mg)
50
25
10
5·0
2·5
1·0
TUBAL
CERVICAL
TUBAL
CERVICAL
TUBAL
CERVICAL
MYOMETRIAL AREA

TABLE 27: A summary of variance analysis of results in Figure 8

Source of Variation	PGF F	PGF P	PGE F	PGE P	6-oxo-PGF$_{1\alpha}$ F	6-oxo-PGF$_{1\alpha}$ P
Saline vs. ACTH:						
Treatment (1,6)[a]	6.401	0.059	0.634	N.S.	4.989	0.067
Uterine area (4,24)	5.652	<0.01	4.426	<0.01	0.853	N.S.
Treatment x area(4,24)	0.290	N.S.	2.414	0.077	3.685	<0.05
Saline vs. ACTH + indomethacin:						
Treatment (1,6)	0.238	N.S.	4.383	0.081	0.165	N.S.
Uterine area (4,24)	2.744	0.052	3.725	<0.05	1.275	N.S.
Treatment x area(4,24)	1.060	N.S.	1.295	N.S.	1.806	N.S.
ACTH vs. ACTH + indomethacin:						
Treatment (1,6)	14.492	<0.01	0.305	N.S.	14.073	<0.01
Uterine area (4,24)	10.243	<0.001	2.944	<0.05	2.795	<0.05
Treatment x area(4,24)	0.773	N.S.	0.907	N.S.	2.098	N.S.

[a]degrees of freedom in numerator, degrees of freedom in denominator for the determination of P values

treated sheep and that concentrations were not significantly elevated during ACTH-induced labour. Analysis of variance also indicated a significant effect of uterine area for both PGE and 6-oxo-$PGF_{1\alpha}$ concentrations in the myometrium (Table 27). For PGE, concentrations were higher in myometrium taken from tubal regions for saline + P.B.- and ACTH + P.B.-treated animals ($P < 0.05$, paired t tests) compared to other areas. Concentrations of 6-oxo-$PGF_{1\alpha}$ were also higher in tubal areas in saline + P.B.- and in ACTH + indomethacin-treated animals (both $P < 0.01$, paired t tests); however, during ACTH-induced labour, concentrations were also significantly higher in myometrium adjacent to the cervix ($P < 0.05$, analysis of variance and paired t tests) compared to samples taken from the middle of the uterus.

The ratios of PGF:PGE in myometrium are summarized in Table 28. Neither indomethacin nor ACTH treatment had significant effects on PGF:PGE ratios; however analysis of variance indicated a significant effect of uterine area in all treatment groups (all $P < 0.01$). The ratio PGF:PGE was higher in tubal and cervical myometrium than in the middle areas (all $P < 0.05$, paired t tests).

Concentrations of PGF, PGE and 6-oxo-$PGF_{1\alpha}$ in the endometrium are shown in Figure 9. Concentrations of PGF and 6-oxo-$PGF_{1\alpha}$, but not PGE, were significantly lower in ACTH + indomethacin-treated animals, compared to those treated with ACTH + P.B. (Table 29). In addition, PGE, but not PGF or 6-oxo-$PGF_{1\alpha}$, concentrations were significantly higher in ewes treated with ACTH + P.B., compared to saline + P.B.-treated sheep (Table 29). Analysis of variance also indicated a significant effect of uterine area on endometrial PGE concentrations

TABLE 28: The ratios of concentrations of prostaglandin F (PGF) to PGE in myometrium of sheep receiving 70 h of intra-fetal ACTH (240 µg/day) or saline and intra-maternal phosphate buffer (P.B.) or indomethacin (25 mg/kg/day) infusions

Treatment	Uterine Area				
	Tubal	Mid-tubal	Middle	Mid-cervical	Cervical
Saline + P.B.	0.541±0.304[a]	0.083±0.032	0.129±0.012	0.127±0.027	0.298±0.083
ACTH + P.B.	0.588±0.094[a]	0.179±0.061	0.194±0.060	0.221±0.088	0.484±0.189
ACTH + indomethacin	0.441±0.114[a]	0.071±0.052	0.098±0.026	0.092±0.041	0.216±0.501

[a]Significantly higher than other uterine areas in the same treatment group ($P < 0.05$; nested analysis of variance)

FIGURE 9

Concentrations of prostaglandin E (PGE), PGF and 6-oxo-$PGF_{1\alpha}$ in samples taken from different areas of endometrium in ewes, 130 days pregnant, receiving either saline + phosphate buffer (P.B.) (—— ·· ——; n = 4), ACTH + P.B. (———— ; n = 4) or ACTH + indomethacin (—— · ——; n = 4) treatments for 70 h.

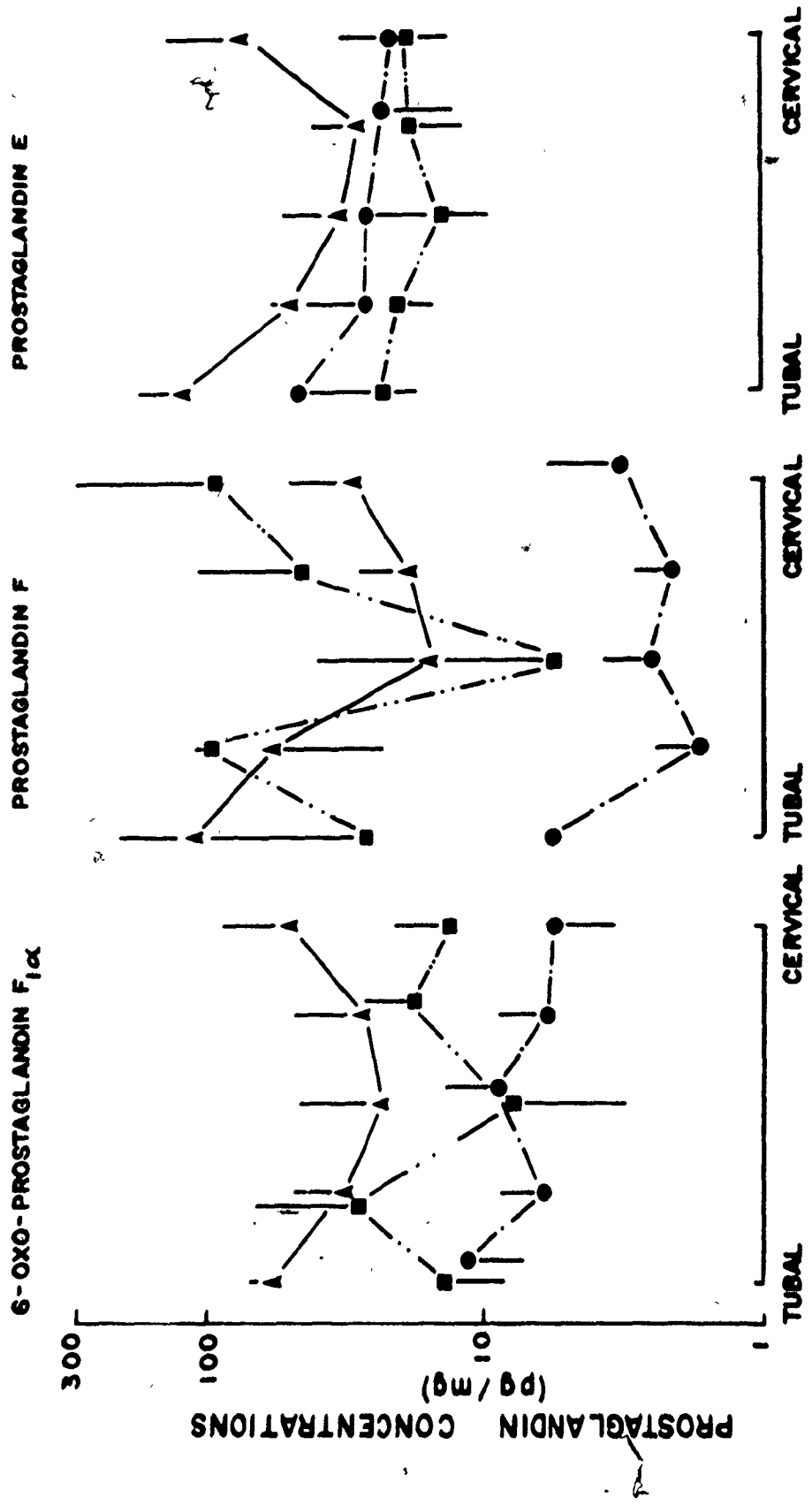

6-OXO-PROSTAGLANDIN $F_{1\alpha}$
PROSTAGLANDIN F
PROSTAGLANDIN E
300
100
10
1
(pg / mg)
PROSTAGLANDIN CONCENTRATIONS
TUBAL
CERVICAL
ENDOMETRIAL AREA

TABLE 29: A summary of variance analysis of results in Figure 9

Source of Variation	PGF		PGE		6-oxo-PGF$_{1\alpha}$	
	F	P	F	P	F	P
Saline vs. ACTH:						
Treatment (1,6)[a]	0.066	N.S.	6.733	<0.05	0.536	N.S.
Uterine area (4,24)	2.762	0.051	2.448	0.074	0.751	N.S.
Treatment x area(4,24)	1.311	N.S.	1.348	N.S.	1.610	N.S.
Saline vs. ACTH + indomethacin:						
Treatment (1,6)	3.334	N.S.	1.365	N.S.	0.008	N.S.
Uterine area (4,24)	1.665	N.S.	0.420	N.S.	1.339	N.S.
Treatment x area(4,24)	1.978	N.S.	0.901	N.S.	0.865	N.S.
ACTH vs. ACTH + indomethacin:						
Treatment (1,6)	16.974	<0.01	1.063	N.S.	7.828	<0.05
Uterine area (4,24)	2.399	0.078	5.348	<0.01	1.959	N.S.
Treatment x area(4,24)	0.865	N.S.	1.762	N.S.	1.261	N.S.

[a] degrees of freedom in numerator, degrees of freedom in denominator for the determination of P values

in ACTH + P.B.-treated animals; concentrations being higher in tubal regions than in other areas of the uterus ($P < 0.05$, paired t tests).

Figure 10 shows PGF, PGE and 6-oxo-$PGF_{1\alpha}$ concentrations in cotyledons taken from saline + P.B.-, ACTH + P.B.-, or ACTH + indomethacin-treated ewes. For PGF, analysis of variance (Table 30) indicated that concentrations in ewes treated with ACTH + P.B. were significantly higher than concentrations in the saline + P.B. group. In addition, concentrations were significantly lower in ACTH + indomethacin-treated ewes than in those treated with ACTH + P.B. or in the saline + P.B.-treated animals. For PGE, concentrations were significantly lower in ACTH + indomethacin-treated animals than in saline + P.B.- and in ACTH + P.B.-treated ewes. However, in ewes receiving ACTH + P.B., PGE concentrations were not significantly higher than those in the saline + P.B. group. For 6-oxo-$PGF_{1\alpha}$, concentrations were significantly higher in ewes receiving ACTH + P.B., compared to those receiving saline. In addition, analysis of variance indicated that concentrations in ACTH + indomethacin-treated animals were significantly lower than those in animals receiving ACTH + P.B. and in the saline + P.B. group. Indomethacin + ACTH treatment resulted in a 10- to 20-fold decrease in the concentrations of all PG's, compared to animals receiving ACTH + P.B. There was no significant effect of uterine area on PG concentrations in the cotyledons.

Concentrations of PGF, PGE and 6-oxo-$PGF_{1\alpha}$ in chorioallantois are shown in Figure 11. Analysis of variance (Table 31) indicated that concentrations of PGF and 6-oxo-$PGF_{1\alpha}$, but not PGE were significantly elevated in ACTH + P.B.-treated ewes, compared to

FIGURE 10

Concentrations of prostaglandin E (PGE), PGF and 6-oxo-$PGF_{1\alpha}$ in samples taken from different uterine areas in cotyledons taken from ewes, 130 days pregnant, receiving either saline + phosphate buffer (P.B.) (—— · · ——; n = 4), ACTH + P.B. (———— ; n = 4), or ACTH + indomethacin (—— · ——; n = 4) treatments for 70 h.

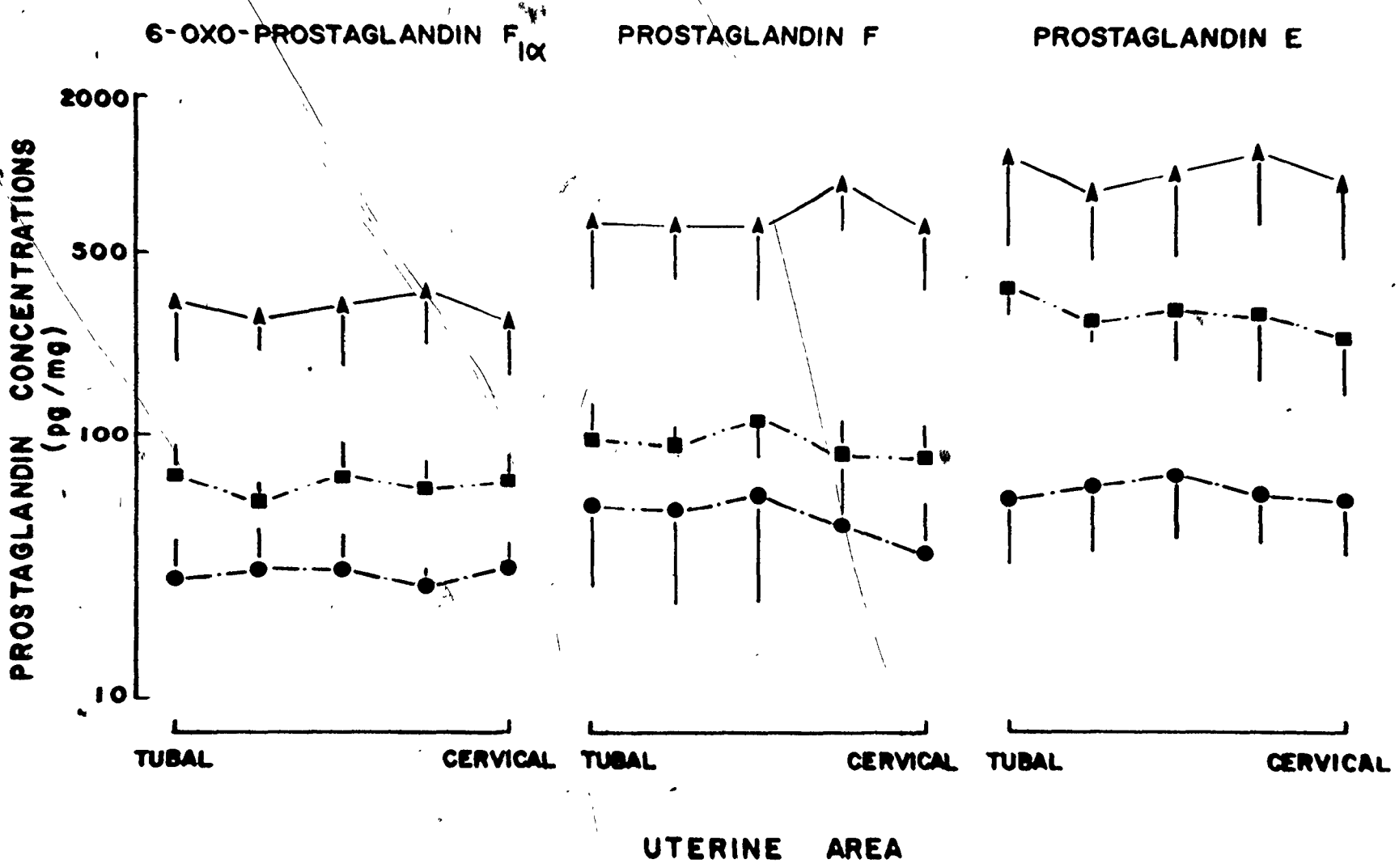

6-OXO-PROSTAGLANDIN F1α
PROSTAGLANDIN F
PROSTAGLANDIN E
PROSTAGLANDIN CONCENTRATIONS (pg/mg)
2000
500
100
10
TUBAL
CERVICAL
TUBAL
CERVICAL
TUBAL
CERVICAL
UTERINE AREA

TABLE 30: A summary of variance analysis of results in Figure 10

Source of Variation	PGF		PGE		6-oxo-PGF$_{1\alpha}$	
	F	P	F	P	F	P
Saline vs. ACTH:						
Treatment (1,6)[a]	10.571	<0.05	2.893	N.S.	9.000	<0.05
Uterine area (4,24)	0.061	N.S.	2.209	0.098	0.852	N.S.
Treatment x area(4,24)	1.107	N.S.	0.657	N.S.	0.548	N.S.
Saline vs. ACTH + indomethacin:						
Treatment (1,6)	51.501	<0.01	16.374	<0.01	17.594	<0.01
Uterine area (4,24)	0.932	N.S.	2.297	0.088	0.217	N.S.
Treatment x area(4,24)	0.551	N.S.	1.773	N.S.	1.801	N.S.
ACTH vs. ACTH + indomethacin:						
Treatment (1,6)	9.185	<0.05	10.458	<0.05	20.988	<0.01
Uterine area (4,24)	0.527	N.S.	1.007	N.S.	0.050	N.S.
Treatment x area(4,24)	0.643	N.S.	1.552	N.S.	0.828	N.S.

[a] degrees of freedom in numerator, degrees of freedom in denominator for the determination of P values

FIGURE 11

Concentrations of prostaglandin E (PGE), PGF and 6-oxo-$PGF_{1\alpha}$ in samples taken from different uterine areas in chorioallantois taken from ewes, 130 days pregnant, receiving either saline + phosphate buffer (P.B.) (——··——; n = 4), ACTH + P.B. (——— ; n = 4), or ACTH + indomethacin (—— · ——; n = 4) treatments for 70 h.

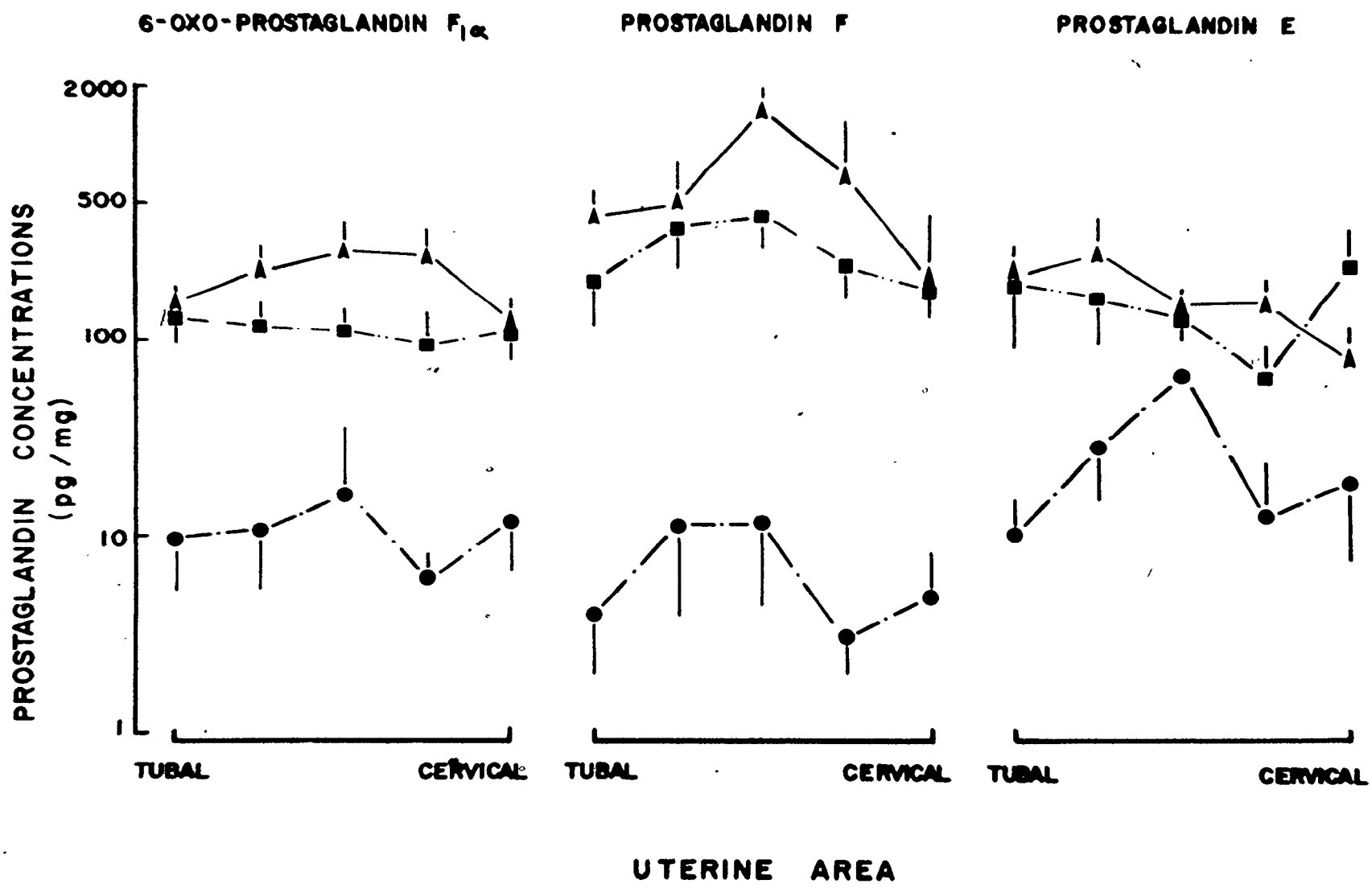

6-OXO-PROSTAGLANDIN $F_{1\alpha}$
PROSTAGLANDIN F
PROSTAGLANDIN E
PROSTAGLANDIN CONCENTRATIONS (pg / mg)
2000
500
100
10
1
TUBAL
CERVICAL
TUBAL
CERVICAL
TUBAL
CERVICAL
UTERINE AREA

TABLE 31: A summary of variance analysis of results in Figure 11

Source of Variation	PGF		PGE		6-oxo-PGF$_{1\alpha}$	
	F	P	F	P	F	P
Saline vs. ACTH:						
Treatment (1,6)[a]	6.670	<0.05	0.034	N.S.	9.341	<0.05
Uterine area (4,24)	2.568	0.064	1.660	N.S.	0.786	N.S.
Treatment x area(4,24)	1.302	N.S.	2.423	0.076	1.096	N.S.
Saline vs. ACTH + indomethacin:						
Treatment (1,6)	85.352	<0.001	5.364	0.060	73.096	<0.001
Uterine area (4,24)	1,641	N.S.	1.773	N.S.	0.153	N.S.
Treatment x area(4,24)	0.415	N.S.	1.163	N.S.	0.204	N.S.
ACTH vs. ACTH + indomethacin:						
Treatment (1,6)	39.645	<0.001	14.687	<0.01	30.087	<0.01
Uterine area (4,24)	3.020	<0.05	2.061	N.S.	1.075	N.S.
Treatment x area(4,24)	0.999	N.S.	2.088	N.S.	1.301	N.S.

[a] degrees of freedom in numerator, degrees of freedom in denominator for the determination of P values

concentrations in saline + P.B.-treated animals. In addition, concentrations of all PG's were significantly lower in ACTH + indomethacin-treated ewes than in ewes receiving ACTH + P.B. PGF and 6-oxo-$PGF_{1\alpha}$ concentrations in ACTH + indomethacin-treated animals were also significantly lower than those in the saline+P.B. group. Analysis of variance indicated a significant effect of uterine area on PGF concentrations in the chorioallantois, concentrations being higher in the mid-uterine regions than in areas close to the utero-tubal junction or the cervix ($P < 0.05$, paired t tests). In this tissue, treatment with ACTH + indomethacin resulted in a 40- to 50-fold reduction in PG concentrations, compared to animals treated with ACTH + P.B.

Figure 12 shows PGF, PGE and 6-oxo-$PGF_{1\alpha}$ concentrations in amnion collected from ewes treated with saline + P.B., ACTH + P.B. or ACTH + indomethacin. Analysis of variance (Table 32) indicated that the concentrations of all PG's were not significantly increased during ACTH-induced labour, and that they were significantly lower in ACTH + indomethacin-treated animals than in the saline + P.B. or ACTH + P.B. groups. In addition, PG concentrations were decreased 10- to 100-fold in these animals, compared to animals receiving ACTH + P.B.

To determine if the changes in PG concentration in intrauterine tissues following ACTH treatment were correlated with the changes in steroid concentrations in these tissues, correlation coefficients and t test of ratios for zero correlation were calculated. The average PG concentration in myometrium, endometrium, cotyledons, chorioallantois and amnion were compared with the average ratio of

FIGURE 12

Concentrations of prostaglandin E (PGE), PGF and 6-oxo-$PGF_{1\alpha}$ in samples taken from different uterine areas in amnion taken from ewes, 130 days pregnant, receiving either saline + phosphate buffer (P.B.) (— • • —; n = 4), ACTH + P.B. (————— ; n = 4), or ACTH + indomethacin (—— • —; n = 4) treatments for 70 h.

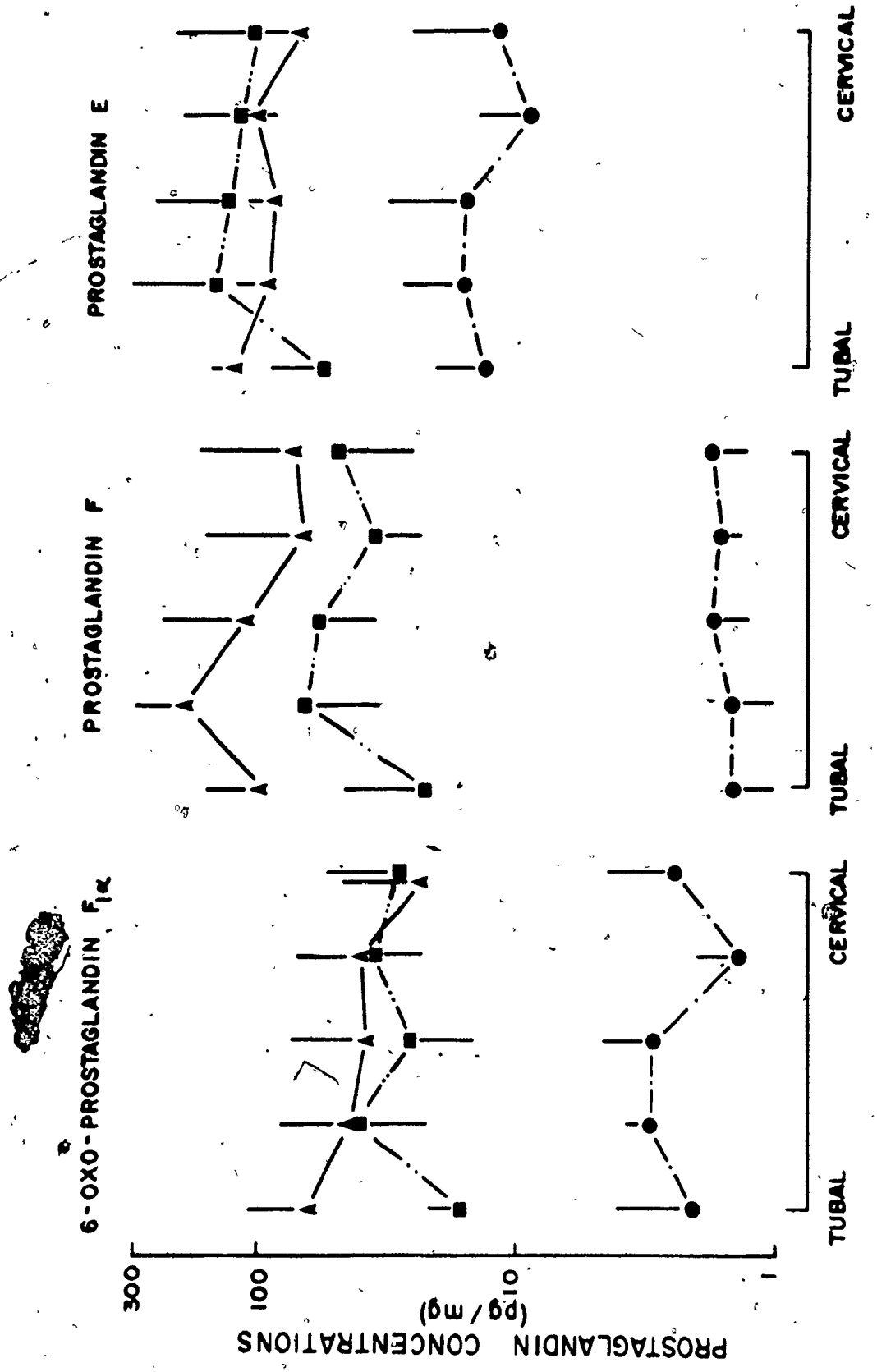

PROSTAGLANDIN E
PROSTAGLANDIN F
6-OXO-PROSTAGLANDIN F1α
CERVICAL
TUBAL
UTERINE AREA
PROSTAGLANDIN CONCENTRATIONS
(pg / mg)
300
100
10
1

TABLE 32: A summary of variance analysis of results in Figure 12

Source of Variation	PGF F	PGF P	PGE F	PGE P	6-oxo-PGF$_{1\alpha}$ F	6-oxo-PGF$_{1\alpha}$ P
Saline vs. ACTH:						
Treatment (1,6)[a]	2.338	N.S.	2.233	N.S.	0.302	N.S.
Uterine area (4,24)	0.778	N.S.	0.362	N.S.	0.365	N.S.
Treatment x area(4,24)	0.245	N.S.	0.686	N.S.	0.979	N.S.
Saline vs. ACTH + indomethacin:						
Treatment (1,6)	10.551	<0.05	5.981	0.050	6.944	<0.05
Uterine area (4,24)	1.016	N.S.	0.959	N.S.	1.316	N.S.
Treatment x area(4,24)	0.112	N.S.	0.081	N.S.	0.131	N.S.
ACTH vs. ACTH + indomethacin:						
Treatment (1,6)	49.626	<0.001	17.800	<0.01	18.601	<0.01
Uterine area (4,24)	0.409	N.S.	0.414	N.S.	0.893	N.S.
Treatment x area(4,24)	0.572	N.S.	0.649	N.S.	1.157	N.S.

[a] degrees of freedom in numerator, degrees of freedom in denominator for the determination of P values

progesterone:estrogen concentrations, average progesterone concentrations, and average estrogen concentrations for each of the saline + P.B.- and ACTH + P.B.-treated sheep. For myometrium and chorioallantois, a significant negative correlation was found between 6-oxo-$PGF_{1\alpha}$ concentrations and the ratio of progesterone:estrogen concentrations (Table 33). In addition, a significant positive correlation between 6-oxo-$PGF_{1\alpha}$ concentrations and the ratios of progesterone:estrogen concentrations was found in the endometrium (Table 33). The correlation between PG concentrations and the ratios of progesterone:estrogen concentrations were not significant for other tissues and PG's examined. Similarly, a significant correlation between PG and progesterone concentrations was not observed in the tissues examined. However, compared to estrogen concentrations, significant positive correlations were found in endometrium for PGE, and in cotyledons and chorioallantois for PGF and 6-oxo-$PGF_{1\alpha}$ (Table 34).

The effects of indomethacin on the frequency and maximum amplitude of uterine contractions during ACTH-induced labour are shown in Figures 13 and 14, respectively. There was a progressive increase in the frequency of contractions in sheep treated with ACTH + P.B., beginning 34-35 h prior to tissue collection (35-36 h after the start of ACTH infusions) (Figure 13). In the animals receiving ACTH + indomethacin, the frequency of uterine contractions did not start to increase until 2-3 h before tissue collection (67-68 h after the beginning of ACTH infusions). At this time, the frequency of uterine contractions was significantly lower in these animals than in those not receiving indomethacin ($P < 0.01$, unpaired t test). There was

TABLE 33: Correlation co-efficients (C.C.) for the relationship between PG concentrations and the ratios of progesterone: estrogen concentrations in intra-uterine sheep tissues (n = 8 animals)

	6-oxo-PGF$_{1\alpha}$		PGF		PGE	
	C.C.	P*	C.C.	P	C.C.	P
Myometrium	-0.778	<0.05	-0.579	N.S.	-0.442	N.S.
Endometrium	0.841	<0.05	0.117	N.S.	-0.503	N.S.
Cotyledons	-0.354	N.S.	-0.612	N.S.	-0.423	N.S.
Chorioallantois	-0.793	<0.05	-0.730	<0.10	0.443	N.S.
Amnion	-0.723	<0.10	-0.327	N.S.	-0.781	<0.10

*determined from the t-test of ratios for zero correlation

TABLE 34: Correlation co-efficients (C.C.) for the relationship between PG concentrations and estrogen concentrations in intra-uterine sheep tissues (n = 8 animals)

	6-oxo-PGF$_{1\alpha}$		PGF		PGE	
	C.C.	P*	C.C.	P	C.C.	P
Myometrium	0.613	N.S.	0.610	N.S.	0.329	N.S.
Endometrium	-0.596	N.S.	-0.242	N.S.	0.870	<0.05
Cotyledons	0.794	<0.05	0.985	<0.001	0.324	N.S.
Chorioallantois	0.824	<0.05	0.912	<0.05	0.682	N.S.
Amnion	0.514	N.S.	0.426	N.S.	0.549	N.S.

*determined from the t-test of ratios for zero correlation

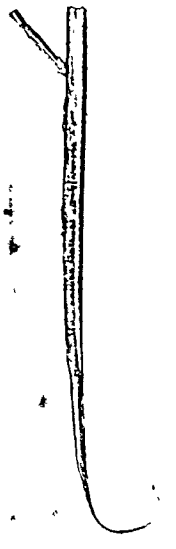


FIGURE 13

The frequency of uterine contractions (contractions/h) in ewes during the continuous infusion of ACTH and indomethacin (n = 4) or vehicle (n = 4).

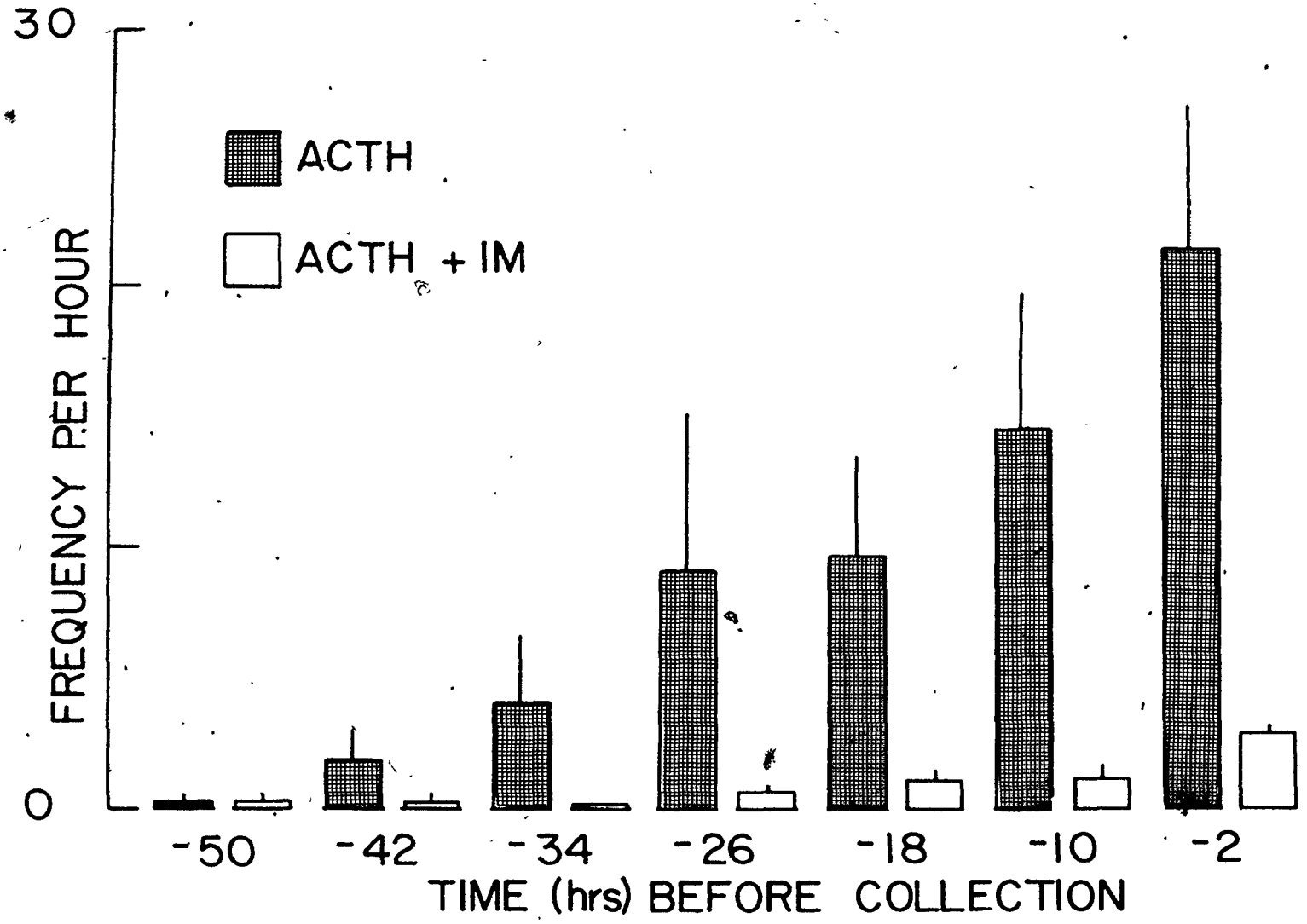
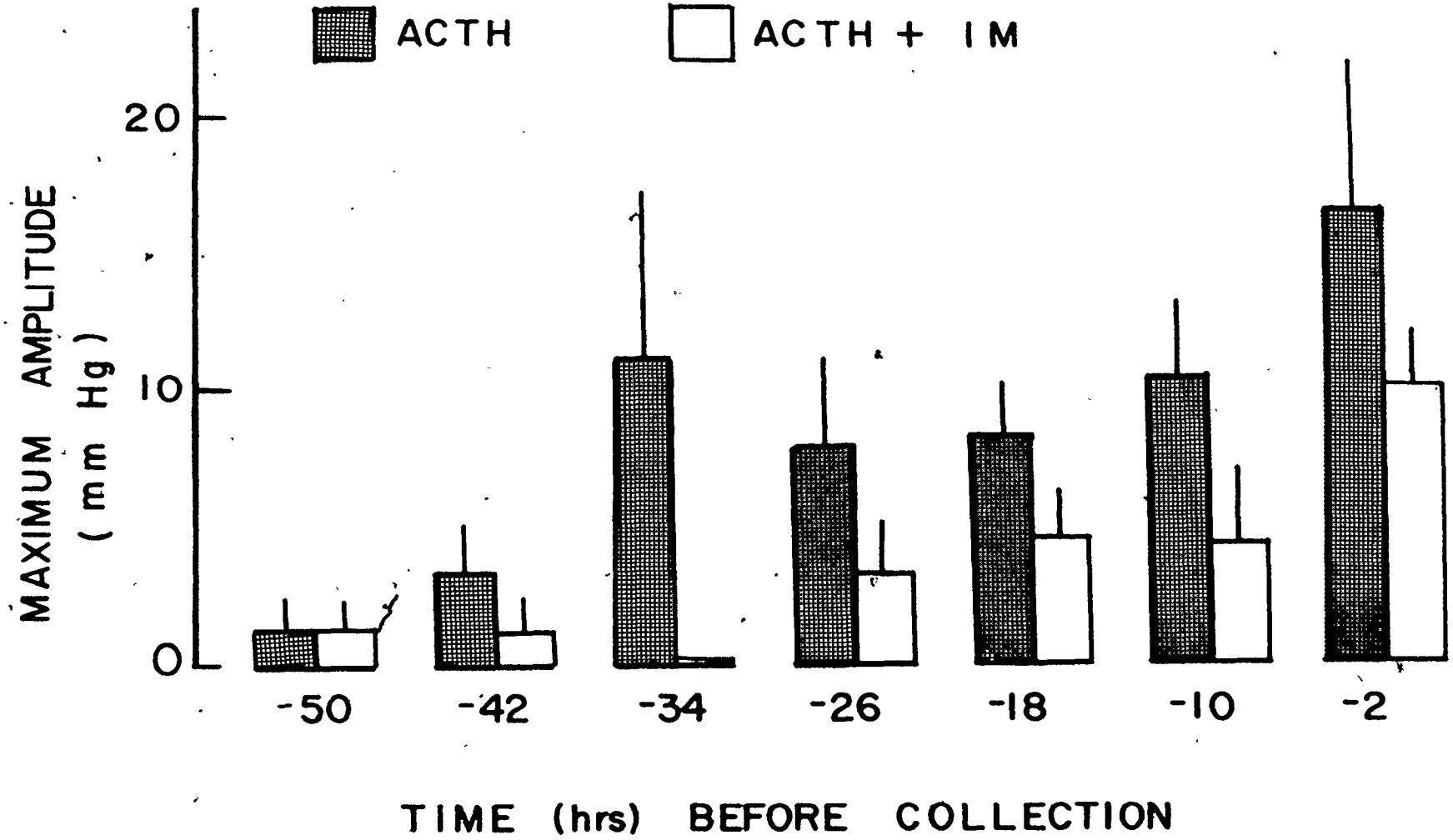

30
0
FREQUENCY PER HOUR
ACTH
ACTH + IM
-50
-42
-34
-26
-18
-10
-2
TIME (hrs) BEFORE COLLECTION

FIGURE 14

The maximum amplitude (mm Hg) of uterine contractions in ewes during the infusion of ACTH and indomethacin (n = 4) or vehicle (n = 4).


ACTH
ACTH + IM
MAXIMUM AMPLITUDE
(mm Hg)
20
10
0
-50
-42
-34
-26
-18
-10
-2
TIME (hrs) BEFORE COLLECTION

also an increase in the maximum amplitude of contractions after the start of ACTH infusions (Figure 14), beginning 34-35 h and 2-3 h prior to tissue collection for animals treated with ACTH + P.B. and ACTH + indomethacin, respectively. Although indomethacin delayed the increase in maximum amplitude of contractions, it did not significantly suppress the maximum amplitude of contractions 2-3 h prior to sacrifice. Uterine contractions in saline + P.B.-treated sheep were not observed in many animals at the times uterine activity was analyzed and when present, were always of low amplitude (5-7 mm Hg); therefore, these data were not included in Figures 13 and 14.

To determine if the effects of indomethacin on uterine activity were due to non-specific effects of the drug on phosphodiesterase activity, myometrial cyclic AMP concentrations were measured. Concentrations in saline + P.B.-treated animals (226.4 ± 18.8 fm/mg wet weight) were significantly higher than concentrations in ewes treated with ACTH + P.B. ($P < 0.01$; 86.9 ± 28.7 fm/mg wet weight) and in ewes treated with ACTH + indomethacin ($P < 0.01$; 51.5 ± 16.4 fm/mg wet weight). However, the concentrations of cyclic AMP in ACTH + indomethacin-treated animals were not different from those in sheep receiving ACTH + P.B.

5.4 Discussion

The changes in plasma steroid concentration in this study are similar to those reported previously during spontaneous (Thorburn et al., 1972) and ACTH-induced parturition (Kendall et al., 1977), suggesting that the sheep receiving ACTH were approaching delivery at the time of sacrifice. Estimates of the frequency and maximum amplitude of uterine contractions and the assessment of cervical dilatation support this conclusion. The highest PG concentrations were found in cotyledons and chorioallantois in saline-treated animals. The concentrations of PGF and 6-oxo-$PGF_{1\alpha}$ in these tissues were elevated significantly after 70 h of intra-fetal ACTH infusion. Similarly, in these tissues, the ratio of concentrations of progesterone:estradiol was significantly reduced following intra-fetal ACTH infusions and the concentrations of PGF and 6-oxo-$PGF_{1\alpha}$ were significantly correlated with estradiol-17β concentrations. Concentrations of 6-oxo-$PGF_{1\alpha}$ were elevated also in myometrium adjacent to the cervix in animals treated with ACTH + P.B., while PGE concentrations were elevated significantly only in the endometrium in these animals. The changes in PG concentrations in these tissues were also significantly correlated with changes in estradiol-17β concentrations. Indomethacin, a potent inhibitor of PG synthesis, reduced uterine PG concentrations but did not alter significantly the changes in plasma steroid or myometrial cyclic AMP concentrations characteristic of ACTH-induced labour. However, the increased frequency and maximum amplitude of uterine contractions observed during ACTH-induced labour were delayed in these animals. Furthermore, the frequency, but not maximum amplitude, of

contractions was significantly attenuated two hours prior to sacrifice in indomethacin-treated sheep.

Liggins and Grieves (1971) observed elevated PGF concentrations in maternal cotyledons and myometrium but not in fetal cotyledons while PGE was not detectable in any tissue during dexamethasone-induced labour in sheep. In contrast, other investigators (Mitchell and Flint, 1977) reported high levels of PGE in fetal cotyledons during late pregnancy and elevated PGF concentrations after delivery in fetal cotyledons, but not myometrium or maternal cotyledons. These seemingly paradoxical results may be due, in part, to the considerable variation among single observations taken from the few animals used in these previous studies. Furthermore, PG determinations in separated fetal and maternal cotyledons may be influenced by the artifactual effects of trauma associated with manual separation (see Chapter 4). In the present study, PG's were measured in whole cotyledons and concentrations of PGF and 6-oxo-$PGF_{1\alpha}$ were elevated significantly during ACTH-induced labour. Concentrations of PGE were elevated in only two of the four animals. Using a superfusion system, PGE output by fetal and maternal cotyledons from three sheep was generally higher than that of PGF (Mitchell and Flint, 1978). This observation is consistent with the present results showing that the mean concentrations of PGE in whole cotyledons generally exceeded those of PGF. It is not possible from previous studies to draw any reasonable conclusions concerning the relative importance of fetal and maternal cotyledons as sites of PG synthesis.

The results from measurements of tissue steroid concentrations indicate that concentrations of progesterone or its metabolites

are lower in cotyledons than in other intra-uterine tissues. This likely reflects the rapid clearance of progesterone from this tissue as a result of the high percentage (83%) of uterine blood flow to this tissue (Rosenfeld et al., 1974). However, since the placenta produces both progesterone and estradiol-17β (Flint et al., 1975b; Thorburn et al., 1977), and only progesterone was present at lower concentrations compared to other uterine tissues, this suggests that mechanisms other than increased clearance rates may be involved. Rawlings and Ward (1976) observed higher concentrations of progesterone and estrogens in myometrial tissue than in the peripheral plasma in sheep and suggested that steroid binding proteins in this tissue may act to effect a local accumulation of steroids. It is possible, therefore, that the placenta might have a lower concentration of a progesterone binding protein. The steroid concentrations in myometrium did not change during ACTH-induced labour in the present study, and were of the same order of magnitude as those reported by Rawlings and Ward (1976). These investigators similarly did not observe changes in myometrial steroid concentrations during the initiation of parturition; however myometrial estrogen concentrations were significantly elevated following delivery. In the present study, the ratio of progesterone to estrogen concentrations was significantly reduced only in the cotyledons and chorioallantois, indicating that these tissues may be sites of steroidogenesis during parturition in sheep. Although this ratio may be important in the regulation of PG production (see Chapter 1), without data concerning steroid receptors in these tissues, the physiological significance of tissue steroid concentrations remains

uncertain. However, the observations that PG concentrations were elevated in the cotyledons and chorioallantois and that the ratio of progesterone to estradiol concentrations in these tissues were reduced, supports the hypothesis that steroid hormones may play a role in the regulation of uterine PG production. In the tissues examined, progesterone and PG concentrations were not significantly correlated and concentrations of PGE in endometrium and of PGF and 6-oxo-$PGF_{1\alpha}$ in cotyledons and chorioallantois were correlated better with estradiol concentrations than with the ratio of progesterone:estradiol concentrations. These results suggest that progesterone does not play a major role in the regulation of intra-uterine PG production. In addition, these results are consistent with a role for estrogens in the regulation of PG production in endometrium, chorioallantois and cotyledons.

Of the tissues examined, the chorioallantois contained the highest concentrations of PGF and 6-oxo-$PGF_{1\alpha}$ in the control animals, and PGF and 6-oxo-$PGF_{1\alpha}$, but not PGE, were elevated after ACTH + P.B. treatment. Grieves and Liggins (1976) have reported previously that phospholipase A activity is elevated in the chorioallantoic membranes of a sheep fetus after ACTH infusion. Taken together, these observations suggest that these fetal membranes are major sites of PG production in late pregnancy and during ACTH-induced labour. Although the concentrations of PGF and 6-oxo-$PGF_{1\alpha}$ were elevated in this tissue during ACTH-induced labour, there was no significant change in PGE concentrations in the present study. This might suggest that although there may be an increase in the

availability of arachidonic acid after ACTH treatment (Grieves and Liggins, 1976), there may also be directed synthesis and/or metabolsim of PG's in this tissue.

In marked contrast to the pattern of PGF and 6-oxo-$PGF_{1\alpha}$ concentrations in the chorioallantoic membranes, significant changes in the concentrations of PGE, PGF or 6-oxo-$PGF_{1\alpha}$ were not observed in the amnion after ACTH + P.B. treatment. Other investigators have reported elevated concentrations of PGE, PGF and 6-oxo-$PGF_{1\alpha}$ in amniotic fluid prior to delivery in sheep (Mitchell et al., 1977, 1978; Challis et al., 1978). However, the possibilities that these PG's are derived from the fetus or uterine tissues other than the amnion, or that PG synthesis is increased in the amnion during later stages of labour than those examined in this study cannot be excluded.

Although a slight delay in the increased maximum amplitude of contractions was observed during ACTH-induced labour in the present study, the primary effect of indomethacin was suppression of contraction frequency. It is possible that the delayed onset of uterine activity in these sheep resulted from the failure of indomethacin to suppress myometrial PGE concentrations after 70 h of infusion. The reason for this, however, is not clear. Myometrial PGF and 6-oxo-$PGF_{1\alpha}$ concentrations were decreased in these animals, which is consistent with an inhibitory action of indomethacin on myometrial PG synthetase activity. It is interesting that the effects of indomethacin on PG concentrations were most marked in the fetal membranes and cotyledons which were also the tissues containing highest PG concentrations.

The results of this study are consistent with those of other workers. The delivery of fetuses, induced by dexamethasone, is delayed in sheep (Mitchell and Flint, 1978) and pigs (Nara and First, 1981) and the duration of gestation is prolonged in the rhesus monkey (Novy et al., 1974) by inhibitors of PG synthesis. However, these investigators also reported high fetal mortality rates in indomethacin (Novy et al., 1974; Nara and First, 1981)- and meclofenamic acid (Mitchell and Flint, 1978)-treated animals. In addition, some inhibition by indomethacin of myometrial phosphodiesterase activity has been reported (Beatty et al., 1976). Therefore, the possibility that the effects of inhibitors of PG synthesis were due to an elevation of myometrial cyclic AMP or to events predisposing fetal death, rather than decreased PG's cannot be excluded. In the present study, myometrial cyclic AMP concentrations were not altered by indomethacin treatment, suggesting that the effects of this drug were not due to cyclic AMP accumulation. In addition, at the time of tissue collection all fetuses in this study were viable.

The contribution and interactions of different arachidonic acid metabolites in the regulation of uterine activity in sheep is unknown. Exogenous $PGF_{2\alpha}$ induces uterine activity only after a time lag of several hours (Liggins et al., 1973), or during delivery induced by dexamethasone (Mitchell et al., 1976a). Some investigators have suggested that in primates prostacyclin inhibits spontaneous myometrial contractions in vitro in a dose-dependent manner (Omini et al., 1979), as well as inhibit those contractions induced by PGF (Bennett and Sanger, 1979). In the rat, however, prostacyclin appears

to be uterotonic (Omini et al., 1977). The role of increased myometrial 6-oxo-$PGF_{1\alpha}$ levels following intra-fetal ACTH in regulating uterine tone in sheep is uncertain. Rankin and co-workers (1979) did not observe any change in uterine activity in a near-term sheep following the administration of 0.7 mg (20 µg/kg) PGI_2; however 0.35 mg PGE_2 was stimulatory. Recently Lye and Challis (1982) have demonstrated a dose-dependent inhibition of uterine activity in sheep after the administration of 50 µg and 200 µg PGI_2. The elevated levels of 6-oxo-$PGF_{1\alpha}$ observed in the present study in myometrium adjacent to the cervix during ACTH-induced labour therefore may exert a local effect on uterine tone and facilitate fetal descent. The cervix produces 6-oxo-$PGF_{1\alpha}$ (Ellwood et al., 1980) and concentrations of 6-oxo-$PGF_{1\alpha}$ increase in the cervical venous drainage (Ellwood et al., 1979) during parturition in sheep. Prostacyclin produced in the lower uterine myometrium may also facilitate cervical ripening, along with PG's produced directly in the cervix.

In the present study myometrial concentrations of PGE and PGF varied with uterine area and this effect was not influenced by indomethacin treatment. In non-pregnant sheep, Rexroad (1980) showed higher PGF concentrations in myometrium and endometrium collected from anterior areas compared to posterior areas and these differences were similarly not affected by indomethacin treatment. In the present study, the ratio of PGF:PGE was greater at the tubal and cervical ends of the myometrium than in the rest of the uterus in all treatment groups. The physiological significance of this observation is uncertain; however this ratio may be important in the

regulation of uterine contractility, perhaps by facilitating the formation of gap junctions (Garfield et al., 1978, 1979). Degeneration of adrenergic nerves has been reported in perifetal tissues during late pregnancy in the guinea pig (Thorbert et al., 1977, 1979). The decreased ratio of PGF:PGE in mid-uterine areas in the present study, therefore, may also be related to regional differences in catecholamine concentrations.

In summary, the salient findings in this chapter include: 1) a 70 h infusion of ACTH (240 µg/day) into the sheep fetus results in the induction of labour; 2) myometrial PGE and PGF concentrations vary with uterine area; 3) 6-oxo-$PGF_{1\alpha}$ concentrations are elevated in myometrium adjacent to the cervix during ACTH-induced labour; 4) PGE concentrations are elevated in endometrium and PGF and 6-oxo-$PGF_{1\alpha}$ concentrations are elevated in chorioallantois and cotyledons during ACTH-induced labour; 5) the tissues having highest PG concentrations were cotyledons and chorioallantois; 6) PG concentrations in these tissues were unrelated with the tissue concentration of estrogen; and 7) infusions of indomethacin into the maternal femoral vein decreased the frequency, but not the maximum amplitude of uterine contractions during ACTH-induced labour, as well as decrease PG concentrations in intra-uterine tissues but had no effect on cAMP or plasma steroid concentrations.

CHAPTER 6

GESTATIONAL CHANGES IN PROSTAGLANDIN (PG) CONCENTRATIONS IN INTRA-UTERINE TISSUES AND FETAL FLUIDS COLLECTED FROM PREGNANT SHEEP

6.1 Introduction

Evidence supporting a role for prostaglandins in the initiation of ovine parturition has been summarized (see Chapter 1). Part of this evidence is that prostanoid (PG) concentrations in maternal and fetal plasma increase at the time of parturition. However, relatively little is known about the biosynthesis and physiological roles of PG's during early and mid-gestation. The reduced levels of PGF in the utero-ovarian vein on Days 14-16 of pregnancy suggest that PGF synthesis may be suppressed at this time (Thorburn et al., 1973; Roberts et al., 1975), although not all evidence is consistent with this notion (Findley et al., 1981). Changes in myometrial activity (Nathanielsz et al., 1980; Van Der Weyden et al., 1981), uterine hemodynamics (Rosenfeld et al., 1974), and allantoic fluid composition (Mellor and Slater, 1971, 1972) have been reported during mid-pregnancy in sheep and may be under the control of PG's. To investigate the possibility that uterine PG production is lower during early pregnancy and to examine when intra-uterine PG production might change during pregnancy, concentrations of PGE, PGF, 6-oxo-$PGF_{1\alpha}$

and thromboxane B_2 (TXB_2) were measured in intra-uterine tissues, and amniotic fluid and allantoic fluid collected on Days 50, 100, 130 and 145 of pregnancy in sheep.

6.2 Materials and Methods

Sixteen sheep of mixed breeds were used. Gestational age was estimated from known insemination dates, and was confirmed by the fetal crown-rump lengths and fetal weights (Barcroft, 1946).

Tissues were collected from four sheep on each of Days 50, 100, 130 and 145 of pregnancy using procedures similar to those described previously (see Chapter 3). General anesthesia was induced with sodium pentothal and was maintained by using a 50:50 nitrous oxide:oxygen mixture with 2-3% halothane delivered at 2-3 l/min. Samples of myometrium, endometrium, cotyledons, chorioallantois and amnion were collected from the anti-mesometrial side of the uterus from tubal, middle and cervical uterine areas as described in Chapter 3. Amniotic fluid (10 ml) and allantoic fluid (10 ml) samples were collected by needle puncture of the intact amniotic or allantoic fluid sacs. The samples were immediately frozen on dry ice and stored at -20°C until assayed.

Prostanoid concentrations in intra-uterine tissues and amniotic fluid and allantoic fluids were estimated by radioimmunoassay, as described previously (see Chapter 3).

The significance of gestation age effects on tissue PG

concentrations was determined by analysis of variance, with variation being partitioned on a between and within animal basis. When there were no differences between sites of sampling, the data were expressed as means ± S.E.M., the latter being based on between animal variation. The significance of gestational age on PG concentrations in amniotic fluid and allantoic fluid was determined by Duncan's New Multiple Range Test. The data were logarithmically transformed prior to statistical analysis when F_{max} tests indicated heterogeneity of variance (Sokal and Rohlf, 1969).

6.3 Results

Concentrations of PGE, PGF, 6-oxo-$PGF_{1\alpha}$ and TXB_2 in samples taken from different areas of myometrium in ewes at different stages of pregnancy are shown in Figure 15. Analysis of variance (Table 35) indicated a significant effect of uterine area for PGE, PGF, 6-oxo-$PGF_{1\alpha}$ and TXB_2, concentrations being lower in mid-myometrial areas compared to samples taken from the tubal end of the uterus (paired t test; $P < 0.05$). For TXB_2, analysis of variance indicated a significant interaction between uterine area and fetal age, concentrations in mid-myometrial areas on Day 145 being significantly lower than on Days 50 and 100 of pregnancy (Duncan's New Multiple Range Test; $P < 0.05$). Analysis of variance did not indicate a significant effect of gestational age on myometrial concentrations of PGE, PGF or 6-oxo-$PGF_{1\alpha}$.

FIGURE 15



Concentrations of prostaglandin E (PGE) (△), PGF (▲), 6-oxo-$PGF_{1\alpha}$ (●), and thromboxane B_2 (○) in samples taken from different uterine areas of myometrium on Days 50, 100, 130 and 145 of pregnancy.

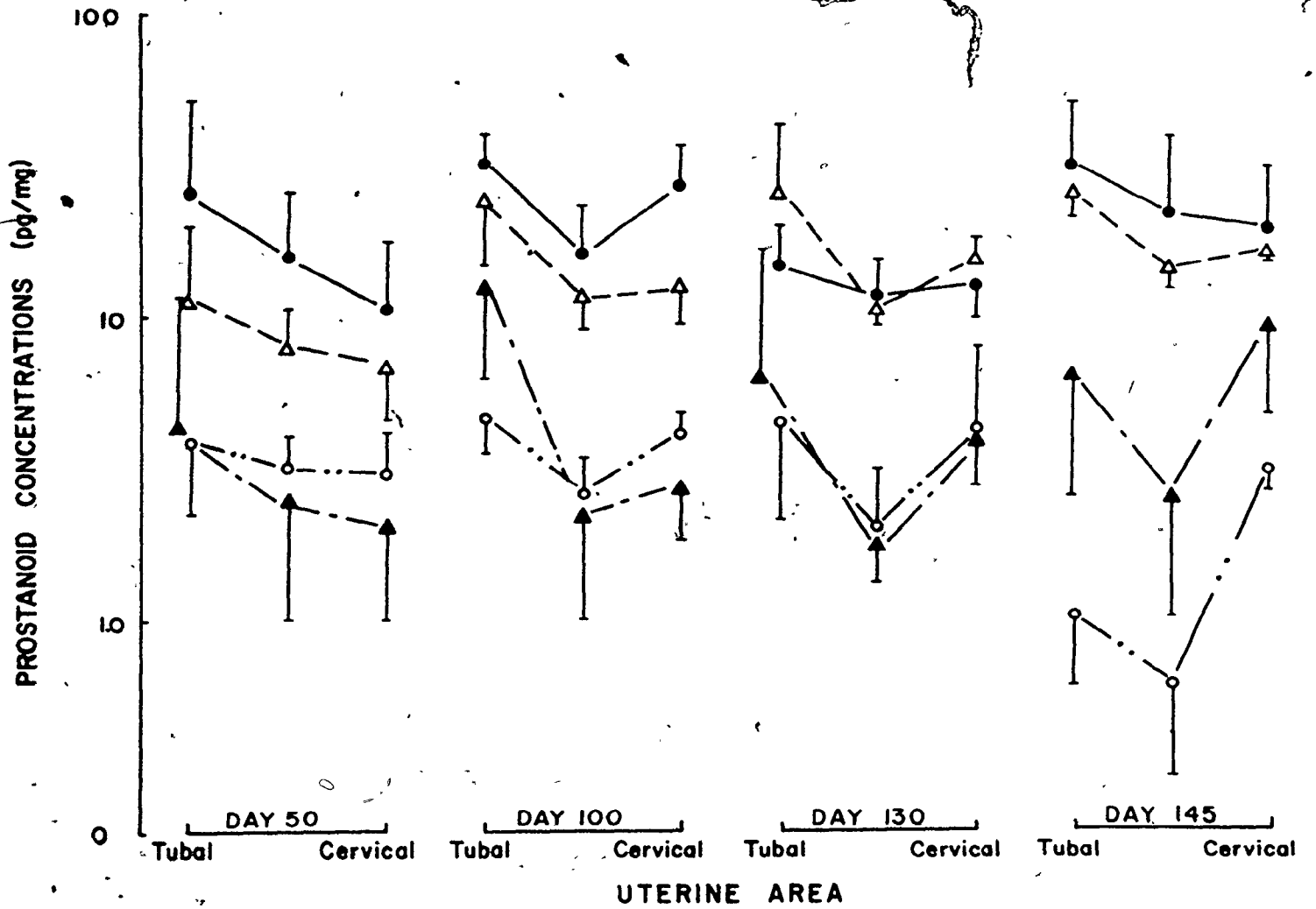

100
10
1.0
0
PROSTANOID CONCENTRATIONS (pg/mg)
DAY 50
DAY 100
DAY 130
DAY 145
Tubal
Cervical
Tubal
Cervical
Tubal
Cervical
Tubal
Cervical
UTERINE AREA

TABLE 35: A Summary of variance analysis of results in Figures 15 and 16

Uterine Tissue	Prostanoid	Source of Variation: Fetal Age F(3,12)	P	Uterine Area F(2,24)	P	Age x Area F(6,24)	P
Myometrium	PGF	0.172	N.S.	7.612	<0.01	1.244	N.S.
	PGE	1.667	N.S.	7.602	<0.01	0.304	N.S.
	6-oxo-$PGF_{1\alpha}$	0.599	N.S.	4.089	<0.05	0.752	N.S.
	TxB_2	1.732	N.S.	13.440	<0.001	4.163	<0.01
Endometrium	PGF	0.840	N.S.	0.794	N.S.	1.169	N.S.
	PGE	0.545	N.S.	1.566	N.S.	1.088	N.S.
	6-oxo-$PGF_{1\alpha}$	1.878	N.S.	0.986	N.S.	1.387	N.S.
	TxB_2	2.125	N.S.	0.150	N.S.	1.478	N.S.

Concentrations of PGE, PGF, 6-oxo-$PGF_{1\alpha}$ and TXB_2 are shown for samples taken from ewes at different stages of pregnancy for endometrium (Figure 16), cotyledons (Figure 17), chorioallantois (Figure 18) and amnion (Figure 19). Analysis of variance (Tables 35 and 36) did not indicate a significant effect of uterine area on PG concentrations in these tissues. There were no significant effects of gestational age (Table 35) on the different PG concentrations in endometrium. For cotyledons, analysis of variance (Table 36) indicated a significant effect of gestational age on concentrations of PGF, PGE, 6-oxo-$PGF_{1\alpha}$ and TXB_2, concentrations being significantly higher on Days 130 and 145 than on Days 50 and 100, respectively (all $P < 0.01$; analysis of variance). For chorioallantois, analysis of variance (Table 36) indicated a significant effect of gestational age on PGF, PGE and 6-oxo-$PGF_{1\alpha}$; significantly higher concentrations were found on Days 100, 130 and 145 than on Day 50 of pregnancy (all $P < 0.00$ analysis of variance). For amnion, analysis of variance (Table 36) indicated a significant effect of gestational age on concentrations of PGE, concentrations being significantly higher on Days 130 and 145 than on Days 50 and 100 of pregnancy ($P < 0.05$; analysis of variance).

Figure 20 shows prostanoid concentrations in allantoic fluid collected from sheep at different stages of pregnancy. The concentrations of PGF, PGE and 6-oxo-$PGF_{1\alpha}$ were significantly (all $P < 0.05$) higher on Day 145 than on Days 50, 100 and 130 of pregnancy. In addition, PGF concentrations were significantly lower on Day 100, compared to other stages of pregnancy ($P < 0.05$). Concentrations of

FIGURE 16

Concentrations of thromboxane B_2 (TxB_2), prostaglandin F (PGF), PGE and 6-oxo-$PGF_{1\alpha}$ in samples taken from endometrium on Days 50 (), 100 (), 130 () and 145 () of pregnancy.

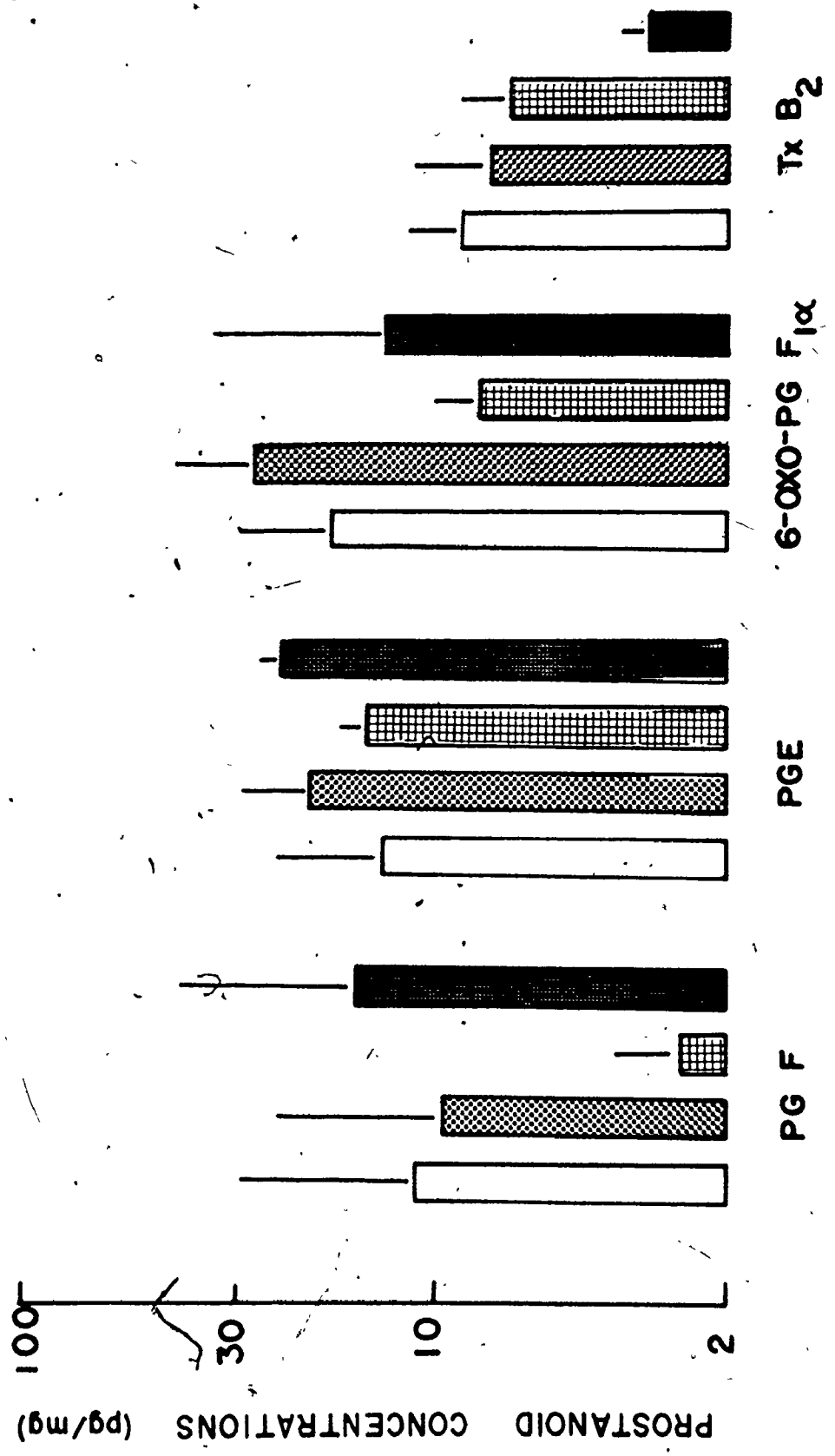

Tx B_2
6-OXO-PG $F_{1\alpha}$
PGE
PG F
100
30
10
2
PROSTANOID CONCENTRATIONS (pg/mg)

FIGURE 17

Concentrations of thromboxane B_2 (TxB_2), prostaglandin F (PGF), PGE and 6-oxo-$PGF_{1\alpha}$ in samples taken from cotyledons (Placentomes) on Days 50 (), 100 (), 130 () and 145 () of pregnancy.

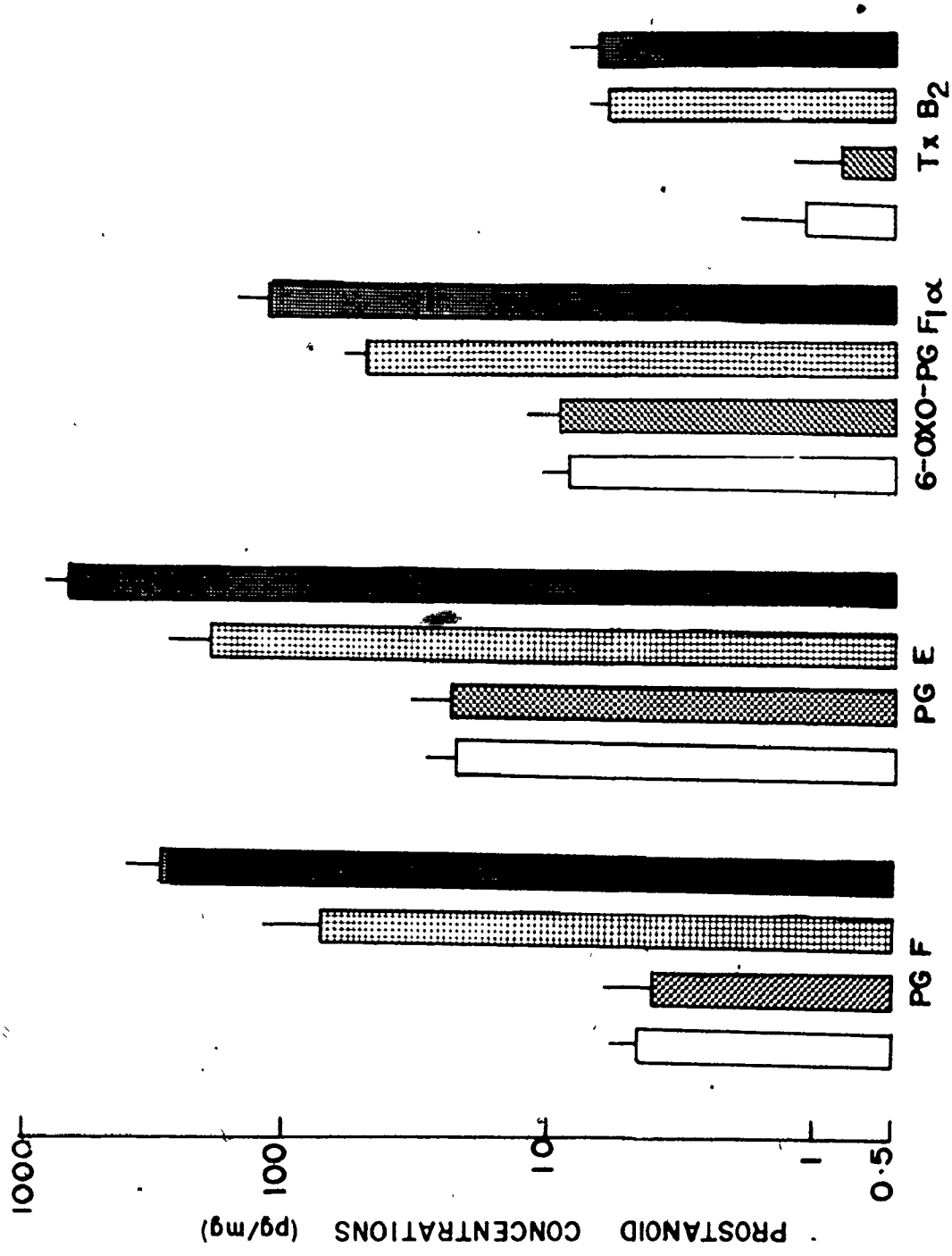

Tx B2
6-OXO-PG F1α
PG E
PG F
1000
100
10
1
0·5
PROSTANOID CONCENTRATIONS (pg/mg)

FIGURE 18

Concentrations of thromboxane B_2 (TxB_2), prostaglandin F (PGF), PGE and 6-oxo-$PGF_{1\alpha}$ in samples taken from chorioallantois on Days 50 (), 100 (), 130 () and 145 () of pregnancy.

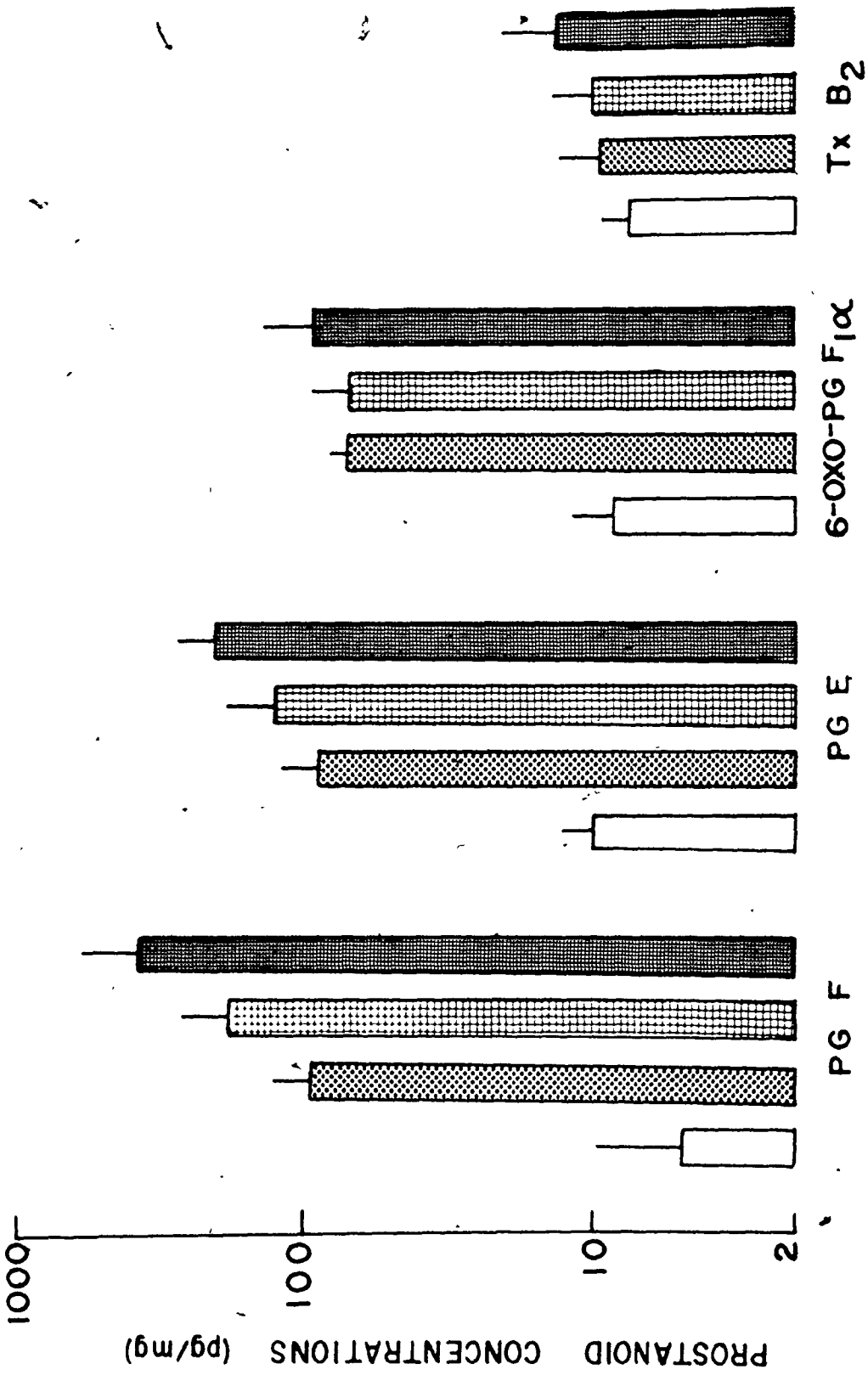
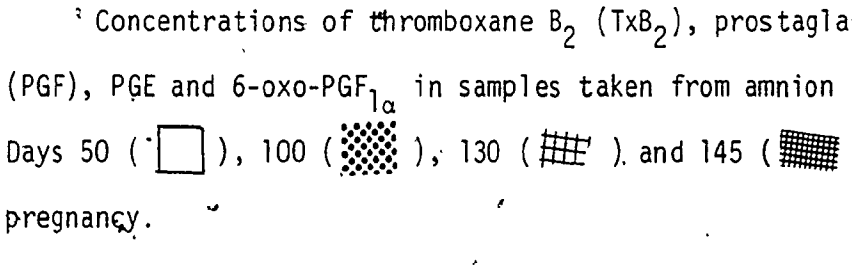

PROSTANOID CONCENTRATIONS (pg/mg)
1000
100
10
2
PG F
PG E
6-OXO-PG F1α
Tx B2

FIGURE 19


Concentrations of thromboxane B_2 (TxB_2), prostaglandin F (PGF), PGE and 6-oxo-$PGF_{1\alpha}$ in samples taken from amnion on Days 50 ( ), 100 ( ), 130 ( ) and 145 ( ) of pregnancy.

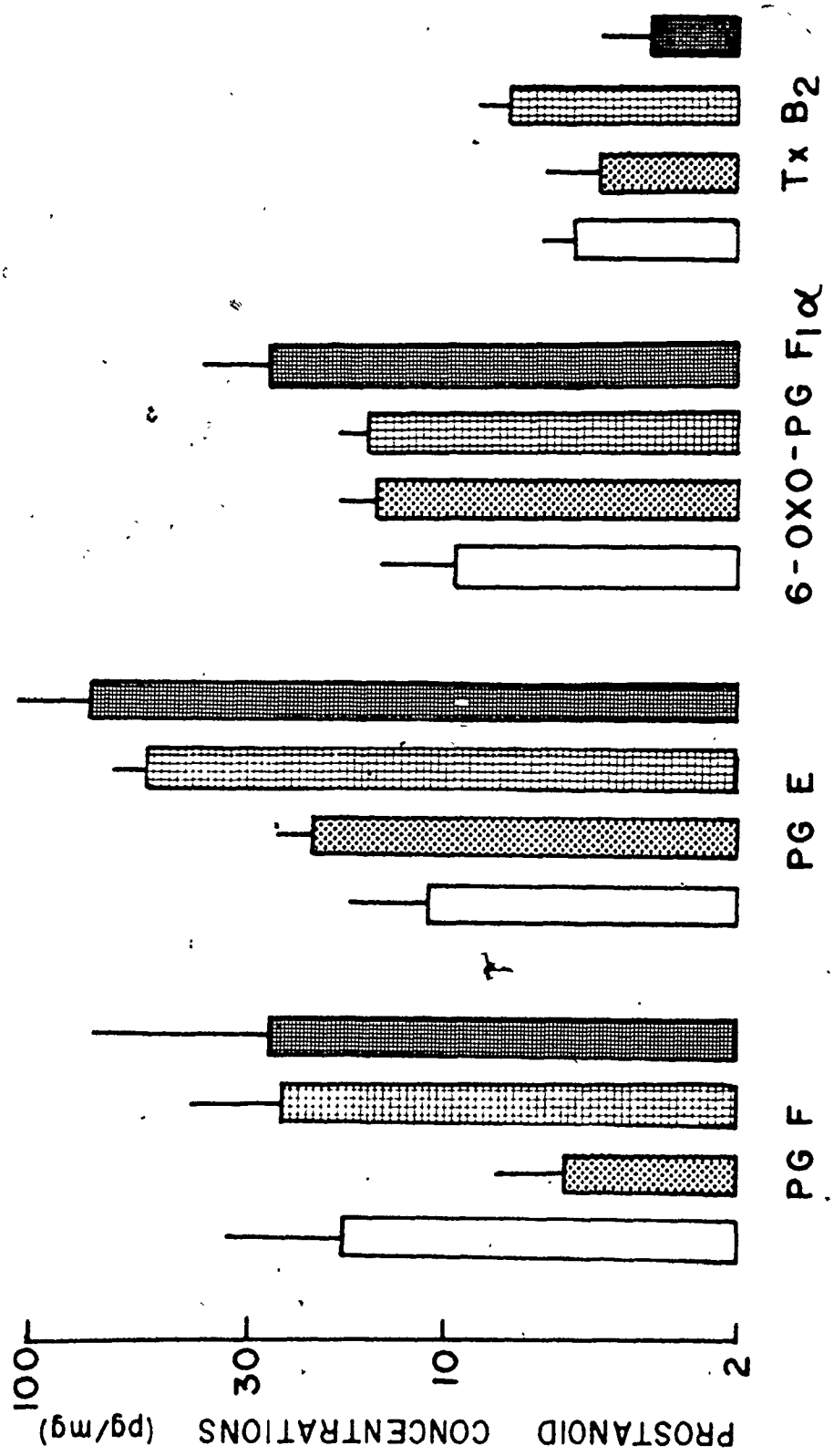
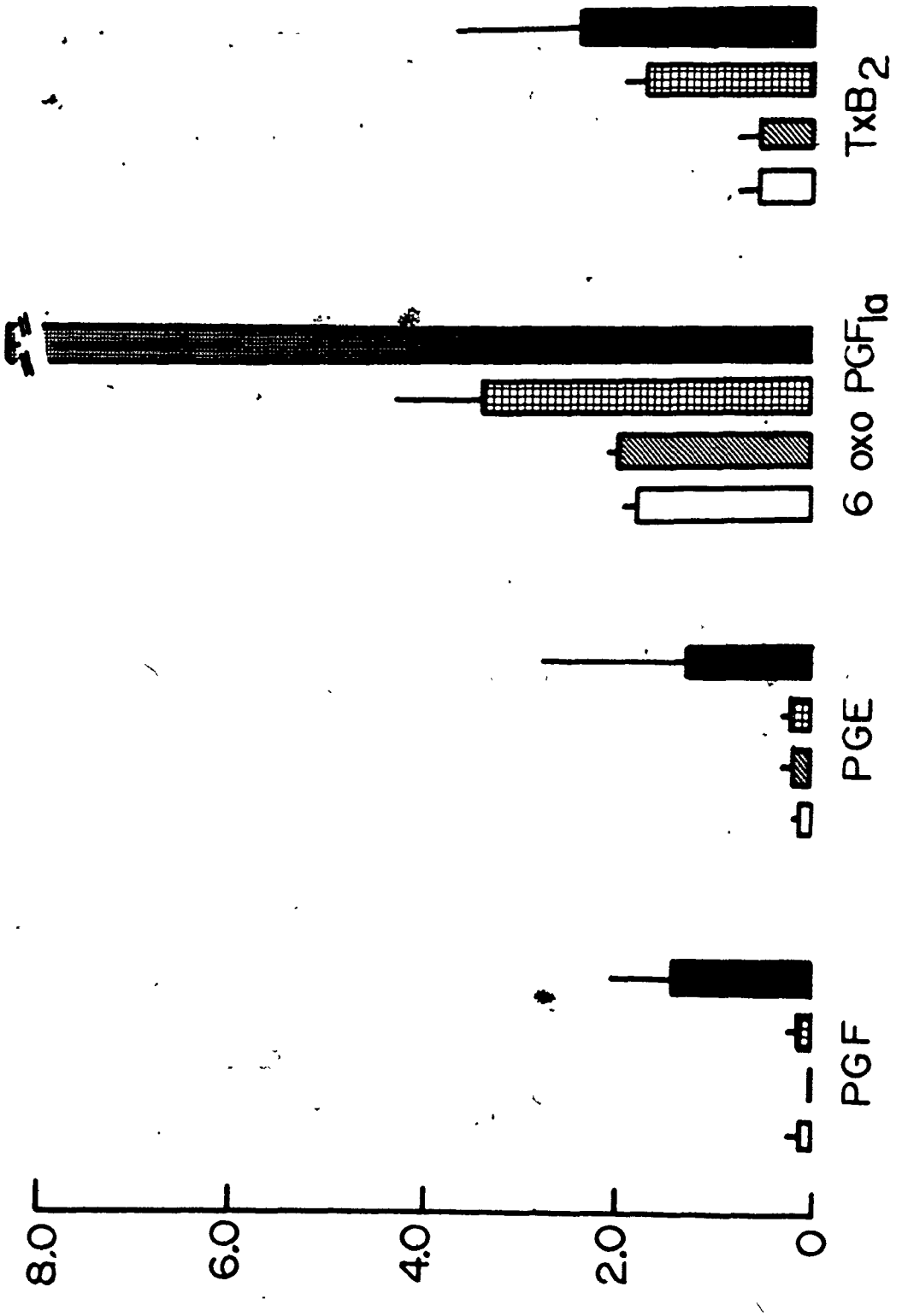

PROSTANOID CONCENTRATIONS (pg/mg)
100
30
10
2
PG F
PG E
6-OXO-PG F1α
Tx B2

TABLE 36: A summary of variance analysis of results in Figures 17-19

Uterine Tissue	Prostanoid	Source of Variation: Fetal Age F(3,12)	Fetal Age P	Uterine Area F(2,24)	Uterine Area P	Age x Area F(6,24)	Age x Area P
Cotyledons	PGF	26.482	<0.001	0.263	N.S.	0.138	N.S.
	PGE	20.842	<0.001	1.098	N.S.	0.177	N.S.
	6-oxo-$PGF_{1\alpha}$	26.823	<0.001	1.165	N.S.	0.122	N.S.
	TxB_2	7.845	<0.01	0.371	N.S.	0.902	N.S.
Chorio-	PGF	18.257	<0.001	2.168	N.S.	0.526	N.S.
allantois	PGE	20.997	<0.001	0.379	N.S.	1.374	N.S.
	6-oxo-$PGF_{1\alpha}$	13.800	<0.001	0.692	N.S.	0.793	N.S.
	TxB_2	0.470	N.S.	0.048	N.S.	0.371	N.S.
Amnion	PGF	1.321	N.S.	1.488	N.S.	1.591	N.S.
	PGE	6.748	<0.01	1.038	N.S.	1.329	N.S.
	6-oxo-$PGF_{1\alpha}$	1.922	N.S.	0.462	N.S.	1.877	N.S.
	TxB_2	1.664	N.S.	0.660	N.S.	1.005	N.S.

FIGURE 20

Concentrations of prostaglandin F (PGF), PGE, 6-oxo-$PGF_{1\alpha}$ and thromboxane B_2 (TxB_2) in allantoic fluid on Days 50 (), 100 (), 130 () and 145 () of pregnancy.


PROSTANOID CONC^n (ng/ml)
8.0
6.0
4.0
2.0
0
PGF
PGE
6 oxo PGF$_{1\alpha}$
TxB$_2$

TXB_2 were significantly higher on Days 130 and 145 than on Days 50 and 100 of pregnancy ($P < 0.05$).

The changes during pregnancy in the concentrations of PGE, PGF, 6-oxo-$PGF_{1\alpha}$ and TXB_2 in amniotic fluid are shown in Figure 21. Concentrations of PGF and TXB_2 were significantly lower (both $P < 0.05$) on Day 100, compared to the rest of pregnancy. PGF concentrations on Day 145 were significantly higher ($P < 0.05$) than during all earlier stages of pregnancy. The mean concentration of PGE on Day 145 was significantly greater than on Day 50 ($P < 0.05$). The mean concentration of 6-oxo-$PGF_{1\alpha}$ on Day 145 was significantly greater than on Days 50 and 100 ($P < 0.05$).

To determine if the differences in PG concentrations in intra-uterine tissues were correlated with the differences in PG concentrations in allantoic and amniotic fluid at different gestational ages, correlation co-efficients and t tests of ratios for zero correlation were calculated. For each animal, the average PG concentration in cotyledons, chorioallantois and amnion were compared with PG concentrations in allantoic and amniotic fluids. Significant positive correlations were found between the concentrations of all PG's in cotyledons and concentrations of these PG's in allantoic fluid (Table 37). Significant positive correlations were also found between PGF and 6-oxo-$PGF_{1\alpha}$ concentrations in the amnion and the concentrations of these PG's in allantoic fluid (Table 37). For PG concentrations in amniotic fluid, significant positive correlations were found with PGE and 6-oxo-$PGF_{1\alpha}$ concentrations in cotyledons and with PGE, PGF and 6-oxo-$PGF_{1\alpha}$ in the amnion (Table 38).

FIGURE 21

Concentrations of prostaglandin F (PGF), PGE, 6-oxo-$PGF_{1\alpha}$ and thromboxane B_2 (TxB_2) in amniotic fluid on Days 50 (), 100 (), 130 () and 145 () of pregnancy.

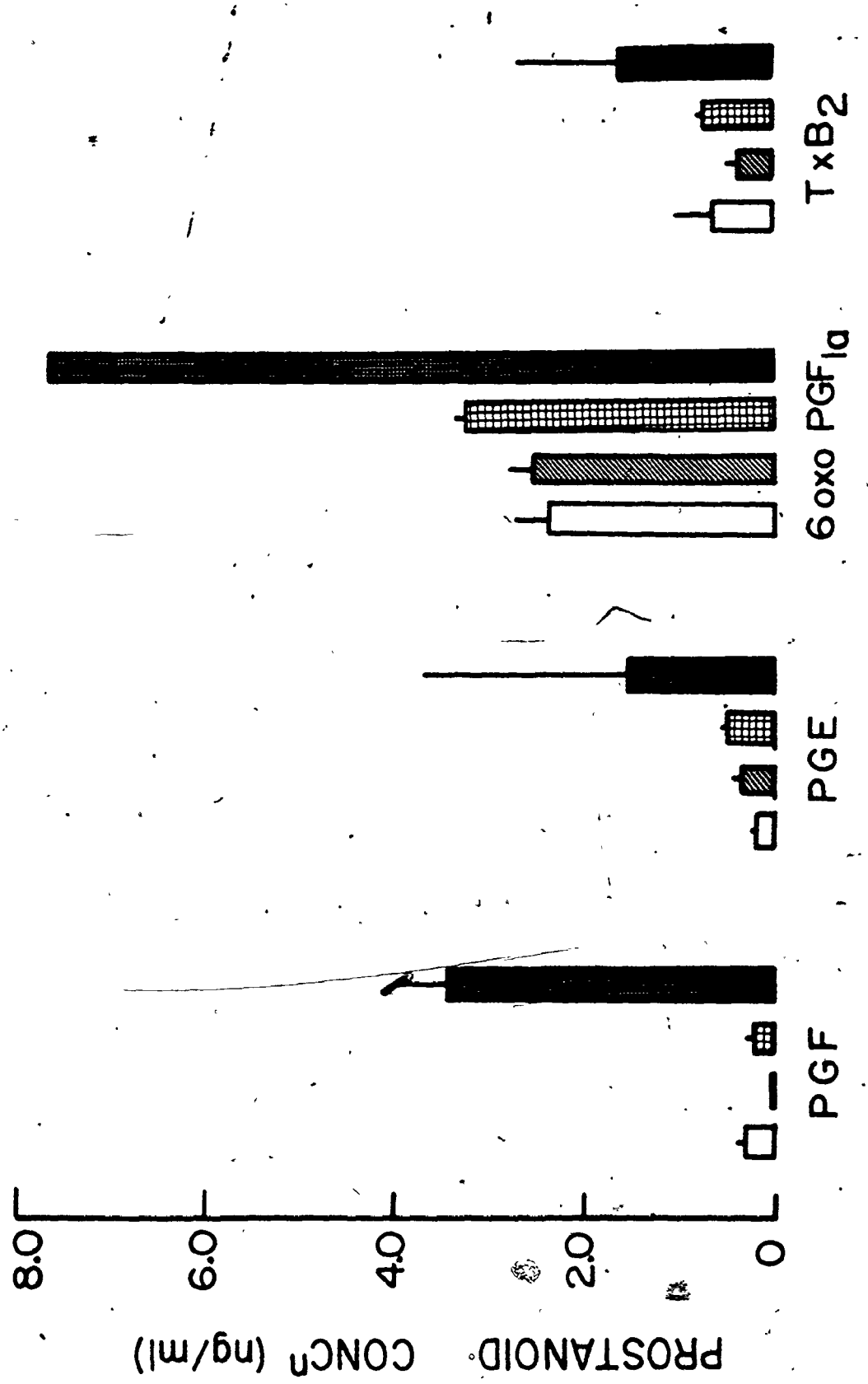

PROSTANOID CONCn (ng/ml)
8.0
6.0
4.0
2.0
0
PGF
PGE
6oxo PGF1a
TxB2

TABLE 37: Correlation co-efficients (C.C.) for the relationship between PG concentrations in cotyledons, chorioallantois, amnion and PG concentrations in allantoic fluid (n = 16 sheep)

	Cotyledons		Chorioallantois		Amnion	
	C.C.	P*	C.C.	P	C.C.	P
Prostaglandin E	0.8502	<0.001	0.588	N.S.	0.592	N.S.
Prostaglandin F	0.890	<0.001	0.152	N.S.	0.963	<0.001
6-oxo-$PGF_{1\alpha}$	0.936	<0.001	0.614	N.S.	0.935	<0.001
Thromboxane B_2	0.813	<0.001	0.587	N.S.	0.044	N.S.

*determined from the t-test of ratios for zero correlation

TABLE 38: Correlation co-efficients (C.C.) for the relationship between PG concentrations in cotyledons, chorioallantois, and amnion, and PG concentrations in amniotic fluid (n = 16 sheep)

	Cotyledons		Chorioallantois		Amnion	
	C.C.	P*	C.C.	P	C.C.	P
Prostaglandin E	0.910	<0.01	0.647	N.S.	0.902	<0.01
Prostaglandin F	0.639	N.S.	0.115	N.S.	0.958	<0.01
6-oxo-$PGF_{1\alpha}$	0.878	<0.01	0.559	N.S.	0.923	<0.01
Thromboxane B_2	0.585	N.S.	0.644	N.S.	-0.343	N.S.

*determined from the t-test of ratios for zero correlation

6.4 Discussion

The results in this study support the suggestion that uterine prostanoid production increases near term in sheep. Concentrations of PGF were elevated in amniotic fluid, and PGE, PGF and 6-oxo-$PGF_{1\alpha}$ concentrations were elevated in allantoic fluid on Day 145, compared to Day 130 of pregnancy. Other investigators have reported elevated PGF concentrations in amniotic fluid during parturition in sheep (Mitchell et al., 1977; Challis et al., 1978), possibly reflecting increased PG production in the fetal membranes. However, caution should be exercised when interpreting results from measurements of PG concentrations in amniotic and allantoic fluids since the fetal kidney may be a source of PG's found in these fluids (Walker and Mitchell, 1978), and PG's in amniotic fluid may be derived from metabolism in the fetal lung (Clyman et al., 1981). In the present study, however, concentrations of all PG's in cotyledons, and of PGF and 6-oxo-$PGF_{1\alpha}$ in amnion were significantly correlated with the concentrations of these PG's in allantoic fluid. Similarly, concentrations of PGE and 6-oxo-$PGF_{1\alpha}$ in cotyledons, and of PGE, PGF and 6-oxo-$PGF_{1\alpha}$ in amnion, were significantly correlated with the concentrations of these PG's in the amniotic fluid. These results suggest that PG's produced in these tissues may be additional sources of PG's found in amniotic and allantoic fluids. In general, the concentrations of prostanoids in cotyledons, chorioallantois and amnion were low in early pregnancy but increased after Day 100 or Day 130.

Significantly lower prostanoid concentrations in myometrium and endometrium on Day 50 of pregnancy were not observed in the present

study. Other investigators have suggested that at Day 15 of pregnancy $PGF_{2\alpha}$ output by endometrial slices may be higher than in non-pregnant sheep (Ellinwood et al., 1979). However, Findlay et al. (1981) reported lower net $PGF_{2\alpha}$ production at this time, possibly associated with synthesis of a PG-binding protein, and lower release into the utero-ovarian vein (Thorburn et al., 1973; Roberts et al., 1975). Detailed studies of the changes in prostanoid production between this time and Day 50, the earliest period examined, are not available.

In the sheep, spontaneous activity of the myometrium is low until the fifth week of gestation. After that time, however, electrical activity occurs in bursts which last 5-10 min and have a periodicity of 1-3 episodes per 2 h until late pregnancy (Nathanielsz et al., 1980; Van Der Weyden et al., 1981). The amplitude, but not the frequency, of electrical discharges increases 10-fold between Days 50 and 100 of pregnancy. The possibility that the changes in uterine activity occurring between Days 50 and 100 of pregnancy resulted from an increase in placental steroid production seems unlikely since plasma progesterone concentrations did not change during this period (Van Der Weyden et al., 1981). In the present study, placental PG concentrations were not significantly elevated until after Day 100, coinciding with the time when placental steroid production increases (Thorburn et al., 1977). In contrast, a 10-fold increase in PG concentrations was observed in the chorioallantois between Days 50 and 100. Although PG produced in the chorioallantois might reach the myometrium and influence uterine activity during this period, it is possible that increased concentration of steroid or PG

receptors and/or myometrial stretch may also contribute to the increased amplitude of contractions. This is supported by the observation that indomethacin inhibits the frequency but not the amplitude of uterine contractions in pregnant sheep (see Chapter 5).

The effects of gestational age on the distribution of ions in amniotic and allantoic fluids in sheep have been reported (McDougall, 1949; Alexander et al., 1958a,b; Mellor, 1970; Mellor and Slater, 1971, 1972). Between Days 50 and 100, concentrations of magnesium and calcium increase, while chloride concentrations decrease in allantoic fluid (McDougall, 1949). Similarly, concentrations of potassium in allantoic fluid increase while sodium decreases after Day 100 of pregnancy (Alexander et al., 1958a; Mellor, 1970; Mellor and Slater, 1971, 1972). The mechanism by which ion concentrations in amniotic and allantoic fluids are regulated is uncertain. It has been suggested that the chorioallantois contains pumping mechanisms operating between allantoic fluid and fetal blood (Mellor, 1970; Mellor and Slater, 1971, 1972). Evidence suggests that PG may be involved in the regulation of similar electrolyte fluxes in the gastrointestinal tract (Robert, 1976). In the present study, between Days 50 and 100 of pregnancy, there was an increase in the concentrations of PGE in amnion, and of PGE, PGF and 6-oxo-$PGF_{1\alpha}$ in chorioallantois. These results raise the possibility that changes in the composition of allantoic fluid between Days 50 and 100 may be related to increased PG production in the fetal membranes. It is known that arachidonate is released in a calcium-dependent process from the adrenal (Laychock et al., 1977) and thyroid (Haye and Jacquemin, 1977)

gland. The possibility that increased PG production in the chorioallantois results from stimulation of arachidonate release by the rising concentrations of calcium and/or magnesium (McDougall, 1949) is intriguing.

In the sheep fetus, urine passes into the allantoic sac predominantly via the urachus up to approximately 90 days of gestation. Thereafter, urine passes progressively more and more into the amniotic sac, owing to gradual occlusion of the urachus and patency of the urethra (Alexander et al., 1957). Whether PG's play a role in this maturational event remains to be determined. However, in the present study, PGF and TXB_2 concentrations were decreased in amniotic and allantoic fluids on Day 100, which might reflect changes in PG production in the fetal urogenital tract.

In summary, the salient findings in this chapter include: 1) myometrial and endometrial PG concentrations did not change with increasing gestational age; 2) PG concentrations in cotyledons were low until Days 100-130, when concentrations increased; 3) PG concentrations in chorioallantois were higher on Days 100, 130 and 145 than on Day 50 of pregnancy; 4) PGE concentrations in amnion were higher on Days 130 and 145 than on Days 50 and 100 of pregnancy; 5) concentrations of all PG's in allantoic fluids and of PGF in amniotic fluid were higher on Day 145 than earlier stages of pregnancy; 6) PG concentrations in allantoic fluid were correlated with concentrations in cotyledons; and 7) PG concentrations in amniotic fluid were correlated with concentrations in cotyledons and amnion.

CHAPTER 7

DEVELOPMENT OF A CELL SYSTEM FOR THE INVESTIGATION OF FACTORS WHICH MIGHT REGULATE INTRA-UTERINE PG PRODUCTION

7.1 Introduction

Evidence supporting a role for hormones in the regulation of intra-uterine prostanoid (PG) production and release in pregnant and non-pregnant ewes has been reviewed (see Chapter 1). The roles of cortisol, relaxin and ovine placental lactogen in the regulation of intra-uterine PG production have not been investigated in sheep. However, *in vivo* studies in pregnant sheep suggest that estrogen stimulates uterine PG production and release while the main effect of progesterone is inhibition of PG release (Challis *et al.*, 1972; Currie *et al.*, 1973; Liggins *et al.*, 1973). PG concentrations were found to be highest in cotyledons and chorioallantois during late ovine pregnancy (Chapters 5 and 6); however, PG concentrations in these tissues were significantly lower during earlier stages of pregnancy (Chapter 6). Similarly, progesterone and estrogen concentrations remain low until approximately Days 70 and 100 of ovine pregnancy (Fevre and Rambauts, 1966; Thorburn *et al.*, 1977). Although these changes in PG and steroid concentrations appear to be related, the *in vitro* responsiveness of intra-uterine tissues obtained from sheep at different gestational ages has not been investigated. In addition,

fetal plasma cortisol concentrations increase during the last two weeks of pregnancy (Bassett and Thorburn, 1969; Nathanielsz et al., 1972). Although evidence suggests that glucocorticoids inhibit the formation of PG's (Hong and Levine, 1976; Blackwell et al., 1978), this possibility has not been investigated in intra-uterine tissues obtained during different stages of pregnancy.

In contrast to pregnant sheep in vivo estrogen treatment had no effect on PGF concentrations in uterine tissues and uterine vein plasma in non-pregnant, ovariectomized ewes (Ford et al., 1975; Louis et al., 1977; Scaramuzzi et al., 1977; Rexroad, 1978). Furthermore, in vivo estrogen treatment either has no effect (Louis, 1977) or inhibits (Findlay et al., 1981) in vitro PGF production in caruncle slices prepared from non-pregnant, ovariectomized sheep. It is possible that estimates of in vitro PG production using tissue slices reflected altered responsiveness to trauma rather than steroid treatment since PGF concentrations were also reduced in endometrium after crushing (see Chapter 4). Alternatively, artifactual PG production may result from unequal distribution of substrates, nutrient and oxygen in these tissues.

In the present study, therefore, the in vitro effects of estrone, progesterone and cortisol on the output of PG's in vitro were examined in cell suspensions prepared from cotyledons, chorioallantois and amnion from sheep of different gestational ages. In addition, the in vitro effects of estradiol-17β, progesterone, relaxin and ovine placental lactogen on PG output in vitro were examined in cell suspension from caruncles in non-pregnant, ovariectomized sheep treated with estradiol and/or progesterone in vivo for 9 days.

7.2 Materials and Methods

Thirty-two sheep of mixed breeds were used. In one study, tissues were collected from the same sixteen sheep of known gestational age that were described in Chapter 6. In the second study, sixteen non-pregnant sheep were ovariectomized using procedures discussed in Chapter 3. General anesthesia was induced with sodium pentothal and was maintained by using a 50:50 nitrous oxide:oxygen mixture with 2-3% halothane delivered at 2-3 l/min. Prophylactic antibiotics were given for 3 days, and beginning one week after surgery the animals were randomly assigned to one of four *in vivo* treatment groups, receiving either 2.5 ml corn oil or estradiol-17β (50 μg) and/or progesterone (50 mg). Daily s.c. injections were given at approximately 0900 h on each of the nine treatment days.

7.2.1 Effects of Steroids and of Gestational Age on PG Output in vitro

Prostaglandin output *in vitro* was determined from samples of cotyledons, chorioallantois and amnion collected from tubal, middle and cervical uterine areas. After excision, the tissues were placed immediately in ice-cold Krebs-Ringer bicarbonate buffer. Samples of each tissue for the different uterine areas were pooled for each animal prior to dispersion into single cell suspension.

The tissue samples of cotyledons, chorioallantois and amnion were minced at 4°C, dispersed into single cell suspensions and washed as described in Chapter 3. The cells were counted on a

hemocytometer and their viability was assessed by exclusion of trypan blue stain. To determine if the relationship between PG output in vitro and cell number per incubation was linear, quadruplicate samples of cotyledon, chorioallantois and amnion cells were incubated at final concentrations of 5×10^4, 10^5 and 2×10^5 cells per incubation in one ewe which was 145 days pregnant. The effects of time of incubation on PG output in vitro was also assessed in this animal by incubating cell suspensions (5×10^4 cells/ml) prepared from cotyledons, chorioallantois and amnion for 0 h, 1 h, 2 h and 4 h. In all subsequent studies, cells were incubated for 4 h at a final concentration of 5×10^4 cells/ml in Krebs buffer containing 0.2% glucose and 0.05% trypsin inhibitor (Krebs GI).

The possibility that the changes in tissue PG concentrations with increasing gestational age (Chapter 6) resulted from changes in responsiveness to steroids was investigated in vitro. Tissue samples of cotyledons, chorioallantois and amnion collected from four sheep on each of Days 50, 100, 130 and 145 of pregnancy were dispersed into cell suspensions. The cells were incubated in vitro in the presence of different concentrations of estrone (0, 1 ng, 10 ng and 100 ng), progesterone (0, 10 ng, 100 ng and 1000 ng) or cortisol (0, 10 ng, 100 ng and 1000 ng). The steroids were diluted in ethanol and 10 µl was aliquoted in duplicate into 12 x 75 mm plastic tubes containing 0.5 ml Krebs GI and mixed by vortexing. Arachidonic acid was not added to the incubations. Cells dispersed from cotyledons, chorioallantois and amnion were diluted in Krebs GI, gently mixed and added to the incubation tubes in 0.5 ml aliquots. Incubations were

performed in a shaking bath at 37°C under an atmosphere of 95% O_2: 5% CO_2. After 4 h incubation the cells were centrifuged at approximately 1500 x g and stored at -20°C until assayed. To determine the PG content prior to incubation, cell suspensions for each tissue were centrifuged at 4°C after dispersion and were stored at -20°C until analysis.

7.2.2 Effects of Steroid Treatments in vivo and of Hormone Treatments in vitro on PG Output in vitro from Cell Suspensions Prepared from Caruncles in Non-Pregnant, Ovariectomized Sheep

Prostaglandin output in vitro was determined from samples of caruncles collected from non-pregnant, ovariectomized sheep treated in vivo with corn oil, or estradiol-17β and/or progesterone. for nine days. On the tenth day of in vivo treatment the tissues were excised and placed immediately in ice-cold Krebs-Ringer bicarbonate buffer. Non-caruncular endometrium was carefully disected and discarded. Approximately 25-50 caruncles from each animal were pooled and weighed prior to dispersion into single cell suspensions, using methods described in Chapter 3.

To determine if the relationship between PG output in vitro and cell number per incubation was linear, quadruplicate samples of caruncular cells from a ewe treated with corn oil were incubated at final concentrations of 5×10^4, 10^5 and 2×10^5 cells per incubation. In subsequent studies, cells were incubated in final concentrations of 3×10^4 - 1.4×10^5 cells/ml in Krebs GI, depending on cell yields.

The possibility that PG output *in vitro* from caruncular cell suspensions may be modified by *in vivo* or *in vitro* hormone treatments was examined. The cells prepared from each animal were counted on a hemocytometer and their viability was assessed before and after a 4 h incubation by exclusion of trypan blue stain. Cells prepared from each animal were incubated *in vitro* in the presence of different concentrations of estradiol-17β (0, 10 ng, 100 ng and 1 μg), progesterone (0, 10 ng, 100 ng and 1 μg), ovine placental lactogen (OPL) (0, 1 ng, 10 ng, 100 ng) or relaxin (0, 10 ng, 1 μg and 100 μg) in the presence or absence of 1 μM arachidonic acid. Duplicate aliquots (10 μl) of estradiol-17β or progesterone in ethanol, and of OPL or relaxin in Krebs GI were added to 12 x 75 mm plastic tubes containing 0.5 ml Krebs GI. Arachidonic acid or ethanol (10 μl) was aliquoted and mixed by vortexing immediately prior to the addition of 0.5 ml caruncle cell suspensions. The incubations were performed in a shaking bath at 37°C under an atmosphere of 95% O_2:5% CO_2. To determine if *in vivo* or *in vitro* hormone treatments influenced the relationship between incubation time and PG output *in vitro*, cells were incubated *in vitro* in the presence of 1 μM arachidonic acid plus either 1 μg estradiol-17β, 1 μg progesterone, 100 ng OPL, 1 μg relaxin or no hormone for 0, 1 h, 2 h or 4 h. All other cells were incubated for 4 h. In addition, duplicate samples containing 1.0 ml Krebs GI with no cells, and 1 μM arachidonic acid were incubated for 4 h in order to estimate PG content in the Krebs GI after the incubation which resulted from auto-oxidation of arachidonic acid. After incubation, the cells were centrifuged and stored at -20°C until assayed. To

determine the PG content prior to incubation, cell suspensions for each animal were also centrifuged at 4°C after dispersion and stored at -20°C until analysis.

7.2.3 RIA's and Statistical Analysis

Prostaglandin output *in vitro* was estimated by RIA in aliquots of Krebs GI following centrifugation, as described in Chapter 3. To determine net output (assuming metabolism equals zero), the PG content of the cells at the start of the incubation period was subtracted from that measured at the end of the incubation in all experiments except the *in vitro* effects of OPL in caruncles.

The data are expressed as mean ± S.E.M. The data were transformed prior to statistical analysis when F_{max} tests indicated heterogeneity of variance (Sokal and Rohlf, 1969). The significance of the effects of gestational age, *in vivo* and *in vitro* hormone treatments on PG output *in vitro* were determined by analysis of variance or Duncan's New Multiple Range Test, as indicated in the Results.

7.3 Results

7.3.1 Effects of Steroid and of Gestational Age on PG Output in vitro

The effects of cell number and incubation time on the in vitro output of PGE, PGF and 6-oxo-$PGF_{1\alpha}$ from cells isolated from chorioallantois, cotyledons and amnion of a ewe 145 days pregnant are summarized in Tables 39 and 40, respectively. The in vitro output of all PG's from chorioallantois, cotyledons and amnion were significantly correlated with the number of cells in the incubation (all $P < 0.01$, t test of ratios for zero correlation). For chorioallantois, analysis of variance indicated a significant effect of incubation time on PGE, PGF and 6-oxo-$PGF_{1\alpha}$ output in vitro (all $P < 0.01$), output increasing significantly until 2 h of incubation (all $P < 0.05$; Duncan's New Multiple Range Test). Similarly, analysis of variance indicated a significant effect of incubation time on the in vitro output of PG's from cotyledons and amnion cells (all $P < 0.05$). The output of PGE and PGF from cotyledon cells in vitro increased significantly until 2 h of incubation (both $P < 0.05$, Duncan's New Multiple Range Test). However, the in vitro output of 6-oxo-$PGF_{1\alpha}$ from cotyledon cells and of all PG's from amnion cells was significantly elevated after 1 h of incubation (all $P < 0.05$, Duncan's New Multiple Range Test), but did not increase further with longer incubation times.

The effects of adding different doses of estrone, progesterone or cortisol to the incubations on the output of PG's in vitro from chorioallantois, cotyledon and amnion cells collected from sheep of different gestational ages were investigated. The effects of

CHAPTER 8

EVIDENCE FOR A ROLE OF PROSTAGLANDINS (PG'S) IN THE INITIATION OF LABOUR

II. PROSTAGLANDIN CONCENTRATIONS AND THE GENESIS OF UTERINE ACTIVITY FOLLOWING FETAL DEATH AND COMPARISON WITH ACTH-INDUCED LABOUR

8.1 Introduction

It is generally accepted that spontaneous parturition in sheep is triggered by fetal adrenal activation which results in changes in the pattern of placental steroidogenesis (see Chapter 1). However, labour ensues in the absence of fetal adrenal activation in sheep bearing dead fetuses. In these animals, as well as in those bearing live fetuses, labour is associated with a fall in the concentration of progesterone in plasma; however, unlike sheep bearing live fetuses, estrogen concentrations do not rise (Carson and Challis, 1981). Delivery of both live and dead sheep fetuses, therefore, is associated with an increase in the estrogen:progesterone ratio in maternal peripheral plasma. An increase in this ratio is thought to stimulate uterine prostaglandin (PG) biosynthesis and release (see Chapters 1 and 5), resulting in uterine contractions. Since degenerative changes such as tissue necrosis and/or vascular congestion following intrauterine death may lead to PG production (Myers et al.,

TABLE 40: Effect of incubation time on PG output *in vitro* (pg/5 x 10^4 cells) from cells isolated from intra-uterine tissues in a ewe, 145 days pregnant (4 incubations/group; mean ± SEM)

	Time of Incubation			
	0 h	1 h	2 h	4 h
Chorioallantois				
PGE	24.6±3.9	98.1±11.2	172.9±23.5	160.8±11.3
PGF	52.5±7.8	187.3±27.7	301.7±42.1	324.3±18.6
6-oxo-$PGF_{1\alpha}$	16.8±4.1	90.1±18.1	184.9±22.0	215.0±24.4
Cotyledons				
PGE	36.6±4.9	77.9±14.2	141.7±19.1	136.6± 9.3
PGF	19.2±3.1	49.9± 5.8	71.0±10.3	60.6± 2.9
6-oxo-$PGF_{1\alpha}$	22.7±3.3	98.2±16.3	132.2±19.6	116.9± 7.9
Amnion				
PGE	41.3±2.9	139.2±19.3	159.8±21.3	154.4±18.2
PGF	16.1±4.3	38.6± 4.6	44.2± 6.4	48.2± 7.0
6-oxo-$PGF_{1\alpha}$	19.2±2.1	71.4±11.9	88.2±11.3	78.1± 9.1

estrone on PG output in vitro from cells isolated from sheep 145 days pregnant are summarized in Table 41. For chorioallantois, analysis of variance indicated that estrone had no significant effect on the output of PGE, PGF and 6-oxo-$PGF_{1\alpha}$. Similarly, analysis of variance indicated that estrone had no significant effect on PG output in vitro from isolated cotyledon and amnion cells. In addition, estrone treatment in vitro had no significant effects (data not shown) on PG output from these tissues collected from animals at other gestational ages (all $P > 0.05$, analysis of variance). Similarly, analyses of variance indicated that progesterone and cortisol treatment in vitro did not significantly alter the basal PG output from these tissues at any gestational age (data not shown). There was no significant effect of steroid treatment in vitro on cell viability, and the percentage of viable cells before and after the 4 h incubation period did not differ significantly ($P > 0.05$, paired t test) for cotyledons (88.7 ± 3.3 (mean ± S.E.M.) vs. 88.4 ± 3.7); chorioallantois (88.5 ± 4.2 vs. 88.3 ± 3.7) or amnion (66.3 ± 3.4 vs. 66.4 ± 3.4) for all animals. The output of PG's from cell suspensions was non-detectable (< 10 pg/5 x 10^4 cells/4 h) on Day 50 of pregnancy for all tissues examined.

The basal output of PG's in vitro from cell suspensions prepared from cotyledons, chorioallantois and amnion, collected at different stages of pregnancy are shown in Figures 22-24. For cotyledons, there was a progressive increase in PGF output with gestational age (Figure 22) in the order: output on Day 50 < Day 100 < Day 130 ($P < 0.05$, Duncan's New Multiple Range Test) < Day 145

TABLE 41: Effects of estrone on PG output *in vitro* (pg/5 x 10^5 cells/4 h) from cells isolated from sheep, 145 days pregnant (n = 4 sheep/group; mean ± SEM)

	Dose of Estrone per Incubation			
	0 ng	1 ng	10 ng	100 ng
Chorioallantois				
PGE	253.5±27.5	273.6±31.7	221.7±81.2	264.9±41.0
PGF	272.3±31.6	259.6±16.8	301.2±62.4	281.4±22.9
6-oxo-$PGF_{1\alpha}$	188.8±19.1	210.2±36.3	152.6±26.5	202.5±19.9
Cotyledons				
PGE	651.6±71.2	609.7±82.4	692.0±70.2	707.9±84.3
PGF	543.8±67.9	621.7±77.9	601.2±52.1	579.3±72.1
6-oxo-$PGF_{1\alpha}$	161.8±18.9	132.7±19.2	144.4±17.7	140.9±19.6
Amnion				
PGE	118.5±74.4	159.2±42.6	129.5±19.2	180.8±61.2
PGF	140.8± 8.1	152.6±21.0	127.9±18.8	136.9±17.1
6-oxo-$PGF_{1\alpha}$	43.1±13.3	57.8±22.1	48.1±17.1	61.0± 7.9

FIGURE 22

The *in vitro* output of prostaglandin F (PGF), PGE and 6-oxo-$PGF_{1\alpha}$ (pg/50,000 cells/4 h) by isolated cotyledon cells on Days 50 (), 100 (), 130 () and 145 () of pregnancy. ND, not detectable.

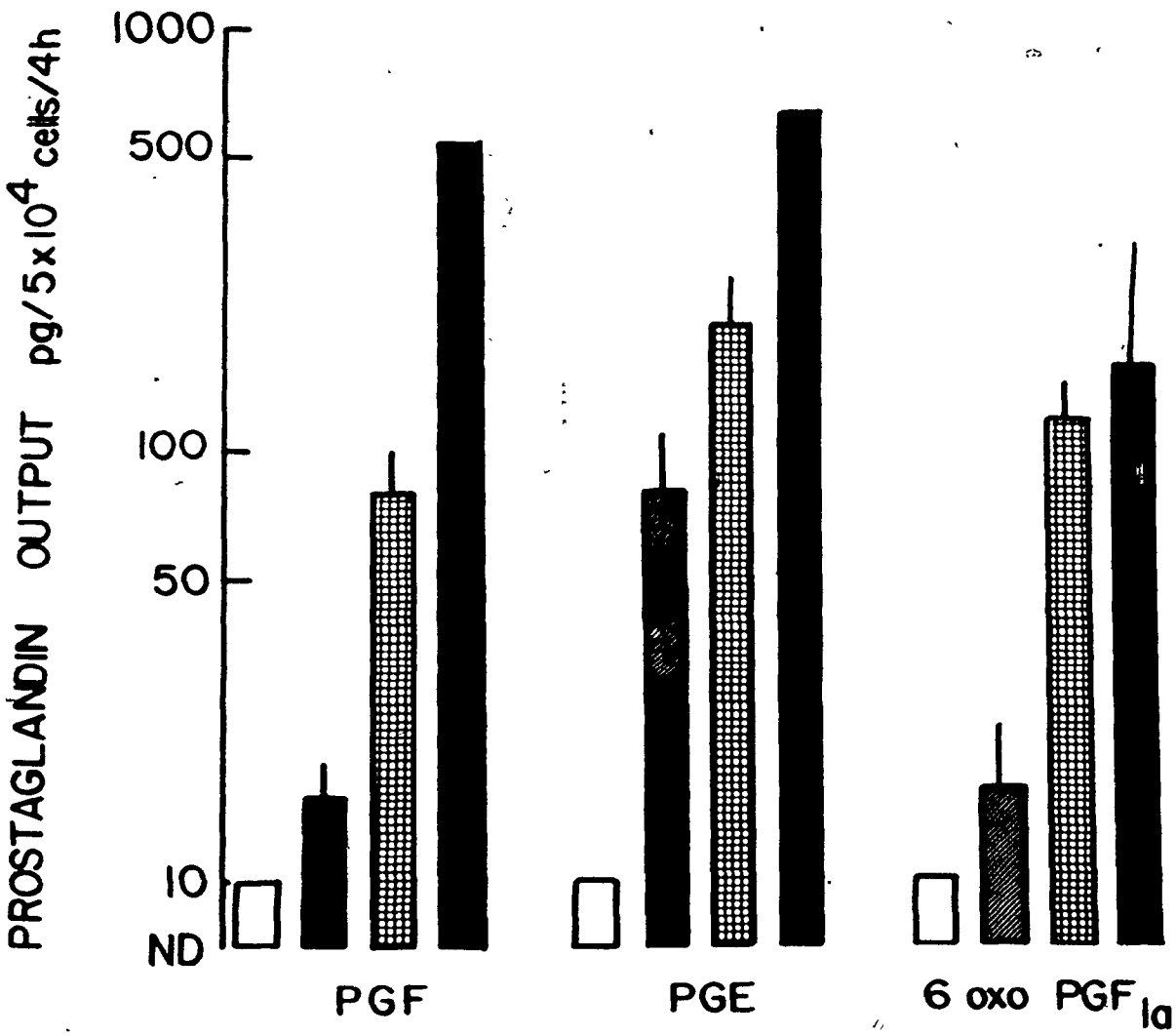

PROSTAGLANDIN OUTPUT pg/5x10⁴ cells/4h
1000
500
100
50
10
ND
PGF
PGE
6 oxo PGF1a

FIGURE 23

Prostaglandin F (PGF), PGE and 6-oxo-$PGF_{1\alpha}$ output *in vitro* (pg/50,000 cells/4 h) by isolated chorioallantois cells on Days 50 ( ), 100 ( ), 130 ( ) and 145 ( ) of pregnancy. ND, not detectable.

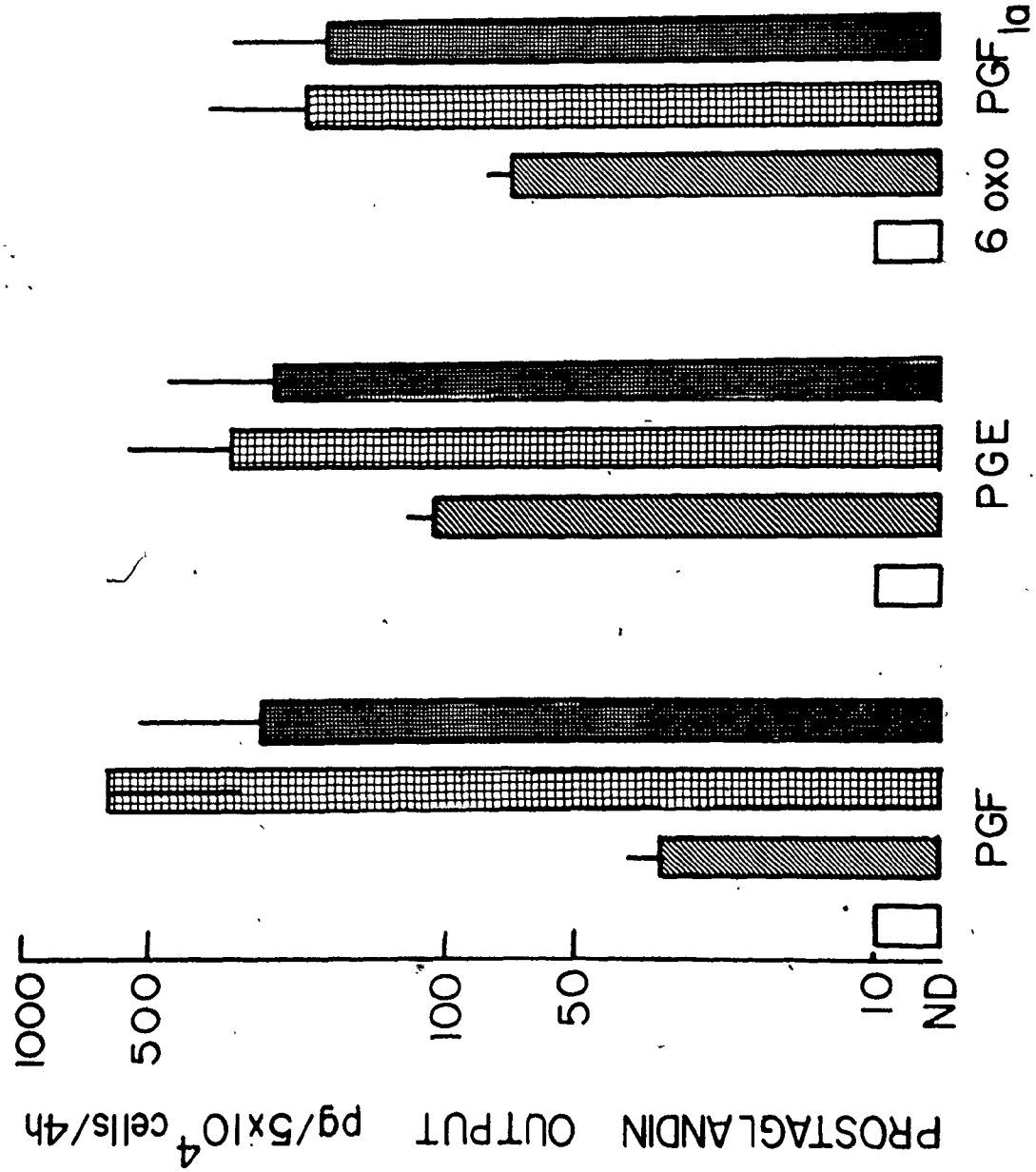
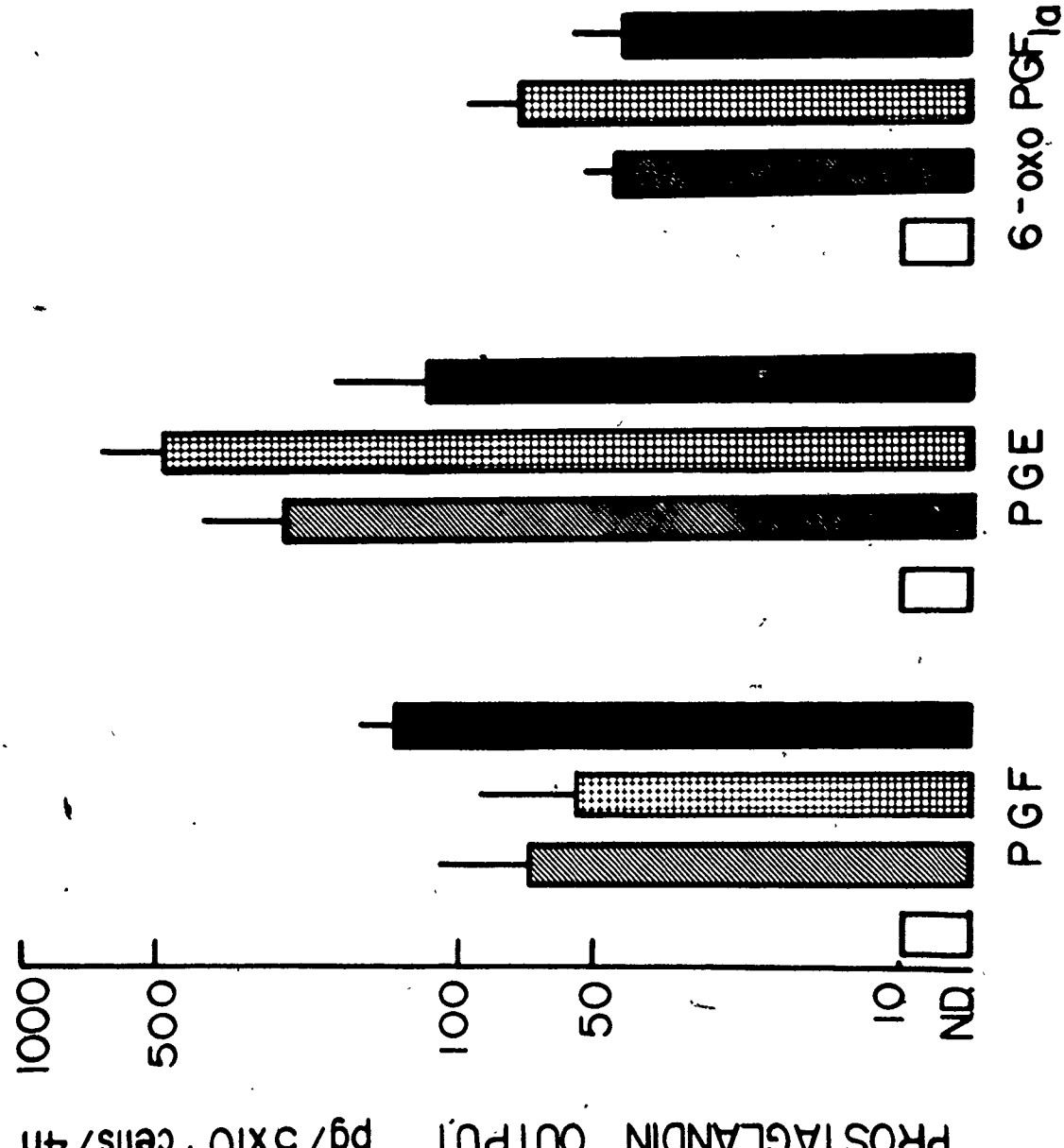

PROSTAGLANDIN OUTPUT pg/5x10⁴ cells/4h
1000
500
100
50
10
ND
PGF
PGE
6 oxo PGF1α

FIGURE 24

Prostaglandin F (PGF), PGE and 6-oxo-$PGF_{1\alpha}$ output *in vitro* (pg/50,000 cells/4 h) by isolated amnion cells on Days 50 ( ), 100 ( ), 130 ( ) and 145 ( ) of pregnancy. ND, not detectable.


PROSTAGLANDIN OUTPUT pg/5x10⁴ cells/4h
1000
500
100
50
10
ND
PGF
PGE
6-oxo PGF1α

($P < 0.05$, Duncan's New Multiple Range Test). The output of PGE and 6-oxo-$PGF_{1\alpha}$ from cotyledon cell suspensions was significantly greater on Day 145 than on Days 50 and 100 of pregnancy (both $P < 0.05$, Duncan's New Multiple Range Test). For chorioallantois cell suspensions, the outputs of PGF, PGE and 6-oxo-$PGF_{1\alpha}$ were greater on Days 100, 130 and 145 than on Day 50 (Figure 23). PGF output on Days 130 and 145 were also significantly greater than on Day 100 of pregnancy (both $P < 0.05$, Duncan's New Multiple Range Test). In suspensions of cells from amnion (Figure 24), the output of PGF, PGE and 6-oxo-$PGF_{1\alpha}$ was greater on Days 100, 130 and 145 than on Day 50.

The basal outputs of PG's in vitro from cell suspensions of cotyledons, chorioallantois and amnion were compared with PG concentrations in these tissues and in amniotic and allantoic fluids collected from these animals (see Chapter 6). Correlation coefficients for the relationship between basal PG output in vitro and PG concentrations in amniotic and allantoic fluids are summarized in Table 42. The basal output in vitro of all PG's from cell suspensions prepared from cotyledons, but not from chorioallantois or amnion, were significantly correlated with PG concentrations in amniotic and allantoic fluids. Similarly, the basal output in vitro of all PG's from cell suspensions prepared from cotyledons and chorioallantois were significantly correlated with PG concentrations in these tissues (Table 43). For amnion, the basal output in vitro of PGF and 6-oxo-$PGF_{1\alpha}$, but not PGE, was significantly correlated with PG concentrations in this tissue.

TABLE 42: Correlation co-efficients (C.C.) for the relationship between PG output in vitro (pg/5 x 10^4 cells/4 h) from cotyledons, chorioallantois, and amnion, and PG concentrations in amniotic and allantoic fluid. (n = 12 sheep)

	Cotyledons		Chorioallantois		Amnion	
	C.C.	P*	C.C.	P	C.C.	P
Allantoic fluid						
PGE	0.781	<0.05	0.598	N.S.	0.247	N.S.
PGF	0.837	<0.01	0.624	N.S.	0.143	N.S.
6-oxo-$PGF_{1\alpha}$	0.869	<0.01	0.619	N.S.	0.362	N.S.
Amniotic fluid						
PGE	0.872	<0.01	0.492	N.S.	0.351	N.S.
PGF	0.792	<0.05	0.563	N.S.	0.426	N.S.
6-oxo-$PGF_{1\alpha}$	0.836	<0.01	0.377	N.S.	0.501	N.S.

*determined from the t-test of ratios for zero correlation

TABLE 43: Correlation co-efficients for the relationship between PG concentration and PG output *in vitro* in cotyledons, chorioallantois and amnion (n = 12 sheep)

	Cotyledons		Chorioallantois		Amnion	
	C.C.	P*	C.C.	P	C.C.	P
PGE	0.798	<0.05	0.772	<0.05	0.289	N.S.
PGF	0.849	<0.01	0.653	<0.05	0.670	<0.05
6-oxo-$PGF_{1\alpha}$	0.864	<0.01	0.648	<0.05	0.658	<0.05

*determined from the *t*-test of ratios for zero correlation

7.3.2 Effects of Steroid Treatments in vivo and of Hormone Treatments in vitro on PG Output in vitro from Cell Suspensions Prepared from Caruncles in Non-Pregnant, Ovariectomized Sheep

The effect of cell number on PG output in vitro from caruncular cell suspensions from one animal are summarized in Table 44. The basal outputs of PGE and PGF were significantly correlated with the number of cells in the incubation (both $P < 0.01$, t test of ratios for zero correlation). The effects of steroid treatment in vivo on caruncle weight and the viability of caruncle cell suspensions before and after a 4 h incubation are summarized in Table 45. Analysis of variance (Table 46) indicated that the mean weight per caruncle was significantly greater in animals treated in vivo with progesterone. In addition, analysis of variance indicated that in vivo steroid treatment did not influence cell viability and that cell viability before and after the 4 h incubations was not significantly different.

The effects of incubation time on basal PG content in caruncle cell suspensions incubated in the presence of arachidonic acid after steroid treatment in vivo are summarized in Table 47. For PGE, analysis of variance (Table 49) indicated a significant effect of steroid treatment in vivo, PGE content being significantly greater in sheep treated with estradiol-17β in vivo than in animals not receiving estradiol-17β ($P < 0.05$, analysis of variance). Analysis of variance also indicated a significant effect of time on PGE content in these incubations. For all in vivo treatments, the content of PGE and 6-oxo-$PGF_{1\alpha}$ was significantly elevated after 1 h of incubation

TABLE 44: Effect of cell number on PG output *in vitro* (pg/4 h) from cells separated from caruncles in an ovariectomized ewe treated with corn oil (4 incubations/group; mean ± SEM)

	Number of Cells per Incubation			Correlation Co-efficient
	5×10^4	10^5	2×10^5	
PGE	142.6±15.8	201.4±24.9	432.6±52.7	0.988
6-oxo-$PGF_{1\alpha}$	251.7±28.7	421.6±57.6	779.7±91.4	0.979

TABLE 45: Effects of estradiol-17β and progesterone treatments *in vivo* on caruncle weight and the percentage of viable cells prepared from the caruncles in these animals, before and after a 4 h incubation period (n = 4 sheep/treatment; mean ± SEM)

Treatment	Wt/Caruncle	Percent Viable Cells	
		Before Incubation	After Incubation
Corn oil	26.3 ± 4.6	93.2 ± 2.8	90.1 ± 1.2
Estradiol	54.0 ± 12.7	89.9 ± 1.7	91.4 ± 1.0
Progesterone	74.1 ± 3.4	90.9 ± 1.1	92.3 ± 1.9
Estradiol + progesterone	100.0 ± 22.5	90.2 ± 0.9	91.7 ± 1.3

TABLE 46: A summary of variance analysis of results in Table 45

Weight/caruncle

Source of Variation		
progesterone	$F(1,12) = 15.198$	$P < 0.01$
estradiol	$F(1,12) = 3.987$	$P < 0.10$
progesterone x estradiol	$F(1,12) = 1.146$	N.S.

Percent viable cells

Source of Variation		
treatment	$F(3,12) = 0.721$	N.S.
time	$F(1,12) = 0.326$	N.S.
treatment x time	$F(3,12) = 0.842$	N.S.

TABLE 47: Effects of incubation time on PG content (pg/5 x 10^4 cells) in caruncle cell suspensions from ovariectomized ewes after steroid treatment in vivo (4 sheep/treatment; mean ± SEM)

Treatment	Time of Incubation			
	0 h	1 h	2 h	4 h
Corn oil:				
PGE	48.3± 7.2	94.6±19.2	131.4±31.4	102.9±20.2
6-oxo-$PGF_{1\alpha}$	101.4±12.6	181.9±41.2	248.9±64.2	232.6±84.3
Estradiol-17β:				
PGE	94.6±21.4	382.0±81.2	184.7±46.2	418.8±214.5
6-oxo-$PGF_{1\alpha}$	72.0±11.2	106.7±22.2	151.9±81.2	156.7± 37.5
Progesterone:				
PGE	122.4±32.6	185.1±64.2	280.0±81.0	294.4±141.7
6-oxo-$PGF_{1\alpha}$	86.4±16.4	126.1±27.7	177.1±51.1	244.5±118.6
Estradiol-17β + progesterone:				
PGE	92.1±31.4	196.6±87.0	321.4±64.7	383.9±152.1
6-oxo-$PGF_{1\alpha}$	131.4±18.9	151.4±32.1	199.0±81.2	224.4± 75.0

TABLE 48: Effects of ovine placental lactogen (OPL) on PG content (pg/5 x 10^4 cells/4 h) in cell suspensions prepared from caruncles in ovariectomized sheep after *in vivo* steroid treatments (4 sheep/*in vivo* treatment; mean ± SEM)

Treatment	Dose of OPL per Incubation			
	0 ng	1 ng	10 ng	100 ng
6-oxo-$PGF_{1\alpha}$:				
Corn oil	232.6±84.3	129.1±30.6	107.6±10.1	63.3±18.1
Estradiol-17β	156.7±37.5	175.5±50.8	120.8±21.2	52.9±22.8
Progesterone	244.5±118.6	163.2±96.3	174.9±85.2	75.4±10.7
Estradiol-17β + progesterone	224.4±75.0	222.6±51.1	259.2±88.6	73.4±31.1
Prostaglandin E:				
Corn oil	102.9± 20.2	85.0±27.1	79.0± 32.9	30.0± 7.3
Estradiol-17β	418.8±214.5	250.5±96.0	314.9±158.4	27.0±15.5
Progesterone	294.4±141.7	242.2±109.4	244.6± 87.7	107.7±32.8
Estradiol-17β + progesterone	383.9±152.1	496.0±150.5	567.6±227.6	46.2± 7.2

TABLE 49: A summary of variance analysis of results in Tables 47 and 48

Effects of incubation time on PG output *in vitro* (Table 47)

	Source of Variation					
	Treatment		Time		Treatment x Time	
	F(3,12)	P	F(3,36)	P	F(9,36)	P
Prostaglandin E	7.346	<0.05	11.947	<0.01	1.741	N.S.
6-oxo-$PGF_{1\alpha}$	1.247	N.S.	13.742	<0.01	1.142	N.S.

Effects of oPL on PG output *in vitro* (Table 48)

	Source of Variation					
	Treatment		Dose		Treatment x Dose	
	F(3,12)	P	F(3,36)	P	F(9,36)	P
Prostaglandin E	5.756	<0.05	45.331	<0.001	3.635	<0.01
6-oxo-$PGF_{1\alpha}$	0.524	N.S.	14.064	<0.001	0.853	N.S.

(all $P < 0.05$, Duncan's New Multiple Range Test), but did not increase further with longer incubation times. This effect of time on PG content was not altered by in vitro hormone treatments.

The effects of estradiol-17β, progesterone, relaxin and ovine placental lactogen on PG output in vitro from cell suspensions prepared from caruncles in non-pregnant, ovariectomized sheep after in vivo steroid treatment were investigated. The effects of OPL on PG content in caruncle cell suspensions incubated in the presence of arachidonic acid are summarized in Table 48. In this experiment, PG content at the end of the incubations was not subtracted from those at the beginning of the incubation (Table 47). For 6-oxo-$PGF_{1\alpha}$, analysis of variance (Table 49) indicated that PG content was significantly decreased by increasing doses of OPL. Analysis of variance also indicated a significant effect of in vivo treatment on PGE content, content being greater in sheep treated with estradiol ($P < 0.05$, Duncan's New Multiple Range Test). In addition, analysis of variance indicated a significant effect of OPL dose on content, content being significantly decreased at 100 ng OPL ($P < 0.05$, analysis of variance). As indicated by the significant interaction between in vivo treatment and dose of OPL in vitro on PGE content, the magnitude of the effect of OPL on PGE content was influenced by in vivo treatment, OPL having a greater effect in animals treated with estradiol in vivo ($P < 0.05$, analysis of variance). PG content was often lower after 4 h incubation in the presence of 100 ng OPL than content at the beginning of the incubation (Table 47). Analysis of variance also indicated that there were no significant effects of in vitro estradiol-17β,

progesterone or relaxin treatments on PG output *in vitro* from caruncle cell preparations (data not shown). PG output *in vitro* was low or non-detectable (< 10 pg/5 x 10^4 cells/4 h) when arachidonic acid was not added to the incubation. The mean arachidonic acid blank was 22.8 ± 4.7 (n = 16) for PGE and 14.7 ± 3.2 for 6-oxo-$PGF_{1\alpha}$.

7.4 Discussion

The results of this study indicate that *in vitro* treatment with estrone, progesterone or cortisol did not alter the output of PG's *in vitro* from chorioallantois, cotyledon or amnion cells prepared from sheep at different gestational ages. However, the basal output of all PG's *in vitro* from these cells was significantly lower during early stages of pregnancy. The present study also indicated that *in vitro* treatment with estradiol-17β, progesterone or relaxin did not alter the output of PG's *in vitro* from caruncle cells prepared from non-pregnant, ovariectomized sheep treated with corn oil or estradiol-17β and/or progesterone *in vivo*. In these animals, the basal output of PGE was significantly greater in sheep treated with estradiol-17β *in vivo* for nine days. Moreover, *in vitro* OPL treatment resulted in a significant decrease in the *in vitro* output of both PGE and 6-oxo-$PGF_{1\alpha}$. For PGE, this effect was more pronounced in sheep which had been treated with estradiol-17β *in vivo*.

In the present study, PG output *in vitro* increased linearly with increasing numbers of cells in the incubation medium;

however, output was not linear with time. The output could not be increased after 2 h, even with the addition of arachidonic acid. This is consistent with the results of other investigators (Louis et al., 1977; Findlay et al., 1981) and suggests that the accumulation of PG's in the incubation medium may inhibit further PG production by these cells. In addition, the extent to which arachidonic acid enters dispersed cells is not known. It is possible, therefore, that PG output, using the present system, was substrate-limiting. Alternatively, the possibility that the trauma of cell separation contributed to the in vitro PG output cannot be ruled out. Furthermore, the present incubations were terminated after 4 h in order to examine the possibility that in vitro steroid treatments alter the time course of PG output. Therefore, PG's measured in the incubation media likely reflect the difference between the release of sequestered (Findlay et al., 1981) or synthesized PG and the metabolism of PG's by these cells.

The failure of in vitro hormone treatments to alter PG output in vitro from cells prepared from pregnant and non-pregnant uterine tissues is consistent with the results of other investigators (Naylor and Poyser, 1975; Kelly and Abel, 1980). In the present study, this may result from inadequate arachidonic acid concentrations in the incubation media or insufficient incubation periods.

The results of this study suggest that the in vitro output of PG's from cell suspensions might reflect in vivo PG production. Significant correlations were found between basal PG output in vitro in cotyledon, chorioallantois and amnion cell suspensions and PG concentrations in these tissues collected from sheep at different

gestational ages. Significant correlations were also found between basal PG output in vitro from cotyledon, but not chorioallantois or amnion, cell suspensions and PG concentrations in allantoic and amniotic fluids. This observation, together with the finding that PG concentrations in cotyledons (see Chapter 6) are also significantly correlated with PG concentrations in these fluids suggest that the cotyledons may be an important source of PG's found in allantoic and amniotic fluids.

In non-pregnant sheep, PGF output in vitro from caruncle slices was significantly increased after in vivo progesterone treatment (Louis et al., 1977; Findlay et al., 1981). Findlay et al. (1981) suggested that this increased output in progesterone-treated animals may result from the release of sequestered PG, possibly resulting from the formation of a PG-binding protein in response to progesterone. In the present study, in vivo estradiol-17β or progesterone treatments did not result in significant changes in 6-oxo-$PGF_{1\alpha}$ output in vitro. However, basal PGE output was significantly higher in ewes treated with estradiol-17β in vivo. In contrast, Findlay et al. (1981) reported a suppression of PGF output in ewes treated with similar doses of estradiol-17β. Although PGF was not measured in the present study, it is possible that estrogen might alter the ratios of PG's produced by intra-uterine tissues. This possibility is supported by the results of studies in other species (Ham et al., 1975; Kelly and Abel, 1980) and casts doubt upon a current hypothesis (Samuelsson, 1970; MacDonald et al., 1978) that intra-uterine PG production is regulated solely by the availability of substrate precursor acids.

Thorburn (1977) proposed that placental lactogen may play a role in the mechanism of parturition, particularly in species which are corpus luteum-dependent. In the goat, the pre-parturient rise in fetal plasma corticosteroid levels appears to switch off placental lactogen production by the placenta (Currie and Thorburn, 1977; Currie et al., 1977). These authors suggested that a small decrease in progesterone levels may result from a fall in placental lactogen and that the decreased progesterone levels facilitate the release of PGF from cotyledons, resulting in final luteal regression. A similar mechanism has been proposed for the initiation of luteolysis and parturition in the rat (Thorburn and Challis, 1979). In the present study, basal output in vitro of both PGE and 6-oxo-$PGF_{1\alpha}$ from caruncle cell suspensions obtained from non-pregnant, ovariectomized sheep were significantly reduced by in vitro ovine placental lactogen treatment. These results suggest that placental lactogen might inhibit PG synthesis during pregnancy, and the fall in ovine placental lactogen concentrations prior to parturition (Chan et al., 1978) might contribute to the elevated PG concentrations in intra-uterine tissues at this time. It would be interesting to see if placental lactogen has a similar effect on goat uterine tissues. In addition, it is possible that the high concentrations of ovine placental lactogen in cotyledons and chorioallantois during early pregnancy (Chan et al., 1978) might contribute to the low PG concentrations (see Chapter 6) and basal PG output in vitro from these tissues, and subsequently to the relative uterine quiescence during this period (Nathanielsz et al., 1980; Van Der Weyden et al., 1981). The mechanism

by which ovine placental lactogen inhibits PG output *in vitro* awaits further studies. However, in the present study, PG output at high doses of ovine placental lactogen was lower than output at the beginning of the incubation, suggesting that ovine placental lactogen may increase the rate of PG metabolism.

In summary, the salient findings in this chapter include: 1) evaluation of a cell system for the investigation of factors which might regulate intra-uterine PG production is described; 2) PG output from cells isolated from cotyledons, chorioallantois and amnion were not affected by estrone, progesterone or cortisol treatments *in vitro*; 3) basal PGF output from cotyledon cell suspensions increased progressively with gestational age and PGE and 6-oxo-$PGF_{1\alpha}$ outputs were greater on Day 145 than Days 50 and 100 of pregnancy; 4) basal PG output from chorioallantois and amnion cell suspensions were greater on Days 100, 130 and 145 than Day 50 of pregnancy; 5) basal PG output from these tissues were correlated with PG concentrations in these tissues; and 6) PG output *in vitro* from caruncle cell suspensions was decreased following *in vitro* OPL treatment and for PGE, the magnitude of this effect was greater in animals treated with estradiol *in vivo*.

CHAPTER 8

EVIDENCE FOR A ROLE OF PROSTAGLANDINS (PG'S) IN THE INITIATION OF LABOUR

II. PROSTAGLANDIN CONCENTRATIONS AND THE GENESIS OF UTERINE ACTIVITY FOLLOWING FETAL DEATH AND COMPARISON WITH ACTH-INDUCED LABOUR

8.1 Introduction

It is generally accepted that spontaneous parturition in sheep is triggered by fetal adrenal activation which results in changes in the pattern of placental steroidogenesis (see Chapter 1). However, labour ensues in the absence of fetal adrenal activation in sheep bearing dead fetuses. In these animals, as well as in those bearing live fetuses, labour is associated with a fall in the concentration of progesterone in plasma; however, unlike sheep bearing live fetuses, estrogen concentrations do not rise (Carson and Challis, 1981). Delivery of both live and dead sheep fetuses, therefore, is associated with an increase in the estrogen:progesterone ratio in maternal peripheral plasma. An increase in this ratio is thought to stimulate uterine prostaglandin (PG) biosynthesis and release (see Chapters 1 and 5), resulting in uterine contractions. Since degenerative changes such as tissue necrosis and/or vascular congestion following intrauterine death may lead to PG production (Myers et al.,

1974), and the pattern of hormone changes preceding abortive and induced labour differ, the identity and intra-uterine source of PG's produced in response to fetal death were of interest. Therefore, concentrations of PGF, PGE and 6-oxo-$PGF_{1\alpha}$ were measured in different uterine tissues obtained from sheep in which fetal death had occurred following fetal catheterization in utero. The concentrations of these PG's were compared with those obtained from sheep bearing live fetuses of similar gestational age and were treated with either intra-fetal saline + phosphate buffer (P.B.) or ACTH + P.B. (see Chapter 5).

8.2 Materials and Methods

In the studies discussed in Chapters 4 and 5, approximately 70-80% of the fetuses were viable two weeks after fetal catherization. In the remaining animals fetal demise occurred, possibly as a result of infection incurred during subsequent sampling. The opportunity, presented, therefore, for an ongoing study on the possible role of PG's in the mechanism of labour associated with the presence of a dead fetus. Accordingly, fetal and maternal vascular cathers, an amniotic pressure catheter and fetal electrocardiogram (ECG) leads were implanted in mixed breed sheep on Days 113 to 117 of pregnancy as described previously (see Chapter 3). Fetal (2 ml) blood samples were collected daily after surgery for blood gas determinations. Beginning on Day 120 of pregnancy, continuous recordings of fetal heart rate and amniotic pressure were made using a Grass Polygraph (see Chapter 3).

The fetal heart rate signal was fed through a Grass EKC Tachograph pre-amplifier (Model 7P4F).

Eight sheep bearing dead fetuses were used. At the time of-sacrifice, fetuses had been dead 12 to 26 h in four sheep, and 34 to 72 h in a second group of four sheep. These two groups were designated fetuses dead less than 30 h and greater than 30 h, respectively. The interval between fetal death and the delivery of dead fetuses was 2 to 5 days in those sheep which were allowed to deliver.

Samples of myometrium, endometrium, cotyledons, chorio-allantois and amnion were collected under general anesthesia from tubal, mid-tubal, middle, mid-cervical and cervical uterine areas (see Chapters 3 and 4). The chorioallantoic and amniotic membranes in sheep bearing fetuses dead greater than 30 h were not collected since in these animals the fetal membranes could not be separated easily from each other. Concentrations of PGF, PGE and 6-oxo-$PGF_{1\alpha}$ were estimated in these tissues by RIA using antisera and techniques described previously (see Chapter 3). The results from this study were compared with those discussed in Chapter 5.

The data are expressed as means ± S.E.M., the latter being based on between animal variation in all cases. For each tissue, average PG concentrations were calculated from the five individual estimates from different uterine areas in each animal. The significance of the effects of fetal death on blood gases and pH were determined by Student's *t* tests. The significance of fetal death on PG levels was determined by analysis of variance, with variation being partitioned on a between and within animal basis. The data were logarithmically transformed prior to statistical analysis when F_{max}

tests indicated heterogeneity of variance (Sokal and Rolf, 1969).

8.3 Results

The timing of fetal death was determined from continuous recordings of fetal heart rate. To confirm that cessation of fetal heart rates were due to fetal death and not due to electronic difficulties, fetal arterial pO_2, pCO_2 and pH were determined (Table 50). The animals suspected dead on the basis of heart rate recordings had a significant reduction in fetal arterial pO_2 and pH (both $P < 0.01$) and elevated pCO_2 ($P < 0.01$) compared to sheep bearing live fetuses of similar gestational age.

Concentrations of PGF in myometrium, endometrium, cotyledons, chorioallantois and amnion are summarized in Figure 25. Analysis of variance (Table 54) indicated that for all tissues, PGF concentrations in ewes bearing fetuses dead greater than 30 h were not different from those bearing fetuses dead less than 30 h; however, there was a significant effect of uterine area in cotyledons collected from these animals. In this tissue, PGF concentrations were significantly higher in samples taken from mid-uterine areas than from other areas of the uterus ($P < 0.05$, paired t tests). Compared to sheep bearing live fetuses which were treated with saline + P.B. (Table 51), PGF concentrations were significantly higher in cotyledons obtained from all sheep bearing dead fetuses and in myometrium in sheep bearing

TABLE 50: Measures of fetal arterial blood gases and pH in fetuses suspected live and dead.

State of Fetus	Number of Sheep	pO_2 (mm Hg)	pCO_2 (mm Hg)	pH
Live***	4	20.6 ± 2.91	44.3 ± 3.21	7.4 ± 0.01
Dead*	8	9.4 ± 1.7**	260.4 ± 97.9**	6.3 ± 0.94**

* Determined within 24 h of the cessation of fetal heart rate.

** Significantly different from measurements from live fetuses (P 0:05; t-test)

*** Determined after 70 h of continuous infusions of saline into the fetus and phosphate buffer (0.12 m, pH 7.4) into the mother, on Day 130 of pregnancy)

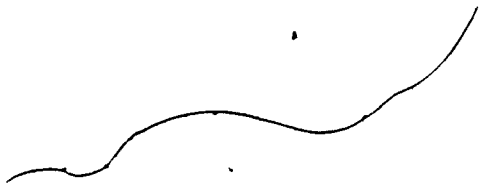
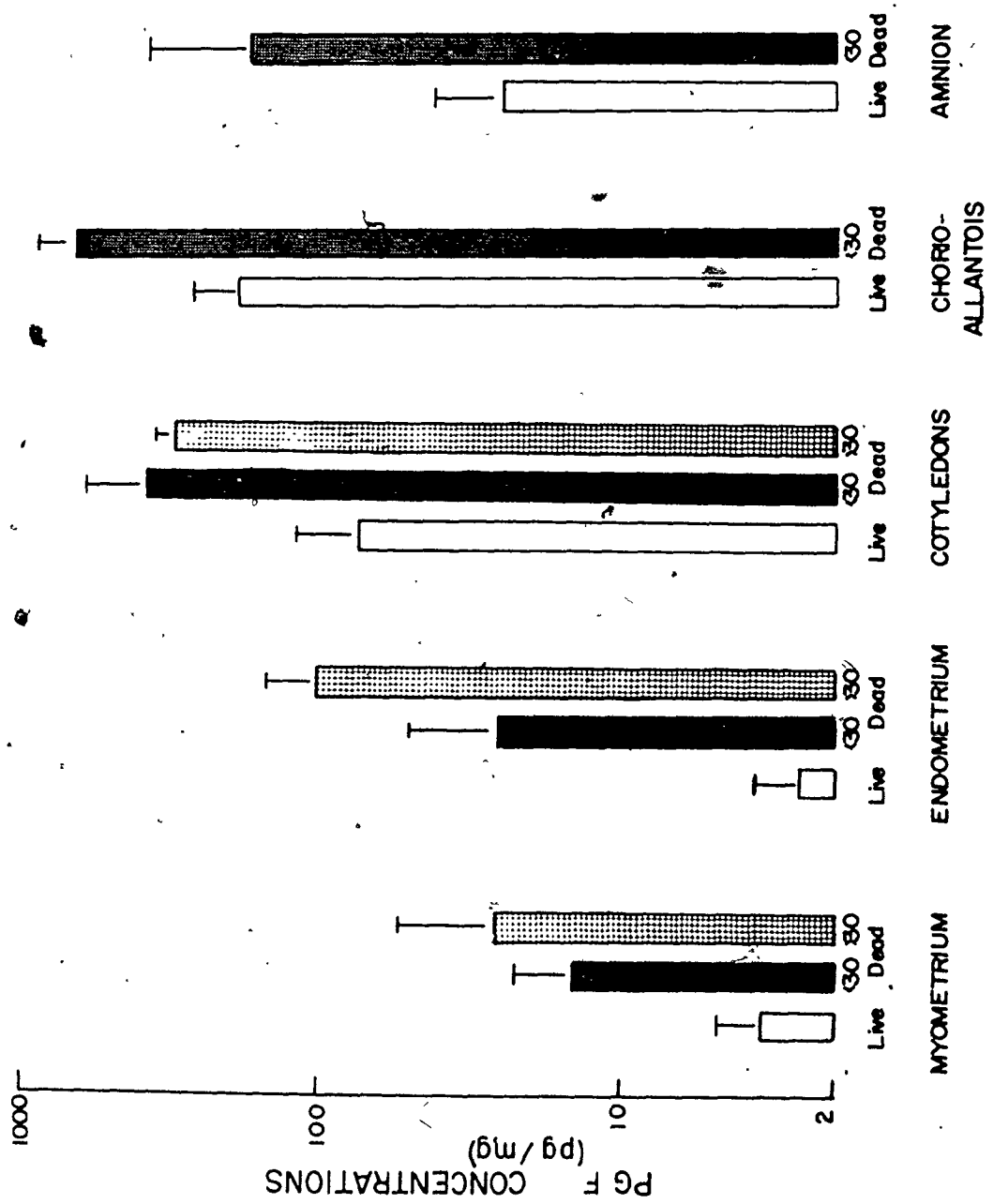


FIGURE 25

Concentrations of prostaglandin F (PGF) in uterine tissues taken from sheep bearing live chronically catheterized fetuses (130 days of gestation; n = 4) and from sheep bearing chronically catheterized fetuses (123-130 days after mating) dead 12-26 h (< 30; n = 4) or 34-72 h (> 30; n = 4).


PG F CONCENTRATIONS
(pg / mg)
1000
100
10
2
MYOMETRIUM
ENDOMETRIUM
COTYLEDONS
CHORIO-ALLANTOIS
AMNION
Live
Dead
30
30
30
30
30
30
30
30
30
30

fetuses dead greater than 30 h. In all animals bearing dead fetuses PGF concentrations in all tissues were not significantly different from those obtained during ACTH-induced labour (Table 51); however, PGF concentrations in the former group were much lower than those found during ACTH-induced labour in cotyledons. As indicated by significant interactions between treatment and uterine area (Table 51), PGF concentrations in myometrium taken from sheep bearing dead fetuses were greater than those taken during ACTH-induced labour. In addition, myometrial PGF concentrations were similar in samples taken from different uterine areas in sheep bearing dead fetuses, but not during ACTH-induced labour.

Results from measurements of uterine PGE concentrations are summarized in Figure 26. Concentrations of PGE were significantly higher (Table 54) in endometrium taken from sheep bearing fetuses dead greater than 30 h, than in those with fetuses dead less than 30 h ($P < 0.01$). In these animals, analysis of variance also indicated a significant effect of uterine area on PGE concentrations in cotyledons, concentrations being lower in samples taken from tubal areas than from other areas of the uterus ($P < 0.05$, paired t tests). Compared to sheep bearing live fetuses which were treated with saline + P.B. (Table 52), PGE concentrations were significantly higher in endometrium taken from all sheep bearing dead fetuses. Analysis of variance also indicated a significant interaction between treatment and uterine area in cotyledons collected from sheep bearing fetuses dead less than 30 h and those receiving ACTH + P.B., indicating that concentrations in the latter group were higher than those in the former group.

TABLE 51: A summary of variance analysis on prostaglandin F concentrations following fetal death (Figure 25), compared to those with live fetuses and those in intra-uterine tissues during ACTH-induced labour.

Source of Variation		F Values				
		Myometrium	Endometrium	Cotyledons	Chorioallantois	Amnion
a) Live[a] vs. Dead Fetuses (less than 30 h)						
Treatment	(1, 6)[b]	4.516	0.206	9.827*	2.761	2.837
Uterine Area	(4,24)	1.824	1.917	1.972	2.368	0.109
Treatment X Area	(4,24)	1.824	1.934	1.761	0.645	1.354
b) Live vs. Dead Fetuses (greater than 30 h)						
Treatment	(1, 6)	7.631*	0.016	19.322**		
Uterine Area	(4,24)	1.824	1.021	1.744		
Treatment X Area	(4,24)	1.642	1.378	0.930		
c) ACTH[a] vs. Dead Fetus (less than 30 h)						
Treatment	(1, 6)	0.693	0.229	0.892	0.432	0.224
Uterine Area	(4,24)	5.387**	1.298	0.651	1.572	0.408
Treatment X Area	(4,24)	4.721**	1.551	0.698	2.188	1.230
d) ACTH vs. Dead Fetus (greater than 30 h)						
Treatment	(1, 6)	3.144	1.628	1.709		
Uterine Area	(4,24)	3.260*	0.912	0.951		
Treatment X Area	(4,24)	4.033*	2.381	0.810		

[a]Sheep (130 days pregnant) receiving Saline + phosphate buffer (P.B.) (live fetuses) or ACTH + P.B. (ACTH)
[b]Degrees of freedom in (numerator), degrees of freedom (denominator)
*$P < 0.05$
**$P < 0.01$

FIGURE 26

Concentrations of prostaglandin E (PGE) in uterine tissues taken from sheep bearing live chronically catheterized fetuses (130 days of gestation; n = 4) and from sheep bearing chronically catheterized fetuses (123-130 days after mating) dead 12-26 h (< 30; n = 4) or 34-72 h (> 30; n = 4).

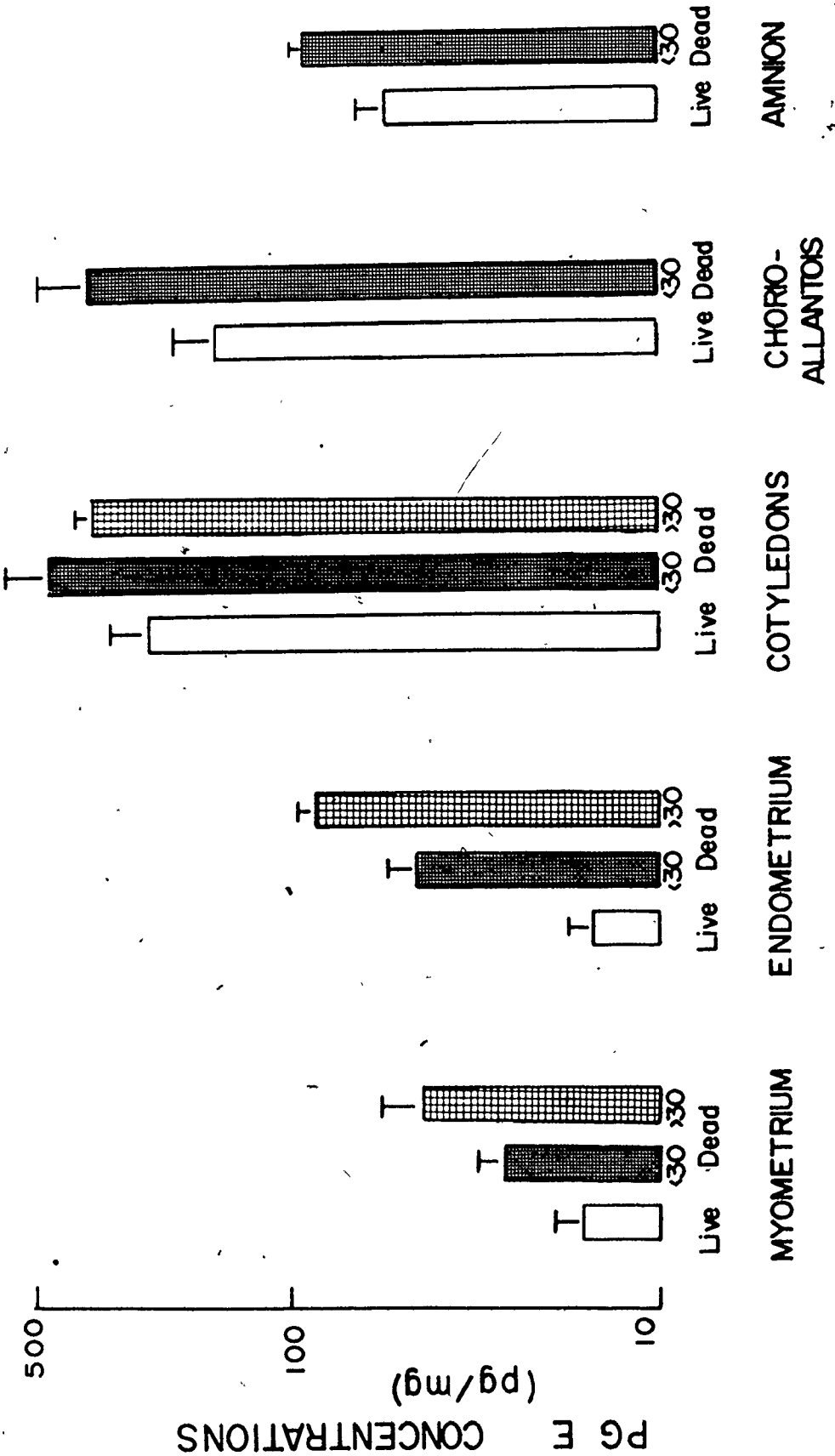

PG E CONCENTRATIONS
(pg/mg)
500
100
10
MYOMETRIUM
ENDOMETRIUM
COTYLEDONS
CHORIO-ALLANTOIS
AMNION
Live
Dead
<30
>30

TABLE 52: A summary of variance analysis on prostaglandin E concentrations following fetal death (Figure 26), compared to those with live fetuses and those in intra-uterine tissues during ACTH-induced labour.

Source of Variance		F Values				
		Myometrium	Endometrium	Cotyledons	Chorioallantois	Amnion
a) Live[a] vs. Dead Fetuses (less than 30 h)						
Treatment	(1, 6)[b]	0.000	15.109**	0.360	1.099	0.363
Uterine Area	(4,24)	4.039*	1.057	1.961	2.675	1.004
Treatment X Area	(4,24)	1.488	0.511	2.515	0.928	0.566
b) Live vs. Dead Fetuses (greater than 30 h)						
Treatment	(1, 6)	1.263	38.576**	0.275		
Uterine Area	(4,24)	1.136	0.712	2.499		
Treatment X Area	(4,24)	0.911	0.770	2.949*		
c) ACTH[a] vs. Dead Fetus (less than 30 h)						
Treatment	(1, 6)	1.169	0.242	1.882	1.993	0.200
Uterine Area	(4,24)	2.123	3.574*	0.484	1.416	2.472
Treatment X Area	(4,24)	1.473	2.065	3.565*	2.198	1.922
d) ACTH vs. Dead Fetus (greater than 30 h)						
Treatment	(1, 6)	0.412	1.690	2.378		
Uterine Area	(4,24)	0.263	1.797	1.011		
Treatment X Area	(4,24)	0.560	3.082*	2.404		

[a]Sheep (130 days pregnant) receiving Saline + phosphate buffer (P.B.) (live fetuses) or ACTH + P.B. (ACTH)
[b]Degrees of freedom (numerator), degrees of freedom (denominator)
*P < 0.05
**P < 0.01

TABLE 53: A summary of variance analysis on 6-oxo-prostaglandin $F_{1\alpha}$ concentrations following fetal death (Figure 27), compared to those with live fetuses and those in intra-uterine tissues during ACTH-induced labour.

Source of Variance		F Values				
		Myometrium	Endometrium	Cotyledons	Chorioallantois	Amnion
a) Live[a] vs. Dead Fetuses (less than 30 h)						
Treatment	(1, 6)[b]	12.334*	6.187*	2.575	7.972*	11.851*
Uterine Area	(4,24)	0.933	1.582	1.815	0.105	0.860
Treatment X Area	(4,24)	0.405	0.422	2.573	0.569	0.715
b) Live vs. Dead Fetuses (greater than 30 h)						
Treatment	(1, 6)	9.639*	29.523**	8.791		
Uterine Area	(4,24)	1.930	0.538	0.799		
Treatment X Area	(4,24)	2.156	1.069	0.574		
c) ACTH[a] vs. Dead Fetus (less than 30 h)						
Treatment	(1, 6)	2.458	1.815	2.836	0.364	1.916
Uterine Area	(4,24)	0.909	0.450	0.643	1.950	1.697
Treatment X Area	(4,24)	0.878	1.119	1.346	2.219	1.253
d) ACTH vs. Dead Fetus (greater than 30 h)						
Treatment	(1, 6)	3.311	18.508*	1.634		
Uterine Area	(4,24)	3.092*	1.070	1.143		
Treatment X Area	(4,24)	2.236	0.981	0.664		

[a]Sheep (130 days pregnant) receiving saline + phosphate buffer (P.B.) (live fetuses) or ACTH + P.B. (ACTH)
[b]Degrees of freedom (numerator), degrees of freedom (denominator)
*P < 0.05
**P < 0.01

Uterine concentrations of 6-oxo-$PGF_{1\alpha}$ are summarized in Figure 27. Analysis of variance (Table 54) indicated that for all tissues, 6-oxo-$PGF_{1\alpha}$ concentrations in sheep bearing fetuses dead less than 30 h and greater than 30 h were not significantly different. Compared to animals bearing live fetuses which were treated with saline + P.B. (Table 53), concentrations were significantly higher in myometrium, endometrium, chorioallantois and amnion from sheep bearing fetuses dead less than 30 h and in myometrium, endometrium and cotyledons from sheep bearing fetuses dead greater than 30 h. Concentrations of 6-oxo-$PGF_{1\alpha}$ in endometrium taken from sheep bearing fetuses dead greater than 30 h were greater than those found during ACTH-induced labour; however, concentrations in other tissues obtained after fetal death did not differ from those collected during ACTH-induced labour. In addition, analysis of variance indicated a significant effect of uterine area on myometrial 6-oxo-$PGF_{1\alpha}$ concentrations in sheep during abortive and ACTH-induced labours, concentrations being significantly higher in myometrium adjacent to the cervix than other areas of the uterus ($P < 0.05$, paired t tests).

Analysis of the amniotic fluid pressure recordings 1 h prior to sacrifice is summarized in Table 55. There was a progressive increase in the maximum amplitude and frequency of uterine contractions following fetal death, and the proportions of animals showing uterine contractions at this time tended to be higher in sheep bearing fetuses dead greater than 30 h than in the saline + P.B.-treated animals. The maximum amplitude and frequency of uterine contractions and the proportions of animals showing uterine contractions in sheep bearing fetuses dead greater than 30 h and in those where labour was

FIGURE 27

Concentrations of 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$) in uterine tissues taken from sheep bearing live chronically catheterized fetuses (130 days of gestation; n = 4) and from sheep bearing chronically catheterized fetuses (123-130 days after mating) dead 12-26 h (< 30; n = 4) or 34-72 h (> 30; n = 4).

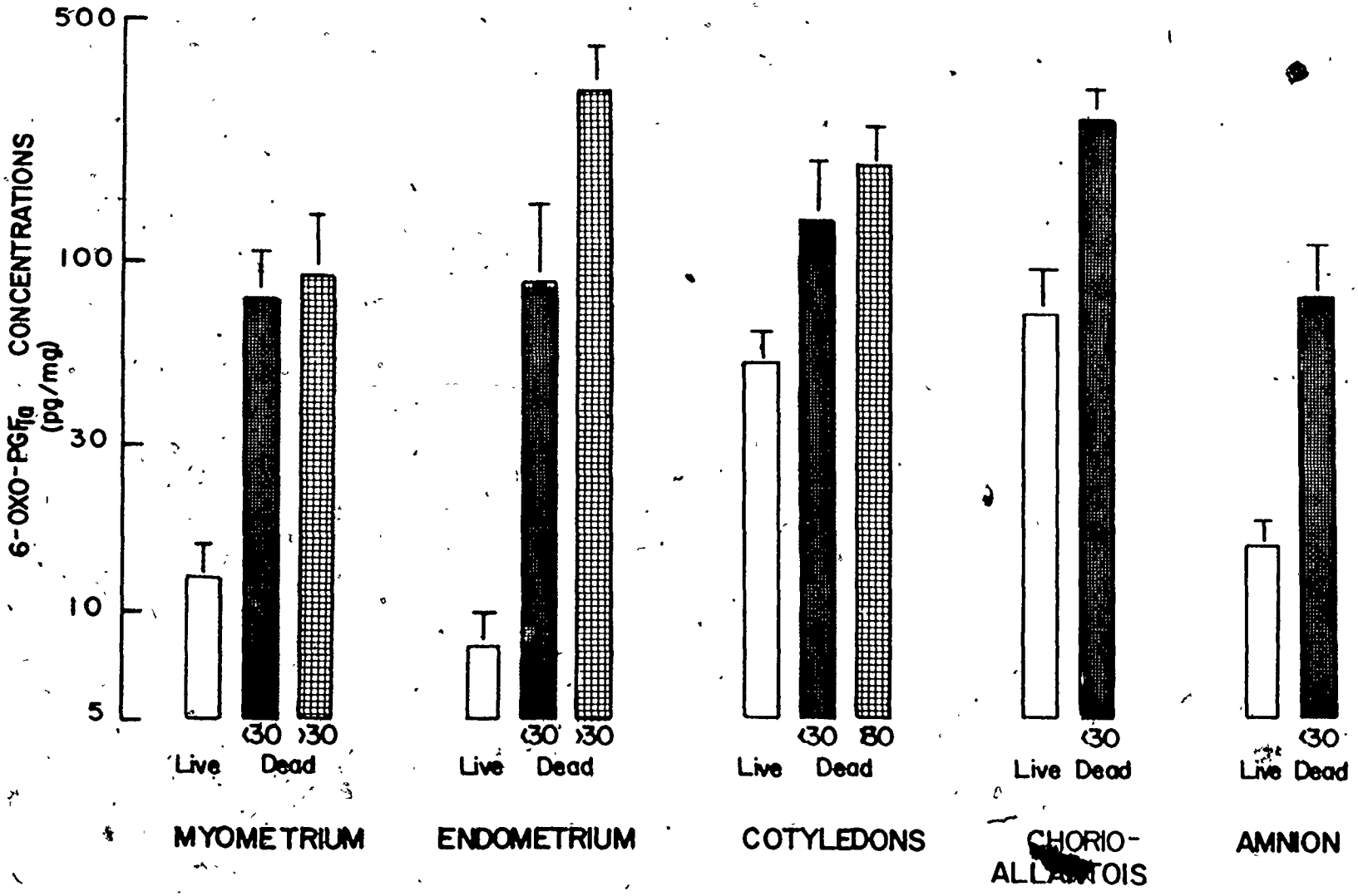

500
100
30
10
5
6-OXO-PGF1α CONCENTRATIONS (pg/mg)
<30
>30
Live
Dead
MYOMETRIUM
ENDOMETRIUM
<30
80
COTYLEDONS
CHORIO-ALLANTOIS
AMNION

TABLE 54: A summary of variance analysis of results in Figures 25-27. PG concentrations in ewes bearing fetuses dead less than 30 h compared to those having fetuses dead greater than 30 h.

Source of Variation		F Values: Myometrium	Endometrium	Cotyledons
Prostaglandin F (Figure 25)				
Treatment	(1, 6)[a]	1.929	1.337	0.410
Uterine Area	(4,24)	0.387	0.433	3.259 (P<0.05)
Treatment X Area	(4,24)	0.493	0.756	0.933
Prostaglandin E (Figure 26)				
Treatment	(1, 6)	1.302	14.787 (P<0.01)	0.046
Uterine Area	(4,24)	1.180	0.954	3.195 (P<0.05)
Treatment X Area	(4,24)	0.152	0.674	1.768
6-oxo-prostaglandin $F_{1\alpha}$ (Figure 27)				
Treatment	(1, 6)	1.259	2.563	0.276
Uterine Area	(4,24)	2.637 (P=0.059)	0.445	1.429
Treatment X Area	(4,24)	1.390	0.754	0.798

[a] Degrees of freedom (numerator), degrees of freedom (denominator)

TABLE 55: The frequency and maximum amplitude of uterine contractions one hour prior to tissue collection from PG determinations in sheep bearing live fetuses receiving saline + phosphate buffer (P.B.; n = 4) and sheep bearing fetuses dead less than (n = 4) or greater than 30 h (n = 4).

Treatment	Proportion with Contractions	Maxmimum Amplitude (mm Hg)	Frequency (Contractions/h)
Saline + P.B.	1/4	(5)[a]	(1)
Dead less than 30 h	3/4	6.0 ± 3.5	12.7 ± 7.5
Dead greater than 30 h	4/4*	18.0 ± 5.7	23.0 ± 7.2

[a] Raw data (one animal)

* Tended to be greater than the saline + P.B. Group ($P < 0.10$; Fisher test)

induced by the administration of ACTH to the fetus were not significantly different ($P < 0.05$, *t* tests and Fisher test).

8.4 Discussion

Results in the present study support the hypothesis that intra-uterine PG production in sheep in response to the presence of a dead fetus stimulated the onset of abortive labour (Gustavii, 1977). This study also suggests that this increased PG production varies with the tissue and PG studied. Prostaglandin E concentrations were elevated only in the endometrium and myometrium of sheep bearing dead fetuses, while PGF concentrations were elevated only in myometrium, endometrium and cotyledons of these animals. Concentrations of 6-oxo-$PGF_{1\alpha}$ were elevated in all tissues after fetal death. Tissue-specific increases in intra-uterine PG concentrations were also observed in sheep during labour induced by the intra-fetal administration of ACTH (see Chapter 5). In contrast to concentrations found during ACTH-induced labour, however, myometrial PGF and 6-oxo-$PGF_{1\alpha}$ concentrations are significantly elevated in the presence of a dead fetus. Myometrial 6-oxo-$PGF_{1\alpha}$ concentrations were also higher in regions adjacent to the cervix while PGF concentrations were fairly uniform throughout the myometrium. It is possible that the regional distribution of PG's within the myometrium during labour is important in the regulation of uterine activity in this tissue. In pregnant sheep, the administration of $PGF_{2\alpha}$ causes uterine contractions

(Liggins et al., 1973; Mitchell et al., 1976a), while PGI_2 causes uterine relaxation (Lye and Challis, 1982). It is possible, therefore, that the high concentrations of 6-oxo-$PGF_{1\alpha}$ in myometrium adjacent to the cervix, compared to other areas during both abortive and ACTH-induced labours ensure that contractions proceed in a tubal-cervical direction.

Although total uterine blood flow does not change after fetal death in sheep (Raye et al., 1971), the changes in the distribution of uterine blood flow are unknown. In the present study, uterine hyperemia was observed in sheep bearing dead fetuses, possibly a consequence of decreased placental blood flow. Since PGI_2 causes myometrial vasodilation in pregnant sheep (Rankin et al., 1979) and increases uterine blood flow in non-pregnant sheep (Resnik and Brink, 1980), the elevated concentrations of 6-oxo-$PGF_{1\alpha}$ in myometrium and endometrium may contribute to the redistribution of uterine blood flow following fetal death. Similarly, increased endometrial PGE concentrations during both abortive and ACTH-induced labours may contribute to the hemodynamic changes in the uterus.

In sheep, the cotyledons may be major sites of PGF and 6-oxo-$PGF_{1\alpha}$ production during ACTH-induced labour (see Chapter 5), and concentrations of these PG's were found to be elevated after fetal death in this tissue. The high concentrations of PG's that were found in cotyledons during ACTH-induced labour were not observed following fetal death, possibly a result of differences in steroid production by these tissues (Carson and Challis, 1981). Alternatively, it is possible that the concentrations of placental lactogen

do not decrease in these animals. If this hormone acts to suppress PG concentrations (see Chapter 7) during pregnancy, then the release from this suppressive influence might not occur following fetal death.

Concentrations of PGF and 6-oxo-$PGF_{1\alpha}$ were elevated in chorioallantois obtained from animals during ACTH-induced labour (see Chapter 5); however, shortly after fetal death, only 6-oxo-$PGF_{1\alpha}$ concentrations were elevated in this tissue. PG concentrations could not be determined in sheep bearing fetuses dead greater than 30 h, due to the difficulty in separating the fetal membranes in these animals. The possibility, therefore, that PGF concentrations are elevated at later times in this membrane cannot be ruled out. Degenerative changes, as indicated by adhesion of fetal membranes and discolouration of fetal fluids, occurred after fetal death. Such degenerative changes may lead to increased PG production directly by labilizing lysosomes (Myers et al., 1974; Gustavii, 1977). Alternatively, changes in the composition of amniotic and allantoic fluids following fetal death might also stimulate PG production in fetal membranes.

In two monkeys bearing dead fetuses, maternal venous plasma concentrations of 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ did not increase until the time of delivery, approximately 25 and 50 days after fetal death (Mitchell et al., 1976). In the present study, the proportion of animals showing uterine activity tended to be higher only in sheep bearing fetuses dead greater than 30 h and the frequency and maximum amplitude of contractions in these animals were not

significantly different from those observed during ACTH-induced labour. However, PG concentrations were elevated in sheep bearing fetuses dead less than 30 h, suggesting that PG's produced in response to fetal death may result in increased uterine activity in these animals. In the monkey, fetal death appears to result in decreased peripheral plasma estrogen with no changes in progesterone concentrations (Mitchell et al., 1976; Challis et al., 1977). After fetal death in the sheep, estrogen levels remain unaltered and progesterone levels decline (Carson and Challis, 1981). The causes of these changes in placental steroid production are uncertain. It is possible that in the monkey, tissue necrosis following fetal death may represent the major stimulus for PG production. In the sheep, however, changes in the relative concentrations of progesterone and estrogen may contribute an added stimulus for PG biosynthesis.

In summary, the salient findings reported in this chapter include: 1) PG concentrations are elevated within 30 h after fetal death; 2) the proportions of animals bearing dead fetuses having uterine contractions were not increased until after 30 h after fetal death.

CHAPTER 9

CONCLUSIONS

9.1 Methodological Considerations

The methodological problems associated with estimating endogenous tissue prostanoid (PG) production have been a concern of many investigators during the last decade. Estimates of PG concentrations in biological fluids such as plasma and amniotic and allantoic fluids, have been made in an attempt to estimate PG concentrations in intra-uterine tissues (Thorburn et al., 1972; Mitchell et al., 1978, 1979). However, such estimates do not differentiate between production rate and the rate of release by intra-uterine tissues, nor do they identify the major site(s) of PG production and/or release. In addition, a number of recent studies have suggested that the interpretation of results from plasma PG determinations may be obscured by technical artifacts. For example, blood collected from both adult and fetal sheep has been shown to contain prostaglandin-9-ketoreductase activity which may result in the interconversion of PGE_2 and $PGF_{2\alpha}$ (Bolla et al., 1977). In addition, the production of TXA_2 and subsequently TXB_2 may be stimulated in platelets during the collection of blood, since this compound is a powerful inducer of platelet aggregation (Moncada and Vane, 1978). Therefore, unless inhibitors of PG-forming cyclo-oxygenase activity are used, measurements of TXB_2 in

plasma may not be meaningful. The potential problems of measuring 6-oxo-$PGF_{1\alpha}$ (the hydrolysis product of prostacyclin) are many. The blood vessels, especially the endothelium, have been shown to be a major site of prostacyclin production (MacIntyre et al., 1978). Therefore, damage to the vascular endothelium such as that occurring during the insertion of catheters or needles for the collection of blood, likely results in increased production of prostacyclin and subsequently 6-oxo-$PGF_{1\alpha}$. In addition, platelets have been shown to contain 9-prostaglandin dehydrogenase activity which may metabolize 6-oxo-$PGF_{1\alpha}$ to 6-oxo-PGE_1 and subsequently reduce immunoassayable 6-oxo-$PGF_{1\alpha}$ concentrations in plasma (Wong et al., 1980). Moreover, 6-oxo-$PGF_{1\alpha}$ has been shown to be unstable during acidification and extraction from plasma with cyclohexane and ethyl acetate (Mitchell et al., 1981). Measurements of 6-oxo-$PGF_{1\alpha}$ in plasma, therefore, likely do not represent endogenous concentrations or biological activity. In addition to the possible artifactual contribution by platelets, and perhaps other blood and vascular elements, PG's have been shown to be released from the sheep uterus in a pulse-like fashion into the utero-ovarian vein (McCracken et al., 1973; Barcikowski et al., 1974). Estimates of PG concentrations in single samples of plasma from this vessel, therefore, probably do not reflect total uterine PG production.

Measurements of PG concentrations in biological fluids such as amniotic and allantoic fluids which contain few cellular elements probably represent the endogenous levels provided there is no contamination with blood (Green, 1979). The results from studies

presented in Chapters 7 and 8 suggest that cotyledons may be the major source of PG's found in these fluids. However, as pointed out earlier (see Chapter 7), PG's produced in the fetal kidney contribute to the PG's measured in these fluids (Walker and Mitchell, 1978) and PG's in amniotic and allantoic fluid may be metabolized by the fetal lung (Clyman et al., 1981) or fetal membranes (Keirse et al., 1975).

Prostaglandin concentrations have been measured in intra-uterine tissues in sheep as an estimate of tissue PG production rates (Liggins and Grieves, 1971; Mitchell and Flint, 1977). However, a large between animal variation was observed and the results in these studies were not comparable, possibly due to differences in methodology. It has recently been suggested that measurements of tissue PG concentrations reflect the capacity for formation rather than endogenous levels (Green, 1979; Liggins et al., 1980), since PG output is stimulated from a number of tissues in response to trauma (Piper and Vane, 1971). However, the effects of trauma on uterine PG production is uncertain. Results from studies presented in Chapter 4 suggest that the responsiveness of sheep uterine tissues to trauma differs between intra-uterine tissues and between different arachidonic acid metabolites. Therefore, the responsiveness of individual tissues to trauma is likely dependent upon the availability of the various PG synthetases prior to trauma. Since these enzymes normally regulate endogenous patterns of arachidonic acid metabolism (Lands, 1979), trauma probably amplifies this pattern by increasing the rate of arachidonic acid release from cell membranes due to rupture and/or distortion. In addition, since crushing after the

tissues were excised from the uterus resulted in changes in PG concentrations in most tissues (see Chapter 4), the capacity for PG production could not have been maximally stimulated during tissue dissection. However, the finding that PG concentrations were higher in cotyledons following the manual separation of fetal and maternal placentomes than in cotyledons which were frozen intact (see Chapter 4), suggests that some PG production may occur during tissue collection and processing. In all other experiments, therefore, PG concentrations were reported for whole cotyledons which were frozen immediately after dissection. In the present study, excised tissues were immediately placed in ice-cold phosphate buffer containing indomethacin, frozen, and homogenized in ethanol. Under these circumstances, PG production is reduced by indomethacin and freezing, and the ethanolic extraction likely results in the denaturation of cyclo-oxygenase, phospholipase, and PG synthetase enzymes. In studies where PG's are extracted from tissues by homogenizing in aqueous buffer, the PG-forming enzymes will not be denatured and PG's are likely produced.

In an attempt to reduce the artifactual production of PG's in response to such tissue injury, some investigators have examined the production and subsequent release of PG's using tissue incubation systems (Abel and Baird, 1980; Satoh et al., 1981). However, the possibility that PG production and release may be reduced by the inhibitory effects of the end-products of arachidonic acid metabolism during prolonged incubation periods (Lands, 1979) prompted other investigators to examine PG output from uterine tissues using a perfusion system (Mitchell and Flint, 1976; Mitchell et al., 1978a;

Liggins et al., 1980). Other investigators have examined PG output and production (the metabolism of radiolabelled arachidonic acid) in microsomes prepared from uterine tissues (Fenwick et al., 1977; Jones et al., 1977; Abel and Kelly, 1979; Alwachi et al., 1980). Although all of these studies make important contributions to the state of knowledge on PG production by intra-uterine tissues, it is difficult to draw comparisons from studies using different techniques. The production and output of PG's by homogenates of uterine tissues likely reflect the capacity of these tissues to produce PG's since phospholipase A_2 and C are no longer rate-limiting and PG production rates in these studies were much higher than those in studies using other techniques. In addition, the production of PG's and the nature of PG's produced using this technique are dependent upon the cofactors added to the incubation medium (Lands, 1979). Similarly, the output of PG's using superfusion or tissue incubation systems may not represent endogenous PG production rates. Under these circumstances, it is possible that the availability of substrates, nutrients and oxygen is not uniformly distributed within the tissues and may result in artifactual PG production. The biphasic responses in PG production rates and the unduly high output of PG's reported in studies using the superfusion technique (Mitchell and Flint, 1976; Mitchell et al., 1978a; Liggins et al., 1980) support this possibility. In an attempt to reduce the possible effects of the non-uniform distribution of essential nutrients to cells within tissues, the output of PG's in vitro was examined in the present study following cell separation (see Chapter 7). For cotyledons, chorioallantois and amnion, the results

suggest that there was a good correlation between tissue PG concentrations and PG output *in vitro*. This observation supports the contention that tissue PG concentrations may provide useful information regarding the net tissue production rates of PG's. On this basis, therefore, it is reasoned that measurements of tissue PG concentrations may provide useful information regarding the overall state of tissue PG turnover.

In the studies which have been presented, myometrial concentrations of PGE, PGF and 6-oxo-$PGF_{1\alpha}$ varied with uterine area in most animals, concentrations being greater in the tubal and/or cervical areas than the rest of the uterus. Although the physiological significance of this observation is uncertain, it suggests that estimations based on single samples may not be representative of the entire uterus. In addition, this finding may partially explain the high between animal variability in PG concentrations in similar studies where PG concentrations were estimated in single samples (Liggins and Grieves, 1971; Mitchell and Flint, 1977). Whether the differences in PG concentrations within animals reflect different rates of production and/or metabolism or clearance remains to be established.

In contrast to the myometrium, substantial between animal variation in PG concentrations persisted in endometrium and amnion when samples were collected from different areas of the uterus (see Chapters 4, 5 and 6). Samples of endometrium were more difficult to dissect from the uterus than other intra-uterine tissues. It is possible, therefore, that results of PG concentrations measured in the endometrium, compared to other intra-uterine tissues, are more

likely to be influenced by factors such as trauma and/or contamination with other tissue types. For the amnion, effects of uterine area were observed in some animals; however, PG concentrations appear to relate more consistently with the local distribution of visible blood vessels than with uterine area between animals. Since the distribution of blood vessels in the amnion varied between animals, significant effects of uterine area on PG concentrations may not have been detected, and for a given uterine area a large variation in PG concentrations was observed between animals.

9.2 PG's and the Initiation of Parturition in Sheep: A New Hypothesis

Evidence suggesting that PG's play a role in the regulation of uterine blood flow, cervical ripening and myometrial activity during ovine pregnancy and parturition has been reviewed in Chapter 1, and is supported by results from the present studies.

Prior to Day 50 of pregnancy in sheep, approximately 50% of uterine blood flow goes to the endometrium and only 27% to the sites of implantation. After this time there is a rapid shift of flow to the placenta which receives about 82% of total uterine blood flow after Day 100 of pregnancy (Rosenfeld et al., 1974). However, during spontaneous (Assali et al., 1958; Greiss, 1965) or oxytocin-induced (Assali et al., 1958) labour, uterine blood flow decreases significantly. In the present studies, PG output in vitro by placental cells on Day 100 of pregnancy was predominantly as PG.

of the E series. Furthermore, PGE output *in vitro* by these cells increased 8-fold between Days 50 and 100, followed by a 10-fold increase in placental PGE concentrations on Day 130 of pregnancy. These results, as well as the results of others (see Chapter 1) suggest that the increased placental production of PGE between Days 50 and 130 of pregnancy may contribute to the shunting of uterine blood flow and to the regulation of placental perfusion during gestation. In addition, results from this study indicate that PGE concentrations are elevated in the endometrium during ACTH-induced labour and during labour associated with the presence of a dead fetus, and that PGF concentrations in these animals were elevated in cotyledons (see Chapters 5 and 8). These changes in PG concentrations might be important in minimizing blood loss following placental separation during labour. For example, it is possible that PGF contributes to the decreased uterine blood flow during labour by mediating placental vasoconstriction. In addition, PGE might contribute to the shunting of blood flow away from cotyledons by mediating vasodilation in the endometrium.

The observation that cervical ripening following intra-fetal ACTH treatment is inhibited by indomethacin, despite the occurrence of increasing estrogen and decreasing progesterone concentrations, supports a role for PG's in the process of cervical ripening. These results are consistent with those of Mitchell and Flint (1978) and others (see Chapter 1), and suggest that estrogen and progesterone are not directly involved in cervical ripening in sheep.

Evidence suggests that PG's play an important role in the regulation of uterine activity during pregnancy and parturition (see

Chapter 1). In the present experiments, myometrial PG concentrations were elevated during labour associated with the presence of a dead fetus, and indomethacin significantly reduced the frequency of uterine contractions during ACTH-induced labour (see Chapters 5 and 8). In addition, PG concentrations were elevated in cotyledons, but not myometrium, during ACTH-induced labour (see Chapter 5). These results are consistent with the findings of Liggins et al. (1972). These investigators found that although PGF concentrations were elevated in the maternal placenta prior to dexamethasone-induced labour, concentrations in the myometrium were not elevated until after labour had started. These authors proposed that the maternal placenta may be the source of PG which is active during the initiation of parturition and that PG of myometrial origin may be more important in the maintenance of uterine activity than in the initiation process. By comparing measurements of PGF in blood samples obtained simultaneously from a cotyledonary vein and the utero-ovarian vein, Liggins et al. (1977) concluded that cotyledons were the major source of estrogen-stimulated PGF release, and that the myometrium was the major source of oxytocin-stimulated release.

At present it is uncertain how, or if, PG's produced in the cotyledons reach and exert their actions on the myometrium. Liggins et al. (1973) suggested that PG's might be selectively transported from the uterine vein to the uterine artery and thus to the myometrium by a counter current mechanism, but failed to obtain experimental support for this hypothesis. More recently, Thorburn and Challis (1979) suggested that the fetal membranes or endometrium may

be major sites of PG production and that these PG's might diffuse to the myometrium. In support of this hypothesis, PG concentrations were found to be high in the chorioallantois during late pregnancy, and these high concentrations were further elevated during ACTH-induced labour and labour associated with the presence of a dead fetus (see Chapters 5 and 8). PG concentrations were also found to be elevated on Day 145 of pregnancy in the allantoic fluid (see Chapter 6). In addition, PG's placed in the amniotic or allantoic cavities of sheep during mid-pregnancy can induce abortion (Thorburn, 1977).

Regardless of the pathway, the suggestions that there is an increase in the delivery of PG's to the myometrium, and the observations that PG concentrations do not change in this tissue during the initiation of labour are difficult to reconcile. It is possible that the rate of PG metabolism or clearance increases in the myometrium during the initiation of parturition. However, in the present study, myometrial PG concentrations did not change with gestational age, even when samples were taken on Day 145, just prior to spontaneous labour (see Chapter 6). In addition, PG concentrations in the myometrium did not appear to be related to PG concentrations or PG output in vitro in cotyledons or chorioallantois, concentrations in the latter tissues being very low on Day 50 of pregnancy while concentrations in the myometrium did not differ from those in preterm animals (see Chapters 6 and 7). There also did not appear to be a relationship between the effects of uterine area on PG concentrations in myometrium and those in cotyledons and chorioallantois. Thorburn (1977) suggested that the modest increase in circulating levels of PG's

during the initiation of labour, combined with an increased sensitivity of the myometrium to these PG's might be sufficient to induce uterine activity. It is possible that PG's present in the uterine and ovarian arteries might contribute to the elevated concentrations of PGE and PGF in the cervical and tubal areas of the myometrium, respectively (see Chapters 4 and 5).

With the notable exception of the possible role of PG's in the mechanism of luteolysis in sheep, Samuelsson (1970, 1978) claims that PG's are generally produced in the tissues where they exert their action. It is possible therefore that the initiation of myometrial activity during labour is not dependent upon PG's from other sources. The presence of myometrial activity in the absence of increased myometrial PGE or PGF concentrations might result from an increase in the number of PG receptors. In the non-pregnant monkey, human, rat and hamster, the concentrations of PG receptors vary with the ovarian cycle and is proportional to the rate of myometrial activity (Goldberg and Ramwell, 1976). It appears, therefore, that PG receptors are regulated by steroid hormones. Prior to the initiation of parturient uterine activity, estrogen concentrations increase and progesterone concentrations decrease in the maternal circulation (see Chapters 1 and 5). These changes in hormone levels might increase the concentrations of PG receptors in the myometrium and initiate uterine activity.

Recently, attention has focussed on the physiological regulation of lysosomal phospholipase activities in the fetal membranes. Grieves and Liggins (1976) demonstrated high phospholipase A_2

activities in amnion and chorioallantois in sheep and suggested that these tissues may participate in PG biosynthesis by releasing stored arachidonic acid. MacDonald and co-workers (1978) have provided evidence suggesting that lysosomal stability during human pregnancy is maintained in part by progesterone and that the appearance of a high affinity progesterone-binding protein in the fetal membranes prior to parturition, effects a local progesterone withdrawal and subsequently increased phospholipase A_2 activity. These workers also proposed that the liberation of arachidonic acid as a result of this increased phospholipase A_2 activity may be a major regulatory step in PG biosynthesis during parturition. In addition, Gustavii (1977) suggests that once PG's are produced and released by the fetal membranes, they may also stimulate PG synthesis in other uterine tissues by labilizing lysosomes. In the present study, the concentrations and in vitro output of PG's increased with advancing gestational age in cotyledons, chorioallantois and amnion (see Chapters 6 and 7). These results suggest that there is a progressive increase in PG production with advancing gestational age in these tissues, possibly due to increased phospholipase activities. In addition, the observations that concentrations of PGF and 6-oxo-$PGF_{1\alpha}$, but not PGE, are elevated in cotyledons and chorioallantois, and that only PGE is increased in endometrium during ACTH-induced labour (see Chapter 5) suggests that phospholipase activity may not be completely rate-limiting and that the synthesis of specific PG synthetase enzymes may be involved.

The role(s) of PG's produced by the chorioallantois is uncertain. Although the possibility that these PG's might influence

uterine activity cannot be ruled out, it seems to this author that they likely exert a more local action. For example, it is possible that PG's might alter the stability of this membrane. For example, the infusion of $PGF_{2\alpha}$ into the lumen of the cervix has been shown to cause local softening and dilation (Liggins et al., 1977). Similarly, Ellwood et al. (1979a,b) and Ellwood and Mitchell (1980) have suggested a role for 6-oxo-$PGF_{1\alpha}$ and prostacyclin in the mechanism of cervical ripening. Assuming these PG's have similar effects on collagen and glycosaminoglycan metabolism in the chorioallantois, elevated concentrations of PGF and 6-oxo-$PGF_{1\alpha}$ may participate in the mechanism of membrane rupture. In addition, $PGF_{2\alpha}$ has been shown to promote water and mucous secretion in the stomach and alter sodium and potassium fluxes in the small intestine (Bennett, 1972; Wilson, 1974). PG's produced in the chorioallantois therefore might be important in the regulation of secretions into the allantoic cavity during pregnancy and parturition. Alternatively, PG's produced in the chorioallantois might contribute to the changes in placental perfusion during pregnancy and parturition.

The factor(s) which promote the directed synthesis of PG's in intra-uterine tissues during labour is uncertain. The elevated concentrations of PGF and 6-oxo-$PGF_{1\alpha}$ in cotyledons and chorioallantois during ACTH-induced labour were correlated best with the estrogen concentrations in these tissues and not with the ratio of estrogen: progesterone concentrations (see Chapter 5). In addition, in vivo treatment with estradiol-17β in ovariectomized, non-pregnant sheep resulted in an increase in the basal output of PGE, but not 6-oxo-$PGF_{1\alpha}$,

in vitro from caruncle cell suspensions (see Chapter 7). These results, and those of others (see Chapter 1), suggest that estrogen may be important in the regulation of PG production in intra-uterine tissues. This conclusion is supported by the observations that PG concentrations and in vitro output in cotyledons and chorioallantois increase between Days 50 and 100 of pregnancy (see Chapters 6 and 7), and that this is associated with an increase in the rate of placental steroidogenesis (Thorburn et al., 1977).

PG content in caruncular cell suspensions was also significantly reduced in the present study following in vitro ovine placental lactogen (OPL) treatment. The concentrations of OPL in cotyledons and chorioallantois are high during early pregnancy (Chan et al., 1978) and these high concentrations might contribute to the low PG concentrations and in vitro output observed in these tissues on Day 50 of pregnancy (see Chapters 6 and 7). After this time, estrogen levels increase (Fevre and Rambauts, 1966), possibly allowing for a partial release from the suppressive effects of OPL on PG concentrations. Prior to parturition, however, OPL concentrations decrease and estrogen concentrations increase (see Chapter 1). The elevated concentrations of PGF and 6-oxo-$PGF_{1\alpha}$ in cotyledons and chorioallantois during ACTH-induced labour, therefore, might result from the combined effects of the release from the inhibitory action of OPL and the stimulatory action of estrogen on PG release from these tissues.

The changes in OPL concentrations following fetal death have not been measured. In the present study, PG concentrations in cotyledons collected from ewes in labour associated with the presence

of a dead fetus were not as high as those collected during ACTH-induced labour (see Chapters 5 and 8). It is possible that in the absence of a rise in glucocorticoid concentrations prior to the initiation of labour in these animals, OPL concentrations are not reduced in the cotyledons (see Currie and Thorburn, 1977; Currie et al., 1977). Hence, release from the inhibitory effects of OPL might not occur in these animals and PG production in intra-uterine tissues may be regulated by the changes in the ratios of estrogen: progesterone concentrations (Carson and Challis, 1981) and possibly also tissue necrosis (Myers et al., 1974).

In the myometrium, progesterone withdrawal associated with parturition in rats has been shown to result in an increase in the concentrations of estrogen receptors (Alexandrova and Soloff, 1980b). It is proposed that an increase in estrogen receptors and their occupancy by estrogen might result in the appearance of PG receptors and result in the initiation of labour. In addition, the preparturient increase in estrogen concentrations may stimulate PG production in the myometrium and increase PG release from the cotyledons and chorioallantois (Liggins et al., 1977). The resulting elevated PG concentrations in the peripheral circulation might augment oxytocin release from the posterior pituitary (Gillespie et al., 1972; Forsling et al., 1979), along with oxytocin released in response to the Ferguson reflex (see Chapter 1). The combined effects of increasing oxytocin and PG concentrations might then be important in the maintenance of uterine activity. In addition, prostacyclin has been shown to inhibit uterine activity in sheep (Lye and Challis, 1982), and

concentrations of 6-oxo-$PGF_{1\alpha}$ were elevated during labour in myometrium adjacent to the cervix (see Chapters 5 and 8). It is possible that the changes in the ratios of stimulatory:inhibitory PG's over different areas of the myometrium might be important in the regulation of the frequency of uterine contractions (see Chapter 5). In addition, PG's may also be important in the generation of synchronized contractions, possibly by facilitating the formation of gap junctions (Garfield et al., 1978).

SECTION III

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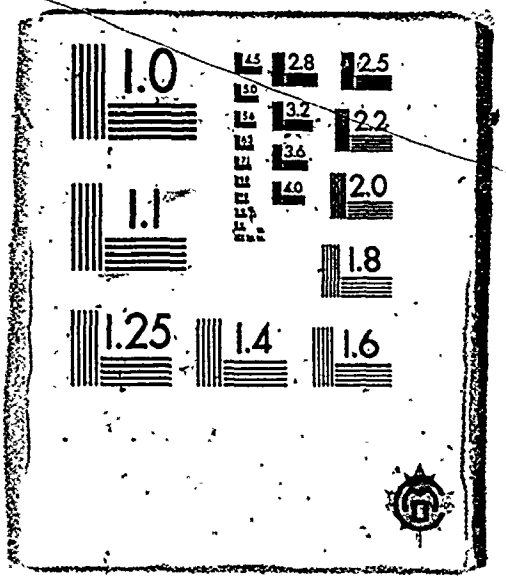

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