IN VITRO AND IN VIVO PROSTAGLANDIN PRODUCTION BY THE GRAVID UTERUS DURING LATE GESTATION IN THE SOW.

BY

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In vitro and in vivo prostaglandin production by the gravid uterus during late pregnancy in the sow.

ABSTRACT

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Prostaglandins (PG) are considered to be final mediators of the events that lead to parturition.

Fifteen sows were randomly divided into 3 groups of 5 each according to gestational age (GA; d 109, d 114 and at labor). Blood from the umbilical artery (FA), umbilical vein (FV), uterine artery (MA) and uterine vein (MV), and amniotic fluid (AMF), fetal urine (U), amnion (AM), allantochorion (ALC) and amniochorion (AMC) were collected from two fetuses per sow per GA.

Prostaglandin concentrations in blood plasma (PL), AMF, U, and 0-4 h incubates from dispersed placental cells were measured by radioimmunoasay. In addition, pre-term amniotic fluid (PAMF), pre-term (FU) or term-fetal urine (TFU) were added to ALC and to bovine seminal vesicle microsomes (BSVM) to assess the effect of these fluids on PG synthesis.

Results have shown that, during labor, plasma PGs rise dramatically but this increase does not follow a uniform pattern for all PGs suggesting that, at parturition, the cyclooxygenase complex is regulated differently than before

labor. This results in larger increases in myometrial stimulatory (PGF_{2a} and PGE_2) than inhibitory (PGI_2) PGs. Increases in the ratio between FV and FA, and MV and MA blood plasma PG levels indicate that the uterus is an important source of PGs during late gestation.

The porcine placenta synthesizes and metabolize PGs. PG production did not vary during the last 5-6 days of gestation, except 6-keto-PGF_{1a} which was produced less by cells collected during labor. Production of PGs by AM was less than ALC or AMC, although that of the latter two did not differ. Treatment of ALC and BSVM with FU, TFU or AMF increased PG synthesis . Fetal fluids, therefore, contain a substance which stimulates PG production.

Production *in vivo* et *in vitro* de prostaglandines par l'utérus gravide à la fin de la gestation chez la truie.

RESUME

Javier Burchard **MSc** **Zootechnie**

Les prostaglandines (PG) sont considérées comme les médiatrices finales des évènements qui mènent à la mise bas.

Quinze truies ont été divisées au hasard en 3 groupes de 5 chacun selon le stage gestationel (GA; j 109, j 114 et pendant le travail). Du sang de l'artère ombilicale (FA), de la veine ombilicale (FV), de l'artère utérine (MA) et de la veine utérine (MV), et du liquide amniotique (AMF), de l'urine fétale (U), l'amnios (AM), l'allantochorion (ALC) et l'amniochorion (AMC) ont été prélevées sur deux fetus par truie pour chaque GA.

Les concentrations des prostaglandines dans le plasma sanguin (PL), AMF, U et des incubations de 0 à 4 heures de cellules placentaires dispersées ont été mesurées par essai radioimmunologique. En plus de liquide amniotique d'avant-terme (PAMF), de l'urine de fétus d'avant-terme (FU)

et à .pa terme (TFU) ont été ajoutés au ALC et à des microsomes de vesicules séminales bovines (BSVM) afin d'évaluer leurs effets sur la synthèse des prostaglandines.

Les résultats ont démontré qu'au cours du travail, les prostaglandines plasmatiques augmentent dramatiquement, mais que cette élévation ne suit pas un modèle uniforme pour chaque prostaglandine, suggérant qu'au moment de la mise bas, le complexe cyclooxygénase est régularisé différemment qu'avant le travail. Cela résulte en des augmentations plus élevées des prostaglandines ayant un effet stimulatoire ($PGF_{2\alpha}$ et PGE_2) qu'inhibiteur (PGI_2) sur le myometrium. Les augmentations des rapports entre le niveau des prostaglandines plasmatiques provenant de la veine ombilicale et de l'artère ombilical, et provenant de la veine utérine et de l'artère utérin indiquent que l'utérus est une source importante de prostaglandines vers la fin de la gestation.

Le placenta porcin synthétise et métabolise les prostaglandines. La production de prostaglandines n'a pas varié pour les 5 à 6 derniers jours de gestation sauf la 6-keto-$PGF_{2\alpha}$ qui était produite en plus petite quantité par les cellules prélevées durant le travail. La production de prostaglandines par l'amnios était moindre que celle de l'allantochorion ou de l'amniochorion qui, cependant ne différaient pas l'une de l'autre. Le traitement de ALC et

prostaglandines. Les liquides fétales semblent donc contenir une substance qui stimule la production des prostaglandines.

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CHAPTER 1

INTRODUCTION

In reproduction, events associated with parturition and the perinatal period influence significantly the efficiency of animal production. Regardless of the domestic species, about 20 % mortality (Randall, 1984) will continue to occur between birth and 72 h after delivery, unless new basic and applied reproductive knowledge is incorporated into the animal production systems.

Although this pathological phenomenon is not considered as a disease in the porcine species, it is the cause of dramatic losses in the swine industry. Therefore, considerable attention should be focussed on this problem and towards the understanding of perinatal physiopathology.

Parturition is a complex phenomenon accomplished by the interrelated action of several hormones which influence the activity of different target organs resulting ,normally, in labor .

Prostaglandins are considered to be the final mediators of the events that lead to parturition; hence, in order to understand their role in the initiation of labor, it is necessary to fully characterize their concentrations in body fluids, production sites, and synthesis regulation during late gestation.

The specific objectives of this research project

were: 1) To measure prostaglandin concentrations and their variations in maternal and fetal blood plasma, amniotic fluid and fetal urine. 2) To determine prostaglandin synthetic capacity of placental tissues and 3) To assess the effect of amniotic fluid and fetal urine upon the regulation of this prostaglandin production throughout late pregnancy .

CHAPTER 2

LITERATURE REVIEW

2.1.- THE ESTROUS CYCLE IN THE PORCINE SPECIES.

Female pigs have estrous cycles of 18 to 21 days (d), ovulation taking place 36-42 h after the initiation of estrous and corpora lutea are detectable by d 4 or 5 of the cycle, when progesterone (P4) secretion is initiated and steadily increasing, reaching its peak secretion by d 12 to 14 of the estrous cycle. It subsequently declines so that by d 20 to 21 plasma P4 is barely detectable . This abrupt decrease in P4 levels is attributed to a luteolytic factor coming from the uterus, specifically from the endometrium. Within this context, Gleeson et al. (1974) and Moeljono et al. (1977), found that elevated prostaglandin F_{2a} (PGF_{2a}) in the utero-ovarian vein in sows during the late luteal phase of the oestrous cycle was transiently associated with declining plasma P4 concentrations. When 10 or 20 mg PGF_{2a} were administered intramuscularly to gilts on d 12 of the estrous cycle, mean weight of corpora lutea was less than in controls (Douglas and Ginther, 1975); however, no gilt returned to estrous or ovulated prior to necropsy on d 16. The above mentioned declines in plasma P4 levels and in corpora lutea weight lead to the conclusion that PGF_{2a} could

be a luteolytic factor in swine and that failure to decrease P4 levels before d 12-14 of the oestrous cycle (Moeljono et al., 1976) may be explained by a refractoriness of early corpora lutea to the luteolytic effects of PGF_{2a}. Apparently, this luteal resistance may last until the time that corpora lutea normally regress due to endogeneous luteolytic factors.

2.2.- INITIATION, RECOGNITION AND MAINTENANCE OF PREGNANCY.

During the luteal phase of an estrous cycle, the presence of embryos in the uterus results in signals that are sent to the ovary to prevent corpora lutea regression. The maintenance of corpora lutea allows the endometrium to continue its secretory and developing activities under the influence of high P4 levels throughout pregnancy (Bazer, 1982)

Maternal recognition of pregnancy is a complicated process which is not fully understood, but one of its most important features is the resultant corpora lutea maintenance after conception. These corpora lutea continue to function for the rest of gestation (114-115 d), regressing only before labor is triggered. Therefore, their functional failure at any stage of pregnancy will result in abortion within 24-36 hours (Belt et al., 1971).

After 60 to 72 h of the onset of estrous, provided that fertilization occurred, the embryos are in the four cell stage and move from the oviduct to the uterus. By d 5, the

zygote reaches the blastocyst stage and sheds its zona pellucida between d 6 and 7 (Giesert et al., 1982a). Synchronization of the P4-stimulated endometrial function and blastocyst development allows maternal recognition of pregnancy to occur by d 11. The initial blastocyst elongation is associated with increased estrogen (E2) content in the uterine lumen, E2 being produced by the blastocyst would stimulate the release of calcium (Ca^{++}) from the endometrium. This, in turn, would increase PGF_{2a} and prostaglandin E_2 (PGE_2) production and the release of secretory proteins into the uterine cavity. These biochemical changes facilitate initial trophoblast attachment to the maternal uterine epithelium by approximately d 13 of gestation (Geisert et al., 1982b). Furthermore, Lewis and Waterman (1983) reported that porcine blastocysts were able to synthetize PGE_2 which would result in : a) corpora lutea continuance through its possible antiluteolytic property (Akinlosotu et al., 1986) and b) an increase in vascular permeability at the site of implantation in order to facilitate passage of nutrients towards the conceptus and blastocyst-induced substances into the maternal blood stream (David et al., 1983; Keys et al., 1986). However, it is unlikely that PGE_2 would cause these effects in isolation, without interacting with other biologically active substances like estrogens and different proteins secreted by the blastocysts. Kraeling et al. (1985) demonstrated that pregnancy was avoided when a prostaglandin synthesis inhibiting com-

pound (indomethacin) was administered to artificially inseminated gilts.

Apart from prostaglandins and estrogens, the rabbit conceptus is also able to secrete proteases which have been implicated in invasive implantation of trophoblastic tissue into the uterine wall (Denker, 1972). However, in swine there is no erosion of the endometrium, and attachment of the conceptus is mainly due to microvilli interdigitation of the chorionic and endometrial opposing surfaces (Steven, 1975). Nevertheless, if the blastocysts are transferred to ectopic sites, such as the kidney capsule, they will show invasive properties (Samuel, 1971). During the elongation phase , the 11 d embryo is able to secrete large amounts of plasminogen activator and possibly other proteases. However, the potential fibrinolytic cascade is suppressed in the pregnant uterus by protease inhibitors secreted by a P4 primed endometrium. In this way, a non-invasive diffuse placentation results, and the delivery of maternal nutrients attached to macromolecules, which must be transfered across several cell layers to reach the embryo, is ensured (Fazleabas et al., 1982).

2.3.- THE DEVELOPMENT OF THE PLACENTAL MEMBRANES.

A fertilized egg is surrounded by the zona pellucida, an acellular glycoproteinaceous layer, within which the zygote divides during its passage through the oviduct,

producing many cells, the blastomeres. This division results in a solid cluster of cells, reaching the morula stage within 4-5 days after fertilization (Lupse, 1973). Once the morula reaches the uterine lumen, the blastomeres lose their spherical appearance and become tightly opposed to each other, a process called compaction. Secretions from the blastomeres are collected within the morula, forming a fluid filled cavity, the blastocoele. At this stage the embryo is known as a blastocyst, and some of the cells surrounding the blastocoele become larger at the point where the embryo will be formed. This enlargement, the embryonic disk, undergoes tangential cell divisions to form the endodermal cells which migrate and become established along the inner surface of the trophoblast. Thus, the blastocoele becomes the yolk sac. Cells surrounding the blastocoele that are not part of the embryonic disk are the trophoblastic cells and their role is to facilitate the absorption of nutrients in the early embryonic stages and later to be part of the extraembryonic membranes and placenta (Perry, 1981). During gastrulation (second week of gestation), the embryonic disk cells form three parallel germ layers, the **ectoderm** (outermost), **the endoderm** (innermost) and one layer that is formed between them, the **mesoderm**, which later splits into an **outer** and inner **mesoderm** (FIG. 1A a-b). At the time of gastrulation, the trophoblast is the extension of the embryonic ectoderm and its combination with outer mesoderm layer is referred to as **somatopleure**. Similarly, the resultant combination of inner

mesoderm and endoderm is called **splanchnopleure**. When the somatopleure and splanchnopleure extend beyond the limits of the embryonic disk area, they are referred to as extraembryonic membranes. Cell division continues in the embryonic and extraembryonic tissues, and the somatopleure surrounds the embryonic disk forming the amniotic folds which later fuse. Thus, the **amnion** (AM) and amniotic cavity are formed. The outer layer of somatopleure constitutes the **chorion** (C) and a permanent chorio-amniotic raphe remains attached to the chorion at the original site of fusion of the amniotic folds. (Fig 1B).

The elevation of the somatopleure and its consequent separation from the splanchnopleure results in the formation of a cavity between the inner and outer mesoderm layers, the exocoele (Fig. 1A d-e) . This separates the yolk sac from the trophoblast and the relative size of the yolk sac decreases gradually, becoming indistinguishable by day 20 of gestation. Conversely, behind the connection of the gut with the yolk sac, an outgrowth of the gut emerges into the exocoele. This extension of the gut will expand and materialize as the allantoic sac, lined by the endoderm and covered by the mesoderm inner layer (Fig. 1A f). By day 25 of gestation, the **allantois** (AL) has filled the entire extraembryonic coelom, except the area dorsal to the embryo where the expanding amnion remains attached to the chorion. The mesoderm covering the dorsal amnion and allantois fuses with that lining the chorion, thus the **amniochorion** (AMC) and

allantochorion (ALC) formed have an outer layer of ectoderm or trophoblast, an inner layer of endoderm and between them, mesodermal tissue (Fig 1A). The mesoderm in the allantois carries extraembryonic blood vessels which branch all over the allantochorionic sac, except at its extremities. This sac will be firmly attached to the uterine epithelium from day 18 of pregnancy until labor is initiated. The yolk sac and its vascular system, which facilitate histotrophic nourishment of the early embryo, is readily replaced by the allantois.

On the basis of the facts presented above, the porcine placenta is classified as diffuse epitheliochorial in which the chorionic or trophoblastic epithelium is in direct juxtaposition with the endometrial epithelium throughout the whole uterine inner surface.

According to Noden and de Lahunta (1985), the development of the porcine placenta originates three zones on the chorionic surface. The **placental zone** is the central chorion that develops transverse folds which fit into similarly oriented grooves in the endometrium. This, and the extrusion of the microvilli on the free surface of the trophoblastic cells, further increase the area of contact. In this zone there are numerous aurolae, which are cup-shaped indentations in the chorion located against the openings of the endometrial glands, and probably are involved in the transfer of material derived from uterine secretions to the fetus. The **paraplacental zones** consist of smooth chorion

without folds or areolae. The third zone is the **necrotic tip or ischemic zones** which are usually brown coloured due to the occlusion of periphereal blood vessels.

Since the porcine embryonic membranes are not invasive, there is no loss of maternal uterine endometrium at the moment of implantation, and both maternal tissue layers and fetal membranes remain intact throughout gestation. Therefore, the placenta is basically composed of six layers, fetal endothelium, connective tissue, chorionic epithelium (trophoblast), endometrial epithelium, uterine connective tissue and maternal endothelium (Noden and de Lahunta, 1985). However, the distance between the maternal and fetal sides is considerably reduced by capillary vessels infiltrated in the maternal and fetal epithelia.(Stevens, 1975).

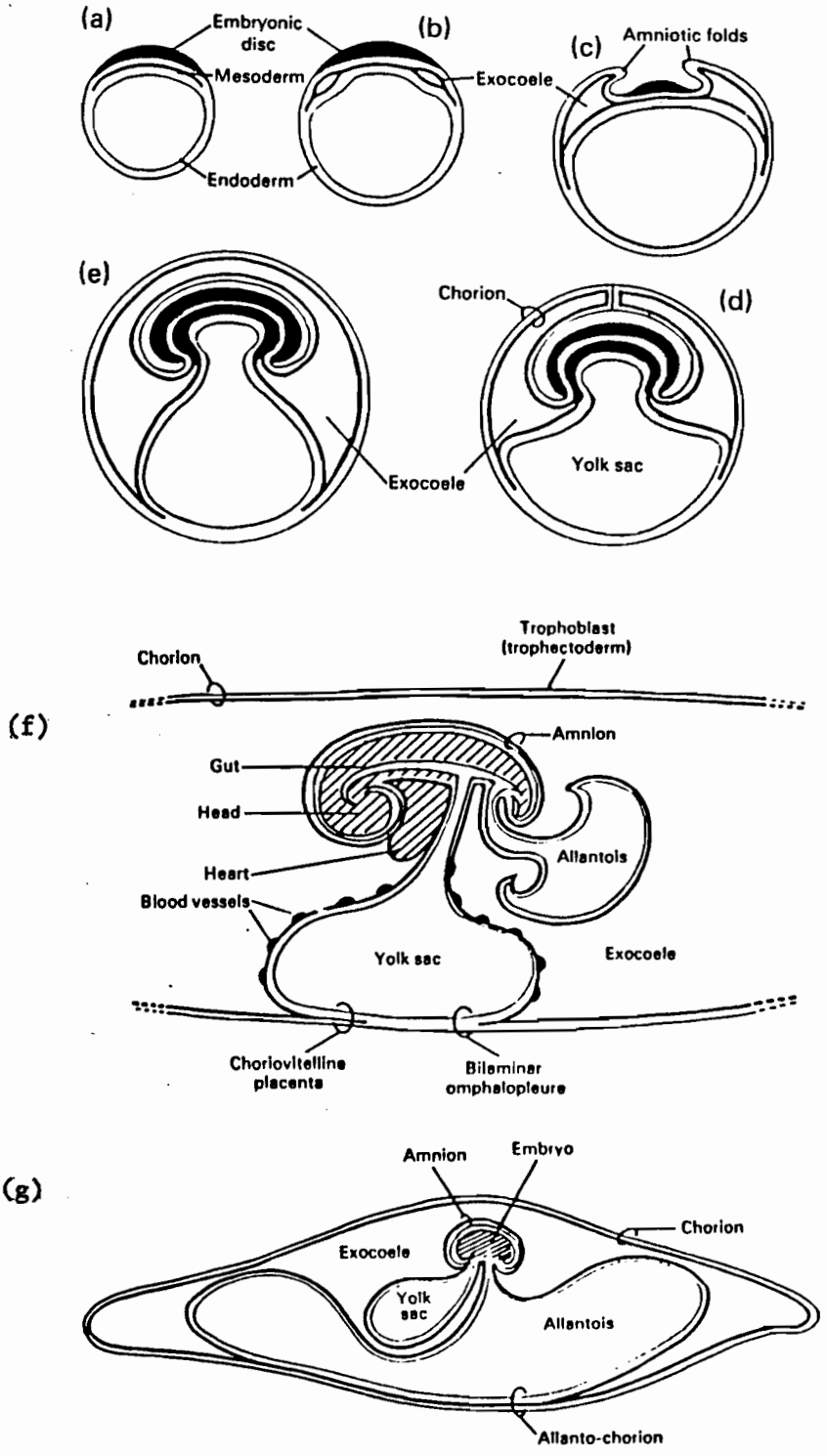


Fig. 1A.- Development of Porcine Fetal Membranes.
(Perry, 1981)

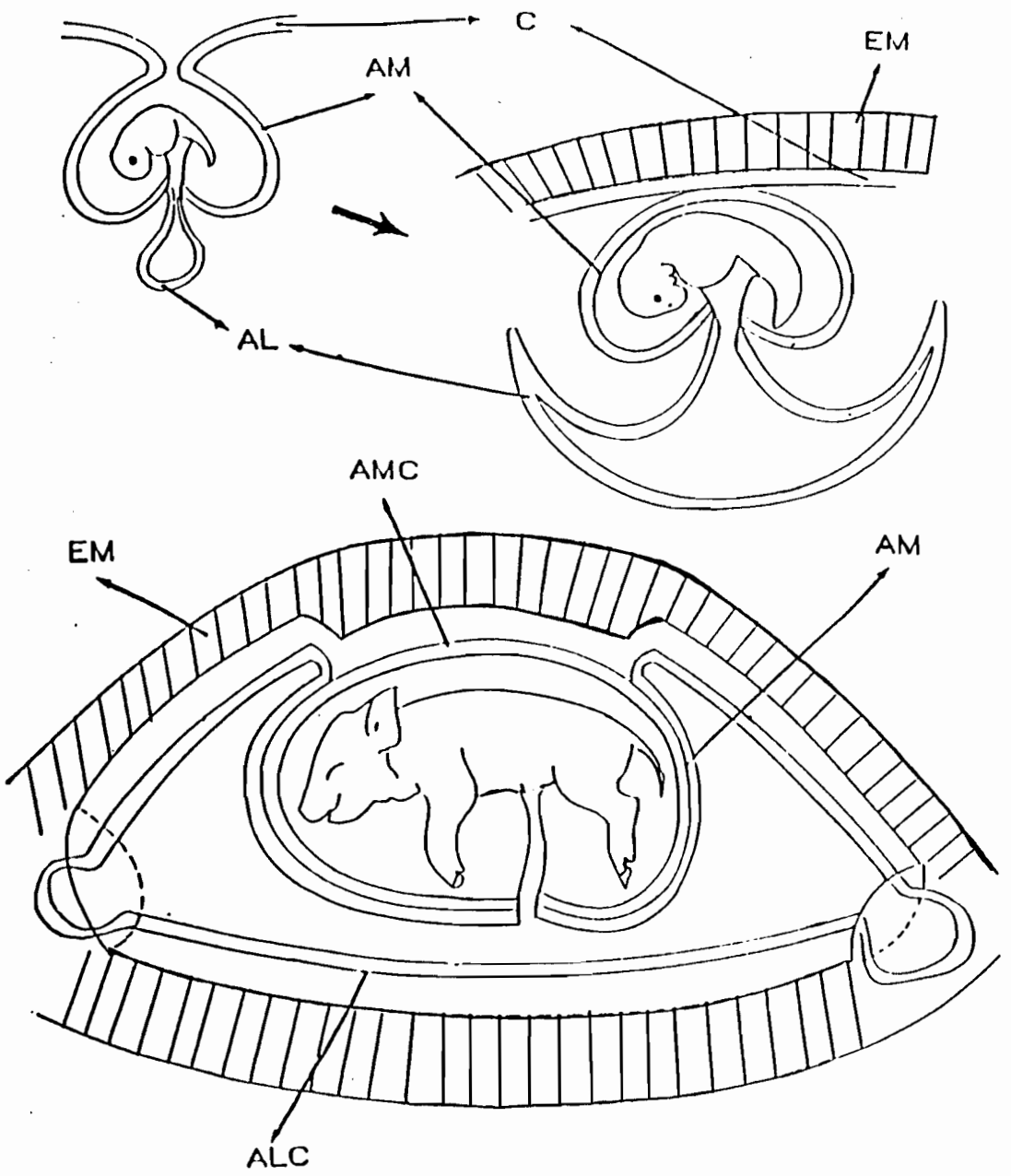


Fig.1B.- Intrauterine disposition of the fetal membranes.
C= Chorion, EM= Endometrium, AM= Amnion,
AL= Allantois , AMC= Amniochorion, ALC= Allantochorion.

2.4.- FETAL AND MATERNAL BLOOD CIRCULATION

Since blood oxygenation and nutrient exchange takes place within the placental circulation rather than in the lungs and digestive tract, the fetal circulatory system differs from that in the adult animal. Arterial blood passes from the fetus through the umbilical arteries to the placenta, where maternal-fetal exchange of CO_2, O_2, nutrients and excretory products takes place. Once the blood has been oxygenated, it returns to the fetus by the umbilical vein. This interchange is achieved with maximum efficiency thanks to three different circulation patterns: a) **counter-current exchange circulation**, where the blood flow in fetal capillaries is opposite to that in the mother, so that the venous outflow within the fetal circulation is in equilibrium with the arterial inflow of the maternal circulation, b) **parallel placental circulation**, where the venous outflow of the fetal circulation is in equilibrium with the venous outflow of the maternal capillaries and c) **perpendicular placental flow**, where the values of blood materials in the umbilical veins are intermediate between the maternal arterial and venous blood within the placental circulation (Breazile, 1971).

2.5.- THE MECHANISM OF INITIATION OF PARTURITION.

Parturition in mammals, is a complex physiological

phenomenon which is accomplished by the interrelation and interactions of several hormones acting on diverse target tissues. Current models of the mechanism of parturition will be briefly described here with emphasis on the sheep and swine. Labor is triggered in ewes and sows after approximately 150 and 114 days of gestation, respectively.

2.5.1.- Sheep.

The hypothesis that the fetus would be able to time its own delivery by means of a mechanism involving the fetal pituitary was tested in the sheep by ablation of the fetal hypophysis. This resulted in a prolonged pregnancy which could be terminated by infusions of adrenocorticotropic hormone (ACTH) into the lamb (Liggins et al., 1966).

During the last 7-10 days of pregnancy fetal adrenal weight doubles (Comline and Silver, 1961). However this weight, in lambs with hypophyseal lesions, is approximatelly half that of intacts (Liggins and Kennedy, 1968). On the other hand corticosteroid concentrations in the fetal plasma increase dramatically during the last days of pregnancy and at labor (Basset and Thorburn, 1969). Infusions of ACTH into pre-term fetal lambs result in premature parturition and in an increased fetal adrenal weight which is equal to or exceeds that of normal lambs (Liggins, 1968). Infusions of cortisol into lamb fetuses also cause premature parturition (Liggins, 1968) even when they are adrenalec-

tomized (van Rensburg, 1967).

Cortisol production by fetal lamb adrenal cells collected during term and incubated with ACTH is greater than that of those collected at day 100 of gestation (Glickman and Challis, 1980). Therefore, the adrenal gland is more sensitive to ACTH during labor. This was shown *in vivo* by Rose *et al.* (1982) since plasma cortisol but not ACTH increases dramatically during the last five days of pregnancy.

Spontaneous (Basset and Thorburn, 1969) and glucocorticoid induced parturition in the ewe (Flint *et al.*, 1974) is associated with a decrease in maternal P4 , and an increase in PGF and estrogens levels (Challis, 1975). The main source of P4 in the sheep during pregnancy is the placenta (Linzell and Heap, 1968) which during spontaneous or dexamethasone induced labor contains enhanced steroid 17-alpha-hydroxylase (Anderson *et al.*, 1975; Flint *et al.*, 1978) and C_{17-20} lyase activities (Steele *et al.*, 1975; John and Pierrepoint, 1975). On the other hand cortisol increases steroid 17-alpha-hydroxylase (Anderson *et al.*, 1978), and parturition is associated with an increment in C_{17-20} lyase activity. Cortisol stimulates steroid aromatase activity in placental explants (Ricketts *et al.*, 1980). Cortisol therefore, can be considered as the biological compound that shifts placental steroid production into estrogens at the expense of P4 at the end of the ovine gestation. This increase in estrogen production takes place in the placenta (Anderson *et al.*, 1975).

Infusions of estrogens into the ovine fetus causes a rise in maternal plasma PGF concentrations (Flint et al., 1974), and estrogens stimulate in vitro and in vivo PGF_{2a} production by the human uterus (Kelly and Abel, 1980; Schatz and Gurpide, 1983; Schatz et al., 1986; Schatz et al., 1987). PGF_{2a} has been associated with an increased uterine contractile activity (see section 2.10.2.) which causes delivery.

2.5.2.- Swine.

Decapitation of pig fetuses between day 40 and 50 of gestation (Bosc et al., 1974; Stryker and Dzuik, 1975; Coggins and First, 1977), and fetal hypophysectomy (Randall and Tsang, 1986) delay labor until day 120. Methallibure, an inhibitor of the hypothalamic function, also increases gestational length (First, 1972).

During the last 10 days of pregnancy pig fetuses double their adrenal weight (Dvorak, 1972) and their plasma corticostroid concentration increases dramatically (Silver et al., 1979; Randall, 1983). Fetal cortisol concentrations are approximately six times lower in either decapitated or hypophysectomized than those in intact fetuses (Randall and Tsang, 1986).

Fetal infusions of ACTH resulted in an increase in both fetal adrenal weight and cortisol levels although, only a small proportion of sows had premature parturition (Randall et al., 1984). Conversely, infusions of

dexamethasone to pig fetuses reduced the adrenal weight and gestational length (North et al., 1973; First and Staigmiller, 1973). Randall et al. (1984) also found that dexamethasone decreased adrenal weight; however this corticosteroid did not produce a significant effect on pregnancy length. This might be attributable to the fact that Randall et al. (1984) used a continuous infusion system while other authors utilized single injections and higher doses of dexamethasone (North et al., 1973; First and Staigmiller, 1973).

When continuously treated with P4, dexamethasone did not induce parturition in intact or ovariectomized sows (First and Staigmiller, 1973). Therefore this corticosteroid may induce labor by affecting the source of P4, and since ovariectomy produces abortion (First and Staigmiller, 1973), this source , probably, is the ovary .

Even though the porcine placenta also has some 17 '-hydroxylase activity , which increases at late gestation (Craig, 1982), Randall and Tsang (1986), when infusing dexamethasone into hypophysectomyzed fetuses, did not find alterations in either P4 or estrogen fetal plasma levels. Nevertheless hypophysectomized fetuses contained higher P4 and lower estrogen concentrations than did intact fetuses. They concluded that, in swine, the role of cortisol and estrogens during late gestation and parturition is still uncertain and requires further investigation.

In the sow maintenance of pregnancy is P4 dependent since its administration prevents spontaneous (First and

Staigmiller, 1973) and induced parturition (Coggins et al., 1977) when endogenous production is very low. On the other hand inhibition of 3-beta-hydroxysteroid-dehydrogenase, which converts pregnenolone to P4 (Martin et al., 1987), removal of corpora lutea (Nara et al., 1981) or ovariectomy (First and staigmiller, 1973), cause a decrease of maternal P4 levels and, consequently, parturition occurs prematurely. Therefore , although P4 is produced by the placenta as well (Craig, 1982; Hagen, 1983), the main souce of P4 throughout porcine pregnancy are the corpora lutea (Nara et al., 1981).

Injections of PGF_{2a} cause a decrease in maternal P4 concentrations and induce parturition in the sow (Coggins et al., 1977). Dexamethasone infusions raise plasma PGF_{2a} concentrations resulting in a simultaneous decrease in P4 and parturition (Coggins et al., 1977; Nara and First, 1981). Inhibition of PG synthesis during late gestation does not allow the decrease of P4 to occur and delay of the onset of labor ensues (Nara and First, 1981; Gooneratne et al., 1982; Taverne et al., 1982).

The gradual decline in plasma P4 before parturition was not associated with increasing PG concentrations (Silver et al., 1979; Randall et al., 1986) probably because luteolysis in the sow takes place in two phases, the first phase being independent of PGs (Gooneratne et al., 1982).

During delivery, maternal and fetal plasma levels of PGF and PGE increase dramatically in swine (Silver et al., 1979; Randall et al., 1986). These changes are as-

sociated with decreased maternal plasma P4 and increased plasma relaxin (Sherwood et al., 1979; Randall et al., 1986), prolactin (Taverne et al., 1982) and oxytocin (Ellendorf et al., 1979ab) values. PGF_{2a} is known to cause release of relaxin (Sherwood et al., 1979) and ,in the rat, it increases the uterine sensitivity to oxytocin at late gestation (Chan, 1983). These hormonal changes together with the effect of PGs upon luteal function (see 2.10.1) and uterine contractile activity (see 2.10.2) result in the initiation of labor. Prostaglandins are therefore, considered as the common mediators of the different events that lead to parturition.

The evidence presented clearly supports the hypothesis that the initiation of parturition is related to the integrity of the fetal hypothalamic-pituitary-adrenal axis. This axis, as mentioned above, has been experimentally manipulated to cause delayed or premature labor in both the ovine and porcine species. However, contrary to the ovine, the sequence of events that lead to parturition after the adrenal activation, and the role of cortisol and estrogens in this phenomenon remains unclear in swine. Therefore, it requires further investigation. Nevertheless, the increase in fetal cortisol levels before labor does occur, and the rise in plasma PG concentrations in maternal and fetal compartments during labor seem to be key features in both species.

2.6.- THE BIOSYNTHESIS AND METABOLISM OF PROSTAGLANDINS.

Prostaglandins are lipid compounds, specifically cyclopentane derivatives, synthetized from polyunsaturated fatty acids by most mammalian, lower vertebrate and, to a lesser extent, invertebrate tissues. The biological actions of PGs are numerous and are of great diversity but, in general, they can be defined as modulators of responses caused by different hormonal, neurohormonal and other stimuli (Lands, 1979).

Although there are several polyunsaturated fatty acids that can be converted into PGs, the scientific attention has been focussed on the synthesis and function of arachidonic acid derivatives which, as far as biological activity is concerned, are the most important.

2.6.1.- Synthesis of Prostaglandins and Related Compounds.

The synthesis of PGs, thromboxanes, lipoxins, and related compounds requires the catalytic action of several enzymes namely, phospholipases, an enzymatic complex known as cyclooxygenase or PG synthetase complex and lipoxygenases (Samuelson, 1975).

2.6.1.1.- Phospholipase A_2.

Fatty acids, which are precursors of prostaglan-

dins, are esterified in phospholipids and triglycerides in cell membranes. Prior to the initiation of PG synthesis, arachidonic acid, the precursor of which is linoleic acid, must be released from the cellular lipids by either phospholipase A_2 or phospholipase C in order to provide substrate for the PG synthetase and lipoxygenase complexes (Granstrom, 1981). Phospholipase A_2 activity may be suppressed by anti-inflamatory steroids, thereby lowering the availability of substrate (Gryglewsky et al., 1975). This decrease is presumably caused by a decline in phospholipid deacylation due to an increase in a 40 K protein named lipocortin, the synthesis of which is stimulated by glucocorticoids (Wallner et al., 1986).

Since linolate is an essential fatty acid, an efficient use of it by the cell should be expected. In respect to this, Hsueh et al. (1977), suggested that the hormone-mediated release of prostaglandins would be possible due to the activation of a specific phospholipase A_2, which would selectively liberate arachidonic acid from phospholipids. Since PGs and free arachidonic acid are not stored in the cell, the arachidonate-containing phospholipid pool plays a key role in the storage of prostaglandin precursors. Therefore, the existence of a reacylation mechanism by which the non-oxydized arachidonate would be reincorporated into the phospholipid pool is possible (Hsueh et al., 1979).

2.6.1.2.- Cyclooxygenase Complex.

Cyclooxygenase is a complex multienzymatic system which is able to oxygenate arachidonic acid producing PG and thromboxanes compounds. Miyamoto et al. (1974) reported the identification of two enzymatic fractions in the microsomes of bovine seminal vesicular cells. In 1976, they were able to purify these fractions and described two different enzymes. One of them, **prostaglandin endoperoxide synthetase** (referred to also as **prostaglandin H synthetase** by Kulmacs, 1985), catalyzes the insertion of two molecules of oxygen into arachidonic acid yielding a cyclopentane peroxide, PGG_2, which is further reduced to PGH_2. The other one, **prostaglandin endoperoxide E isomerase**, converts PGH_2 to PGE_2. Christ-Hazelhof et al. (1976), described **glutathione-s-transferase** in the sheep lung. This enzyme, in the presence of glutathione, catalyzes the formation of PGD_2 from PGH_2 and, since it is present many organs (Hayakawa et al., 1979), it may play an important role in the metabolism of endoperoxides.

Uterine cell microsomes are very efficient in reducing PGH_2 to $PGF_{2\alpha}$. This led Wlodawer et al. (1976) to conclude that, in the uterine tissues, an enzymatic pathway with PGH_2 reductase activity was the predominant one. This enzymatic fraction is referred to as **prostaglandin endoperoxide reductase** (Samuelson et al., 1975).

Microsomes from rabbit aortas are able to transform

PGG_2 and PGH_2 to an unstable compound which is able to relax some blood vessels and prevent platelet aggregation (Moncada et al., 1976). The formation of this compound, Prostaglandin I_2 (PGI_2) or prostacyclin, from endoperoxides is catalyzed by **prostacyclin synthetase** (Lands, 1979) or as described by Samuelson et al. (1975), **prostaglandin endoperoxide I isomerase.** This enzyme has been purified be DeWitt and Smith (1983) and immunofluorescene studies indicate that it is located in the plasma and nuclear membranes of a wide variety of smooth muscle cells (Smith et al., 1983).

Other products synthetized from arachidonic acid by the prostaglandin synthetase complex are the thromboxanes, which formation from PGG_2 and PGH_2 is catalyzed by the enzyme **tromboxane synthetase** or **prostaglandin endoperoxide tromboxane synthetase** (Hammarstrom and Falardeau, 1977).

For a long time, PGF_{2a} and PGE_2 were thought not to be interconvertible; however, the existence of an enzyme that catalyzes the formation of PGF_{2a} from PGE_2 and vice versa has been reported. **PGE_2-9-ketoreductase** activity was found in different tissues of pigeons, monkeys (Lee and Levine, 1974), humans (Niesert et al., 1987), cattle (Friedman et al., 1976) and sheep (Bolla et al., 1977).

The oxygenation of arachidonic acid through the lipoxygenase pathway results in a number of biologically active compounds. For example, one of them, 5-hydroperoxy-6,8,11,14-eicosatetraenoic acid (5-HPETE) which may be further converted into leukotrienes, seems to act as mediator in

hypersensitivity reactions and inflammation (Serhan et al., 1985). It has also been suggested that these lipoxygenase products are involved in reproductive processes such as ovulation and luteolysis (Milvae et al., 1986).

2.6.2.- Metabolism of Prostaglandins.

Biologically active PGs are synthetized and metabolized very quickly. Therefore, it is doubtful that they could be long distance acting hormones (Lands, 1979). According to the evidence presented above, it is probable that different tissues have diverse prostanoid synthetic and metabolic capacities. However, the significance of the catabolism of PGs at their production sites is not clear (Lands, 1979). PGs are regarded as freely diffusable compounds although facilitated transport processes have been reported in the rabbit (Bito et al., 1977) and rat lung (Eling and Anderson, 1976;) and rabbit vagina (Bito, 1975).

Prostaglandin biological activity is partially lost when the 15-hydroxyl group is oxidized by the catalytic action of 15-hydroxydehydrogenase (Anggard et al., 1965). This is the major pathway for cellular inactivation of PGs and has been reported to be present in most mammalian tissues, especially in lung and placenta (Lee and Levine, 1975). The activity of this enzyme appears to be very short-lived and its rapid turnover is modulated by several hormonal and other factors (Hansen, 1976; Lee and Levine, 1975). The remaining 15-keto-prostaglandin biological activity is subsequently

decreased by the 13-reductase action. Thus 13,14-dihydro-15-keto-prostaglandins are produced. These pathways show different levels of activity in different tissues and species. The rat and guinea pig lungs, for example, have a very high PG metabolic capacity, being able to remove 90-95% of either PGF_{2a} or PGE_2 from the circulation in one passage of the blood through the lungs (Eling and Anderson, 1976; Anggard et al., 1965). Conversely, when PGI_2 (prostacyclin) and its non-enzymatic formed metabolite 6-keto-PGF_{1a} pass through the lung, no inactivation occurs because their chemical structure does not allow them to match the lung carriers geometric configuration, thereby avoiding their transport into the lung cells to be inactivated (Hawkins et al., 1978). Another reason for prostacyclin delayed metabolism is that blood serum has a stabilizing effect on PGI_2 (Jorgensen et al., 1979) due to PGI_2 binding to plasma glycoproteins. Bound PGI_2 not only retains its biological activity for an extended period of time but it is equally as potent as free PGI_2 (Papp et al., 1985).

Liver cells play an important role in the degradation of PG metabolites as well, where 13,14-dihydro-15-oxo-prostaglandins are metabolized ultimately to dinor and tetranor compounds by the B and W oxidation pathways. Williams et al. (1984) reported the purification of the isoenzyme 450_{PG-w} in rabbit lung microsomes. This izoenzyme catalyzes the w-hydroxidation of prostaglandins. Its activity and levels are increased many fold during pregnancy in the

rabbit but not in the hamster or rat (Powell and Solomon, 1978; Powell, 1978; Powell, 1980). However, the significance of this W-hydroxydation pathway in the physiological processes throughout pregnancy is unknown.

2.7.- BIOLOGICAL ACTIONS OF PROSTAGLANDINS.

The ubiquity of PGs in animal tissues, the complexity of their synthesis, metabolism and regulatory processes, together with conflicting results reported in the literature about the effect of the different PGs in a variety of biological systems, makes it very difficult to review their physiological actions globally. Consequently, this review will be focussed on the effect of the different PGs in the reproductive tract and, specifically, those related to the initiation of parturition.

2.7.1.- Prostaglandins and the Corpus Luteum Function

PGF_{2a} has been reported to be luteolytic in the cycling sow although luteal cells where shown to be resistant to this effect when exogeneous PGF_{2a} is infused during the first 10-12 days of the estrous cycle (Moeljono et al., 1976; Connor et al., 1976). Contrary to this, Krzymowski et al. (1978) reported that PGF_{2a} had a partial luteolytic effect as early as 6 days after estrous in the pig, inhibiting normal corpus luteum growth and preventing the rise of plasma P4

levels. A decrease in cAMP and fatty degeneration of the luteal cells were also found, supporting the hypothesis that PGF_{2a} acts on the luteal cells by antagonizing the luteinizing hormone (LH) induced conversion of adenosine triphosphate (ATP) into cAMP. This anti-LH action may be caused by an increase in the CA^{++} influx or a release of intracellular bound CA^{++} which would prevent adenylate cyclase activity (Behrman, 1979). If cAMP is not produced, phosphatase transforms cholesteryl estearase to its inactive form resulting in a decline in P4 production (Henderson and McNatty, 1976). This action would be reversible in the short term (0-8 h) because, in spite of the presence of high affinity LH receptors, LH accumulation in the rat luteal cell is reduced during this period. However after 8 h there is a permanent reduction in LH receptor.

In rats, prolactin prevents reduction in LH uptake and the loss of LH receptors induced by PGF_{2a}. However, PGF_{2a} does not allow prolactin accumulation within the corpus luteum. The initial suppression of luteal function, therefore, could be caused by a reversible decrease in cAMP levels. The subsequent reduction in LH receptors could be due to the PGF_{2a} preventive action exerted over prolactin. This loss of receptors would make luteolysis irreversible (Behrman et al., 1978; Diekman et al., 1978).

The concept of PGF_{2a} as a luteolysin is also supported by the presence of PGF_{2a} receptors on the luteal cell surface in man, sheep, cattle, horse and rat (Keyes et al.,

1983).

Kehl and Carlson (1981) have shown that 13,14-dihydro-PGF_{2a} is four times more potent in the rabbit as a luteolysin than PGF_{2a} suggesting that metabolites, either produced within the ovary or resulting from peripheral catabolism, should be taken into account when considering luteolytic factors.

It was found that injections of PGI_2 directly into bovine corpora lutea on d 12 or 13 of the estrous cycle resulted in large increases of periphereal plasma P4 concentrations within 5 min, and levels tended to be higher than controls for at least 14 h. Similarly, P4 production by dispersed luteal cells incubated with PGI_2 was increased. Since PGI_2 has a half-life of 2 min in the blood or physiological buffer and 6-keto-PGF_{1a} did not have any effect on plasma P4 concentrations, it was concluded that PGI_2 must act through one of its metabolites (probably 6-keto-PGE_1) which elevates cAMP longer than PGI_2 (Milvae and Hansel, 1980). Nevertheless, the possibility that PGI_2 is more stable in plasma than in buffer solutions should be considered (Jorgensen et al., 1979). It appears that PGI_2 can bind to plasma glycoproteins which would stabilize prostacyclin, allowing it to be active for a longer period of time before being converted into 6-keto-PGF_{1a} (Papp et al., 1985). This binding to glycoproteins could explain the prolonged effect obtained by Milvae and Hansel (1980).

Luteal cells from early in the estrous cycle (d 5)

showed higher *in vitro* PGI_2 synthetic capacity (as measured by 6-keto-PGF_{1a}) than those cells collected in the late luteal phase in the cow, and corpora lutea collected from pregnant animals maintained PGI_2 production characteristic of the early cycle (Milvae and Hansel, 1983). The fact that the bovine corpus luteum has a very high PGI_2 synthetic activity (Sun *et al.*, 1977) and that PGI_2 and 6-keto-PGE_1 increase cAMP levels, indicate that PGI_2 could play a major luteotrophic role. On the other hand, 5-HETE, one of the hydroxyeicosatetraenoic acids produced by a lipooxygenase pathway of arachidonic acid metabolism, produces a dose related reduction in the synthesis of P4 and PGI_2 but not PGF_{2a}. It appears, therefore, that 5-HETE inhibits PGI_2 synthetase in bovine luteal tissues, indicating a luteolytic action of these derivatives. Inhibition of the lipoxygenase pathway *in vivo* with intrauterine infusions of nordihydroguaiaretic acid (NDGA), an inhibitor of the lipoxigenase pathway, resulted in delayed luteolysis and lengthening of the estrous cycle. Luteal cells collected from NDGA treated heifers on d 18 of the estrous cycle were able to produce eight times more 6-keto-PGF_{1a} than the controls (Milvae *et al.*, 1986).

A luteotrophic role for PGI_2 is also supported by *in vitro* studies done in rabbits by Abramowits and Birnbaumer (1979) when they reported a significant activation of luteal cell membrane adenylate cyclase by additions of PGI_2.

Simultaneous infusion of PGE_2 with PGF_{2a} into corpora

lutea of autotransplanted ovine ovaries antagonizes the luteolytic action of PGF_{2a} as reflected by P4 concentrations in the ovarian venous plasma (Henderson et al., 1977). It is probable that PGE_2 has an antiluteolytic property which could be mediated by an increase in adenyl cyclase activity (Abramowits and Birnbaumer, 1979). A PGE_2 antiluteolytic effect has been proposed in the ewe (Pratt et al., 1979; Reynolds et al., 1986), and in the cow (Gimenez and Henrick, 1983). Nevertheless, intrauterine administration of 5 mg of PGE_2 from d 15 to 19 of the estrous cycle twice a day, failed to show any antiluteolytic effect in heifers (Dalla-porta and Humblot, 1983). In the same context, intrauterine infusions of PGE_2 from d 12 of the estrous cycle until estrous, failed to maintain the luteal function in non-pregnant gilts (Schneider et al., 1983). Contrary to this, intrauterine administration of PGE_2 from d 7 of the estrous cycle resulted in prolongation of the cycle length in gilts, suggesting that PGE_2 has antiluteolytic properties (Akinlosotu et al., 1986). Moreover, higher PGE_2 concentrations in utero-ovarian plasma in early pregnant than in non-pregnant ewes (Ellinwood et al., 1979; Silvia et al., 1984) support the suggestions that PGE_2 is an antiluteolysin. Whether or not this increase in PGE_2 levels is a requisite for the maintenance of the luteal function is still unclear.

Estradiol-17B and PGE_2, both of which are produced by the bovine conceptus and secreted from the gravid uterus, may act synergistically to maintain luteal function during

early pregnancy (Reynolds et al., 1983).

2.7.2.- Prostaglandins and Activity of the Myometrium.

PGE_2 and $PGF_{2\alpha}$ are believed to be important mediators of the events that lead to an increased uterine contractile activity during labor (Novy and Liggins, 1980). Transient associations between the rise in plasma concentrations of PGE_2 and PGFM ($PGF_{2\alpha}$ metabolite) with an increased myometrial activity at parturition in the pig suggest that PGE_2 and $PGF_{2\alpha}$ are myometrial stimulatory compounds (Randall et al., 1986). However, their molecular actions at the cellular level, beyond the receptor interaction, are still unclear.

Myometrial contractile activity depends on the concentration of intracellular free CA^{++} which is regulated by a CA^{++}-transport system that maintains along the myometrial cell membrane, a large electrochemical gradient for ionized CA^{++}. This pump keeps CA^{++} cytoplasmic concentrations at a level of 1/10.000 of that in the extracellular space (Portzehl et al., 1964).

Possible mechanisms leading to the increased myoplasmic CA^{++} necessary to achieve uterine muscle contraction are stimulation of CA^{++} entry into the cell, inhibition of CA^{++} output from the cell, and an increased CA^{++} release from storage sites on the cell surface, sarcoplasmic reticulum and mitochondria (Deliconstantinos and Fotiou,

1986).

In 1982, Soloff and Sweet suggested that the plasma membrane of myometrial cells contains a (CA^{++} + Mg^{++})-ATPase which may be part of a CA^{++} extrusion system energized by ATP hydrolysis. The activity of this enzyme is increased in myometrial cells after the administration of estradiol to intact rats. Oxytocin, PGF_{2a} and PGE_2 associate with myometrial cells *in vitro* and inhibit (CA^{++}+Mg^{++})-ATPase activity decreasing the membrane capacity to control the influx of CA^{++} (Deliconstantinos and Fotiou, 1986). Once CA^{++} is in the cell, it binds to regulatory sites on the contractile proteins, namely actin and myosin, allowing expression of the myosin ATPase activity and thereby contraction.

PGE_2 and PGF_{2a} can elicit contractions of estrogen-treated rat myometrium; however, only PGE_2 stimulated cAMP accumulation in myometrium and endometrium (Vesin *et al.*, 1979). Therefore, the decrease in (Ca^{++} + Mg^{++})-ATPase activity and the consequent Ca^{++} influx into the myometrial cell caused by PGE_2, and also by PGF_{2a}, suggest an alternative mechanism of action of PGs to that involving variations in intracellular cAMP. PGF_{2a} may inhibit (Ca^{++} + Mg^{++})-ATPase, suppressing the extrusion of intracellular Ca^{++} in swine granulosa cells. However, PGE_2 induced an increase in the Ca^{++} exchange rate without any significant alteration in Ca^{++} pool sizes (Veldhuis *et al.*, 1987).

Gap junctions between myometrial cells are considerably increased *in vivo* during parturition in rats,

guinea pigs and humans (Garfield, 1979). The hormonal milieu at the end of gestation, apparently, facilitates the formation of gap junctions. Estrogens and P4 specifically, seem to regulate the formation of them in the mouse myometrium. Immature animals, exposed to low endogenous levels of steroids, developed fewer gap junctions than when they were treated with estrogens. Conversely, P4 by itself was not effective in increasing the number of gap junctions, but was able to inhibit estrogen stimulation when added to *in vivo* or *in vitro* systems. These experiments, done by Garfield *et al.* (1980), also provide evidence which suggests that PGs play an important role in the formation of gap junctions since the inhibition of cyclooxygenase by indomethacin decreases their number. However, they do not suggest which specific prostaglandin may be involved.

It has been suggested that PGI_2 may decrease uterine contractile activity (Omini *et al.*, 1978; Omini *et al.*, 1979; Lye and Challis, 1982). The addition of PGI_2 to human myometrial explants resulted in inhibition of contractile activity. In contrast , studies *in vivo* showed that repeated intravenous injections of PGI_2 to non-pregnant and pregnant women had no effect on contractile activity of the uterus (Wilhelmsson *et al.*, 1981). Lumsden and Baird (1986) were not able to reduce myometrial activity by continually infusing PGI_2 into the human uterine cavity. In the sow, however, evidence supporting a specific role of PGI_2 on myometrial activity during pregnancy and labor has not yet

been produced.

Omini et al. (1979), demonstrated an ability of PGI_2 to reduce the spontaneous motility of isolated pregnant human myometrium. This, and the accumulation of cAMP in the myometrial fibres, led them to suggest that PGI_2 stimulates adenylate cyclase activity. Along this line, Lye and Challis (1982) infused PGI_2 into the abdominal aorta of ovariectomized sheep achieving inhibition of spontaneous myometrial activity. This suggested that, under physiological conditions, PGI_2 uterine inhibition during pregnancy may be overcome at term by oxytocin and/or $PGF_{2\alpha}$.

Other cyclooxygenase and lipoxygenase products have been implicated in the activity of myometrium , but including them here is beyond the scope of this literature review.

2.8.- CONTROL OF PROSTAGLANDIN PRODUCTION BY INTRAUTERINE TISSUES AT LATE GESTATION.

2.8.1.- Prostaglandin Production by the Gravid Uterus.

Prostaglandin production by the uterus has been well documented and it varies according to the uterine physiological status (Thorburn and Challis, 1979). Higher PG concentrations in the utero-ovarian venous blood, either at the end of the estrous cycle or at the end of pregnancy in the ewe, suggest that the uterus is the main source of PGs. In the non-pregnant sheep, the endometrium appears to be the

most important site of prostanoid production. In the pregnant uterus, 80% of the blood flow goes to the cotyledons, 13-14% to the endometrium and 3-4% to the myometrium. The myometrium, therefore, is unlikely to contribute significantly to PG levels in the uterine vein during late pregnancy (Thorburn and Challis, 1979).

In the swine species, higher PGFM and PGE_2 concentrations in the uterine vein than in the uterine artery at late gestation clearly point to the gravid uterus as a source of PGs (Silver et al., 1979; Randall et al., 1986).

Abel and Kelly (1979) examined the in vitro prostaglandin production from ^{14}C arachidonic acid by human endometrium and myometrium, both separately and combined. The endometrium appeared to be rich in cyclooxygenase; however, it lacks the enzyme to synthetize PGI_2. Conversely, myometrial tissue has the ability to transform prostaglandin endoperoxide to PGI_2 when combined with endometrium.

Maternal cotyledons obtained from sheep after labor have a higher PGF and PGE synthetic capacity than those obtained in late pregnancy. This capacity is also enhanced after labor in fetal cotyledons and myometrium (Mitchell and Flint, 1978). Conversely, Evans et al. (1982) did not find any differences in PGE, PGF and 6-keto-PGF_{1a} concentraton in ovine myometrium and endometrium that could be attributable to gestational ages. However, higher prostanoid tissue concentration and in vitro outputs were found from cotyledons, chorioallantois and amnion sampled at late gesta-

tion than at early pregnancy (Evans et al., 1982; Risbrigder et al., 1985). This could be caused by the induction of phospholipase A_2 and C (Okazaki et al., 1981b) and cyclooxygenase enzymes (Elliot et al., 1984) at the end of gestation, in order to prepare the myometrium for parturition. Similar PG production tendencies in in vitro experiments were observed in human (Olson et al., 1983) and rabbit (Elliot et al., 1984) intrauterine tissues.

2.8.2.- Prostaglandin Concentrations in Intrauterine Fluids at Late Gestation.

PGF_{2a}, PGFM, PGE and 6-keto-PGF_{1a} amniotic fluid concentrations increase at late gestation, reaching their maximum levels at labor in sheep (Mitchell, 1977; Evans et al., 1982; Olson et al., 1984 and Olson et al., 1985), monkey (Walsh et al., 1984) and human (Mitchell, 1981). Similarly, PGF_{2a}, PGE_2 and 6-keto-PGF_{1a} allantoic fluid concentrations increase dramatically at the end of gestation in sheep (Evans et al., 1982) and swine (Silver et al., 1979).

2.8.3.- Control of Prostaglandin Production within the Gravid Uterus.

Manzai and Liggins (1984) found that human amniotic cells had a greater PGE_2 production capacity than cells obtained from endometrium near term but before labor. This PG

production was higher in tissues obtained at labor. Nonetheless, when both types of tissues were obtained before labor and incubated together, PG production by endometrial cells decreased by 25%, suggesting that a substance that inhibits endometrial production is secreted by amniotic cells. The activity of this substance appears to be decreased at labor. In an analogous manner, bovine caruncle extracts obtained before labor inhibited PG synthesis by cotyledon cells, but when caruncles were obtained at term, this inhibitory activity was absent. Similarly, caruncle cells harvested from pre-term placentomes and co-cultured with fetal cotyledon cells produced suppression of prostanoid synthesis by the fetal cells. This inhibition, however, was not observed when using term caruncle and fetal cotyledon cells, suggesting a pre-term PG synthesis inhibition factor (Shemesh et al., 1984a).

Additions of mid-gestation bovine placental extracts to bovine seminal vesicle microsomes, which are able to synthetize PGs resulted in a greater inhibition of prostanoid production than when term placental extracts were added (Shemesh et al., 1984b).

Data presented by Risbridger et al. (1985), showed an increased ability of isolated ovine trophoblastic cells to synthetize PGs with increasing gestational age. Saeed and Mitchell (1982), using term but pre-labor cytosolic fractions from human intrauterine tissues and bovine seminal vesicle microsomes, found that cytosol homogenates stimulated

PG synthesis. Using this same microsome assay to test the effect of human amniotic (Saeed et al., 1982 and Cohen et al.., 1985) and ovine allantoic fluids (Leach-Harper and Thorburn, 1984) upon PG synthesis, it was found that those fluids obtained during early gestation had the property of producing greater PG synthetic inhibition than those fluids collected at late gestation. Furthermore, when amniotic fluid was passed through a membrane with molecular weight exclusion of 30,000, both an inhibitory and a stimulatory fraction were isolated. This inhibitory fraction was more active in samples obtained either during early pregnancy or before labor than during labor. In contrast, the stimulatory fraction activity was greater in amniotic fluid obtained at term than in that collected during early pregnancy (Cohen et al., 1985).

Treatment of either bovine seminal vesicle microsomes or human amnion cultured cells with human adult or fetal urine resulted in a dramatic increase of PG production. This stimulation was greater when urine was obtained from fetuses delivered at term by cesarean section after the initiation of labor than when the urine was collected during cesarean section prior to labor (Strickland et al.., 1983). Further biochemical studies suggest that a low molecular weight polypeptide is contained in the urine, and once transferred to the amniotic fluid at the end of gestation, acts on the amniotic epithelium increasing PG synthesis (Casey et al., 1983; Casey et al., 1984a). This fac-

tor has also been described as a growth factor-like substance produced by the fetal kidney (Casey et al., 1984b).

The presence of B-adrenergic receptors in the human amnion, the stimulation of arachidonate mobilization by B-adrenergic compounds, the transitory accumulation of cAMP and the enhanced production of PGE_2 at the end of pregnancy are facts that, together with the increase of catecholamines in amniotic fluid during the third trimester of gestation, indicate that catecholamines may be regulators of PG synthesis by amnion during parturition (Di Renzo et al., 1984).

The presence of pregnancy-associated PG synthetase inhibitors , identified in the amniotic epithelium before the onset of human labor, indicates that these epithelial cells play a local role in triggering parturition (Mortimer et al., 1985).

The activity of 15-hydroxy-PG-dehydrogenase increases significantly as a function of gestational age in human placenta (Keirse et al., 1985). This, and the increase of PG endoperoxide synthetase in human pregnant myometrium, when compared to non-pregnant uterus, demonstrate enzymatic changes related to pregnancy . These changes may, in some way, control the production of PGs during pregnancy (Moonen et al., 1984). Studies with ovine intrauterine tissues revealed that maternal cotyledons contained more 15-hydroxy-PG-dehydrogenase than fetal cotyledons or myometrium. At parturition, this enzymatic activity decreased in myometrium but increased in maternal and fetal tissues. Notwithstanding

these changes *in vitro*, a consistent release of PGFM into the utero-ovarian vein *in vivo* did not occur, suggesting that this enzymatic activity may have a localized effect during parturition (Keirse *et al.*, 1977). P4 concentration in human placental tissue is positively correlated with 15-hydroxy-PG-dehydrogenase; therefore, this enzyme could be controlled by P4 (Falkay and Sas, 1978; Braithwaite *et al.*, 1975).

Based on this literature review, a study to investigate PG production in swine during late pregnancy was proposed. Specifically, this project was divided into three experiments. In the first part, the concentrations of PGFM, PGE_2 and 6-keto-PGF_{1a} in maternal/fetal plasma, amniotic fluid and fetal urine were measured at three time periods in late pregnancy. This facilitated the establishment of temporary changes in PG levels in late gestation, the proportional changes in the stimulatory:inhibitory PG ratio during these periods and PG sites of production. In the second part, the *in vitro* prostaglandin production capacities of three different placental tissues were examined at the same time periods. The effect of fetal fluids on prostanoid output by one of these tissues was also assessed. Finally, in the third experiment, the effects of fetal fluids on prostaglandin synthesis by a microsome enriched preparation obtained from bovine seminal vesicles, was investigated.

CHAPTER 3

EXPERIMENT I

PROSTAGLANDIN CONCENTRATION IN MATERNAL AND FETAL BLOOD PLASMA, AMNIOTIC FLUID AND FETAL URINE AT LATE GESTATION IN THE SOW.

3.1.- INTRODUCTION.

This experiment was designed to study variations in blood plasma, fetal urine and amniotic fluid PGF_{2a}, PGF_{2a} metabolite (PGFM), PGE_2 and 6-keto-PGF_{1a} concentrations in the pregnant sow at three different gestational stages, namely, 109, 114 days of gestation and during labor. Differences between arterial and venous PG concentrations in the uterine and umbilical vessels would suggest a predominant site of prostaglandin production during late gestation.

3.2.- MATERIALS AND METHODS.

3.2.1.- Experimental design.

Fifteen Yorkshire gilts averaging 160.9 ± 17.3 kg

body weight were divided into three groups of five each according to gestational age, namely, 109, 114 days of gestation and during labor. The animals were housed in collective pens in the same building and fed commercial pellets containing 15 % protein. Supply of water was ad ad libitum. Those gilts in the at labor group were penned individually 48 h before the due day. Sows were considered in labor once the first piglet was delivered vaginally, and pre-labor sows were those that did not show parturition behaviour before sampling (Randall et al., 1986). In order to obtain blood samples from the umbilical artery, umbilical vein, amniotic fluid and fetal urine, cesarean sections were performed, and two piglets were randomly chosen to be sampled from each sow. Blood samples from a uterine vein and a uterine artery were collected as well. All samples were extracted and PG concentration measured by radioimmunoassay (RIA)(Fig.1C). The animals utilized in this experiment were the same as those used in experiments 2 and 3 of this thesis.

3.2.2.- Surgical Procedures.

General anesthesia was induced in the gilts with a dose to effect (approximately 10 mg/kg) of sodium thiopental injected intravenously and maintained with 5% halothane in O_2. In order to collect samples, cesarean sections by a midline laparotomy under asceptic conditions were carried out. Before opening the uterine wall, blood samples from a middle uterine artery and middle uterine vein were taken. Immediately after the incision of the uterus, blood samples from the umbilical artery and umbilical vein as well as amniotic fluid were collected. Once the fetuses were delivered, fetal urine was collected by direct puncture of the urinary bladder. Samples were transferred to chilled EDTA tubes, centrifuged at 1500 x g for 15 minutes and plasma stored at -20°C pending RIA.

3.2.3.- Extraction of Samples.

Samples were diluted with 2.2 volumes of twice distilled deionized water, acidified with 0.5 volume of 2 M citric acid and extracted once with 10 volumes of ethyl acetate. Samples were shaken for 10 min and centrifuged at 800 x g for 5 min. Ethyl acetate layers were then transferred to 12x75 mm borosilicate tubes and evaporated under air at room temperature. Once ethyl acetate was evaporated,

extracted samples were resuspended in 200 ul of 2 M phosphate buffered saline (PBS) containing 0.1% gelatin.

In order to estimate the extraction efficiency, about 3000 radioactive counts per minute (CPM) of PG tracer in 100 ul PBS were added to tubes containing 100 ul buffer solution which were then extracted using the above mentioned procedure. Extraction efficiency for PGF_{2a}, PGFM, PGE_2 and 6-keto-PGF_{1a} tracers were 68%, 67%, 69% and 53%, respectively.

3.2.4.- Radioimmunoassay.

Standard solutions ranging from 12.5 to 500 pg of PGFM were prepared in 200 ul PBS at pH 6.9 with 0.1 % gelatin in borosilicate culture tubes. Antiserum against PGFM and tritium labelled PGFM were added successively in 100 ul aliquots, so that each tube contained about 5000 CPM. Tubes were agitated and incubated overnight. In order to separate the antibody bound PGFM from the free fraction, 0.5 ml of 2 M PBS containing 0.05 % dextran T-70 and 0.5 % charcoal was added to the tubes which were then centrifuged at 1500 x g for 10 min; 0.6 ml of the resultant supernatant was pipetted into plastic scintillation vials, mixed with 4.5 ml of scintillation fluid (Scintverse I) and counted for 5 min in a Rackbeta 1215 LKB liquid scintillation counter.

Once the unknowns were extracted, 200 ul PBS were added to the tubes and subsequently treated like the standards as above. In order to determine PGFM medium concentra-

tions, CPM resulting from counting the radioactive bound fraction in the unknown samples were compared with those obtained in the standards. The same assay procedure was repeated for PGE_2, PGF_{2a} and 6-keto-PGF_{1a} using the appropiate antibodies.

Intra-assay coefficients of variation for PGFM, PGE_2, PGF_{2a} and 6-keto-PGF_{1a} tests were 1.4% (n=4), 2.5% (n=4), 3.0% (n=4) and 2.5% (n=4), respectively. All samples were analyzed in a single assay for each prostaglandin; hence, there was no inter-assay variation. When plasma (QC) known to contain 50, 100 and 200 pg/ml of each of PGFM, PGE_2, PGF_{2a} and 6-keto-PGF_{1a} were assayed for recovery, the estimated concentrations (pg/ml) were as follows:

QC	PGFM	PGE_2	PGF_{2a}	6-keto-PGF_{1a}
50	108.8	50.3	77.4	49.2
100	174.5	110.7	123.4	103.5
200	263.1	263.3	244.3	228.5

PGFM, which is PGF_{2a} stable metabolite, was measured in blood plasma samples as an indicator of PGF_{2a} concentration. (Salmon and Karim, 1976). Antibody cross-reactivities are presented in Appendix 1.

3.2.5.- Statistical Analysis.

Analysis of variance was performed using the

General Linear Model procedure of the Statistical Analysis System Statistics Package (SAS, 1982).

The linear model used was a nested design, where the effect of sow was nested within gestational age and the effect of fetuses was nested within sow within gestational age. The effect due to different samples was cross-classified with fetus within sow within gestational age. This resulted in the following linear model:

$Y_{ijkl} = u + A_i + B_{ij} + C_{ijk} + D_l + G_{ijkl}$

u = overall mean of the response variable.

A_i = effect of i^{th} gestational age.

B_{ij} = effect of j^{th} sow within i^{th} gestational age. $B_{ij} \sim NID(0, {}^2_{sow})$.

C_{ijk} = effect of k^{th} fetus within j^{th} sow within i^{th} gestational age $B_{ijk} \sim NID(0, {}^2_{fetus})$.

D_l = effect of l^{th} sample.

e_{ijkl} = error term $NID(0, {}^2)$

i = 1,..,3.

j = 1,...,5.

k = 1,2.

l = 1,..,4

Sow and fetus were considered as random effects, hence tests of gestational ages were against sow within gestational age. Furthermore, sow effect was tested against fetus within sow. In order to make multiple comparisons within effects a LSM "t" test was performed.

Significant differences were declared at probability levels less than 5% (Steel and Torrie, 1980).

3.3.- RESULTS.

Least-squares means (LSM) of PG concentrations in plasma and fetal fluids are presented in Table 1.

LSM "t" test revealed, in both maternal and fetal blood samples, higher PGFM, PGE_2 and 6-keto-PGF_{1a} plasma concentrations at labor than at d 114 or at d 109 of gestation. Exceptions to this were PGE_2 concentrations in fetal and maternal arterial plasma which did not increase during labor.

On the other hand, PGFM, PGE_2 and 6-keto-PGF_{1a} concentrations in samples collected during labor were higher in plasma from the umbilical vein than that from the umbilical artery. Similarly, uterine vein plasma tended to have higher ($p < 0.1$) PGFM and PGE_2 but not 6-keto-PGF_{1a} concentrations than uterine artery plasma during labor. However, these differences were not significant in plasma obtained at either d 109 or d 114 of gestation.(Figs. 2-4).

Ratios between venous and arterial plasma concentrations for PGFM, PGE_2 and 6-keto-PGF_{1a} at d 109, d 114 and during labor in umbilical and uterine blood are presented in Table 2. This ratio is greater in samples obtained from fetal and maternal vessels during labor than in those collected either at d 109 or d 114 of gestation. In

turn, samples taken at d 114 have a greater ratio when contrasted with those collected at d 109. On the other hand, 6-keto-PGF_{1a} does not follow this tendency in fetal blood samples collected during labor since the venous-arterial concentration ratio remains analtered during labor (Table 2 and Figs. 5-6).

In Table 1 it can be seen that fetal urine collected during labor contained more PGFM, PGE_2 than that sampled either at d 109 or d 114. In contrast, 6-keto-PGF_{1a} and PGF_{2a} concentrations remained unaltered (Table 1 and Fig. 7).

In amniotic fluid, PGFM and PGE_2 levels increased considerably during labor, PGF_{2a} did not change and 6-keto-PGF_{1a} decreased when compared with d 114. However, only PGE_2 and 6-keto-PGF_{1a} had higher levels at d 114 than at d 109. PGFM and PGF_{2a} levels did not differ between the latter two periods. Concentration of 6-keto-PGF_{1a} in amniotic fluid at d 109 was equal to that obtained during labor, both of them being lower than at d 114 (Table 1 and Fig. 8).

TABLE 1 Least-squares means of PGFM (FM), PGE2 (E2), 6-keto-PGF_{1a} (6K-PG) and PGF_{2a} (F2) concentrations (ng/ml plasma) in the umbilical artery (FA), umbilical vein (FV), uterine artery (MA), uterine vein (MV), fetal urine (FU) and amniotic fluid (AMF) at different gestational stages.

Gest. Stage	day 109 (10)				day 114 (10)				LABOR (10)			
Sample	FM	E2	6K-PG	F2	FM	E2	6K-PG	F2	FM	E2	6K-PG	F2
FA	2.6 ±0.4	1.1 ±1.4	1.4 ±0.2	--	b 3.4 ±0.4	1.2 ±1.4	1.6 ±0.2	--	8.8* ±0.4	3.4 ±1.4	2.2* ±0.2	--
FV	2.6 ±0.4	2.2 ±1.4	1.8 ±0.2	--	b 3.9 ±0.4	2.9 ±1.4	2.0 ±0.2	--	14.3* ±0.4	13.3* ±1.4	a 3.1 ±1.1	--
MA	1.5 ±1.3	1.0 ±1.1	0.8 ±0.2	--	b 4.1 ±1.3	1.2 ±1.1	1.0 ±0.2	--	16.7* ±1.3	3.1 ±1.1	a 1.5 ±0.2	--
MV	1.4 ±1.3	1.1 ±1.1	0.7 ±0.2	--	b 4.8 ±1.3	1.5 ±1.1	0.8 ±0.2	--	20.1* ±1.6	6.1* ±1.3	1.6* ±0.2	--
FU	10.4 ±1.6	2.3 ±0.8	5.7 ±0.7	1.2 ±0.7	13.7 ±1.9	3.6 ±0.9	7.1 ±0.9	2.3 ±0.6	23.3* ±2.0	8.0* ±0.9	8.2 ±0.9	2.5 ±0.6
AMF	6.2 ±0.9	1.4 ±0.5	3.4 ±1.9	0.7 ±0.6	b 9.8 ±1.1	b 3.1 ±0.6	a 10.3 ±2.2	2.1 ±0.4	13.9* ±1.8	7.2* ±1.0	8.0 ±3.8	1.8 ±0.6

(n) Number of fetuses.

* $p < 0.05$ when compared with d 109 or d 114.
a $p < 0.05$ when compared with d 109.
b $p < 0.05$ when compared with d 109 or labor.

TABLE 2 Ratio between venous (V) and arterial (A) prostaglandin plasma concentrations in the umbilical (F) and uterine (M) vessels collected at day 109, day 114 of gestation and during labor.

	PGFM			PGE_2			6-keto-$PGF_{1\alpha}$		
	109	114	LABOR	109	114	LABOR	109	114	LABOR
FV/FA	1.00	1.15	* 1.63	2.00	2.42	* 3.91	1.29	1.25	1.41
MV/MA	0.93	1.17	* 1.20	1.10	1.25	* 1.97	0.88	0.80	* 1.07

* $p < 0.05$ when compared with d 109 or d 114

TABLE 3 Ratio between PGFM-PGE_2 combined and 6-keto-$PGF_{1\alpha}$ concentrations in the umbilical artery (FA), umbilical vein (FV), uterine artery (MA), uterine vein (MV), fetal urine (FU) and amniotic fluid (AMF) sampled at day 109, day 114 of gestation and during labor.

	109	114	LABOR
FA	2.6	2.9	* 5.5
FV	2.7	3.4	* 8.9
MA	3.1	5.3	* 13.3
MV	3.6	7.9	* 16.4
FU	2.2	2.4	3.7
AMF	2.2	1.3	2.6

*$p < 0.05$ when compared with d 109 or d 114

FIG. 2 PGFM VALUES IN THE UMBILICAL ARTERY (FA), UMBILICAL VEIN (FV), UTERINE ARTERY (MA) AND UTERINE VEIN (MV) AT DIFFERENT GESTATIONAL STAGES.

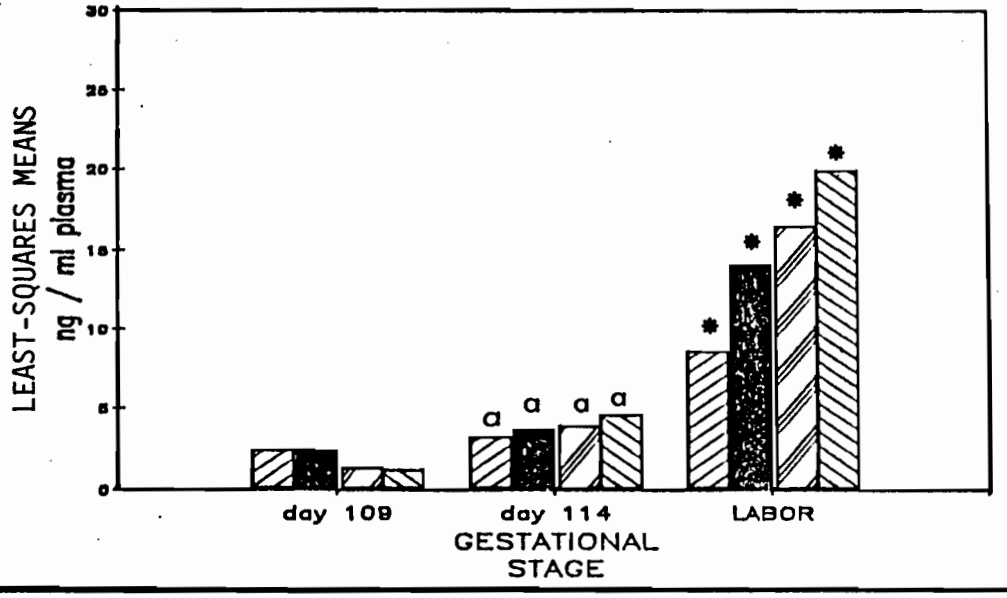
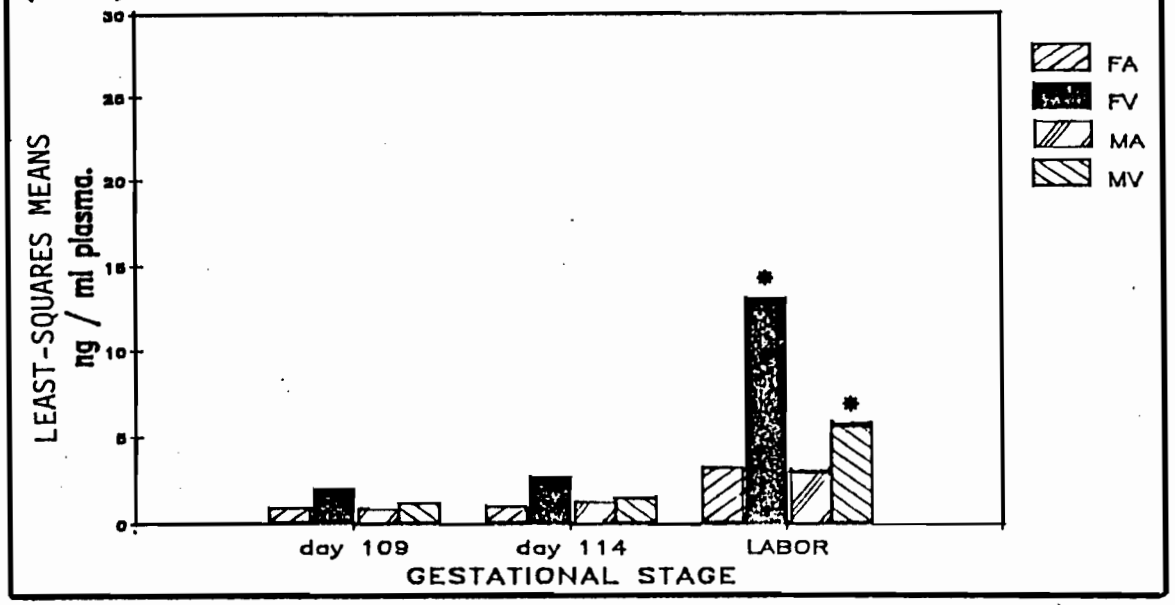


FIG. 3 PGE2 VALUES IN THE UMBILICAL ARTERY (FA), UMBILICAL VEIN (FV), UTERINE ARTERY (MA) AND UTERINE VEIN (MV) AT DIFFERENT GESTATIONAL STAGES.



a $p < 0.05$ when compared with d 109

* $p < 0.05$ when compared with d 109 or d 114

FIG. 4 6-KETO-PGF1$_\alpha$ VALUES IN THE UMBILICAL ARTERY (FA), UMBILICAL VEIN (FV), UTERINE ARTERY (MA) AND UTERINE VEIN (MV) AT DIFFERENT GESTATIONAL STAGES.

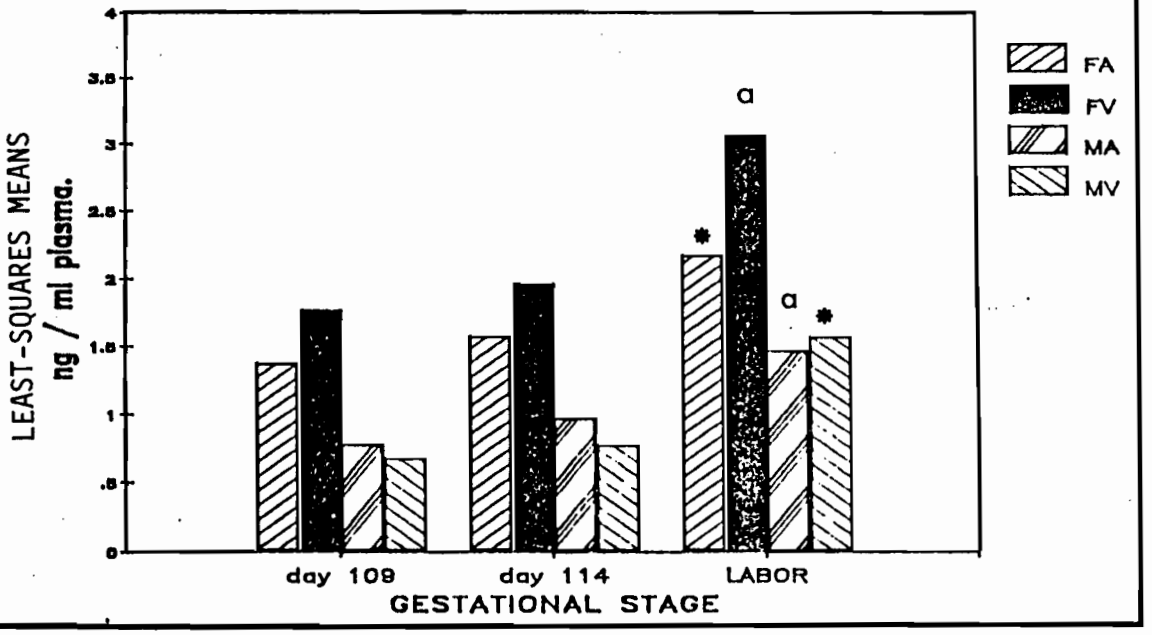
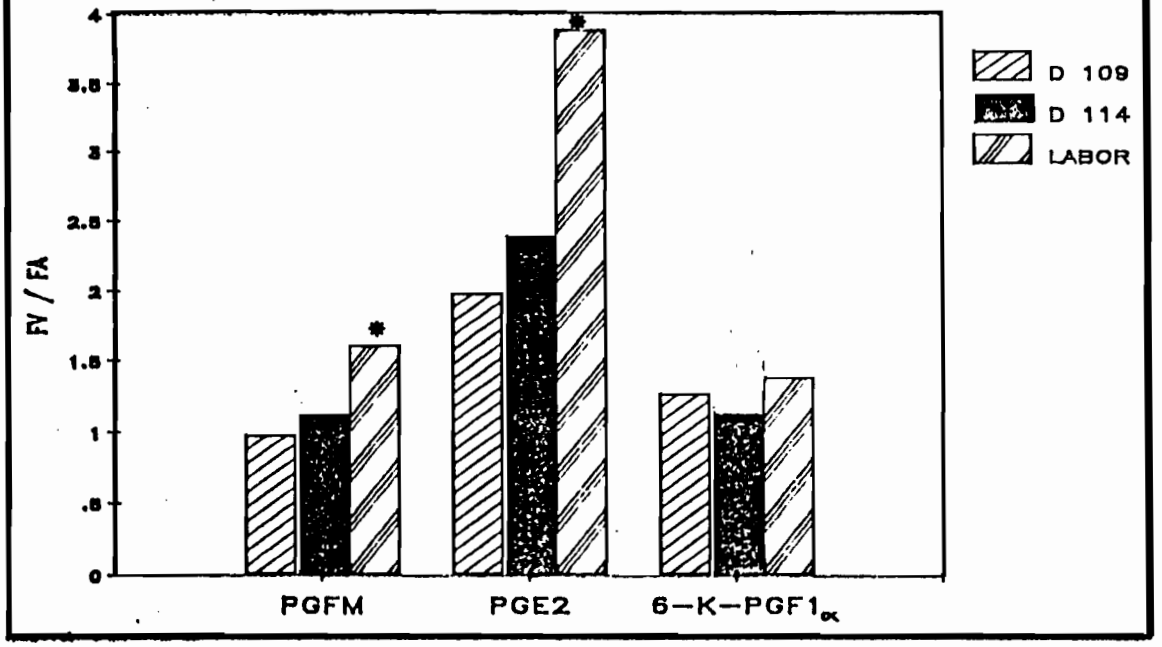


FIG. 5 RATIO BETWEEN VENOUS AND ARTERIAL PLASMA PROSTAGLANDIN CONCENTRATIONS IN THE UMBILICAL VEIN (FV) AND UMBILICAL ARTERY (FA) AT DIFFERENT GESTATIONAL STAGES.



* $p < 0.05$ when compared with d 109 or d 114

a $p < 0.05$ when compared with d 109

FIG. 6 RATIO BETWEEN VENOUS AND ARTERIAL PLASMA PROSTAGLANDIN CONCENTRATIONS IN THE UTERINE VEIN (MV) AND UTERINE ARTERY (MA) AT DIFFERENT GESTATIONAL STAGES.

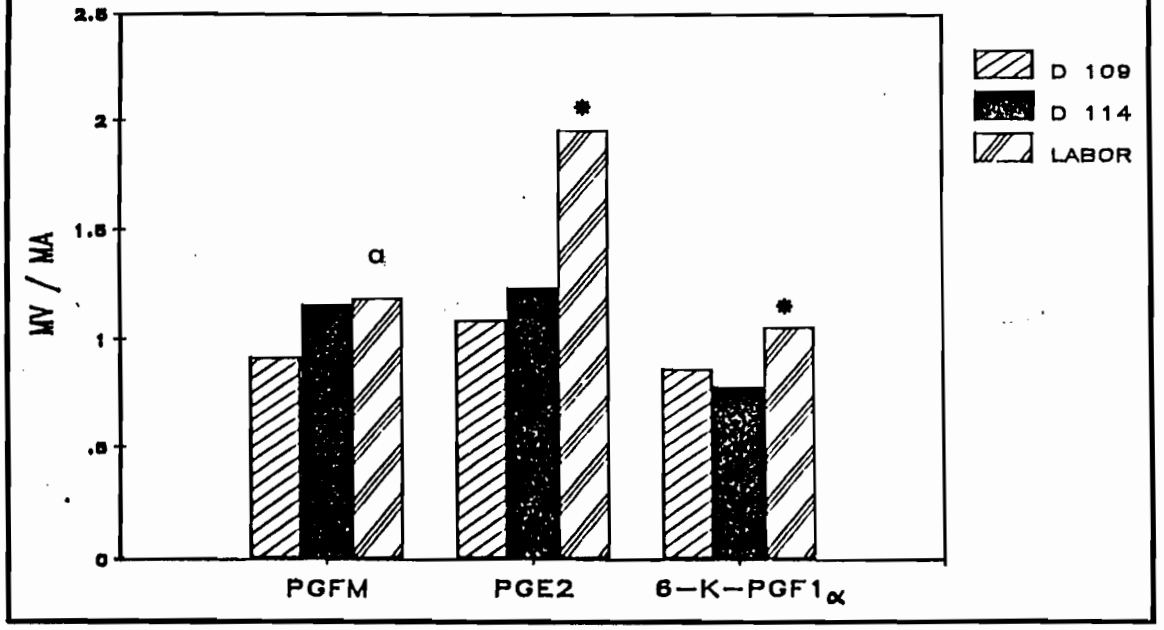
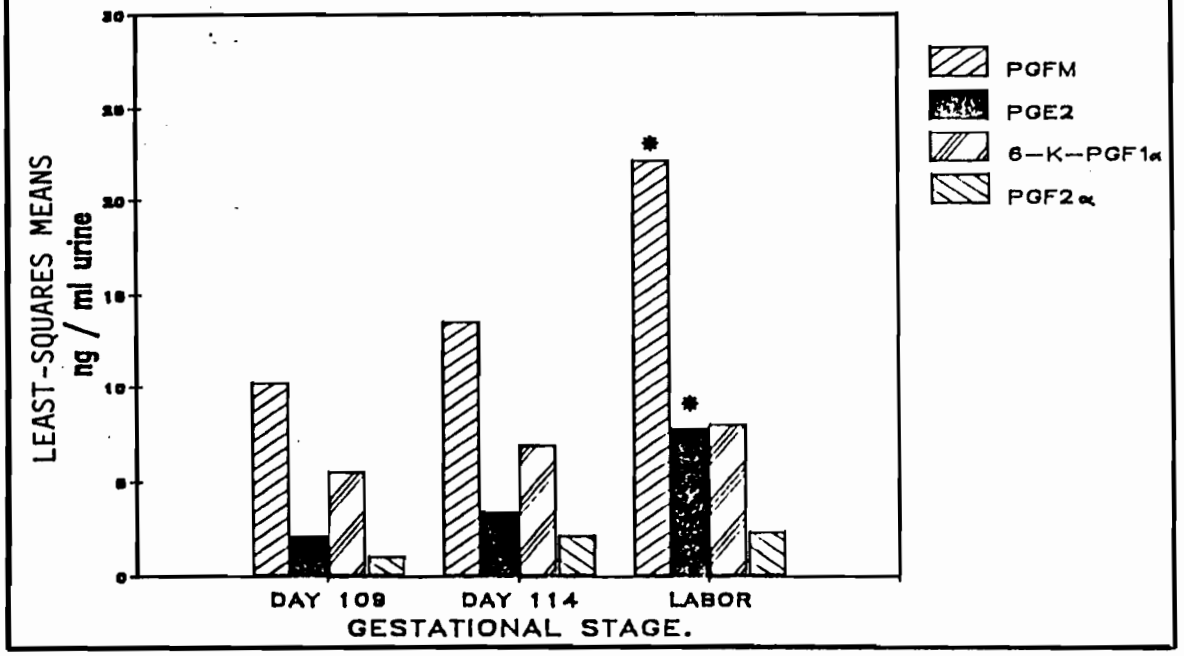


FIG. 7 PROSTAGLANDIN CONCENTRATIONS IN FETAL URINE COLLECTED AT DIFFERENT GESTATIONAL STAGES.



a $p < 0.05$ when compared with d 109

* $p < 0.05$ when compared with d 109 or d 114

FIG. 8 PROSTAGLANDIN CONCENTRATIONS IN AMNIOTIC FLUID COLLECTED AT DIFFERENT GESTATIONAL STAGES.

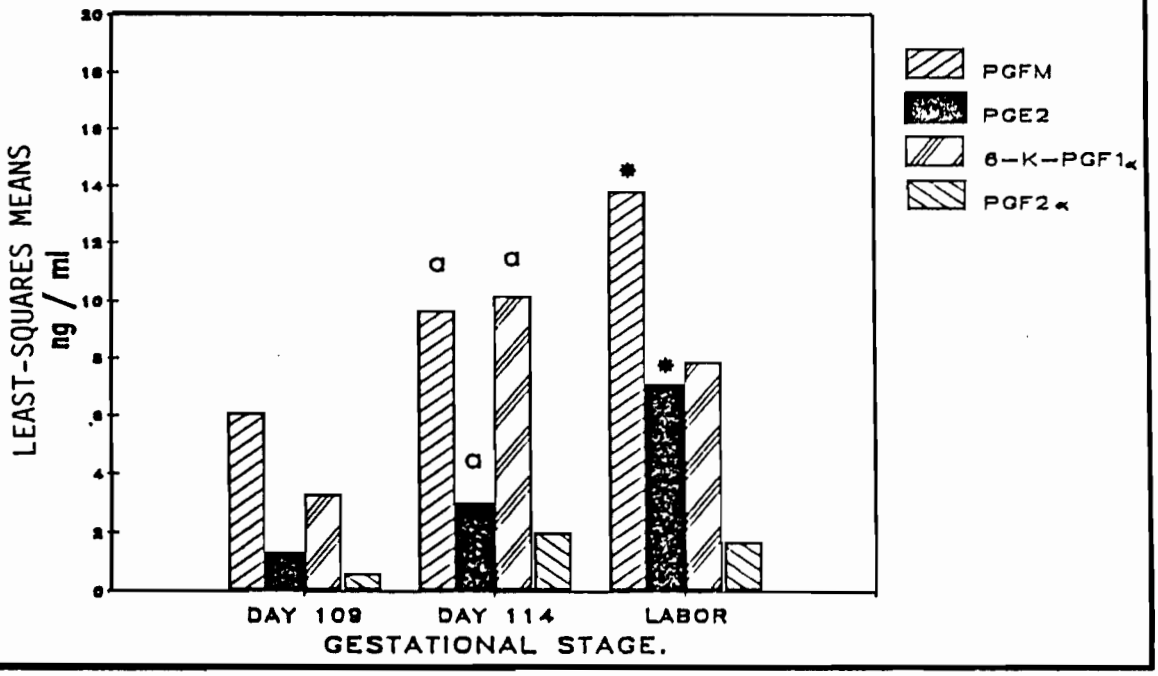
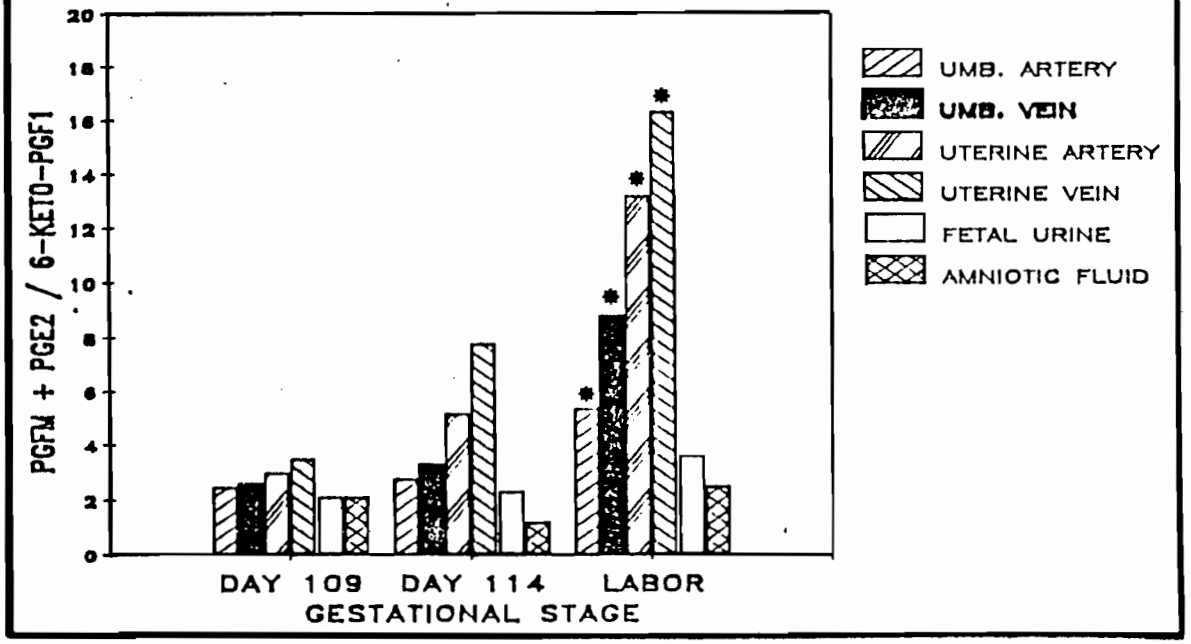


FIG. 9 RATIO BETWEEN PGFM-PGE2 COMBINED AND 6-KETO-PGF1α CONCENTRATIONS IN FETAL AND MATERNAL VESSELS, AND FETAL FLUIDS.



a $p < 0.05$ when compared with d 109

3.4.- DISCUSSION.

This study demonstrated that blood PG levels experience a significant change during labor in both maternal and fetal compartments (Table I Figs. 2-6, and 9). This tendency was reflected in fetal urine and amniotic fluid (Table 1 and Figs. 7-9).

Dramatic increases of blood PGFM and PGE_2 concentrations during labor, when compared to d 109 and d 114 of gestation, are in agreement with results obtained by Silver et al. (1979) and Randall et al. (1986). However data on 6-keto-PGF_{1a} have not been reported previously in swine.

Changes in plasma PGFM concentrations during parturition probably reflect an increase in PGF_{2a} production (Salmon and Karim , 1976) which may be involved in increasing uterine contractile activity once parturition is triggered (Randall et al.,1986). Presumably at that stage of gestation, luteolysis has already taken place and PGF_{2a} secretion is mainly directed toward contraction of the myometrium. Although PGFM is higher at d 114 of pregnancy both in maternal and fetal vessels than at d 109 of gestation, its levels are much lower than during labor. Since luteolysis, as reflected by plasma P4 concentrations, appears to begin 2-4 days before the initiation of labor (Randall et al., 1986), it could be suggested that, if PGF_{2a} is the luteolytic factor at the end of pregnancy, levels of PGF_{2a}

required to cause luteolysis are probably far lower than those required to stimulate the myometrium during labor. In support of this were the intense uterine contractions observed only when cesarean sections were performed in gilts already in labor. This activity was not detected either at d 109 or at d 114 of gestation, and at these stages behavioral signs of labor were not detected either. Whether PGFM is a cause or the result of this myometrial activity is still unclear, since muscular contraction may cause transient anoxia which, paradoxically, stimulates PG synthesis (Lands, 1979). It also should be taken into account that when labor is triggered there are other hormones like cortisol, estrogens, oxytocin and relaxin (First et al., 1982) which undergo dramatic changes in concentrations at this stage and could also influence uterine contractile activity.

Plasma PGE_2 levels in maternal and fetal compartments do not differ between d 109 and d 114. However, as in the case of PGFM, there is a marked PGE_2 increase during labor (Table 1), except in maternal and fetal arterial plasma in which the increase is not significant. These results are consistent with those obtained by Randall et al. (1986) and Silver et al. (1979).

In contrast to PGF_{2a}, PGE_2 has antiluteolytic and luteotrophic properties due to its capacity of stimulating cAMP production (Abramowits and Birnbaumer, 1979; Pratt et al., 1979; Reynolds et al., 1986). However, both PGE_2 and PGF_{2a}, have been associated with increased myometrial ac-

tivity (Vesin et al., 1979; Novy and Liggins, 1980; Randall et al., 1986). They are expected, therefore, to be increased during labor. This uterine activity could be increased due to the inhibition of a muscle cell membrane ($Ca^{++}+Mg^{++}$)ATPase (Soloff and Sweet, 1982) resulting in a rapid influx of Ca^{++} into the cell and thereby contraction.

On the other hand, the enzyme PGE_2-9-ketoreductase, which catalyzes the formation of PGF_{2a} from PGE_2 and vice-versa, may also be activated during labor in order to increase the availability of PGF_{2a} (Niesert et al., 1987). To what extent this formation of PGF_{2a} from PGE_2 contributes to PGFM plasma concentrations during labor, can not be determined from these data.

Since PGI_2 is a very unstable compound in plasma and buffered solutions, its metabolite 6-keto-PGF_{1a} is widely accepted as an estimative measure of PGI_2 concentrations in different fluids (Olson et al., 1985).

Maternal and fetal plasma 6-keto-PGF_{1a} concentrations increase during labor when compared with either of those at d 109 or at d 114 of gestation. Nevertheless, this increase is proportionally smaller when contrasted to PGFM and PGE_2 levels during labor. This is consistent with studies done in the sheep (Olson et al., 1985).

PGI_2 has been shown to be luteotrophic in the cow (Milvae and Hansel, 1983; Milvae et al., 1986). This property, in rats, has been attributed to its capacity to increase cAMP in luteal cells (Abramowits and Birnbaumer,

1979). This results in an increase in P4 secretion which, together with the intrinsic property of PGI_2 of decreasing uterine activity (Omini et al., 1978; Omini et al., 1979; Lye and Challis, 1983), would provide the necessary quiscent uterine environment during pregnancy. Notwithstanding, in vivo studies in human species have produced controversial evidence regarding the role of PGI_2 upon myometrial activity (Lumdsen and Baird, 1986; Wilhelmson et al., 1981).

In this experiment, although maternal and fetal 6-keto-PGF_{1a} plasma concentrations increased during labor, the ratio between the combination of PGFM-PGE_2 and 6-keto-PGF_{1a} values (Table 3 and Fig. 9) shows very clearly that, if PGI_2 is an inhibitor of myometrial contractile activity, its effect would probably be easily overidden by the high PGF and PGE_2 production during labor.

The ratio between venous and arterial PG concentrations in fetal blood was always equal to or greater than one and tended to increase proportional to gestational age (Table 2), being highest at the moment of labor. The only exception to this is 6-keto-PGF_{1a} in which this ratio does not change considerably. Nevertheless, these fetal ratios suggest that the fetal compartment is recieving PGs produced by the uterus, the placental membranes or another maternal compartment organ. Futhermore, this ratio in the maternal blood also tended to increase with gestational age and, at least in the case PGFM and PGE_2, is always greater than one indicating, therefore, that the gravid uterus is an important source of

these prostanoids. This is in agreement with previous studies in the ovine (Thorburn and Challis, 1979) and in the porcine species (Silver et al., 1979; Randall et al., 1986). Although that we have demonstrated in experiment 2 of this thesis the capacity of the porcine placenta to produce PGs, the endometrial and myometrial PG synthetic capacity in this species still remains to be investigated . The ovine myometrium is unlikely to contribute significantly to PG levels in the uterine vein (Thorburn and Challiss, 1979).

Higher 6-keto-PGF_{1a} concentrations in the umbilical vein than in the umbilical artery suggest that this prostanoid is secreted from the gravid uterus towards the fetal compartment. It is also possible that, since the 6-keto-PGF_{1a} venous:arterial ratio in the maternal vessels is always less or equal to one, 6-keto-PGF_{1a} may be transferred from the maternal to fetal compartment. It is probable as well that during labor 6-keto-PGF_{1a} might be further metabolized in the uterus to 6-keto-PGE_1 which has been shown to increase myometrial contractile activity (Lye et al., 1983).

PG concentrations in fetal urine and amniotic fluid are considerably higher than in either maternal or fetal plasma at d 109 or at d 114 (Table 1 and Figs.7-8). PG levels are 2-4 times greater in fetal urine and 1-3 times higher in AMF than in either maternal or fetal plasma. This increase in PG levels could be due to the urine concentrating capacity of the fetal kidney and to the kidney's capacity to produce PGs (Bohman, 1977; Walker and Mitchell, 1978).

However, it is interesting to note that, once labor is triggered, the stimulatory-inhibitory PG ratio (PGFM+PGE_2:6-keto-PGF_{1a}) in fetal urine and in amniotic fluid does not vary as dramatically as in blood plasma (Table 3 and Fig. 9). Since 15-hydroxy-PG-dehydrogenase activity is not present in amniotic fluid (Okazaki et al., 1981a), it may be possible that PGs do not undergo rapid changes once they are stored in the amniotic cavity and urinary bladder.

In the human, subject amniotic fluid PGs are derived mainly from the placental membranes rather than from fetal urine (Reddi et al., 1985) . Nevertheless, in swine, fetal urine can pass from the urinary bladder either through the urethra to the amniotic sac or through the urachus to the allantoic cavity (Noden and de Lahunta , 1985). This together with high PG concentrations found in the fetal urine suggest that, even though the placental membranes are able to produce PGs (Experiment 2, Tables 4-7), fetal urine in effect might contribute to amniotic and allantoic fluid PG levels.

High levels of PGFM in the fetal urine may also reflect levels in fetal plasma, which in turn may be influenced by maternal PGFM and PGF_{2a} concentrations. Consequently, the clearence of PGFM by the fetal kidney at the moment of parturition may be also increased. The porcine placenta appears to have the capacity to transform PGF_{2a} into PGFM (Experiment 2, Fig.10 and 12). This could also contribute to high PG levels in fetal fluids.

In summary, in this experiment it has been

demonstrated that : **a)** PG concentrations in maternal and fetal plasma, fetal urine, and amniotic fluid increase dramatically during labor; **b) the stimulatory** (PGFM + PGE_2) to inhibitory (6-keto-PGF_{1a}) PG ratio also increases considerably during labor, meaning that the cyclooxygenase enzymatic complex is regulated differentially by an unknown mechanism resulting in the production of those PGs which increase myometrial contractile activity; **c)** based on venous and arterial PG concentrations, it can be concluded that venous blood plasma draining from gravid uterus has higher PG levels compared to arterial plasma. Therefore, the gravid uterus can be considered as an important PG production site during late pregnancy.

CHAPTER 4

EXPERIMENT 2

IN VITRO PROSTAGLANDIN PRODUCTION BY LATE GESTATION PORCINE PLACENTAL CELLS NON-TREATED AND TREATED WITH FETAL FLUIDS.

4.1.- INTRODUCTION.

The literature review presents information related to the capacity of fetal membranes obtained from different species, other than swine, to produce PGs. Based on this evidence an experiment was designed to assess PG synthetic capacity of each of three different porcine placental tissues. The specific objectives of this study were to determine which of the selected tissues had the greatest ability to produce PGs in vitro and how this capacity varies with gestational age. The effect of pre-term and term fetal urine and pre-term amniotic fluid upon PG production by placental cells was also studied.

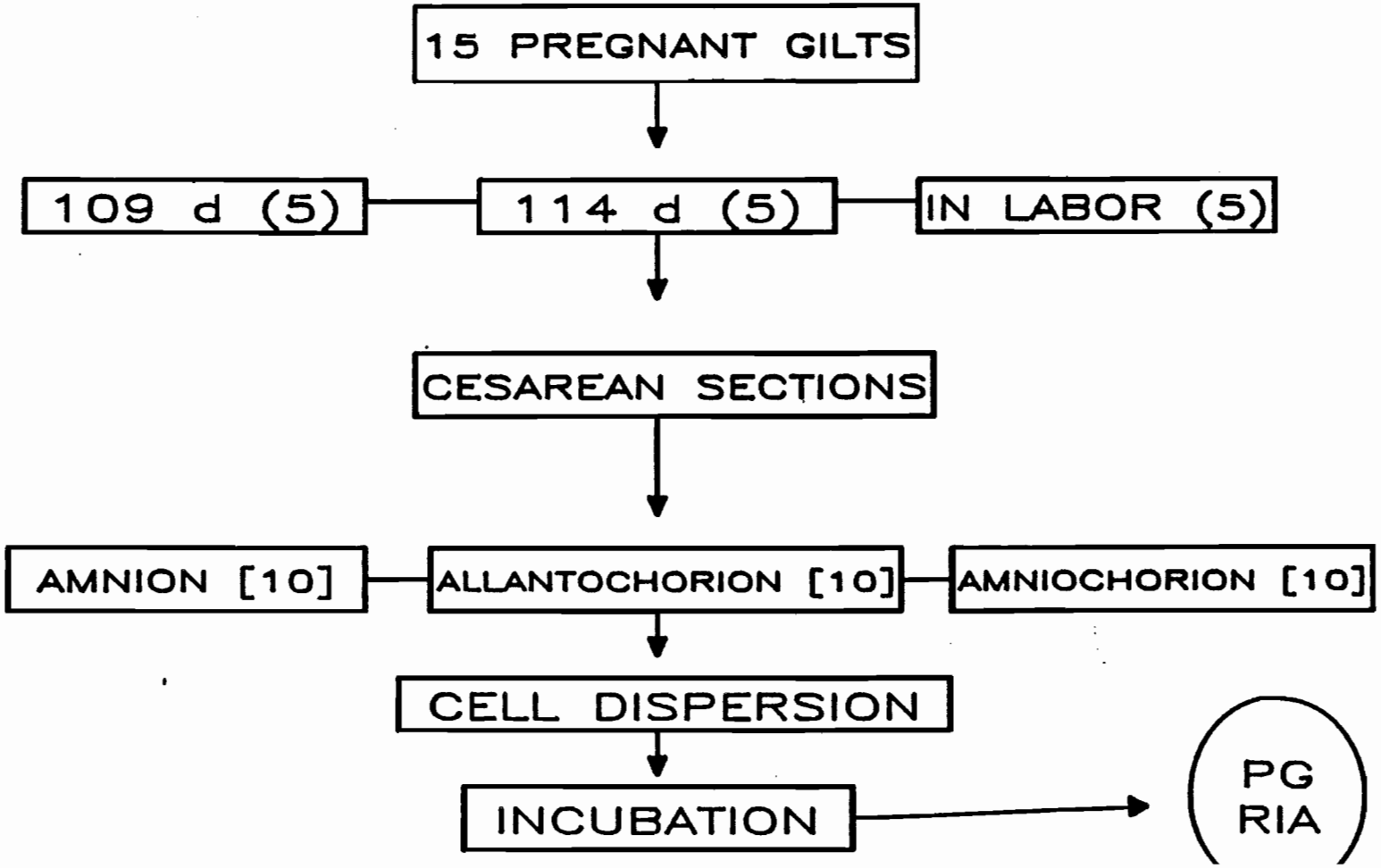
4.2.- MATERIALS AND METHODS.

4.2.1.- Experimental design.

Fifteen Yorkshire gilts with an average weight of 160.9 kg ranging between 133 to 186 kg, were divided into three different groups of five each according to gestational age. Group one: 109 days of gestation , Group two: 114 days of gestation and group three: in labor. Sows were housed and fed according to Experiment 1 (see section 3.2.1.). Two fetuses per sow were randomly chosen in order to collect samples of amnion (AM), allantochorion (ALC), amniochorion (AMC), fetal urine and amniotic fluid. Placental tissues were enzymatically dispersed and incubated. ALC was also treated simultaneously with fetal fluids. After incubation, medium PG concentrations were measured by RIA (Fig. 1D). Gestational ages were estimated according to the date of breeding and a gilt was considered to be in labor when it presented labor behaviour and the first piglet was vaginally delivered.

FIG. 1D

EXPERIMENT II



4.2.2.- Surgical procedures.

Anesthetic and cesarean section procedures have been described in section 3.2.2. Before each fetus was removed, amniotic fluid, was aseptically taken. Once the fetuses were delivered, fetal urine was collected directly from the urinary bladder. At this point, approximately 30 cm^2 of amnion were sampled. Similar amounts of allantochorion and amniochorion were peeled from the endometrium and transferred to plastic containers (4041 Falcon) with ice cooled Hank's balanced salt solution (HBSS) (450-1200 Gibco) at pH 7.3 containing 0.035 % sodium bicarbonate. Fetal fluid samples were collected aseptically and used either immediately for cell treatments or stored at -20° C for future cell treatments and RIA.

4.2.3.- Cell dispersion.

Tissues were transferred to petri dishes (8-757-13 Fisher) containing HBSS, minced into pieces of ca. 6 mm^2 and transferred in the medium to culture tubes (2099 Falcon). Tubes were centrifuged 7 min at 180 x g, the supernatant decanted and tissues resuspended in HBSS (4 ml/ml packed tissue) containing 0.5 % collagenase type II, 0.1 % protease type XIV and 0.1 % hyaluronidase type I-S (C-6885, P-5147, H-3506 Sigma), and incubated in a shaking water bath (210 oscillations per min) at 37 °C for 2 h. After 1 h of incubation

ca. 1 mg deoxyribonuclease type I (D-4527 Sigma) was added into the suspension. Once the incubation was finished, fetal calf serum was added (20 % v/v). The suspension was then diluted with an equal volume of HBSS, filtered twice through a four layer gauze sponge and centrifuged at 180 x g for 10 min in a horizontal rotor and the supernatant discarded. In order to remove contaminating red blood cells, pellets were resuspended in isosmotic HBSS containing 52 % v/v colloidal PVP coated silica (P-1644 Sigma) and centrifuged at 1750 x g for 15 min. This retained placental cells on top of the suspension whereas red cells sedimented. Placental cells were transferred to culture tubes and washed three times in HBSS by successive centrifugations (180 x g at 5 °C for 7 min) and supernatant decantings. Finally, cell pellets were resuspended in Eagles' minimum essential medium (MEM) (410-1100 Gibco), supplemented with 1.0% v/v 100x non-essential aminoacid solution, 50,000 units of penicillin G sodium, 50 mg streptomycin sulfate and 625 ug amphotericin B (320-1140, 600-5145, 600-5295 Gibco), per litre of MEM. Final cell suspension volume was 5 ml (Marcus et al., 1984).

4.2.3.1.- Estimation of Cell Viability.

Viable cells have the ability to hydrolyze fluorescein diacetate which is a non-polar compound that readily penetrates to them. Once they are hydrolyzed by the cell enzymatic system, fluorescein esters produce fluorescein which

due to its polar properties accumulates in cells with intact membranes. This results in fluorochromasia when exposed to ultraviolet light (Rotman and Papermaster, 1966).

On this basis, 200 ul of the last cell suspension were diluted in 4.8 ml MEM containing 0.001 % fluorescein diacetate (F-7378 Sigma) (prepared from a 0.25% stock solution in acetone stored at -20°C). A sample of this suspension was taken to estimate cell number and viability. For this purpose, a hemacytometer chamber and a Zeiss photomicroscope in both bright light (for total cell number) and transmitted ultraviolet light (for cell viability) modes were used. In order to obtain exciting fluorescence, the microscope exciter filter BG3 (II) and barrier filters 50/44 were set up. Cell viability was estimated counting four samples of each diluted cell suspension. A neofluor PH 16 objective and an 10x ocular were used, giving a magnification of 160x.

4.2.3.2.- Cell Incubation

Cell suspensions were diluted with MEM to $8x10^5$ viable cells/ml. Half a ml of this suspension (Approx. 4×10^5 cells) plus either 0.5 ml of MEM or 0.5 ml of MEM containing pre-term amniotic fluid, pre-term fetal urine or term fetal urine (50 % v/v) were incubated in four one-well replicates in 24 well plates (3040 Falcon) at 37°C (95% O_2; 5% CO_2) for 0, 1, 2, 3 and 4 h. Only allantochorionic cells

were treated with fetal fluids and incubated for 4 h. Cell suspensions were then transferred with Pasteur pipettes to polystyrene tubes, and stored at -20°C pending RIA.

4.2.4.- Radioimmunoassay.

Prostaglandin concentrations in culture media were estimated without extraction and using specific radioimmunoassay methods for PGFM, PGE_2, 6-keto-PGF_{1a} and PGF_{2a} (Olson et al., 1983).

Four replicates of standard solutions ranging from 12.5 to 500 pg of PG were prepared in 100 ul of MEM at pH 7.4 and added to borosilicate culture tubes containing 100 ul 2 M phosphate buffered saline (PBS) at pH 6.9 with 0.1 % gelatine. Subsequently, 100 ml PG antiserum and 100 ml [^{3}H]-PG in PBS-gelatin were added successively so that each tube contained about 5000 radioactive counts per minute (CPM). Tubes were agitated and incubated overnight at 5 °C. Antiserum cross-reactivities are presented in Appendix 1.

In order to separate the antibody bound PG from the free fraction, 0.5 ml of 2 M PBS containing 0.05 % dextran T-70 and 0.5 % charcoal was added to the tubes which were then vortex and centrifuged at 1500 x g for 10 min; 0.6 ml of the resultant supernatant was pipetted into plastic scintillation vials, mixed with 4.5 ml of scintillation fluid and counted for 5 min.

Samples containing unknown concentrations of PG

were thawed in ice water, and centrifuged at 2000 x g for 15 min to sediment cellular debris. From each incubated wel, a 25-100 ul aliquot was assayed in the same way as the standards mentioned above. To determine PG medium concentrations, CPM resulting from counting the radioactive bound fraction in the test samples were compared with those obtained from the standards.

Known amounts of PG dissolved in MEM were used as quality control tubes (QC). In this way, when 50, 100 and 200 pg/ml controls were assayed, estimated PG concentrations, and inter (inter cv) and intra-assay (intra cv) coeficients of variation were as follows:

QC	Estimated PG Concentration (pg/ml)			
pg/ml	PGFM	PGE_2	6-keto-PGF_{1a}	PGF_{2a}
	(n=19)*	(n=13)	(n=12)	(n=13)
50	52.9	54.7	53.8	52.1
100	103.2	108.9	112.0	100.0
200	198.2	200.9	200.4	196.0
Inter cv (%)	20.2	26.7	28.5	26.6
Intra cv (%)	2.2	1.9	3.6	1.9

*= Number of RIA.

4.2.5.- Protein Content in Cell Incubates.

Protein content was measured by a protein-dye binding assay (Bradford, 1976) to provide an indicator of cell

counting precision. This assay was performed in 18 randomly chosen placental cell incubates. Mean protein content was 50.3 ug/ml cell suspension with a coefficient of variation of 17%.

4.2.6.- Statistical Analysis.

Statistical analysis was carried out using the general linear model procedure of the Statistical Analysis System Statistics Package included in McGill University Computing System (SAS, 1982).

The linear model was a nested design, where the effect of sow was nested within gestational age and the effect of fetus was nested within sow within gestational age. The tissue, incubation period and treatment effects were cross-classified with fetus within sow within gestational age. Sow and fetus were considered as random effects, thus test of gestational age was against sow within gestational age. Furthermore, sow effect was tested against fetus within sow. This resulted in the following linear model:

$$Y_{ijklm} = u + A_i + B_{ij} + C_{ijk} + D_l + E_m + (AB)_{ij} + (AE)_{im} + (DB)_{lij} + (DC)_{ijkl} + (EB)_{ijmt} + (EC)_{ijkm} + (DE)_{lm} + (EAD)_{ilmt} + (EDB)_{ijlm} + e_{ijklm}.$$

u = overall mean of the response variable.

A_i = effect of i^{th} gestational age.

B_{ij} = effect of j^{th} sow within i^{th} gestational age.

$B_{ij} \sim NID(O, {}^2_{sow})$.

C_{ijk} = effect of k^{th} fetus within j^{th} sow within i^{th} gestational age. $B_{ijk} \sim NID(O, {}^2_{fetus})$.

D_l = effect of l^{th} tissue.

E_m = effect of m^{th} incubation period or m^{th} treatment for section 4.3.2.

$(A\ B)_{ij}$ = interaction effect of A and B associated with the ij^{th} AB combination.

$(A\ E)_{im}$ = interaction effect of A and E associated with the im^{th} AE combination.

$(D\ B)_{lij}$ = interaction effect of D and B associated with the lij^{th} DB combination.

$(D\ C)_{ijkl}$ = interaction effect of D and C associated with the $ijkl^{th}$ DC combination.

$(E\ B)_{ijm}$ = interaction effect of E and B associated with the ijm^{th} EB combination.

$(E\ C)_{ijkm}$ = interaction effect of E and C associated with the $ijkm^{th}$ EC combination.

$(D\ E)_{lm}$ = interaction effect of D and E associated with the lm^{th} DE combination

$(EAD)_{ilm}$ = interaction effect of E, D and D associated with the ilm^{th} EAD combination.

$(EDB)_{ijlm}$ = interaction effect of E, D and B associated with the $ijlm^{th}$ EDB combination.

e_{ijklm} = error term NID $(0, e^2)$.

where:

$i = 1,..,3$

j = 1,..,5

k = 1,2.

l = 1,.,3

m = 1,..,4

In order to make comparisons within effects LSM "t" tests were carried out. Significant differences were declared at probability levels less than 5 % (Steel and Torrie, 1980).

4.3.- RESULTS

4.3.1.- In vitro Prostaglandin Production by Placental Cells

Production by placental tissues at different incubation periods are expressed as net PGs produced by 4 x 10^5 cells in a specified period of time. Net PG production is defined as the PG concentration found in the cell suspensions after the incubation less concentrations measured at the beginning of the incubation (Time 0).

4.3.1.1.- PGFM

Least-squares means (LSM) of PGFM net production by amnion (AM), allantochorion (ALC), and amniochorion (AMC) obtained at d 109, d 114 and during labor after 1, 2, 3 and 4 h incubation are presented in Table 4.

LSM "t" tests revealed smaller PGFM net production by AM than ALC and AMC. The latter two did not differ. This was consistent for all four incubation periods.

No significant differences due to gestational stage among the three tissues at each of the incubation periods were detected. An exception to this was the higher PGFM production at d 109 when compared to d 114 after one hour of incubation.

4.3.1.2.- PGE_2

Net PGE_2 production by AM, ALC and AMC obtained at d 109, d 114 and during labor after 1, 2, 3, and 4 h of incubation are shown in Table 5 and Fig 11.

The analysis of LSM using the "t" test detected lower net PGE_2 production in AM than in ALC and AMC in all four incubation periods and, although AMC produced more in some of the incubation periods at d 109, 114 and labor than did ALC, in more than half of the incubation periods AMC did not differ from ALC.

The differences between gestational ages were not significant in the three tissues at the different incubation periods. An exception to this was the higher net PGE_2 production by AMC at d 109 after 2 and 3 h of incubation.

4.3.1.3.- PGF_{2a}

Testing LSM of net PGF_{2a} production by AM, ALC and AMC at different gestational ages and after different incubation periods demonstrated that AMC has a higher PGF_{2a} synthetic capacity than AM. ALC was not different from AM at any incubation period at d 109 nor at labor after incubations of 1, 3 and 4 h, but produced more PGF_{2a} than AM and the same as AMC at d 114 after 1, 2 and 3 h of incubation. (Table 6 and Fig 12).

Different gestational stages did not differ in net PGF_{2a} production by AM, ALC and AMC after 1, 2, 3 or 4 h of incubation.

4.3.1.4.- 6-keto-PGF_{1a}

Net 6-keto-PGF_{1a} production (LSM) by AM, ALC and AMC at d 109, d 114 of gestation and at labor after 1, 2, 3 and 4 h of incubation are shown in Table 7.

LSM "t" test revealed lower PG production by AM after 1, 2, 3 and 4 h of incubation than by either ALC or AMC although ALC and AMC did not differ.

Dispersed cells from AM, ALC and AMC collected at d 109 revealed a higher 6-keto-PGF_{1a} production capacity than those cells collected either at d 114 or at labor.

Figures 10, 11, and 12 show net PGFM, PGE_2 and PGF_{2a} outputs after 1, 2, 3 and 4 h of incubation, respectively. These data were pooled by gestational age since this

effect did not affect significantly PG outputs. Data pertaining to 6-keto-PGF_{1a} , which was produced more at d 109 than at any other gestational stage, were not pooled.

4.3.2.- PG production by Allantochorionic (ALC) cells treated with pre-term (FU) and term fetal urine (TFU) and pre-term amniotic fluid (AMF).

In order to assess the effect of fetal fluids sampled at different pregnancy stages net PG productions by ALC collected at different gestational ages were pooled .

Fetal fluids contain PGs (see table 1) therefore, PG were measured in wells which contained the different treatments but no cells. These and those values obtained at 0 h of incubation were substracted from total PG output by treated ALC. Resultant values were defined, only for this section, as net PG output.

LSM of net PG production by allantochorionic cells treated and not-treated with different fetal fluids are presented in Table 8.

Addition of pre-term or term fetal urine or pre-term amniotic fluid to ALC resulted in a significant stimulation of PGF_{2a}, PGE_2 and 6-keto-PGF_{1a} productions when compared to controls. PGFM production did not change with the addition of either pre-term fetal urine or amniotic fluid. Con-

versely, term fetal urine caused an inhibiton of PGFM production. (Fig. 16-17).

TABLE 4 : Least-squares means ± SEM of PGFM net productions (pg/4x10⁵ cells) by placental tissues at different gestational stages and after different incubation periods.

	AMNION			ALLANTOCHORION			AMNIOCHORION		
Gestational Stage	109	114	Labor	109	114	Labor	109	114	Labor
Incubation (Hr)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
1	50.4 ±9.7	* 0.04 ±14.7	-4.2 ±7.4	117.1 ±9.2	* 49.3 ±15.1	73.7 ±8.3	117.8 ±9.7	* 42.8 ±14.7	71.3 ±7.7
2	22.4 ±15.6	36.5 ±13.0	-18.6 ±12.2	221.3 ±15.1	126.8 ±13.0	142.8 ±12.9	208.2 ±17.0	107.9 ±14.1	151.7 ±11.9
3	41.9 ±18.5	40.1 ±16.5	23.1 ±16.0	296.4 ±19.2	207.1 ±16.5	222.9 ±15.9	260.4 ±18.7	223.6 ±18.1	254.9 ±15.0
4	56.2 ±27.6	67.5 ±18.6	80.9 ±21.4	462.7 ±27.6	286.2 ±18.6	352.4 ±20.9	112.2 ±27.6	261.5 ±20.4	382.2 ±21.2

(n) Number of fetuses

* $p<0.05$ when compared with day 109

TABLE 5 : Least-squares means ± SEM of PGE_2 net productions (pg/4x10^5 cells) by placental tissues at different gestational stages and after different incubation periods.

	AMNION			ALLANTOCHORION			AMNIOCHORION		
Gestational Stage	109	114	Labor	109	114	Labor	109	114	Labor
Incubation (Hr)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
1	941.0 ±147.1	332.4 ±161.1	301.8 ±131.9	1497.6* ±149.1	998.2* ±156.7	990.2* ±163.6	2137.8* ±153.7	1339.9* ±171.9	1636.6* ±145.8
2	955.3 ±176.4	291.2 ±160.7	300.6 ±104.4	2367.8* ±174.0	1571.0* ±158.5	1297.5* ±115.4	3584.8* ±191.1	1976.6* ±173.8	1532.3* ±108.0
3	1300.0 ±321.2	494.8 ±190.9	277.5 ±116.0	1835.5 ±312.5	1619.8* ±182.5	1747.2* ±124.8	3695.7* ±316.8	2412.4* ±203.3	2074.4* ±117.6
4	868.1 ±235.8	568.1 ±210.1	185.5 ±145.9	1777.5* ±239.1	1670.9* ±204.3	2500.1* ±1480	3782.6* ±239.1	2298.9* ±227.5	2487.0* ±148.0

(n) Number of fetuses

* $p<0.05$ when compared with amnion

TABLE 6: Least-squaresmeans ± SEM of $PGF_{2\alpha}$ net productions (pg/4x10^5 cells) by placental tissues at different gestational stages and after different incubation periods.

	AMNION			ALLANTOCHORION			AMNIOCHORION		
Gestational Stage	109	114	Labor	109	114	Labor	109	114	Labor
Incubation (Hr)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
1	600.9 ±57.3	131.4 ±24.3	224.3 ±34.5	300.1 ±58.1	223.2 ±24.3	251.9 ±40.2	686.1 ±59.9	222.8 ±26.5	304.2 ±38.2
2	706.9 ±109.7	36.5 ±55.2	146.1 ±35.1	643.1 ±111.2	557.1 ±59.4	292.8 ±38.2	1058.7 ±117.1	493.3 ±58.6	412.7 ±36.3
3	643.5 ±80.6	8.9 ±49.6	121.4 ±43.9	543.0 ±89.1	428.2 ±53.7	221.2 ±46.1	951.1 ±89.1	465.4 ±53.8	222.2 ±43.3
4	240.4 ±70.3	167.8 ±56.0	-44.9 ±40.3	355.7 ±71.2	113.7 ±55.5	-96.3 ±40.8	625.5 ±70.3	345.3 ±58.0	37.5 ±40.9

(n) Number of fetuses

TABLE 7: Least-squares means ± SEM of 6-keto-$PGF_{1\alpha}$ net productions by placental tissues at different gestational ages and after different incubation periods.

	AMNION			ALLANTOCHORION			AMNIOCHORION		
Gestational Stage	109	114	Labor	109	114	Labor	109	114	Labor
Incubation (Hr)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
1	394.1 ±104.7	304.7 ±144.7	86.9 ±42.0	907.3 ±104.7	996.9 ±141.4	415.8 ±49.0	811.6 ±108.0	1155.1 ±141.4	509.9 ±45.2
2	379.9 ±129.8	411.0 ±95.3	157.5 ±55.1	1004.1 ±129.8	655.8 ±126.2	715.3 ±59.2	1256.8 ±143.0	619.5 ±126.2	673.8 ±60.2
3	482.7 ±125.8	550.4 ±160.1	163.1 ±68.7	1239.8 ±125.8	1414.8 ±162.3	1145.8 ±699.0	1425.0 ±125.8	2236.8 ±189.8	1170.6 ±66.8
4	1090.6 ±109.8	a 320.0 ±153.1	* 109.5 ±76.1	2520.9 ±109.8	1722.4 ±154.9	* 1013.1 ±75.9	2513.4 ±109.8	1981.8 ±166.3	* 896.1 ±74.8

(n) Number of fetuses

a $p < 0.05$ when compared with d 109 or labor

* $p < 0.05$ when compared with d 109 or d 114

FIG. 10 PGFM NET OUTPUT BY AMNION (AM), ALLANTOCHORION (ALC) AND AMNIOCHORION (AMC) AT DIFFERENT INCUBATION PERIODS.

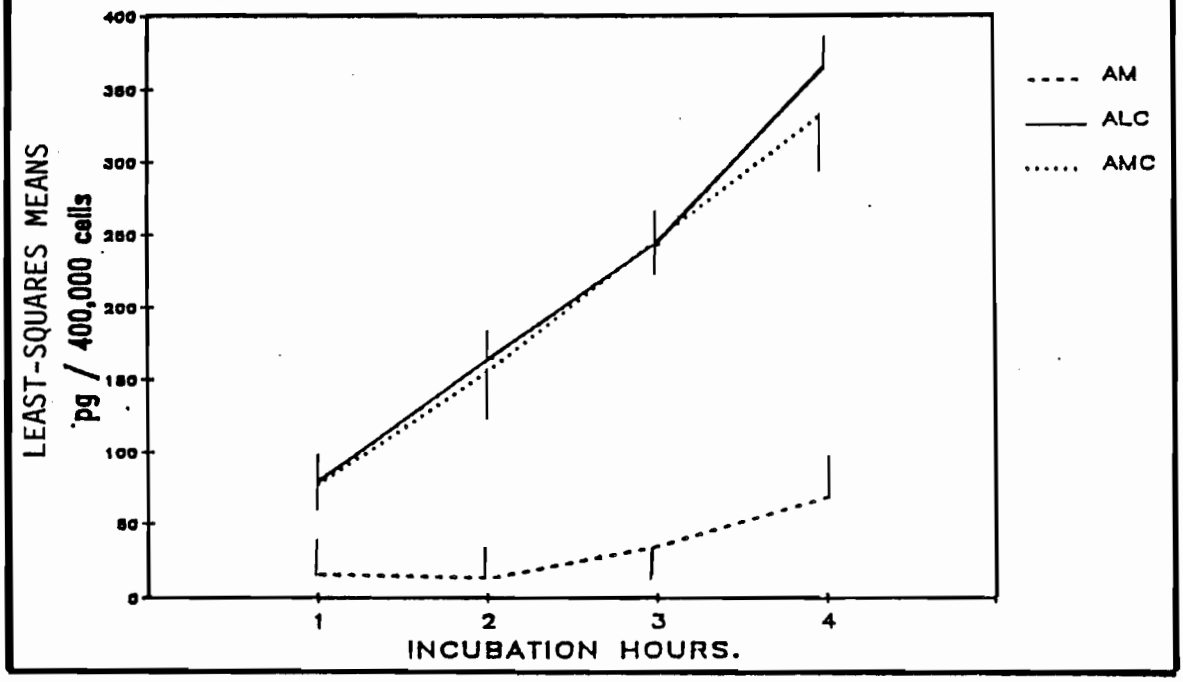


FIG. 11 PGE2 NET OUTPUT BY AMNION (AM), ALLANTOCHORION (ALC) AND AMNIOCHORION (AMC) AT DIFFERENT INCUBATION PERIODS.

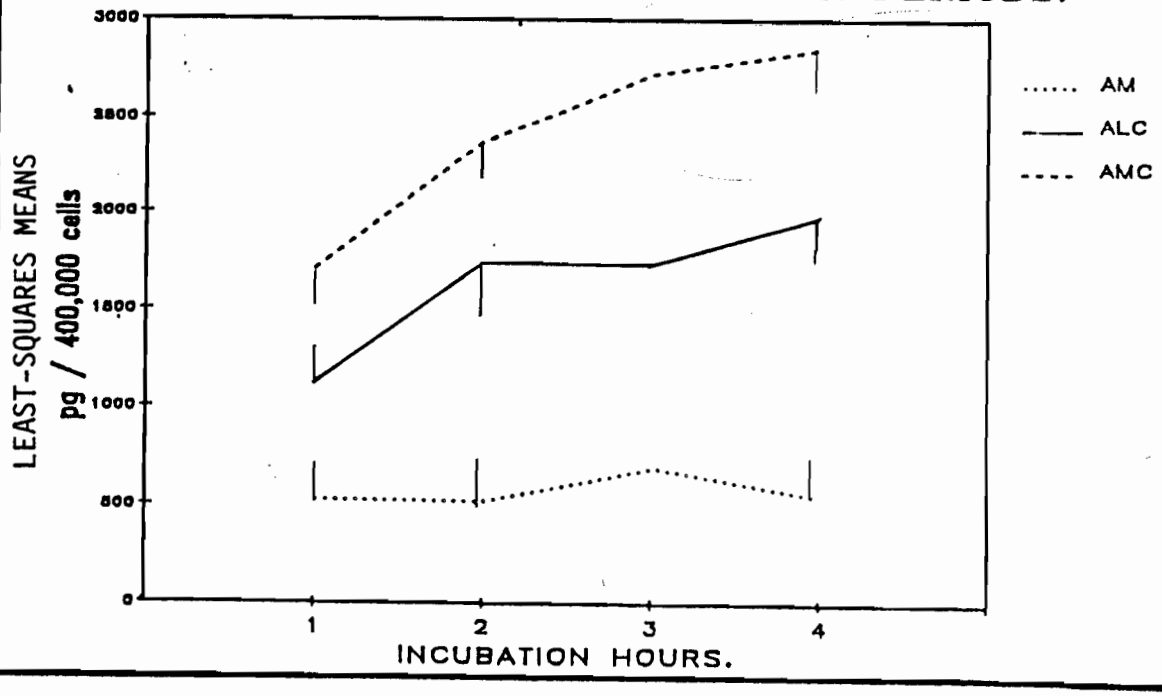


FIG. 12 PGF2$_{\alpha}$ NET OUTPUT BY AMNION (AM), ALLANTOCHORION (ALC) ANDAMNIOCHORION (AMC) AT DIFFERENT INCUBATION PERIODS.

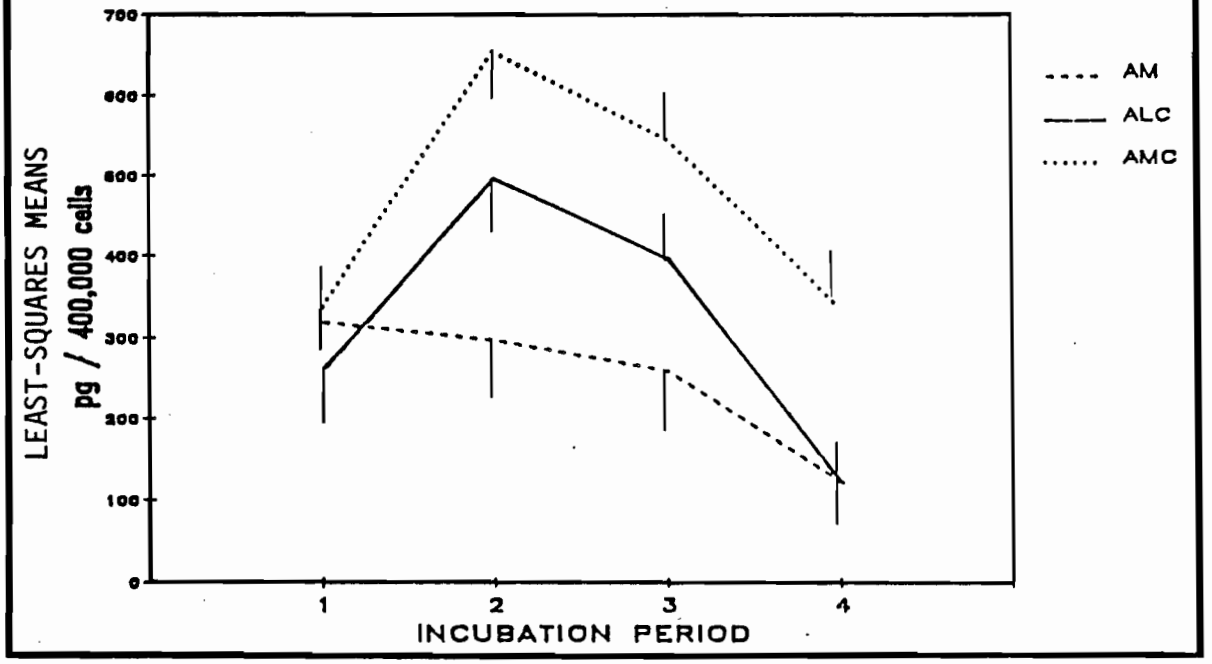
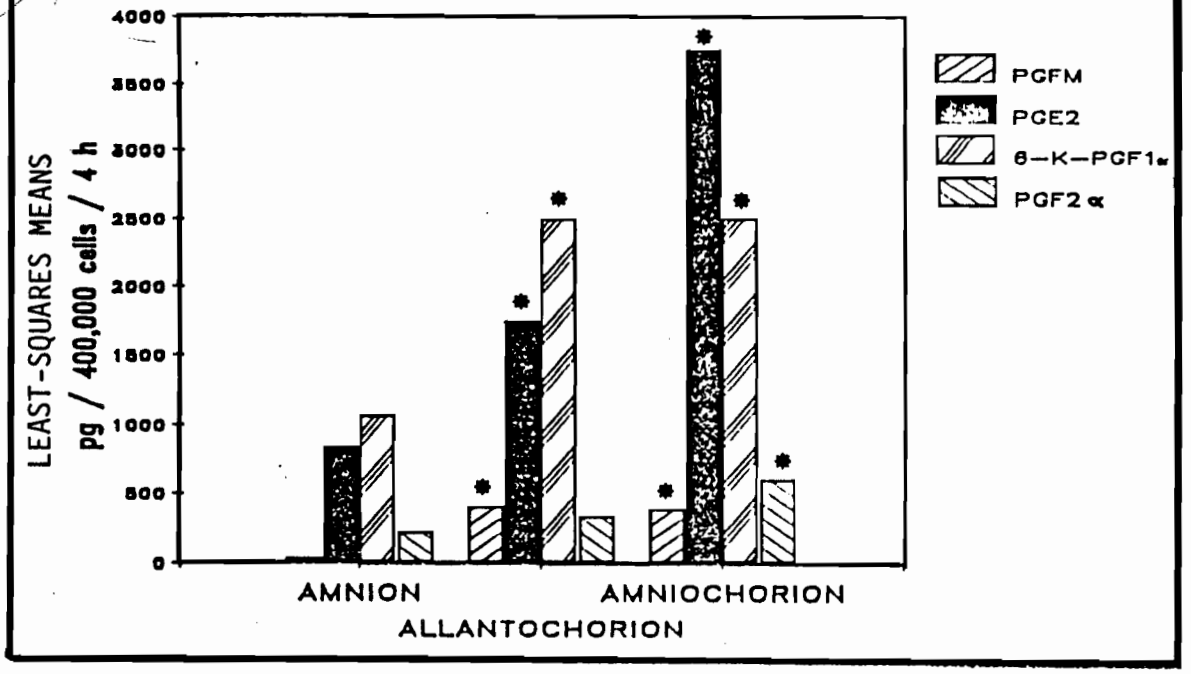


FIG. 13 PROSTAGLANDIN NET PRODUCTION BY PLACENTAL CELLS COLLECTED AT DAY 109 OF GESTATION.



* $p < 0.05$ when compared with amnion

FIG. 14 PROSTAGLANDIN NET PRODUCTION BY PLACENTAL CELLS COLLECTED AT DAY 114 OF GESTATION.

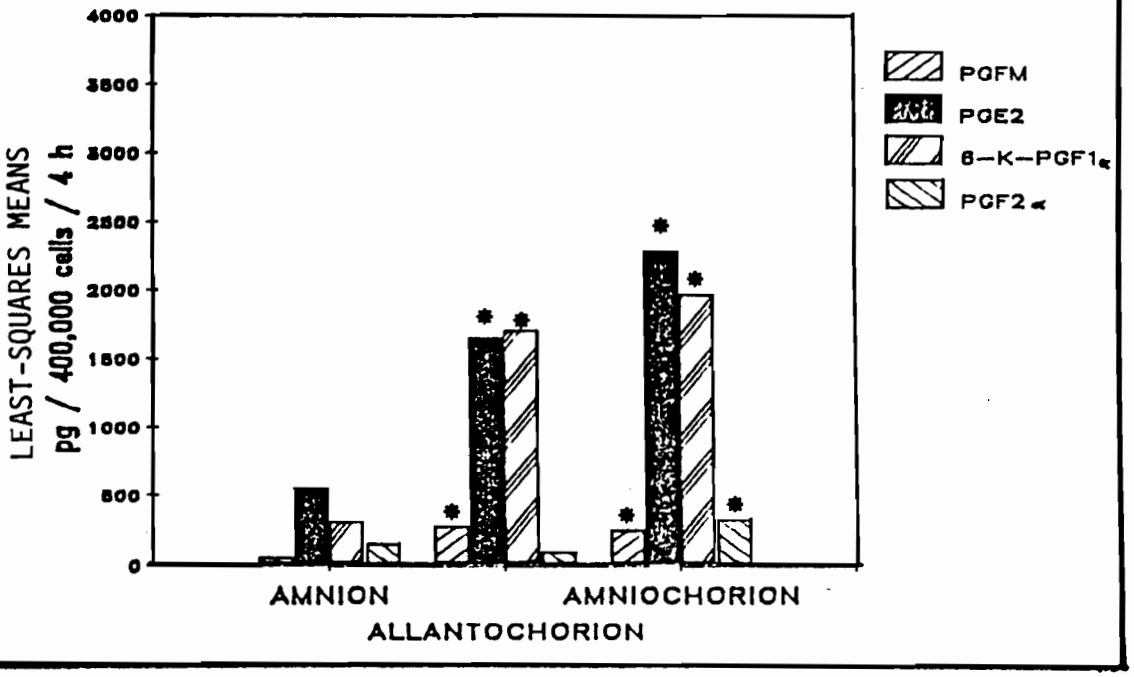
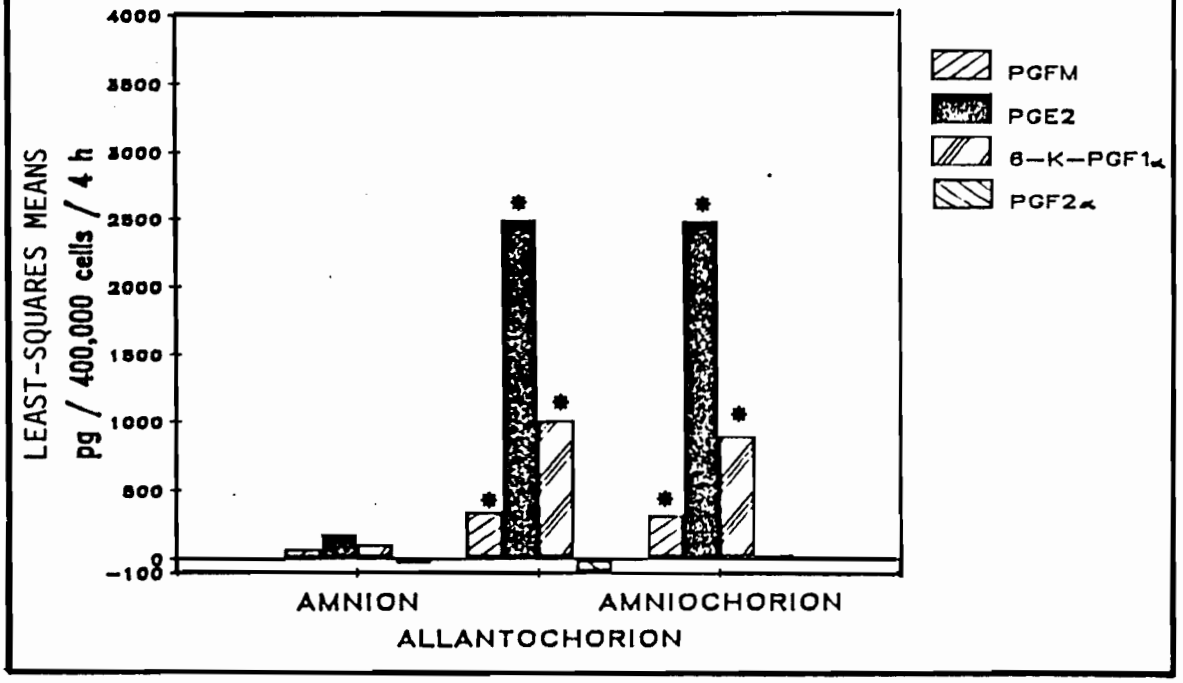


FIG. 15 PROSTAGLANDIN NET PRODUCTION BY PLACENTAL CELLS COLLECTED DURING LABOR.



* $p < 0.05$ when compared with amnion

TABLE 8 : Least-squares means ± SEM of prostaglandin (PG) net output (pg/4x10^5 cells/4 h) by allantochorionic cells non-treated (C) and treated with either fetal urine (FU), amniotic fluid (AMF) collected before labor or fetal urine obtained during labor (TFU).

PG	C [10]	FU [10]	TFU [10]	AMF [10]
FM	355.4 ±72.8	338.4 (-5) ±121.5	* -237.3 (-167) ±145.4	443.5 (25) ±137.3
E_2	1791.6 ±255.8	* 5916.7 (230) ±438.4	* 7143.3 (299) ±445.6	* 5781.5 (223) ±534.8
$F_{2\alpha}$	145.8 ±67.0	* 906.5 (521) ±115.6	* 691.7 (374) ±117.5	* 938.4 (544) ±133.4
6-k-$F_{1\alpha}$	1687.5 ±101.3	* 2921.5 (73) ±174.8	* 2555.5 (51) ±117.7	* 2838.8 (69) ±198.1

* $p < 0.05$ when compared to C

[n] Number of fetuses.

(y) percentage of variation with respect to controls.

FIG. 16 PGFM AND PGF2α NET OUTPUT BY AL-LANTOCHORIONIC CELLS TREATED WITH PRE-TERM FETAL URINE (FU) OR AMNIOTIC FLUID (AMF) OR FETAL URINE COLLECTED DURING LABOR (TFU).

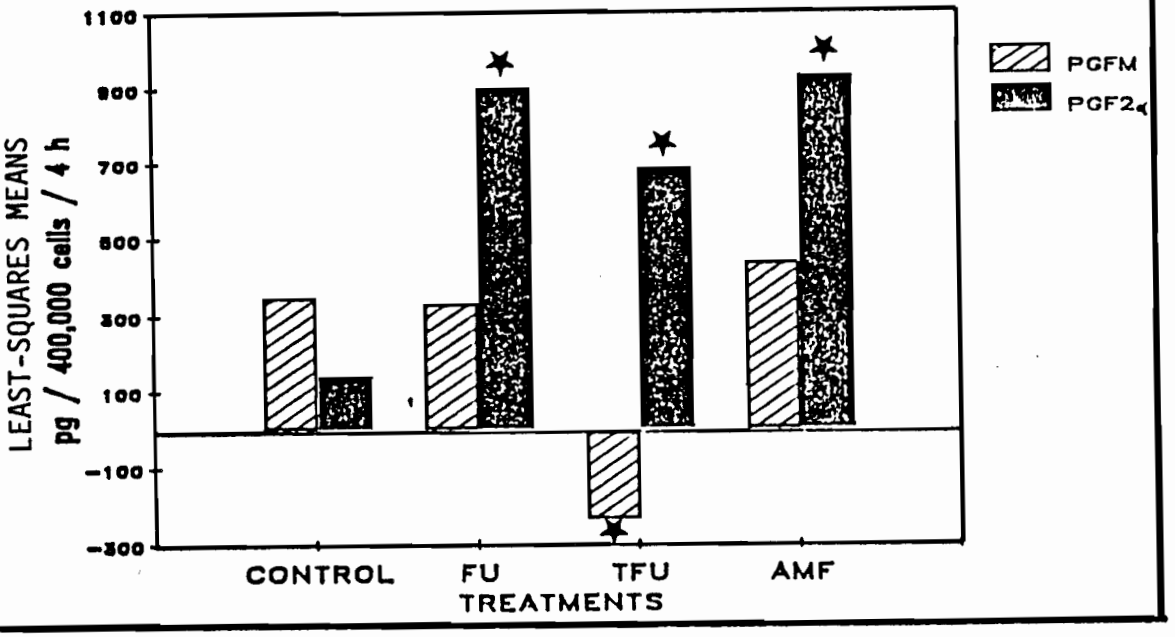
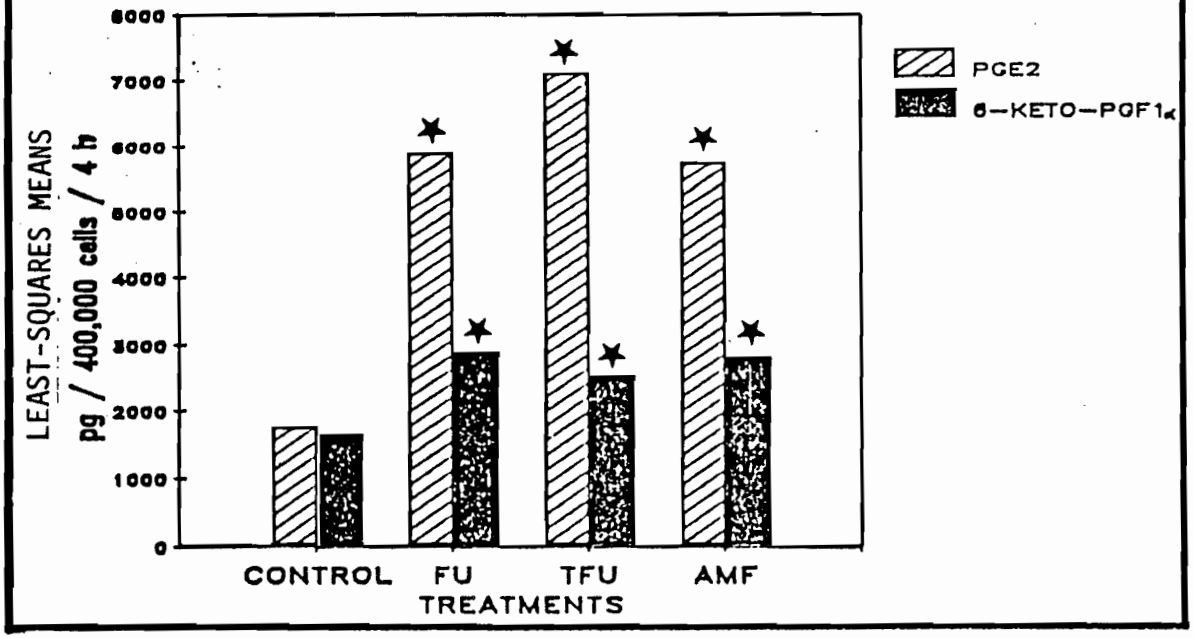


FIG. 17 PGE2 AND 6- K-PGF1 NET OUTPUT BY AL-LANTOCHORIONIC CELLS TREATED WITH PRE-TERM FETAL URINE (FU) OR AMNIOTIC FLUID (AMF) OR FETAL URINE COLLECTED DURING LABOR (TFU).



★ $p < 0.05$ when compared with control

4.4 DISCUSSION

It was shown here that porcine placental membranes have an intrinsic ability to produce PGs , confirming observations of Silver et al. (1979) and agreeing with previous studies done in the ovine species (Mitchell et al., 1978; Evans et al., 1982; Risbridger et al., 1985; Olson *et al.*, 1985). It also has been shown that PGE_2 is the major PG produced in vitro by placental tissues followed by 6-keto-PGF_{1a}, PGF_{2a} being the least produced.

Variations in net PG production within the different incubation periods show the synthetic and catabolic capacity of placental cells. In Fig. 12, it can be seen that, in all cell types, PGF_{2a} incubate concentrations are decreased at 3 h of incubation while PGFM concentrations tend to increase (Fig. 10). This suggests that all cell types have 15-dehydrogenase-13-14 reductase activity which physiological significance is not clear.

PGE_2 and 6-keto-PGF_{1a} reached a plateau after 3 h of incubation; this plateau could be due to a shortage of substrate, further prostanoid catabolism, or PG accumulation which could cause a negative regulatory feed-back upon the cyclooxygenase complex (Lands, 1979). Apparently PG synthetase is one of the most short-lived enzymes in mammalian cells (Fagan et al., 1986), and a molecule of this enzyme is capable of producing only a limited amount of product, avoiding in this way overproduction of these compounds

(Lands, 1979). Natural oxidants released during the enzymatic reduction of hydroperoxides on PGG_2 can rapidly block the production of PGs which can be decreased by treatment of tissues with inhibitors of protein and mRNA synthesis (Fagan et al., 1986). A short half-life of this mRNA coding for PG synthetase, and the self-catalyzing properties of this enzyme would facilitate large short term adaptive changes which agree with the evanescent properties and actions of prostanoid compounds. (Ferreira and Vane, 1967) .

These experiments also show that cells from amnion (AM), allantochorion (ALC) or amniochorion (AMC), collected at d 109, do not differ in their respective PG synthetic capacity at the various gestational stages. An exception to this is the decrease in net 6-keto-PGF_{1a} output by all tissues during labor. This does not agree with results of studies done in the ovine (Risbridger et al., 1985; Olson et al., 1984; Olson et al., 1985) and human species (Olson et al., 1983) where it was found that, in general, those placental membranes collected during labor had a greater in vitro PG synthetic capacity than those collected before the initiation of labor. However, our results are in agreement with those of Mitchell (1981) who was not able to find differences in PG output by human placental cells that could be attributable to gestational age. An exception to this was 6-keto-PGF_{1a} which was produced more by the amnion after the initiation af labor. However, since there are major differences between the human placenta and that of the swine,

equivalent tissues may differ in their PG synthetic capacity. Moreover the ability of the swine placental membranes to produce PGs has not been evaluated previously.

It appears that all tissues collected during labor produce less quantity of PGs that are inhibitory to myometrial activity inhibitory PGs than at the other gestational stages studied in this experiment. This low 6-keto-PGF_{1a} production would be expected if a decrease in uterine relaxation during labor is to occur. Similarly, if an increase in uterine activity is awaited during labor, placental cells might be expected to increase their PGE_2 and PGF_{2a} synthetic capacity at that time. This did not happen in this study, where no significant changes were detected across gestational ages.

Apparently PGI_2 production, as measured by 6-keto-PGF_{1a}, is selectively suppressed resulting in a relative increase in stimulatory prostanoid net output by placental cells collected after the initiation of parturition. This suggests that the regulatory mechanism of PGI_2 synthetase is different from that which controls the production of PGF_{2a} and PGE_2 and, probably, there are other products of the oxidization of arachidonic acid that could act as enzymatic regulatory compounds (Milvae *et al.*, 1986).

The interpretation of the results obtained in this experiment should be made very cautiously since, during late gestation, placental tissues interact *in vivo*, with a completely different hormonal milieu from that one existing in

the in vitro system utilized here.

Another important conclusion extracted from this experiment is that those cells composing the different placental zones defined for this trial show a remarkable PG synthetic capacity. PGE_2 is the major PG produced in all tissues while PGF_{2a} and its metabolite (PGFM) are produced in very small quantities when compared to PGE_2 (Figs. 14-16). However, in Experiment 1, it has been shown that plasma PGFM levels rise sharply during labor. Therefore it may be possible, as occurs in the human subject (Niesert et al., 1987), that this PGE_2 formed by the placenta is transformed into PGF_{2a} by PGE_2-9-keto-reductase when an increased contractile activity is needed. This enzymatic activity has been reported in the human endometrium and myometrium (Niesert et al., 1987).

Significant variation among AM, ALC or AMC PG net production occurs, with AM having a lower synthetic capacity at all gestational stages than either ALC or AMC.

Since PGs are secreted near their target tissues, it is reasonable to expect them to serve as local hormones affecting the corpus luteum and myometrium during parturition. If the deployment of the fetal membranes at the end of gestation is taken into account (Fig. 1B), it is possible to observe that those placental zones considered in this study that have the highest in vitro net production are the ones that are in direct juxtaposition to the endometrium, namely, the allantochorion and amniochorion (Noden and de Lahunta,

1985). Conversely, the amnion, which is virtually floating in the allantoic cavity, is not in apposition with any tissue except its dorsal portion which is attached to the chorion. For the purpose of this experiment, this part of the placenta has been defined as amniochorion.

In order to test the effect of fetal urine and amniotic fluid upon PG production, allantochorion was chosen as the placental tissue to be challenged with fetal fluids. These treatments, in general, increased net prostanoid production except in the case of PGFM. Production of the latter was not affected by either pre-term fetal urine (FU) or pre-term amniotic fluid (AMF), whereas term fetal urine (TFU) inhibited it completely.

Cells treated with either FU, TFU or AMF experienced a 3-5 fold increase in PGF_{2a} production; whether this is due to a reduction in PGF_{2a} catabolism, an increased synthesis or a combination of both, can not be determined from these data. The inhibition of PGFM production by these fluids suggests that a decrease in PGF_{2a} conversion to PGFM may contribute to the total effect of fetal fluid upon PGF_{2a} net production.

Even though the proportional increase of PGE_2 and 6-keto-PGF_{1a} in response to the fluid treatments was much lower than that of PGF_{2a}, in absolute terms, PGE_2 and 6-keto-PGF_{1a} had a greater response. In an analogous manner, non-treated cells produced much more PGE_2 and 6-keto-PGF_{1a} than did either PGF_{2a} or PGFM.

Therefore, it can be said that both fetal urine and amniotic fluid, regardless of gestational stage, have the capacity of stimulating PG production. However, the specific compound responsible for producing these effects, cannot be elucidated from this experiment. Other authors (Casey et al., 1983; Strickland et al., 1983) found that human fetal urine was able to increase in vitro PG production. Urine obtained from fetuses during labor had a greater capacity to stimulate PG synthesis than that collected before labor. They suggested the presence of a protein-associated (Casey et al., 1983) or growth factor-like substance (Casey et al., 1984b) in the fetal urine that would stimulate PGE_2 production.

It also should be born in mind that urine and amniotic fluid contain a considerable amount of estrogens during late gestation (Choong and Raeside, 1974; Robertson et al., 1985) and estrogens have been shown to stimulate in vitro and in vivo PGF_{2a} production by the human endometrium (Kelly and Abel, 1980; Schatz and Gurpide, 1983; Schatz et al., 1986; Schatz et al., 1987;).

In summary, this experiment demonstrated that, indeed, the swine placental tissues produce and metabolize PGs and that those tissues which, during pregnancy, are in direct apposition to the endometrium (AMC and ALC), have greater PG synthetic capacity than AM. This PG production does not vary among the different gestational stages condidered in this experiment, excepting the case of 6-keto-PGF_{1a} where production decreases in cells collected during

labor. This results in a relative increase in stimulatory PGs. It also has been shown that PGE_2 is quantitatively the major PG produced *in vitro* by placental tissues followed by PGI_2, and then $PGF_{2\alpha}$.

CHAPTER 4

EXPERIMENT 3

EFFECT OF FETAL URINE AND AMNIOTIC FLUID UPON PG SYNTHESIS BY MICROSOMES OF BOVINE SEMINAL VESICLES.

5.1.-INTRODUCTION.

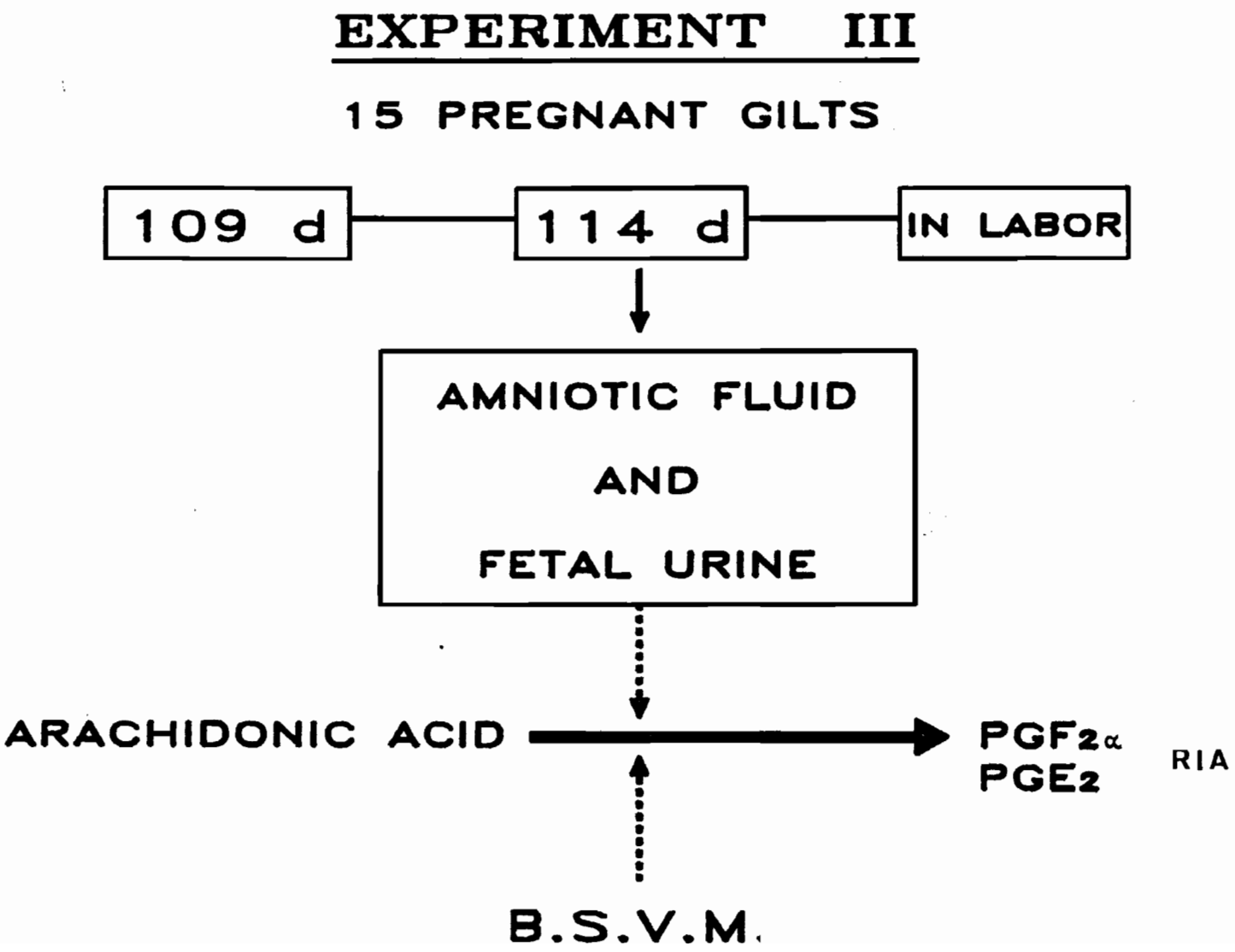
The objective of this experiment was to study the effect of fetal urine and amniotic fluid collected at different gestational stages upon the synthesis of $PGF_{2\alpha}$ and PGE_2 by the cyclooxygenase enzymatic complex contained in microsomes isolated from bovine seminal vesicles.

5.2.- MATERIALS AND METHODS.

5.2.1.- Experimental Design.

Fetal urine and amniotic fluid collected from fetuses used in experiments 1 and 2 were divided into 3 groups of five each according to gestational age, namely 109 and 114 days of gestation and in labor. These fluids were added to a bovine seminal vesicle microsome (BSVM) preparation which was incubated. Afterwards PG concentrations in BSVM incubates were measured by RIA (Fig.1E).

FIG. 1 E


EXPERIMENT III
15 PREGNANT GILTS
109 d
114 d
IN LABOR
AMNIOTIC FLUID
AND
FETAL URINE
ARACHIDONIC ACID
PGF2α
PGE2
RIA
B.S.V.M.

5.2.2.- Prostaglandin Synthetase Assay.

5.2.2.1.- Isolation of Microsomes from Bovine Seminal Vesicles.

Bovine seminal vesicles (BSV) were collected on ice at slaughter from mature bulls with mean age and weight of 3.1 years and 793 kg, respectively. Trimmed of fat and connective tissues, BSV were blended in O.1 M Tris-HCL (1 gr:3 ml) for 1.5 min at speed Nº 10 in a Sorval blender. The resultant homogenate was centrifuged at 12,000 x g for 10 min, the supernatant filtered twice through a two layer gauze and centrifuged at 80,000 x g for 2.5 h. The supernantant was discarded, the precipitate was smeared into a 50 ml serum bottle (06-406 G Fischer), lyophilized and stored at -20º C (Flower et al., 1973; Takeguchi et al., 1971). Microsome yield was 7.68 mg/g of BSV with 36 % being protein as measured by the method of Bradford (1976).

5.1.2.2.- Prostaglandin Synthesis and Assay Validation.

In order to validate this assay, 40 uM Arachidonic Acid sodium salt (A7156 Sigma), 400 uM Epinephrine Bitartrate (E4375 Sigma), 400 uM reduced glutathione (G4251 Sigma) and BSVM in Dulbecco's phosphate buffered saline (310-4200 GIBCO) solution (1 ml final volume) were incubated for **a)** 0-60 min with 26 ug BSVM (Fig.19), **b)** 35 min with 0.6-

39 ug BSVM (Fig. 20), c) 35 min with 26 ug BSVM and 0-30 uM indomethacin (Fig 21). For this purpose, a water bath at 37° C and at 210 oscillations per min was used. Incubation was stopped by the addition of 5 ml ethyl acetate, and incubates were immediately extracted for PGF_{2a} and PGE_2 radioimmunoassays according to procedures described in Experiment 1 (3.2.3. and 3.2.4))

Consequently, 26 ug of BSVM were incubated with the fetal fluids to be tested (20 % v/v) for 35 min as a standard assay procedure.

5.2.3.- Statistical Analysis.

Analysis of variance was performed using the General Linear Model procedure of the Statistical Analysis System Statistics Package (SAS, 1982).

The linear model used was a nested design where the effect of sow was nested within gestational age. The effect due to different samples was cross-classified with sow within gestational age. This resulted in the following linear model:

$Y_{ij} = u + A_i + B_{ij} + e_{ijkl}$

u = overall mean of the response variable.

A_i = effect of i^{th} gestational age.

B_{ij} = effect of j^{th} sow within i^{th} gestational age.

e_{ij} = error term NID $(0, \sigma^2)$

i = 1,..,3.

j = 1,...,5.

In order to make comparisons within effects, LSM "t" tests were carried out. Significant differences were declared at probability levels less than 5 % (Steel and Torrie, 1980).

5.3.-RESULTS

Results are presented as the percentage of change in PG synthesis when compared to the synthetic capability of non-treated microsomes.

Treatment of BSVM with fetal urine collected at d 109, d 114 of gestation and during labor resulted in 136%, 48% and 57% of increase in PGF_{2a} synthesis and 289%, 94% and 50% increases in PGE_2 synthesis respectively.

The addition of amniotic fluid collected at d 109, d 114 or during labor to the microsomes increased PGF_{2a} production by 16% , 26% and 36 % respectively. However these increases were not significant when compared to non-treated microsomes. Unfortunately, due to the size of the amniotic fluid samples, it was not possible to test the effect of this fluid upon PGE_2 synthesis.

FIG. 18 PROSTAGLANDIN SYNTHESIS BY B.S.V. MICROSOMES INCUBATED FOR DIFFERENT PERIODS OF TIME.

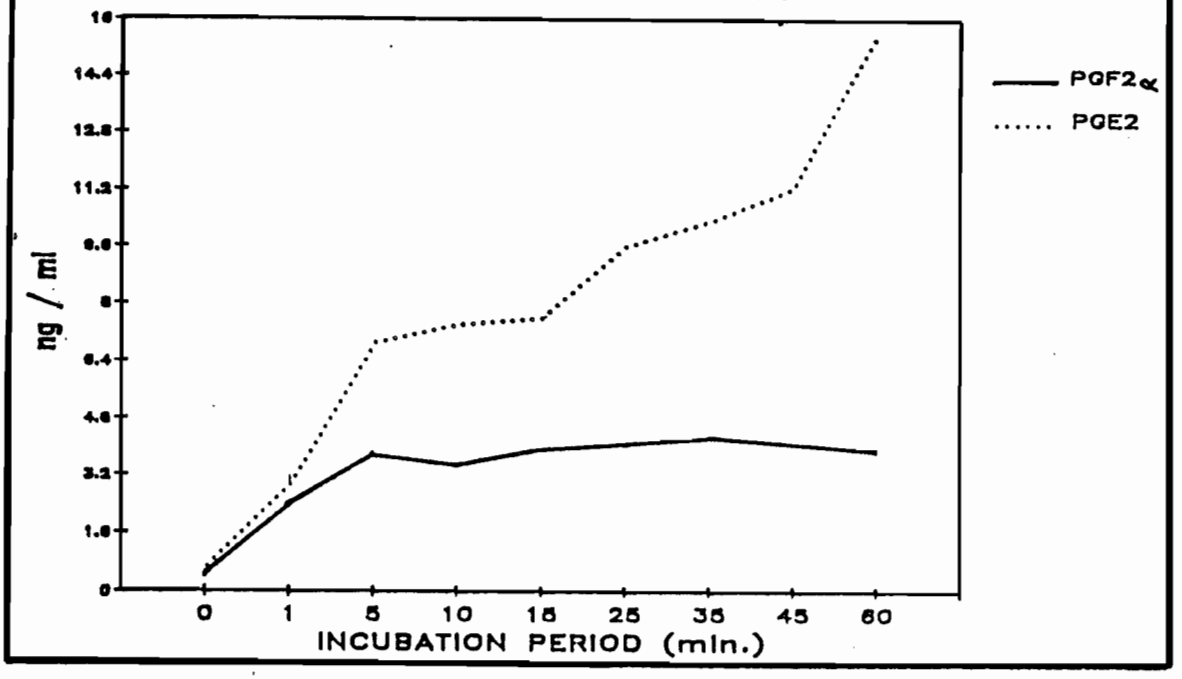


FIG. 19 PROSTAGLANDIN SYNTHESIS BY DIFFERENT AMOUNTS OF B.S.V. MICROSOMES.

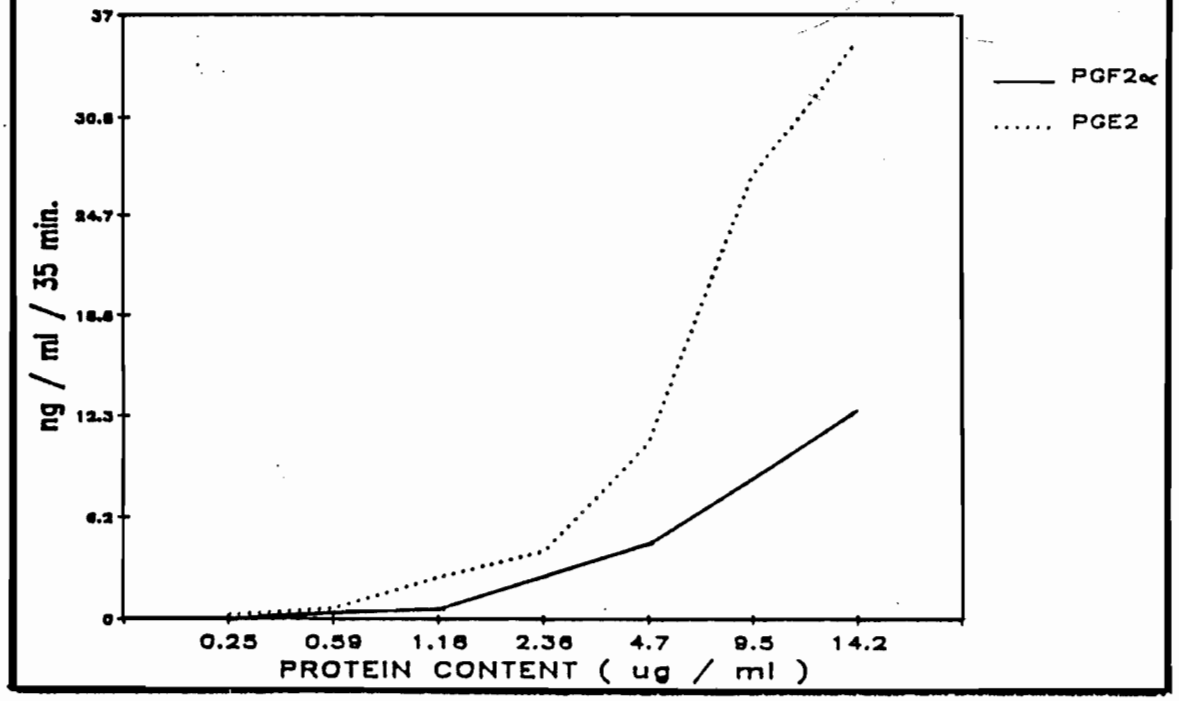


FIG. 20 EFFECT OF INDOMETHACIN UPON PGF2 SYNTHESIS BY B.S.V. MICROSOMES.

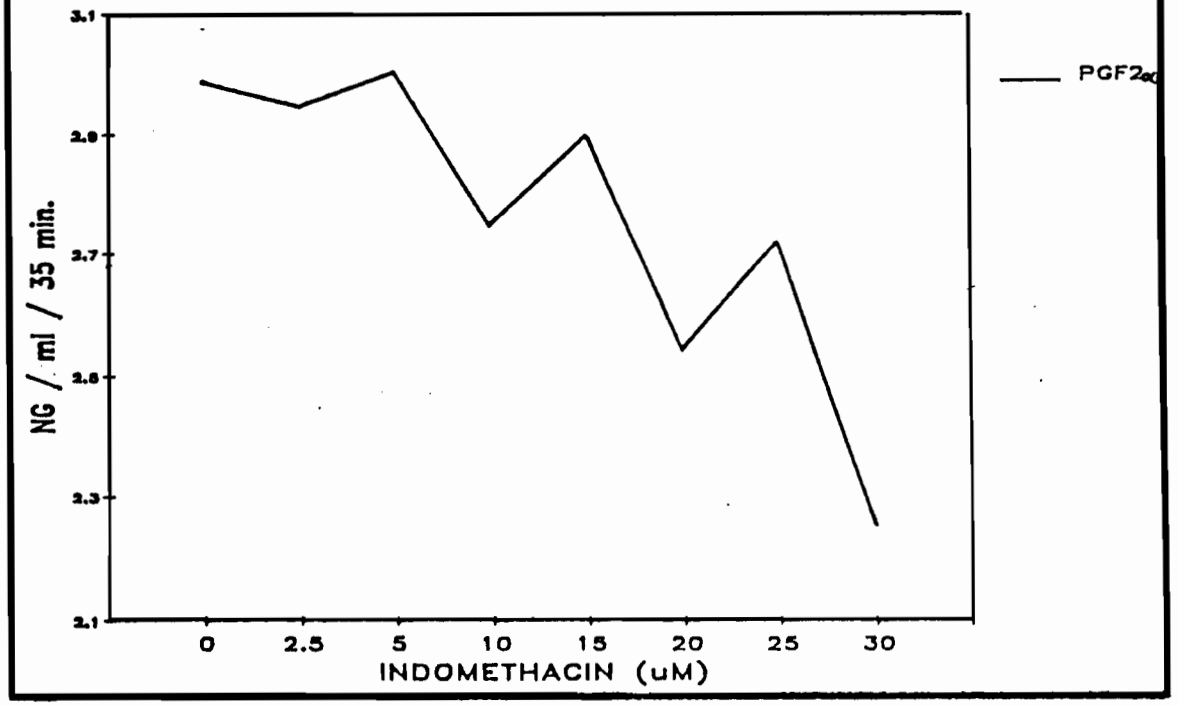


TABLE 9 Percentage of increase of prostaglandin synthesis by bovine seminal vesicle microsomes treated with either fetal urine (FU) or amniotic fluid (AMF) collected at day 109, day 114 of gestation and during labor.

	$PGF_{2\alpha}$			PGE_2		
	109	114	labor	109	114	labor
FU	136 (12)	48 (10)	57 (11)	289 (10)	94 (8)	50 (10)
AMF	16 (11)	26 (5)	33 (4)			

(n) Number of fetuses

5.4.- DISCUSSION

Fetal urine collected at d 109 of gestation has the ability to increase PGF_{2a} and PGE_2 synthesis. However, the effect produced by fetal urine in this experiment was far smaller than that obtained by Strickland et al. (1983) using a similar system and by Casey et al. (1983) and Casey et al. (1984) using an amnion monolayer culture. Nevertheless, it should be borne in mind that tissues and fluids utilized in these experiments were of human origin.

The increase in PG synthesis caused by fetal urine was greater when the samples were collected at d 109 than those obtained either at d 114 or during labor. This is not consistent with the situation observed in allantochorion cells in Experiment 2 (4.3.2.) where pre-labor and at labor fetal urine stimulated PGF_{2a} and PGE_2 production similarly. The BSVM preparation is very sensitive to changes in pH, substrate and product concentrations and, in fact, if arachidonate concentration is increased in the urine collected either at d 114 or during labor, it can decrease PG formation (Flower et al., 1973). Since PG concentrations increase dramatically in fetal urine and amniotic fluid during labor (see Exp.1 Table 1), they could produce a negative feedback in the BSVM preparation due to an excess of product already present in these fluids.

On the other hand, it is also possible that PG syn-

thesis is regulated in a different fashion *in vivo* than *in vitro*.

Amniotic fluid did not increase PGF_{2a} synthesis and its effect in PGE_2 production could not be evaluated. However, AMF produced a notable increase in PGF_{2a} production by allantochorionic cells.

In general, results obtained with the BSVM preparation do not relate to those obtained using allantochorionic cell in experiment 2 (4.3.2.). Even though fetal urine treatments in both experiments resulted in an increased PG synthesis, the cell response was far greater with allantochorionic cells than when using BSVM.

The results obtained treating the microsomes with the different fetal fluids may be due to physico-chemical changes (Yoshimoto *et al.*, 1970) such as pH, substrate availability, and different chemical concentrations rather than a biologically active component in the urine that might require the complete cell in order to manifest its effect.

CHAPTER 6

GENERAL CONCLUSIONS

Parturition is an intriguing phenomenon that appears to be triggered by the harmonious interaction of several hormones resulting in the activation of the prostaglandin cyclooxygenase complex. This enzymatic system catlyzes the formation of those PGs which, eventually, are necessary to increase myometrial contractile activity during labor.

In this study it has been shown that even though there is a dramatic increase in PG concentrations in maternal and fetal blood that drains from the gravid uterus during labor, the increment does not follow a uniform pattern for all PGs. For example, 6-keto-PGF_{1a} plasma levels show smaller increments than either PGFM or PGE_2 during labor, suggesting that the hormonal milieu at parturition regulates the cyclooxygenase either to increase the formation of those prostanoids that stimulate myometrial activity or to increase the catabolism of those that limit this activity.

With regard to the site of PG production, venous-arterial concentrations in both uterine and umbilical blood plasma strongly suggest that the gravid uterus is an important source of PGF and PGE during labor. However, maternal

plasma 6-keto PGF_{1a} levels indicate that although placental cells are able to synthetize PGI_2, this PG is also produced in an extrauterine maternal compartment. Otherwise, production must be insufficient to be detected in the uterine vein or simply is being metabolized within the uterus.

In swine, intrauterine tissues have a considerable ability to generate PGs. After 4 h of incubation, decreasing PGF_{2a} and increasing PGFM medium concentrations indicate that placental cells have a prostanoid metabolic capacity. However, the in vitro formation of PG by these cells did not change during the last 6-7 days of gestation except in the case of PGI_2 which decreased during labor. This resulted in a proportional increase in stimulatory PG which must be required for muscular contraction to occur. It should be emphasized that the in vivo hormonal environment of intrauterine tissues during labor is absolutely different from that existing in the in vitro experiments of this project. For example, the addition of either fetal urine or amniotic fluid to this cell system increased PGF_2 and PGE_2 but not 6-keto-PGF_{1a} production dramatically demonstrating that these fluids contain biologically active substances which alter prostanoid synthesis. However, these fluids did not show the same effects when applied to the bovine seminal vesicle microsomes preparation, even though it must be recognized that this preparation is very susceptible to physicochemical conditions like pH, substrate availability and temperature. In this system biologically active substances which

may need complete cells or membrane receptors to have an effect, would be in direct contact with the enzymatic complex rather than having to act through an intermediary messenger. Therefore, this system might not be appropiate to test the effect of biological substances that probably require cell integrity.

Prostaglandin synthetic capacity varies with different placental membranes. Incubation of amniotic, allantochorionic and amniochorionic cells revealed that those tissues in direct juxtaposition to the endometrium , namely, allantochorion and amniochorion, have a greater production capacity than the amnion, which is virtually floating in the allantoic cavity. In this way, PGs could easily be transferred from the allantochorion and amniochorion to the myometrium in order to exert their effects during pregnancy.

As a future line of research aimed at the elucidation of the effects of late gestation hormonal milieu on PG production by fetal membranes, it will be necessary to challenge either allantochorionic or amniochorionic cells with combination of those hormones that change dramatically at the moment of the initiation of parturition.

APPENDIX 1

Cross-reactivities of the antibodies used to measure the different PGs in this thesis.

	Relative cross-reaction (%)			
PG measured	**PGF_{2a}**	**PGFM**	**PGE_2**	**6-keto-PGF_{1a}**
PGF_{2a}	100.0	0.1	<0.5	<0.1
PGF_{1a}	53.6	-	-	-
15-keto-PGF_{2a}	0.22	20.0	-	-
Arachidonic acid	<0.04	-	-	-
PGE_3	<0.04	-	-	-
PGE_2	<0.04	-	100.0	<0.1
PGE_1	<0.04	-	16.8	-
PGA_1	-	-	<0.5	-
PGA_2	<0.04	-	2.8	-
13,14-dihydro-PGF_{2a}	-	0.5	-	-
13,14-DH-15-O-PGF_{2a}	-	100.0	<0.5	<0.1
15-keto-PGE_2	-	-	<0.5	-
6-keto-PGF_{1a}	-	-	-	-

PGFM and PGF_{2a} antibodies (Ab) supplied by Dr. K.T. Kirton, Upjohn Company, Kalamazoo, Michigan. PGE_2 and 6-keto-PGF_{1a} Ab supplied by Drs. N.R. Mason, Lilly Research Lab, Indianapolis, Indiana, and C.W. Leffler, University of Tennessee, Memphis, Tennessee; respectively.

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