PORCINE INTRAUTERINE STEROIDOGENESIS: LUTEAL VS. INTRAUTERINE PROGESTERONE AS A MEDIATOR OF PRENATAL SURVIVAL, CONCEPTUS DEVELOPMENT AND IN VITRO STEROID PRODUCTION BY THE PLACENTA AND ENDOMETRIUM

by

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(ABSTRACT)

This investigation is a series of four experiments that examine the role of intrauterine steroidogenesis in the pregnant gilt. In chapter 3, ovariectomy (OVX) and medroxy-progesterone acetate (MPA) treatment at two stages of gestation (d19-29 or d59-69) resulted in maintained pregnancy, normal fetal survival rates, normal conceptus development and typical plasma estrogens when compared to intact, control gilts.

In chapter 4, intrauterine steroid synthesis was quantitated by incubating placenta (PLAC) and endometrium (ENDO) from the control and MPA-treated gilts of chapter 1. Placental P_4, estrone (E_1) and estrone sulfate (E_1SO_4) concentrations were significantly higher than ENDO. Progesterone (P_4) production increased between d30 and 70 of pregnancy while E_1 and E_1SO_4 decreased. The addition of pregnenolone (P_5) to the incubation medium enhanced P_4 but not E_1 or E_1SO_4 release. MPA-treatment had no effect on in vitro steroid production.

In chapter 5, OVX gilts from 9 stages of gestation (d20, 25, 30, 35, 40, 45, 55, 60, 80 and 90) were

administered P_5 until undergoing hysterectomy (10d after OVX). Only 1 of 10 gilts OVX on d20 or 25 was able to maintain pregnancy for the entire treatment period. The pregnancy rate was variable (67-100%) for gilts OVX between d30-45 of gestation and 100% for gilts OVX subsequent to d45. All measures of whole litter survival and conceptus development for gilts that maintained pregnancy were equivalent to those expected under ovarian-intact, untreated conditions. Plasma steroid levels were relatively normal but allantoic P_4 failed to increase late in gestation.

In chapter 6, in vitro steroid synthesis by PLAC and ENDO from gilts treated in chapter 5 was evaluated. Placental P_4 production increased as gestation progressed while ENDO P_4 production was low throughout. The addition of P_5 to the incubation medium resulted in increased P_4 synthesis for both tissues at most stages of gestation. Extending the incubation period also resulted in increased P_4 production at several stages of pregnancy. In vitro estrogen production increased markedly as gestation progressed past d65. The addition of P_5 and extended incubation time enhanced E_1 but not E_1SO_4 synthesis. Overall, data indicate that the PLAC and ENDO have a large capacity for steroid synthesis and estrogen synthesis can occur de novo in the absence of ovarian precursors.

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Chapter One

Introduction

A variety of economic, political and environmental factors have had far-reaching effects on agriculturally-related enterprises over the last several decades. Large costs and decreasing profit margins have made it exceedingly difficult for such businesses to remain profitable. Producers attempting to stay solvent must accommodate by either increasing the size of their operations and/or becoming more efficient in their production practices.

The swine industry is no exception and may, in fact, encounter some of the largest problems within the field of animal production. Swine are fed expensive, grain-based diets and are largely unable to take advantage of relatively inexpensive, high roughage content feeds. In addition, many costly drugs and vaccinations are needed to prevent and treat a multitude of prevalent diseases and conditions that have a negative effect on production efficiency. Most important, modern swine production is disadvantaged by large overhead costs associated with total-confinement-type facilities. The advent of confinement operations has been brought about in an attempt to improve the efficiency of swine production and

hopefully increase profit margins. However, enormous investment costs in this type of system make the potential advantages difficult to realize.

Improving the reproductive efficiency of the sow would be one approach modern swine producers could use to increase their profit margins. The best way of quantitating reproductive efficiency of the sow is by the number of pigs weaned per unit time. Nationally, the average number of pigs weaned per litter (8-9) has not improved over the last several decades. Given the large number of viable embryos present at the onset of pregnancy (14-16 average) there is much potential for improvement in this area. However, many researchers have attempted to increase fetal survival using a variety of nutritional, genetic, managerial and surgical approaches and have met with little success.

The three major pieces of the complex puzzle of prenatal mortality are the endometrium, fetus and placenta. The placenta is the life-support system of the fetus, and it would follow that a properly-functioning placenta is essential for fetal survival. Therefore, prenatal mortality (which is a major cause of sub-optimal litter size) may be a function of placental deficiencies that occur during gestation. Unfortunately, relatively few studies have investigated porcine placental function. A key to understanding the dynamics of fetal-maternal

interactions (and therefore fetal survival) would be to increase our understanding of the biochemical events occurring within the cells of the placental and uterine tissues. One biochemical pathway that may have a role in the growing conceptus is the pathway used in the synthesis of steroid hormones. Steroids have a multitude of functions in reproductive processes but their role in the pregnant uterus is largely unknown. This investigation will attempt to futher elucidate the role of intrauterine steroids on pregnancy maintenance, prenatal survival and conceptus development. More specifically, this investigation will provide information on:

1) The ability of the pregnant porcine uterus to function normally at two physiologically important stages of gestation when ovarian progesterone is replaced by a non-aromatizable progestogen (medroxy-progesterone acetate). This treatment will allow for an analysis of the role of ovarian progesterone on estrogen synthesis by the intra-uterine tissues.
2) The ability of the pregnant uterus to function normally when ovarian progesterone is replaced by the steroidal precursor,

pregnenolone. This treatment will test the ability of the intrauterine tissues to produce sufficient steroids for both local and systemic control of pregnancy-related events.

3) The synthesis (and/or release) of various steroid hormones (progesterone, estrone, and estrone sulfate) by the placenta and endometrium of gilts under the above experimental conditions. These studies will allow for quantification of steroid activity in tissues subjected to the described systemic environments.

Chapter Two

Review of Literature

Estrous Cycle

Female swine are polyestrous with estrous cycles occurring approximately every 21 d. These cycles commence at puberty which normally occurs between 4 and 9 months of age. Many factors including nutrition, growth rate, season of the year, and social environment can affect the age at puberty (Christenson and Ford, 1979). Once estrous cycles begin, they usually continue throughout the female's life, interrupted only by pregnancy, lactational anestrus, or endocrine dysfunction.

The estrous cycle is marked by the estrous period which will last for 48-72 h. Estrus is characterized by a series of behavioral and physiological changes that define sexual receptivity for the boar. Ovulation occurs 36-42 h after the onset of estrus and mating should occur prior to this time.

Following estrus the female enters the luteal phase of the cycle. After ovulation, the walls of the recently ovulated follicles undergo luteinization during which the granulosal and thecal-cell layers of these walls reorganize to form luteal tissue. The resulting structures are known as corpora lutea (CL). The reproductive system which was under the domination of estrogen (E) during estrus is now

under the influence of progesterone (P_4), the chief steroid product of CL.

The scenario of follicular growth, ovulation and CL formation will occur regardless of whether the female becomes pregnant. If pregnancy does not occur the CL will undergo luteolysis and decrease the production of P_4 beginning about d 16 post-estrus (Hansel et al., 1973). The uterine production of the luteolysin prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is responsible for CL regression. Progesterone levels continue to decrease and reach basal levels about d 20 of the cycle (Hansel et al., 1973). At this time new follicles are undergoing growth and development for the subsequent estrous cycle.

However, if pregnancy does occur, the CL will be rescued from luteolysis. Luteal regression will not occur (due to a process discussed below) and the luteal tissue persists for the length of gestation (114 d).

Establishment of Pregnancy

Swine are unique among domestic farm species in that they are polytocous (litter bearing). Females ovulate between 10 and 25 oocytes during the estrous period of each cycle, with each oocyte having the potential of being fertilized (Wrathall, 1971).

Fertilization of the oocytes within the oviduct marks the beginning of gestation. Under normal conditions of appropriate timing of insemination and adequate numbers of spermatazoa, the fertilization rate will be between 90 and 95% (Perry and Rowlands, 1962; Wrathall, 1971).

Embryos enter the uterus about 48 h post-ovulation when they are at the 4-cell stage and remain at the cranial end of the uterine horn until about d 6 (Dziuk, 1985). During this period the embryo is growing, first advancing to the morula stage, which is characterized as a solid clump of cells, and then by d 5 to the blastocyst stage (Bazer et al., 1979). The blastocyst consists of an outer layer of trophoblastic cells, an inner cell mass, and a central cavity known as the blastocoel (Anderson, 1978). This structure is completely surrounded by the zona pellucida, a tough mucopolysaccharide layer that protects the developing embryo. Between d 6 and 7 the blastocyst will emerge from the zona pellucida by a process called hatching (Perry and Rowlands, 1962).

A phenomenon known as intrauterine migration also begins concomitant with hatching on d 6 (Dziuk, 1985). This process evenly distributes the blastocysts throughout the length of each uterine horn and from one uterine horn to the other (Warwick, 1926; Dhindsa et al., 1967). Lasley et al. (1963) found intrauterine migration to be a common occurrence upon examination of litters from sows

slaughtered at mid-gestation. They reported that about 40% of the time there were more fetuses in a uterine horn than CL on the ipsilateral ovary . Litters in which intrauterine migration had taken place had more pigs and a lower death loss than litters in which migration was not evident. The migratory process seems to be dependent on the presence of estrogen (E) within the uterus. Pope et al. (1982a) found that silicone beads were distributed throughout the uterus in a similar manner to embryos. Beads implanted with estradiol (E_2) traveled more efficiently than those not implanted or those implanted with cholesterol. Pope et al. (1982a) also suggested that histamine had a role in the movement of embryos in the uterus. Estrogens are probably eliciting their effect by stimulating the myometrium (the muscular layer of the uterus) to contract either directly or through the production of prostaglandins by the endometrium (Pope et al., 1982b). Failure of the system to distribute embryos so that contact is made in both uterine horns results in the loss of that pregnancy (du Mesnil du Buisson and Rombauts, 1963; Dziuk, 1985). Also, the presence of fewer than five blastocysts within the uterus terminates pregnancy resulting in a cycle of extended length (Polge et al., 1966; Dziuk, 1985). However, pregnancy can continue with fewer than five blastocysts if the balance are removed on d 14 or later (Dziuk, 1985). Alternatively, pregnancy

can be maintained with as few as one fetus or in only one horn as long as any portion of the uterus not surrounding fetuses is removed by d 14 (du Mensil, du Buisson and Rombauts, 1963). These findings suggest that there is a local fetal-maternal signal within the uterus. Through this system small litters which would be energetically inefficient can be prevented.

Intrauterine migration is complete by d 11 (Waite and Day, 1967; Dziuk, 1985). Just prior to the cessation of movement, the blastocysts begin to undergo a marked morphological change. The most dramatic changes occur between d 10-12 when the embryos grow from a spherical (3-10 mm diameter) to a tubular (10 to 50 mm long) and then to a thread-like filamentous form (>100 mm in length; Perry and Rowlands, 1962; Anderson, 1978; Bazer et al., 1979; Geisert et al., 1982a). By d 16 the filamentous blastocysts have attained a length of up to 1 m. However, due to the intimate relationship of the embryo with the highly folded endometrium, there is no overlap of adjacent embryos (Anderson, 1978; Dziuk, 1985).

As the expanding blastocyst increases in length it also increases in protein content at an exponential rate, with filamentous embryos incorporating protein at the highest rate (Anderson, 1978). However, prior to d 12 there is little or no increase in DNA or RNA content of the blastocyst. This suggests that growth is taking place due

to cellular remodeling and hypertrophy and not because of cellular hyperplasia (Geisert et al., 1982b). A dense band of cells develop in the ovoid blastocyst extending from the inner cell mass to the top of the embryo (Geisert, 1982b). This band or elongation zone is composed of both trophectodermal and endodermal cells. The remarkable growth phase of the blastocyst takes place through alterations in cellular microfilaments and junctional complexes of the trophectoderm and the formation of filapodia by the endoderm (Geisert, 1982b).

The uterine environment has an important role in the development of the early blastocyst (Pope, 1988). O'Neill and Quinn (1981) showed synchrony between the blastocyst and the uterus to be important by demonstrating that uterine flushings from various stages of the estrous cycle had an inhibitory effect on mouse blastocyst growth in vitro. Bazer et al. (1969) also indicated timing to be critical by showing that transferred porcine embryos which were developmentally younger than indigenous embryos by 1 d or less had a decreased survival rate when compared to the more mature embryos. Also, the uterus is a specific environment for embryonic development since restriction of fertilized ova to the oviductal environment with ligatures impairs development by d 5 (Murray et al., 1971). However, blastocysts restricted only to the ampullary portion of the

oviduct showed few signs of retardation up to the blastocyst stage (Pope and Day, 1972).

Changes in the secretory activity of the endometrium are coincident with blastocyst elongation. The production of various proteins and other compounds by the endometrial epithelium is cyclic in nature with increases starting around d 12 of the estrous cycle and peaking near d 15, regardless of the presence of blastocysts (Ulberg and Rampacek, 1974). Therefore, the production of these proteins appears to be under the control of steroid hormone levels. Exogenous P_4 administered to intact gilts induced greater development of uterine glands with higher mucus and glycogen content compared to controls (Reddy et al., 1958). Experiments using hormone replacement therapy of P_4 and E in various combinations administered to ovariectomized gilts have shown the amount of P_4 injected to be responsible for the amount of protein produced in uterine secretions for both pregnant (Knight et al., 1974a) and nonpregnant animals (Knight et al., 1973; Knight et al., 1974b).

The presence of the embryo in the uterine lumen seems to also have a local effect on endometrial secretions. Heap et al. (1979) reported the incorporation of radioactive leucine into pre-albumin in co-cultures of endometrium and blastocysts, but not of blastocyst cultures alone. Also, animals which were unilaterally pregnant had

lower incorporation of leucine into macromolecular material in the nongravid horn as compared to the gravid horn (Basha et al., 1980). The production of E by the elongating blastocyst is probably the embryonic factor responsible for enhancing uterine secretions. Perry et al. (1976) documented the synthesis of high quantities of E, specifically estrone (E_1), by the d 12 blastocyst. Geisert et al. (1982a) also showed that E in uterine flushings increased markedly as the blastocyst grew from the spherical into the filamentous stage. The filamentous blastocyst continued to produce high levels of E until about d 14 of pregnancy. The timing of production of estrogenic steroids by the blastocyst corresponded directly to the chronology of the presence of protein in uterine flushings (Ulberg and Rampacek, 1974; Geisert et al., 1982a). The mechanism of action of E of embryonic origin on endometrial secretions appears to be through an increase in free intrauterine calcium. The calcium increase probably results from an increase in prostaglandin synthesis by the uterine epithelium (Geisert et al., 1982a; Bazer and Roberts, 1983).

The composition of uterine secretions has received attention by researchers. Energy sources such as glucose and fructose have been detected in the uterine flushings (Bazer and Roberts, 1983), as well as several large molecular weight components. Most of these large molecules

are protein in nature and have a wide range of functions (Bazer et al., 1981; Bazer and Roberts, 1983; Geisert et al., 1982a). Hydrolytic enzymes are present in the fluids and probably function as either a bacteriocide or an inhibitor of the various proteases produced by the blastocyst (Bazer et al., 1981). These proteases allow for implantation of the blastocyst into the endometrial wall; a process that must be prevented in swine (Bazer and Roberts, 1983; Bazer et al., 1981). Two vitamin A carriers are present, retinol and retinoic acid binding protein, presumably to transport vitamin A to the quickly-developing fetus (Bazer et al., 1981).

Most of the research involved with endometrial secretions has centered around the protein uteroferrin. Uteroferrin, also known as porcine purple acid phosphatase, has high acid phosphatase activity and a purple opalascence due to the presence of an iron molecule (Bazer, 1975; Roberts and Bazer, 1980; Bazer and Roberts, 1983; Bazer et al., 1981). The function of this protein appears to be in the transportation of iron to the fetus since radiolabeled uteroferrin can be detected in fetal tissues (Renegar et al., 1982) and in the allantoic fluid pool (Buhi et al, 1983). Also, intravenous injection of radioactive iron isotopes into the gilt will reach the conceptus quickly, with labeled uteroferrin appearing in both uterine flushings and in allantoic fluid samples within 24 h

(Ducsay et al, 1982). Uteroferrin reaches a maximum concentration in the uterine flushings by about d 60-75 of gestation and is about one-third of the total protein produced by the endometrium (Buhi et al., 1983).

Role of the Corpora Lutea

As previously mentioned, in order for pregnancy to be maintained past d 14 in swine, luteal function must persist for the duration of pregnancy. In fact, pregnancy will be terminated within 24-36 h when CL function ceases lost at any stage of pregnancy (Jainudeen and Hafez, 1987). Progesterone secretion by the CL reaches a peak early in pregnancy (around 35-40 mg at d 12), and then decreases until about d 25 of gestation (10-25 mg/ml) (Guthrie et al., 1972). Progesterone levels then remain fairly constant until around d 100 (Knight et al., 1977; Hafez, 1987). There is then a gradual decline in plasma P_4 levels until a few days before parturition when luteal steroid production declines sharply (Ash et al., 1973; Baldwin and Stabenfeldt, 1975; Ash and Heap, 1975).

It appears that there are no morphological, histological, or endocrinological differences between the CL of pregnancy and those of the estrous cycle (Bazer and First, 1983). The factor that has the most influence on either the demise or persistence of luteal tissue seems to

be the anatomical relationship of the ovary to the uterus. Anderson and Melampy (1966) found that in unilaterally pregnant pigs the CL ipsilateral to the non-gravid horn regressed at the time appropriate for a cyclic animal (d 14) while CL associated with the gravid horn did not. However, removal of the non-pregnant horn prevented luteolysis. Unilateral modification of the utero-ovarian relationship in pregnant swine results in regression of CL on the ipsilateral ovary and an overall reduction in conception rate (Longeneker and Day, 1973). These results indicate that factors produced by the uterus and/or conceptus must be communicating with the ovary, and therefore, determining the fate of the luteal tissue. The specifics of this communication will be discussed in the subsequent section that discusses the maternal recognition of pregnancy.

Swine are unique in that removal of the ovarian source of P_4 at any stage of gestation will cause a rapid termination of pregnancy. Females of other livestock species such as the ovine, and equine, and woman gain the capacity to continue gestation after removal of their ovaries. The ability to continue gestation after ovariectomy (or luteectomy) occurs at about d 50 for the ewe, d 170 in mares, and as early as d 24 in women (Jainudeen and Hafez, 1987). In these species the placenta

takes over as the progestagen- producing tissue so that systemic P_4 levels remain high enough to support pregnancy.

Partial luteectomy, that is removal of a portion of the luteal tissue, has been performed in swine by several researchers. Results show that removal of CL early in pregnancy (d 30) either by unilateral ovariectomy or luteectomy stimulates an increase in luteal weight of the remaining CL when compared to the CL of control gilts (Hagen et al., 1984). Maternal plasma P_4 levels, which are depressed for a time after luteectomy, rebound and are equivalent to those of controls (Hagen et al., 1984; Thomford and Dziuk, 1982; Nase et al., 1985). Progesterone concentrations are also depressed in both allantoic and amniotic fluid pools (Hagen et al, 1984). The removal of CL resulted in an increase in embryonic mortality and loss of pregnancy in some of the luteectomized females. This suggests that P_4 fell below a critical level in these animals. Thomford and Dziuk, (1982) determined that as long as sufficient luteal tissue remained as to maintain maternal plasma P_4 at 6 ng/ml or more, normal pregnancy would continue.

Alternatively, the induction of extra CL during gestation can prolong the length of pregnancy (Martin et al., 1977). Additional luteal structures induced with PMSG and HCG have a minimum lifespan of approximately 15 days. Therefore, if new CL are induced after d 100, parturition

will be delayed. It is unclear why these structures are unresponsive to the signals that normally cause luteal regression at the time of parturition. It is interesting to note that the lifespan of these supernumerary CL is of a similar duration to CL of the estrous cycle. This finding supports the theory of Rothchild (1981) which hypothesizes CL to be autoregulatory.

The role of pituitary hormones in luteal function has been examined. Hansel et al. (1973) in a review of this research reported that gilts which were hypophysectomized immediately after estrus had normal ovulations and luteal formation. The infusion of ovine anti-LH antiserum between d 7-11 of the cycle also had no effect on luteal weight or plasma P_4 concentration. Therefore, the single ovulatory surge of LH that occurred prior to these treatments was sufficient for CL to be maintained for one cycle. However, when anti-LH antiserum was administered to pregnant gilts, luteal structures regressed and in most cases, pregnancy was terminated. This suggests that there is a different method of control of the CL of pregnancy than those of the estrous cycle. The injection of LH sources promoted luteal persistence in hysterectomized-hypophysectomized sows but not in sows who were hypophysectomized only (Hansel et al., 1973; Anderson and Melampy, 1966). Obviously, the presence of the non-pregnant uterus makes it impossible for the CL

to persist whether the neuroendocrine system is altered or not.

Maternal Recognition Pregnancy

The factor that is produced by the non-pregnant uterus and is responsible for luteolysis has been identified as prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). In several species, $PGF_{2\alpha}$ is produced by the endometrium and travels to the ovary via a vascular countercurrent system to exert its effect (Hansel et al., 1973; Rothchild, 1981). However, there is some question as to how $PGF_{2\alpha}$ elicits its effect on the luteal tissue of swine. Research with species other than swine has shown $PGF_{2\alpha}$ to effect the luteal cell by: reducing cyclic adenosine monophosphate (cAMP) activation (Torjesen and Aakvaag, 1977), reducing the number of LH receptors (Diekman et al., 1978) and by changing the fluidity of the luteal cell membranes (Carlson et al., 1982). Prostaglandin $F_{2\acute{A}}$ may also reduce blood flow to CL and cause them to degenerate (Niswender et al., 1976).

The pig is unusual compared to other species in that porcine CL are unresponsive to exogenous $PGF_{2\alpha}$ until about d 10 of the cycle. Luteal structures of other domestic farm animals (cow, ewe, mare) will regress with exposure to exogenous $PGF_{2\alpha}$ after about d 4 or 5 of the cycle. Rothchild (1981), in a review of the regulation of the

mammalian corpus luteum, concluded that while $PGF_{2\alpha}$ is the inevitable cause of regression of the CL, it is the ability of the CL to secrete P_4 that determines when that event will occur. In other words, P_4 inhibits the production of prostaglandins by the endometrium during the luteal phase of the cycle. It is unclear what factor or factors are responsible for the initial decrease of P_4 that is necessary for this scenario to take place.

Maternal recognition of pregnancy refers to the phenomenon whereby the pregnant uterus promotes the CL to continue to produce P_4 past the normal time of regression in the cycle and does not allow the female to enter into a follicular phase. The method of communication used by the pregnant uterus to signal the ovary has received extensive study. It appears that the chemical signal for the cow and ewe is protein in nature (Bazer and First, 1983). These proteins, produced by the early blastocyst may elicit their effects by preventing the production and/or release of $PGF_{2\alpha}$ by the endometrium, thus allowing the corpus luteum to persist and leaving the reproductive system under the domination of P_4.

The signal for pregnancy in the pig is quite different. Ball and Day (1982) reported that infusing the non-gravid horn of a unilaterally- pregnant gilt with embryonic extracts allowed pregnancy to continue and maintained CL on the ipsilateral side. Heat treatment of

these extracts did not affect their ability to signal the ovary. However, charcoal extraction negated their influence on the luteal structures and pregnancy was rapidly terminated. These findings led researchers to believe that this chemical signal was steroidal in nature.

As mentioned, the early pig conceptus has the ability to produce significant amounts of estrogenic compounds (especially E_1) in situ (Perry et al., 1973; Heap et al., 1975, 1979; Gadsby et al., 1980). These steroidal compounds are particularly high at d 12 and are positively correlated with uterine protein secretions (Geisert, 1982a). The timing of this increase in estrogenic steroids led researchers to originally conceive a hypothesis whereby E traveled from the uterus to the ovary to either override or neutralize the effects of $PGF_{2\alpha}$ (Perry et al., 1976). However, subsequent research revealed that injection with E_2 benzoate at this time decreased the levels of $PGF_{2\alpha}$ in the utero-ovarian vein and increased the $PGF_{2\alpha}$ content of the uterine lumen (Bazer and Thatcher, 1977; Frank et al., 1977). This led Bazer and Thatcher (1977) to theorize that E from the rapidly-growing blastocyst was not directly communicating with the ovary but was redirecting the travel of $PGF_{2\alpha}$ from the endometrium to the uterine lumen rather than to the uterine vasculature. Geisert et al. (1982a) supported this theory when they reported a positive correlation between E levels and $PGF_{2\alpha}$ or prostaglandin E_2

(PGE_2) in uterine flushings before d 14. In a review of the maternal recognition process, Bazer and Roberts (1983) suggested that the production of estrone (E_1) on d 11-12 of pregnancy by the blastocyst promotes the exocrine secretion of $PGF_{2\alpha}$. Estrogen in turn promotes greater secretion of uterine proteins through increased intraluminal $PGF_{2\alpha}$ and free calcium levels. Prostaglandins and calcium probably also play a role in water and electrolyte transport by the early conceptus, as well as in capillary permeability.

The theory that E is the chemical signal of pregnancy is supported by research concerning the pseudopregnant condition. Injection of E sources between d 11-15 of the cycle extends the luteal phase to greater than 60 d, simulating a pregnant condition (Bazer and Thatcheret al., 1977b; Basha et al., 1980; Etienne and Jemmali, 1982). Ford et al., (1982) found this effect to be locally-controlled since unilateral infusion of E_2 benzoate into the uterine lumen maintained CL on the ipsilateral side and increased P_4 levels in the uterine vein on that side. However, no differences could be found in $PGF_{2\alpha}$ concentrations in the uterine vein on d 14-15 for treated and untreated horns. The incubation of endometrium from pregnant and nonpregnant gilts resulted in 4-6 times higher production of $PGF_{2\alpha}$ by the nonpregnant uterine tissue (Guthrie and Lewis, 1986). The high intraluminal $PGF_{2\alpha}$ levels that have been reported for the pregnant female may

be of a placental source. Guthrie and Lewis (1986) hypothesized that placental prostaglandins may be rapidly metabolized within the uterus rendering them inactive as luteolytic agents. Whatever method is used within the pregnant uterus it is clear that intraluminal estrogen is the primary signal that prevents $PGF_{2\alpha}$ from reaching the corpora lutea.

Steroidogenesis

Steroidogenesis is the process whereby cells, using cholesterol as a substrate, produce the various steroid hormones by way of a series of enzyme-dependent pathways. The particular hormone produced by a cell is dependent on several factors including the tissue and species of origin (Martin, 1985). The basic structure of steroid hormones is the perhydro-cyclopentanephenathrene ring (Speroff et al., 1978). The three major groups of hormones are formed by alteration of this basic ring structure. Adrenal corticoids and progestins (including pregnenolone and P_4) share a 21-carbon or pregnane nucleus. All androgens possess a common androstane nucleus which contains 19 carbons. Lastly, E are derived from the 18 carbon estrane nucleus. In the steroidogenic pathway, the number of carbons is decreased, but never increased. Specific steroids derive both their names and activities by

substituents that are located on any six centers of assymmetry (Speroff et al., 1978). A steroid molecule is nearly flat with some substituents situated either above (alpha) or below (beta) the plane of the ring skeleton (Speroff et al., 1978).

Cholesterol is the 27-carbon precursor for steroid hormones. It is stored within the cell in the form of cholesterol esters which are hydrolyzed upon stimulation of the cell by protein hormones (Martin, 1985). Pregnenolone (P_5) is the first cholesterol-derived steroid, produced in a three-step reaction, with the first two reactions catalyzed by the enzyme cytochrome P-450 (Speroff et al., 1978; Bohinski, 1987). These two steps result in the hydroxylation of the cyclopentane side chain. The third reaction cleaves this hydroxylated six carbon side chain (Speroff et al., 1978; Bohinski, 1987). One of the enzyme systems required for the transformation of cholesterol into the various steroids is the cholesterol side chain cleavage enzyme (or pregnenolone synthetase) located within the mitochondria (Speroff et al., 1978; Martin, 1985). Other enzymes are associated with the endoplasmic reticulum amd others are free within the cytoplasm. Therefore, the formation of a steroid hormone involves the shuttling of precursors and intermediates across organelle membranes (Martin, 1985). The pathway used in the formation of a steroid hormone is governed by the cell in which it is

produced; the presence or absence of the various steroidogenic enzymes control the outcome (Speroff et al., 1978).

After a steroid hormone is produced, it may be secreted into the bloodstream. A majority of steroids in the blood are bound either to specific or nonspecific binding proteins (Karlson et al, 1975). Speroff et al. (1978) reported that 80% of E_2 was bound to a specific steroid binding beta globulin (steroid hormone binding globulin). They found the balance of E_2 to be loosely bound to serum albumin, except for about 1% which was free floating. Gorrill and Marshall (1986) also showed most of the E_2 in the bloodstream to be bound. However, they reported the proportion bound to albumin to be greater than that bound to the steroid globulin. In either case, very little hormone was found to be unbound in the bloodstream. Binding proteins increase the efficiency of transport of steroid hormones and probably have a negative influence on their metabolism, therefore preventing breakdown into inactive metabolites (Speroff et al., 1978). The biological activity of a hormone also may be limited by binding to a protein (Speroff et al., 1978). This system tempers the effects of a hormone and prevents dramatic changes in systemic concentrations.

Free steroids can readily enter cells via rapid diffusion (Karlson et al., 1975; Speroff et al., 1978).

However, in order to exert its effect a steroid molecule must be bound to a receptor. Therefore, only target tissues with cells which contain specific receptors for a particular hormone can react to that stimulus (Speroff et al., 1978). The original theory of steroid hormone action is as follows: 1) the molecule enters the cell and is bound by a cytoplasmic protein, the "steroid receptor"; 2) the receptor transports the steroid to the nucleus; 3) the steroid is attached to a smaller receptor within the nucleus, either an entirely nuclear receptor or possibly a fragment of the cytoplasmic receptor; 4) the hormone receptor complex within the nucleus stimulates gene transcription, i.e. the production of mRNA (Karlson et al., 1975). The last step occurs either as a positive response of RNA polymerase to the binding of the complex directly to DNA itself or by the interaction of the complex with a repressor protein. Interaction with the repressor protein causes its removal or derepression (Karlson et al., 1975). A newer model of steroid receptor interaction proposes nuclear estrogen receptors to be immobilized within the nuclear fraction (Gorski et al., 1984). The results of this study showed receptors, whether bound or unbound, to be in the nucleus at all times. The association of the unbound receptor to the target site in the nucleus was hypothesized to be the critical factor in defining the nature of response to the estrogenic hormones. In this

model, binding of the steroid causes conformational changes of the receptor to initiate its effect. Other research has shown the level of cytoplasmic receptors to be critical to the degree of response of a cell. Katzenellenbogen et al. (1977) found the uterine responsiveness to E_2 to be dependent not on total receptor levels but on those found in the cytosol. They reported that administration of antiestrogenic compounds decreased tissue responsiveness by depleting the number of cytoplasmic receptors. The researchers theorized that this occurred because the antagonists bound to the receptors and were translocated to the nucleus, as is the naturally occurring hormone. Therefore, cytosolic receptor levels were depressed without elicitation of a physiologic effect (Katzenellenbogen et al., 1977; Ferguson and Katzenellenbogen, 1977).

Steroidogenesis by the Early Conceptus

There is much evidence that the early mammalian conceptus is capable of steroidogenic activity. Research with laboratory species has shown enzymes of the steroid pathway to be present as early as the one cell stage for the mouse (Wu and Matsumoto, 1985), rat (Wu and Matsumoto, 1985), and hamster (Wu, 1985). However, it does not appear that all of the enzymes necessary for the blastocyst to synthesize steroids de novo are present. The

preimplantation rabbit blastocyst has the ability to metabolize P_4 (Angle and Mead, 1979) and androgens (Singh and Booth, 1979) but cannot synthesize P_4 when P_5 is added as a precursor (Angle and Mead, 1979). Just prior to implantation the rabbit blastocyst gains the ability to produce large amounts of estrogens with aromatase activity reaching peak activity about d 6 (Singh and Booth, 1979; Hoversland et al., 1982; Wu and Lin, 1982). One of the most important steroidogenic enzymes, 3-ß-hydroxysteroid dehydrogenase (3-ß-HSD), does not appear until the post-implantation period for the rat (Dickmann and Dey, 1974) or mouse (Sherman and Atienza, 1977). However, 3-ß-HSD is detectable in hamster embryos as early as d 1 (Wu, 1985). 17-ß-hydroxysteroid dehydrogenase (17-ß-HSD), an important enzyme in E metabolism, has high activity at d 1 in the rat but in the mouse it is not present in significant quantities until about d 4 (Wu and Matsumoto, 1985). The administration of an aromatase inhibitor to the pregnant rabbit had no effect on blastocyst size or on implantation (Wu and Lin, 1982). Conversely, the addition of an E antagonist to rabbit embryos in vitro inhibited their growth from the morula to the blastocyst stage (Paria et al., 1984).

Steroidogenic activity of the early conceptus in domestic farm species has also been studied. As discussed, the early blastocyst of the cow and ewe produce protein

substances that are important in the maternal recognition of pregnancy. It is apparent that the early conceptus in each of these species has some steroidogenic capabilities as well. 3-ß-HSD activity has been detected as early as d 18 in the ovine conceptus (Flood and Ghazi, 1981). Bovine blastocysts cultured in vitro produce significant amounts of P_4 between d 13-16, also suggesting 3-ß-HSD activity (Shemesh et al., 1979; Shemesh and Hensel, 1983; Reimers et al., 1985). Estrogen production by the early bovine and ovine conceptus occurs only to a minor extent (Shemesh et al., 1979; Heap et al., 1979).

Steroid production by the early porcine conceptus has received considerably more study than that in other farm species. Early research revealed the level of E in urine significantly increased by d 15 of pregnancy in gilts (Bowerman et al., 1964). Since ovarian E production should be low during gestation when P_4 from the CL is dominating the reproductive system, this result suggested that steroid metabolism was occurring within the pregnant uterus. Perry et al. (1973) demonstrated the early pig conceptus was capable of E production in situ. They showed free E to be produced from the conversion of ^{3}H-dehydroepiandrosterone (^{3}H-DHEA), androstenedione (A), P_4, and estrone sulfate (E_1SO_4). These results were confirmed by similar studies in which the early blastocyst was found to be a dynamic steroidogenic entity with E_1 as its chief product (Raeside,

1963; Heap et al., 1975; Perry et al., 1976; Heap et al., 1979; Gadsby et al., 1980).

The metabolic capability of the blastocyst to convert steroidogenic precursors into E_1 reaches peak activity at d 12, a period of time critical for maternal recognition of pregnancy (Flood, 1974; Heap et al., 1975; Perry et al., 1976; Heap et al., 1979, Bazer et al., 1979). Heap et al. (1975) found little conversion of cholesterol or P_5 into estrogenic compounds, but significant androgen compounds were present. These results indicate that the blastocyst may not be capable of de novo E_1 synthesis. In a subsequent experiment, Heap et al. (1979) reported evidence that the porcine blastocyst was able to convert acetate (the building block of cholesterol), cholesterol, P_4, DHEA, androstenedione, and E_1SO_4 to E_1 and E_2. In yet another study, Heap et al. (1981) found normal E levels and embryonic development when systemic P_4 was replaced by a non-aromatizable synthetic progesterone (Medroxyprogesterone acetate, MPA) early in gestation in ovariectomized gilts. This series of investigations indicates that either the blastocyst aromatizes steroid precursors that are not of an ovarian source or that the early conceptus is capable of synthesizing E de novo. One anatomically logical source of steroid precursors for the blastocyst is the endometrium. This tissue has been shown to be steroidogenically-active early in gestation, but to a

much lesser degree than the embryo (Flood, 1974; Heap et al., 1975; Fischer et al., 1985).

An important enzymatic role of the endometrium is its ability to conjugate sulfate groups to steroid produced by the blastocyst. Heap et al. (1975) found very little aromatase activity in either the endometrium or myometrium, but a high amount of sulfotranferase activity in both of these tissues. However, Hopkins (1987) found endometrial aromatase activity to be greater than that of the placenta at d 30, 60, and 90. Perry et al. (1976) proposed that E (namely E_1) of conceptus origin may be sulfated by the endometrium and then travel to the ovary to be hydrolyzed and have a luteotrophic effect. Although it was later discovered that the luteotrophic effect of E produced by the blastocyst did not act in this manner, this hypothesis was a significant step in the process of elucidating the role of conjugated E during early pregnancy. It was previously believed that steroids were sulfated for subsequent inactivation and elimination in the urine. Perry et al. (1976) discounted this belief with their theory proposing a physiological role for conjugated steroids. Subsequent research found sulfotransferase activity to be high during the diestrus phase of the estrous cycle (Brooks and Rozhin, 1978) and to remain high during early pregnancy until about d 30 (Dwyer and

Robertson, 1980). Therefore, it can be concluded that the sulfation of E_1 requires systemic P_4 (Pack et al., 1979).

Estrone sulfate is detectable in the plasma of pregnant gilts and sows only and not in cycling females (Robertson et al., 1978). Conjugated E are produced as early as d 12 (Geisert et al., 1982a), are found in the plasma as early as d 17 (Robertson et al., 1978; Hattersley et al., 1980; Horne et al., 1983), and increase to approximately d 30 where they approach a level of 2-3 mg/ml (Hattersley et al., 1980). The level of E_1SO_4 in the plasma is positively correlated with litter size, giving further credence to the hypothesis that circulating E are of embryonic origin (Horne et al., 1983). Sulfatase, the enzyme responsible for hydrolyzing sulfate groups from the E_1, has high activity in the early porcine blastocyst (Heap et al., 1975) and in the endometrium (Dwyer and Robertson, 1980). Knight (1977) suggested that conjugated E_1 may serve as physiological systemic pool of E for local effects at the site of the conceptus.

The presence of local E within the early gravid uterus has multiple and varied functions. The effects of E on luteostasis and on the synthesis and secretion of macromolecules by the endometrium has been discussed. Other functions attributed to E are increasing uterine blood flow (Bazer et al., 1979; Ford and Christenson, 1979), enhancing water, carbohydrate and amino acid

transport to the placenta (Kirkland et al., 1977; Goldstein et al., 1980), and increasing uterine weight through an increase in DNA synthesis and RNA polymerase activity (Kirkland et al., 1977; Whelly, 1985). Pupkin et al. (1975) studied uterine blood flow in the pregnant ewe and found the flow of blood to increase within 90 min of the E peak caused by the administration of E or E precursors. Also, restricting the conceptus to only the pregnant horn of the ewe decreases total uterine blood flow (Caton et al., 1984). The uterine vasculature is affected by the presence of E with an increase in permeability of the vessels starting about d 12 (Keys et al., 1986). Increased vascular permeability allows for improved transport of water and other nutrients to the conceptus. This effect is probably responsible for the rapid increase of fluid within the allantoic compartment of the porcine placenta that occurs between d 20 and d 30 (Knight et al., 1977). Dickmann (1979) proposed a somewhat different theory for the interaction of the early conceptus and the P_4 dominated uterus. He suggested that a local inflammatory-like reaction takes place with E from the embryo relieving the area immediately surrounding the conceptus from P_4 dominance.

Steroidogenesis Beyond the Early Period of Gestation

Although it is clear that the early pig conceptus is steroidogenically-active, it has been long believed that the porcine placenta is unable to produce appreciable quantities of steroid hormones after the early stage of pregnancy. This belief mostly stems from the fact that the pregnant sow is unable to maintain pregnancy once the ovarian source of P_4 is removed. Researchers have concluded from these results that the placenta is unable to produce steroids.

The role of the placenta as an endocrine gland in other species has been substantiated. The sheep is a good example of an animal that is able to continue pregnancy after ovariectomy (Hafez, 1987). Ovine placental tissue produces both E and P_4 in vitro (Perrepoint et al;., 1970; Pupkin et al., 1975; Koligian and Stormshak, 1976; Beal et al., 1986; Power and Challis, 1987) and production is enhanced by precursors such as P_5 (Koligian and Stormshak, 1976; Beal et al., 1986; Power and Challis, 1987), various androgens (Perrepoint et al., 1970; Pupkin et al., 1975), and conjugated E (Power and Challis, 1987). The placenta is also stimulated by the addition of gonadotropic hormones such as LH or HCG (Koligen and Stormshak, 1976; Beal et al., 1986). Similar results have been obtained in the

monkey (Hagemenas and Kittinger, 1974), human (Laplante et al., 1983; Siler-Khodr et al., 1986), rat (Matt and Macdonald, 1982; Legrand et al., 1984; Matt and Macdonald, 1984; Macdonald and Matt, 1984; Jackson and Albrecht, 1985; Bassett and Pepe, 1987) and cow (Evans and Wagner 1981); all species which are able to continue pregnancy without their ovaries at various points of gestation.

In recent years, there has been mounting evidence that the porcine placenta is steroidogenically competent. The ability of the placenta to produce steroids may not be of sufficient degree to compensate for a loss in ovarian sources, but may be of a critical physiological importance. Barnes et al. (1974) were among the first to suggest placental production of P_4 based upon the large umbilical vein minus umbilical artery differences in P_4 concentration. These findings were validated by Hagen et al. (1983) who reported similar results on d 80 of gestation and concluded that this difference was much greater than that which could be attributed to maternal transfer across a concentration gradient. Knight et al. (1977) found uterine artery (UA) minus uterine vein (UV) concentrations of P_4 to be positive and UA-UV E_1 differences to be negative for 11 stages of gestation between d 20 and 100, suggesting that P_4 of luteal origin was being metabolized by the pregnant uterus to form estrogenic compounds. This hypothesis is supported by evidence from several other studies in which pregnant gilts, ovariectomized on d 4 of gestation were treated with exogenous P_4 to maintain pregnancy either to d 40 (Knight

et al., 1974a) or d 60 (Bazer et al., 1979). Gilts which failed to maintain pregnancy under this protocol had plasma P_4 concentrations that were as much as 20 times higher than their pregnant counterparts. This again suggested that the pregnant uterus has a marked effect on P_4 metabolism. Other studies from our laboratory have revealed positive UA-UV differences in P_4 concentration (Dalton and Knight, 1983) and negative differences in E_1 concentration (Kukoly, 1984). Nase et al. (1985) confirmed these findings in a study utilizing partially luteecomized gilts. They also showed positive UA-UV and negative umbilical artery - umbilical vein differences in P_4 concentration at various stages of gestation for all treatment groups.

Evidence from the in vitro incubation of placental tissue has validated the theory that the porcine conceptus has significant steroidogenic activity. Heap et al (1981) showed the placenta of d 28 of pregnancy to produce 10 times as much P_4 as the endometrium in intact gilts. Placentas from ovariectomized gilts maintained with MPA produced less P_4 and more E than control gilts in this study. Results from other studies (Fischer et al., 1985; Kukoly, 1984) also revealed a much higher output of steroids by the placenta as compared to the endometrium. Kukoly (1984) found that the in vitro production of P_4 by the placenta steadily increased throughout gestation, except for a short lag phase between d 40 and 50.

Production of E_1 was biphasic with a peak at d 30 followed by a period of low activity until d 70 when the output of E_1 started an increase that continued until term. There was no additional appearance of steroids in the incubation media after 1 h incubation, suggesting a deficiency of metabolizable substrates in the culture. In a follow-up study Jeantet (1985) detected an augmentation of P_4 production by both the placenta and endometrium (d 30, 60, and 90 of gestation) with the addition of the precursor P_5 to the incubation media. The placentas of d 60 and 90 gilts produced significantly more E_1 with the addition of P_5 while the endometrium only increased E_1 formation with the addition of precursor at d 90 of gestation.

Steroid hormones within the maternal plasma changes dramatically as gestation progresses. It has been known for some time that E can be found in the urine of the pregnant sow and that E concentration increases as pregnancy goes to term (Velle, 1958; Raeside, 1963; Bowerman et al., 1964; Anderson and Melampy, 1966). In fact, E in the maternal blood appear to change in a triphasic fashion with peaks on d 10 to 12 (Stoner et al., 1981) d 16 to 30 (Robertson and King, 1974; Knight, 1977; Stoner, 1981) and from d 60 to term (Robertson and King, 1974; Knight, 1977). The ratio of conjugated to unconjugated E fluctuates as well. Hopkins (1987) found the E_1SO_4:E_1 ratio in the UV plasma to decrease from a

level of 15:1 on d 30 to 4:1 on d 60 and 1:2 on d 90. As previously suggested, the changing ratios of these hormones may have significant physiological significance in controlling the biological availability of the E in the maternal plasma pool.

Plasma P_4, which is fairly steady in concentration throughout gestation (Knight, 1977; Kukoly, 1984; Jeantet and Knight; 1985), starts a gradual decline about 1 week before parturition (Ash et al., 1973; Ash and Heap, 1975; Baldwin and Stabenfeldt, 1975). During the last 24-48 h before parturition there is a sharp decline in progestagens so that together with the increasing levels of E_1 a dramatic change in the systemic P_4:E ratio is evidenced. It is this change that is thought to initiate the cascade of events involved in parturition (Ash et al., 1973; Ash and Heap, 1975; Baldwin and Stabenfeldt, 1975).

Placental Development and Function

The mammalian placenta is an organ that differs from all other organs in several significant respects. Four important differences were outlined in a review by Steven and Morris (1975) and are as follows:

1) the placenta is formed as a result of various degrees (depending on species) of interaction

between fetal and maternal tissues within the pregnant uterus;

2) it is situated outside the body of the embryo to which it is connected by a cord of blood vessels;
3) it is a disposable organ with a strictly limited lifespan; and,
4) it exhibits a wide variety of structural modifications, so much so that major differences may be found between closely-related species.

The porcine placenta when classified by histological appearance falls into the category of a epitheliochorial type placenta (Grosser, 1909). This type of placenta has the maximum number of tissue layers that can be present, therefore, presenting a significant barrier between fetal and maternal blood. The six layers of tissue dividing the two blood systems are: 1) the endothelium of the fetal capillaries, 2) fetal connective tissue, 3) fetal chorionic epithelium, 4) maternal uterine epithelium, 5) maternal connective tissue, and 6) endothelium of maternal capillaries (Grosser, 1909). The elongated blastocyst that is resultant of cellular remodeling begins the process of placental attachment approximately d 14 (Crombie, 1970; Allen, 1975). This process, referred to as placentation, is different from the invasive type of implantation that occurs in many species such as rodents and humans (Samuel and Perry, 1972). The importance of endometrial proteins

in preventing this invasive type of attachment in the porcine has already been discussed. At this time the trophoblast will begin to follow the contours of the endometrium. The epithelial layers of both the fetal and maternal sides begin to form short microvilli and may begin to interlock (Crombie, 1970). Crombie (1970) described this attachment to be well advanced by d 18 with two distinct cell types visible: tall columnar cells that are somewhat separated from the endometrium and a low cuboidal cell type that is in close apposition to maternal epithelium. By this point, the endometrium has developed an extensive vascular plexus along with the large increase in uterine glandular growth. Attachment is complete by d 24 with the placenta being held to the endometrium by a well developed microvillous system and what is described as an electron-dense maternal. There is a significant degree of folding of both the endometrium and placenta that occurs during the placentation process. Distension of these endometrial folds, known as rugae, by the extensive blood and lymph system also aids in keeping the placenta in close apposition to the uterine wall (Crombie, 1970; Allen 1975).

Concominant with trophoblastic attachments to maternal tissues, the trophoblast and cells of the inner cell mass are differentiating into the major layers of the placenta. The somatopleure, the outermost layer of the growing conceptus, is formed by the trophoblastic ectoderm and a

layer of embryonic mesoderm (Steven and Morris, 1975; Jainudeen and Hafez, 1987). This will become the outer placental layer, the chorion. The amniotic sac is completely formed by d 18 and develops by way of dorsal folds of the somatopleure which envelope the growing embryo and fuse above its mid-dorsal surface (Steven and Morris, 1975). The yolk sac, a vesicle of endodermic origin, is also developing by this point. It is a very transitory structure that appears to function as a temporary vascular bridge between the chorion and the embryo (Steven and Morris, 1975). The placenta is categorized as a chorio-vitelline type while the yolk sac is serving in this capacity (Steven and Morris, 1975). Lastly, the allantois is formed as an outpocketing of the embryonic hindgut (Jainudeen and Hafez, 1987) and is first visible by d 14 of gestation (Steven and Morris, 1975). The tissue is mesodermal in origin and is very dynamic, reaching a size as large as the embryo itself by d 17 (Friess et al., 1980) and making contact with the chorion by d 19. By this time the yolk sac is regressing and the allantois is replacing it as the vascular bridge between the chorion and embryo. By d 30 the chorion has become extensively vascularized from blood vessels of the allantois which have contacted all but the chorionic extremities (Steven and Morris, 1975). The allantois has become fused with the chorion at this juncture so that the outer placental membrane can now

be referred to as the chorio-allantois. The fetus is connected to the allantois via the urachus, a duct that is part of the umbilical cord and is continuous with the fetal bladder (Renegar et al., 1982).

The placenta can be morphologically divided into three distinct areas by d 40. These regions are: 1) the large placental zone that directly surrounds the fetus, 2) the paraplacental areas that lie on either side of the placental zone, and 3) the avasucular extremities or necrotic tips (Steven and Morris, 1975). The necrotic tips are formed in the extreme ends of the chorion where the allantois has failed to make contact. Blood flow is restricted in this area rendering the tissue non-functional, thus preventing possible malformative dangers that might occur due to the vasuclar anastomosis of adjoining fetal units (Hughes, 1929). The paraplacental zone is adjoined to the avascular extremities via an annular constriction and can be identified by the parallel arrangement of allantoic blood vessels that run along the long axis of the placenta and beneath its surface (Steven and Morris, 1975). The placental zone is easily identified by the numerous rugae that are present and by a high concentration of specialized absorptive structures known as areolae (Steven and Morris, 1975).

The areolae develop from the tall columnar cells of the chorion that are seen separated from the endometrium on

d 18 (Crombie, 1970). These dome-shaped structures are visually evident by d 30 and appear on points opposite to the endometrial glands (Allen, 1975). Histologically, areolar cells have long microvilli, many coated vessicles, and a well developed apical tubular system. This cellular arrangement implies that these structures have an absorptive function which is substantiated by the fact that the lumen of areolae are usually filled with endometrial gland secretions (Friess, 1981). Absorption of these uterine secretions of histotrophe is undoubtedly essential for placental development and growth of the fetus. The number of areolae increases as gestation advances to about d 50 and the number then remains constant until term. However, individual areolar growth continues until d 100 with the growth rate being lower after d 50 (Knight et al., 1977).

The interareolar regions of the chorio-allantois also undergoes changes as gestation progresses. The fetal capillaries are separated from the maternal epithelium by about 40 μm at d 30 (Friess et al., 1980; Crombie, 1970). The effectiveness of the placental barrier is reduced by the fact that the endometrial capillaries are fenestrated providing for a higher proportion of substrates to be filtered from the maternal vascular system (Crombie, 1970). Perfusion fixation of porcine conceptuses has shown placental thickness to decrease as gestation proceeds

towards term (Friess et al., 1980). This change was found to be due to indentation of the chorio-allantois and the endometrium by the capillary network. Capillary indentation was seen to occur predominantly on the lateral sides and on the summits of chorionic ridges which would be in apposition to the troughs of the endometrial rugae. By term the distance between fetal capillaries and the maternal epithelium has been reduced to as little as 2 μm (Friess et al., 1980). Therefore, it can be concluded that as the fetus is growing and requiring nutrients at an increased rate, the physical impediment imposed by the placenta is accommodating this circumstance by decreasing its effective thickness as gestation progresses.

The placental compartments function as a reservoir for the fetal fluids. The amniotic membrane that directly surrounds the fetus is filled with amniotic fluid that serves several important functions. These functions include: 1) a shock absorber to protect the fetus from external forces, 2) a medium to prevent adhesion of the fetus to the amniotic membrane, and 3) lubrication of the birth canal during parturition (Jainudeen and Hafez, 1987). Amniotic fluid is first detectable about d 30 of gestation and increases in volume steadily until about d 70. After d 70 the volume does not significantly increase and becomes quite variable between littermates (Wislocki, 1935). There is little change in the composition of the amniotic fluid

of the pig during gestation (Wales and Murdoch, 1973; Knight et al., 1977; Bazer et al., 1979), with its main constituents being sodium, chloride, and phosphate ions as well as water and fructose (Jainudeen and Hafez, 1987). Amniotic fluid seems to originate mostly from the maternal circulation, although it is partially derived from the respiratory tract, buccal cavity and fetal urine (Jainudeen and Hafez, 1987). The amnion is a quite impermeable membrane, a characteristic that is very important in determing the composition of the amniotic fluid (Paton et al., 1907; Mellor and Slater, 1971). Early research led to the hypothesis that the fetal fluids were mostly of renal origin (Paton et al., 1907). However, the relative size of the fetus during early gestation and the fact that the urinary system merely redistributes water rather than produces it led researchers to reject this theory (Bazer, et al., 1979). However, urine does become a significant component of the amniotic fluid during late gestation in the sheep (Mellor and Slater, 1971). This occurs as the urethra of the developing lamb becomes patent and capable of transporting urine. It is not clear if this occurs in the fetal pig.

Within the chorio-allantois is the other major fetal fluid pool, the allantoic fluid. In contrast to the amniotic fluid, the allantoic fluid is much more of a dynamic entity (Bazer et al., 1979). Although variable,

the volume of allantoic fluid of the pig exhibits two distinct peaks, one at d 30 of gestation and the other around d 60 (Wislocki, 1935; Knight et al., 1977).

Knight et al. (1977) hypothesized the early peak of allantoic fluid to be important for the rapid expansion of the allantochorion within the uterus. Expansion of the allantoic fluid compartment would therefore allow for maximum contact of the chorion with the endometrium. Placental length and weight increase rapidly between d 20 and 30 of gestation coinciding with the large increase of allantoic fluid. The placenta continues to grow until about d 60, but at a much slower rate. The majority of fetal growth occurs after d 60 but placental expansion prior to this time is probably a critical factor in determining fetal survival and fetal size at term. Knight et al. (1977) further theorized E within the pregnant uterus to be the essential factor necessary for fluid imbibition by the fetal membranes.

Bazer et al. (1979) supported the theory of Knight et al. (1977) by proposing a model which explained the controlling factors involved in fluid accumulation within the placenta. This theory postulates that it is the P_4:E ratio that is responsible for the transport of water and electrolytes across the chorio-allantois. Prior to the first period of fluid accumulation, a sodium-potassium activated transport ATPase initiates the pumping of sodium

out of the allantoic fluid. This produces a hypotonic solution within the allantois relative to the maternal plasma. The hormonal environment at this time renders the chorio-allantois "leaky" thus allowing water and ions to diffuse into the allantoic fluid. Because of the large concentration gradient produced by active transport, sodium passes into the allantoic cavity bringing water and an anion (Cl^-) along with it. Sodium is pumped out of the allantoic fluid faster than it can diffuse in, therefore maintaining this concentration gradient. Changes in the hormonal environment at around d 30 decrease the permeability of the chorio-allantois to these electrolytes. However, continued operation of the sodium pump maintains the electrical potential of the membrane so that when the steroidal hormones return to the proper ratio, allantoic fluid can again accumulate.

Subsequent research has supported the hypothesis of Bazer et al. (1979). Intense sodium-potassium ATPase activity has been detected within the areolar chorionic epithelium with little activity found elsewhere in the placenta (Firth, 1984). This suggests that areolae are the principle sites for placental sodium transport. The presence of a sodium concentration gradient as well as a short-circuit current has been reported by several researchers (Crawford and McCance, 1960; Boyd et al., 1984; Boyd et al., 1985). The potential difference was affected

by various factors in these experiments including several neurotransmitters, pH, CO_2, O_2, temperature, and stage of gestation.

Due to the active transport system already discussed allantoic fluid has low levels of sodium and chlorine. The allantoic fluid also has little phosphate or glucose. Fructose, a readily available energy source, is in abundance in the allantoic fluid as it is in the amniotic fluid (Wales and Murdoch, 1973; Jainudeen and Hafez, 1987). However, high levels of creatinine, uric acid and urea are also seen in allantoic fluid due to its function as a storage site for fetal excretory products (McCance and Widdowson, 1960; Jainudeen and Hafez, 1987). As mentioned, the allantois serves as a depository for fetal wastes until patency of the urethra allows for elimination into the amniotic compartment (Mellor and Slater, 1971).

Steroidal hormones have been reported to be present within the fetal fluid pools (Knight et al., 1977; Kukoly, 1984). Since steroidal hormones are non-polar in nature and freely traverse cell membranes, the source of these steroids could be maternal, placental and/or fetal in origin. There appears to be a disparity in the steroid concentration of the two fluid pools with the allantoic fluid being much more of a dynamic entity. Kukoly (1984) found a moderate increase of P_4 in allantoic fluid between d 25 (.93 ng/ml) and d 110 (5.43 ng/ml), but almost no

change in the mean P_4 concentration within the amniotic compartment throughout gestation (1.67 ng/ml). Estrone changed even more dramatically with concentrations within the allantoic fluid increasing from d 25 (1.6 ng/ml) to d 30 (9.08 ng/ml), remaining fairly constant to d 70 (7.79 ng/ml), and finally showing a dramatic increase to d 110 (3727.76 ng/ml). Estrone increased within the amniotic fluid after d 70 but to a much lesser degree. The functional significance of steroids within the fetal fluid pools of the developing pig is unknown. However, evidence from research with laboratory animals has implicated these hormones in sexual differentiation and fetal growth (Von Saal and Bronson, 1978; Kinsley et al., 1986). Conjugated steroids in the fluid pools may serve as readily-metabolizable storage molecules for their unconjugated forms. Challis and Greeblatt (1980) substantiated this premise for the rabbit when they found radiolabeled E_1 and E_2 in various tissues after infusion of tritiated E_1SO_4 into the amniotic fluid.

Prenatal Mortality

Embryonic and fetal mortality during gestation is a common event in mammalian species and has been attributed to many factors including genetics, inbreeding, nutrition, age, short postpartum interval, disease, and metabolic

disorders (Boyd, 1965; Wrathall, 1971). In species that normally only produce one offspring per gestation, a return to estrus at an abnormally long interval will often identify cases in which mortality of the fetus has occurred. Pregnancy wastage is more difficult to identify in a polytocous species since partial litter losses commonly occur and in the pig are more common than cases of whole litter loss (Wrathall, 1971).

Normally, there is a 30-50% loss of potential offspring during gestation in swine (Perry and Rowlands, 1962; Boyd, 1965; Wrathall, 1971; Pope and First, 1985). Most of this loss occurs prior to d 25, except in situations where the number of offspring is abnormally high (Dziuk, 1968; Bazer et al., 1969a; Bazer et al., 1969b; Fenton et al., 1972; Rampacek et al., 1975) or when uterine space is restricted (Fenton et al., 1970; Webel and Dziuk, 1974; Monk and Erb, 1974; Knight et al., 1977; Hagen et al., 1984)

Loss prior to d 25 is referred to as primary embryonic mortality. By d 18 there is usually a significantly lower proportion of live embryos, with reports of embryonic death being as high as 28% (Perry and Rowlands, 1962; Anderson, 1978). Researchers have studied the requirements of the early embryo for space within the uterus by altering the amount of area available to each conceptus and therefore inducing either a crowded or spacious uterine environment.

Crowded conditions can be accomplished by either increasing the number of conceptus units (superinduction or superovulation) or by decreasing the amount of uterine space. Uterine tissue can be removed via a unilateral hysterectomy ovariectomy (UHOX), a manipulation that results in approximately half the uterine area. Ovulation rate is equivalent between UHOX and control sows due to compensation by the remaining ovary (Fenton et al., 1970; Ulberg and Rampacek, 1974; Webel and Dziuk, 1974; Monk and Erb, 1974; Knight et al., 1977; Hagen et al., 1984).

Although primary death losses increase when the number of conceptus units is above normal (Bazer et al., 1969a; Wrathall, 1971; Fenton et al., 1972), it does not appear that uterine space is limiting prior to d 30. Several workers have reported that a reduction in uterine space does not result in increased embryonic mortality before d 30 when the number of potential embryos is in the normal range (Dziuk, 1968; Fenton et al., 1970; Ulberg and Rampacek, 1974; Webel and Dziuk, 1974; Knight et al., 1977; Hagen et al., 1984; Wu et al., 1988b). Wu et al. (1988a) found that embryos elicit a signal to increase uterine dimensions such as length, weight, and diameter but not until after d 18 of pregnancy. Again this indicates that early embryonic death is not a function of uterine space.

Conversely, ligation of one of the oviducts to prevent the entry of embryos into the uterus (and hence cause their

death) will result in an animal with approximately half the potential number of embryos compared with an intact gilt. Therefore unilateral oviductal ligation provides a spacious uterine environment but this condition does not affect embryonic survival in the early developmental stages (Webel and Dziuk, 1974; Hagen et al., 1984).

Intrauterine competition between elongating blastocysts may be a major factor influencing embryonic losses, with embryos which are lagging behind developmentally being at a major disadvantage (Bazer et al., 1969b; Ulberg and Rampacek, 1974). Growth at this early stage does not seem to be influenced (positively or negatively) by the growth of adjoining conceptus units (Anderson, 1978). Those blastocysts that grow at a reduced rate may not be able to elicit the proper signal for development to continue (Geisert, 1982a) Pope (1988) suggests that considerable embryonic mortality occurs when uterine secretions become altered in such a manner that they are asynchronous with the developing embryos. The theory of embryo-uterine asynchrony as a cause of primary embryonic mortality is supported by research with the prolific Chinese-Meishan pig, which have conceptuses that grow more uniformly at the early stages of pregnancy (d 10-11) and have much lower embryonic death losses (Bazer et al., 1988). The uniform growth of these early blastocysts may reduce losses which occur in other breeds due to

improper signaling. Also, the uterus of a Chinese-Meishan sow may be more efficient in transporting nutrients since it can support large litters despite being smaller than that of the domestic sow.

It can be concluded that uterine space does not exert a major influence on early conceptus development, however, it may be a factor of paramount importance during later stages of growth. Embryonic wastage that occurs during the later stages of growth are known as secondary death losses and under normal conditions are fairly low. Increasing ovulation rate through either natural or artificial means substantially decreases embryonic survival (Wrathall, 1971).

There are dramatic increases in embryonic loss in the UHOX sow after d 30 when compared to controls (Fenton et al., 1970; Webel and Dziuk, 1974; Knight et al., 1977; Hagen et al., 1984). Knight et al. (1977) suggested placental insufficiency caused mortality during this developmental stage since placental mass in UHOX sows was significantally less than that in control sows. Although the uterus has a remarkable capacity for growth (Perry and Rowlands, 1962; Rigby, 1968) and is able to undergo compensatory hypertrophy when a portion is removed (Ulberg and Rampacek, 1974) or if the number of fetuses is increased, the growth rate does not increase linearly with

litter size (Wu et al., 1987). Therefore, it is probable that space does become limited in abnormally-large litters.

Intrauterine migration is another factor affecting secondary death losses that functions by maximizing the efficient use of available uterine space (Lasley et al., 1963). Under normal conditions, distributing conceptus units evenly within the uterine horns decreases embryonic mortality. However, under conditions of larger-than-normal litters this process may increase the chances of whole litter losses if every conceptus is allotted an area inadequate for development to proceed to term . A lack of migration in these cases may be beneficial as far as the whole litter is concerned because at least a few embryos are likely to receive adequate space for normal development. However, in cases of severe embryonic mortality the consequence may be a small litter with low birth weight since surviving embryos are unable to take advantage of the vacated space (Wrathall, 1971).

The steroidogenic capabilities of placentas that are abnormally small due to uterine crowding has received little attention. Research has not correlated litter size with plasma P_4 levels even though breed differences in plasma steroid concentrations have been reported (Wettemann et al., 1980). However, E_1SO_4 levels were positively correlated to litter size and were reduced when embryonic mortality was intentionally inflicted (Horne et al., 1983).

Monk and Erb (1974) and Knight et al. (1977) found no difference in systemic P_4 levels between UHOX and control gilts. However, plasma E_1 levels were positively correlated to the number of fetuses present and therefore lower for the UHOX gilts. Also, abnormally small human infants have placentas that are deficient in aromatase and consequently produce higher quantities of androgens (Thoumsin et al, 1982).

Exogenous Hormones

There have been many attempts to improve embryonic survival and to augment conceptus development through the administration of exogenous hormones. Results from these experiments have been inconsistent and are difficult to interpret since they are variable in factors such as: times of administration, physiological state of the animal (i.e. ovariectomized vs. intact), types of hormones injected, combinations of hormones, dosages of injections, ratio of hormones injected, and interactions of these factors. The identification of positive effects of this type of treatment could be beneficial to swine producers, since this might be a relatively inexpensive way to increase reproductive efficiency in their herds. This information would also be of great benefit to researchers

trying to elucidate the role of endocrine parameters on fetal growth.

In general, intact pregnant swine do not respond negatively when they are supplemented with exogenous P_4 that is administered either alone or in combination with E. Negative effects are seen only when high E dosages alone are given (Miller, 1978; Etienne and Jemmali, 1982; Long and Diekman, 1984; Pope et al., 1986) or if P_4 dosages are extremely high (Davis and Sorenson, 1959; Spies et al., 1959; Morrissette et al., 1963). Some of the negative effects reported with excessive exogenous administration of P_4 are: decreased embryonic survival (Davis and Sorensen, 1959), decreased luteal function (Spies et al., 1959) and increased incidence of cystic ovaries (Morrissette et al., 1963). Alternatively, high E results in increased embryonic mortality (Long and Diekman, 1982: Pope et al., 1986) and a high percentage of pseudopregnant animals (Etienne and Jemmali, 1982). At lower dosages, the supplementation of steroids showed some beneficial effects with the greatest effects occurring with a 2000:1 P_4:E ratio (Reddy et al., 1958; Wildt et al., 1976; McGovern et al., 1981; Knight et al., 1983; Dalton and Knight, 1983). These dosages increased litter size (Reddy et al., 1958; Wildt et al., 1976; Knight et al., 1983), resulted in more developed uterine glands (Reddy et al., 1958), expanded

chorionic surface area (McGovern et al., 1981), and improved areolar formation (Dalton and Knight, 1983).

Hormone replacement of ovariectomized animals produced similar results. Since systemic P_4 levels in the pig are dependent on ovarian production it is important that hormone replacement be at a high enough level to maintain pregnancy. Gentry et al. (1973) found gilts unable to maintain pregnancy when less than 40 mg P_4 was injected per d. Estradiol benzoate administered in concert with 80 mg P_4/d resulted in the highest embryonic survival rates. When the E dosage was increased an adverse effect on fetal mortality rates resulted. Subsequent research has revealed that a P_4:E ratio of 2000:1 most closely mimics the natural hormone environment and minimizes embryonic death (Spies et al., 1960; Day et al., 1959). Conversely, Knight et al., (1974a) found conceptuses to have longer placentas and more allantoic fluid when the ratio was maintained at a ratio of 6000:1 compared to 2000:1. However, the ratio differed because different amounts of P_4 were used as estrogen was kept constant. It is unclear whether increasing the progesterone levels or widening the P_4:E_2 ratio was responsible for the beneficial effects. Hormone replacement in the ewe has also resulted in increased allantoic fluid when pregnancy was supported by P_4 alone; the addition of a small amount of E_2 negated this effect. However, it must be cautioned that the large steroidogenic

capabilities of the ovine placenta makes comparison of these results with the pig difficult (Alexander and Williams, 1968). Alternatively Knight et al., (1974b) showed that increasing the dosage of a 2000:1 P_4:E_2 solution had negative effects on uterine protein secretion. They suggested that the increase in E_2 levels was the factor responsible for the adverse response. This hypothesis is supported by research with the rat that has found that the uterus becomes refractory after prolonged exposure to E_2 (Stormshak et al., 1976). Estrogens do lessen the P_4 requirement for maintaining pregnancy in the mouse and may have an important role in this respect (Miller, 1978).

The maintenance of normal gestation in ovariectomized animals with non-aromatizable progestagens is of significant importance. If we assume that the aforementioned functions of E in conceptus development are accurate, then supporting a normal pregnancy with a synthetic progesterone source such as MPA would allow us to conclude that the porcine placenta is steroidogenically-active. When ovariectomized goats were treated with MPA during mid-gestation an immediate drop in plasma P_4 was noted. However, P_4 levels increased three-fold by late gestation and a negative uterine artery minus uterine vein difference was manifested at this time; all suggesting placental P_4 production (Sheldrick et al., 1980). Heap et

al. (1981) reported the porcine placenta had similar capabilities with normal fetal survival when gilts were treated between d 10-28 with MPA.

Supporting pregnancy with a synthetic progestagen source such as MPA only allows us to conclude that the porcine placenta is steroidogenically capable. We are not able to quantitate the in situ capability of the placenta to produce steroids with this approach. The possibility of supporting a normal gestation with steroid precursors might allow us to make more meaningful conclusions as to the physiological role of the placenta in steroid production.

Chapter Three

PORCINE INTRAUTERINE STEROIDOGENESIS. I. LUTEAL VS. INTRAUTERINE PROGESTERONE AS A MEDIATOR OF CONCEPTUS DEVELOPMENT AND PRENATAL SURVIVAL.

Introduction

Prenatal mortality is common in domestic mammals and has been attributed to a wide range of factors including breed of the dam, lethal genetic defects revealed through inbreeding, deficient or excessive levels of essential nutrients, advanced age, rebreeding in the early postpartum period, and numerous diseases and metabolic disorders (Boyd, 1965; Wrathall, 1971). Fetal loss is more difficult to identify in polytocous compared with monotocous species since it is generally not an "all or none" phenomenon. Partial litter losses in polytocous animals are quite normal while whole litter losses occur only rarely after the first trimester (Wrathall, 1971).

There is typically a 30-50% loss of potential offspring during gestation in swine (Perry and Rowlands, 1962; Boyd, 1965; Wrathall, 1971; Pope and First, 1985). Most of this loss normally occurs prior to d 30 of gestation and is referred to as primary death loss (Pope and First, 1985). Primary fetal losses occur independent of the availability of uterine space (Dzuik, 1968; Fenton et al., 1970; Ulberg and Rampacek, 1974; Webel and Dzuik, 1974; Knight et al., 1977; Hagen et al., 1984; Pope and

First, 1985) and are probably a function of asynchrony between embryos and the uterine environment (Bazer et al., 1969; O'Neill and Quinn, 1981). Embryonic survival prejudicially favors more-developed, steroidogenically-capable embryos within the litter, presumably due to the action of estrogens on the local uterine environment (Pope, 1988).

In contrast, secondary fetal loss transpires subsequent to d 30 and is relatively low, except in situations where the number of potential offspring is abnormally high (Dzuik, 1968; Bazer et al., 1969a; Bazer et al., 1969b; Fenton et al., 1972; Rampacek et al., 1975; Wu et al., 1988b) or when uterine space is artificially restricted (Fenton et al. 1970; Webel and Dzuik, 1974; Monk and Erb, 1974; Knight et al., 1977; Hagen et al., 1984; Wu et al., 1988a). Most approaches attempting to increase litter size in the pig (e.g., superovulation, embryo superinduction) have generally resulted in an increase in secondary death loss, presumably by creating a crowded uterine environment (Knight et al., 1977). However, the manner by which uterine crowding translates into fetal death is unknown.

The placenta must be functioning properly for the fetus to survive. Therefore, prenatal mortality may be a function of placental deficiencies. A key to understanding the dynamics of conceptus-maternal interactions is an

increased understanding of the steroidogenic capabilities of the placenta and endometrium.

Knight et al. (1977) reported a positive progesterone (P_4) and negative estrone (E_1) uterine artery minus uterine vein difference in ovarian-intact gilts , suggesting that P_4 of ovarian origin is utilized within the pregnant uterus as a substrate for subsequent conversion to estrogens. Also, when exogenous P_4 was administered to bilaterally-ovariectomized gilts in order to maintain pregnancy, gilts which did remain pregnant had significantly lower plasma P_4 values than their non-pregnant counterparts (Knight et al., 1974; Bazer et al., 1979). Again, this evidence suggests that pregnancy has a marked affect on P4 metabolism. More recent evidence from our laboratory clearly indicates that the placenta and endometrium are capable of producing P4 and E_1 in vitro (Kukoly, 1984; Jeantet and Knight, 1985).

Estrogens have important localized effects on water and electrolyte movement (Goldstein et al., 1980), cell permeability (Szego and Sloan, 1961), and blood flow (Ford and Christenson, 1979; Bazer et al., 1979). Therefore, it would follow that events such as the rapid growth of the placenta in early gestation and the inbibition of fluids into fetal membranes would be under estrogenic control. However, it is not known if estrogen production is dependent on the conversion of P_4 of ovarian origin or on de novo synthesis by the intrauterine tissues. Heap et al. (1981) reported that fetal development appeared normal when

medroxyprogesterone acetate (MPA) was administered to ovariectomized gilts early in gestation. The ability to maintain normal gestation in ovariectomized gilts with a non-aromatizable progestagen (such as MPA) strongly suggests that the placenta and/or endometrium is capable of providing the estrogens that are necessary for the placental events previously mentioned. However, the ability of the placenta to produce steroids in vivo past d 28 of gestation has received little attention. Therefore, the objective of this study was to determine the role of luteal P_4 as a precursor for intrauterine steroidogenesis by assessing pregnancy maintenance and conceptus development in OVX gilts administered MPA.

Materials and Methods

Animals Forty-one pregnant gilts were utilized for a study of porcine placental function at d 30 and d 70 of gestation. Gilts of similar weight (100-120kg) and genetic background (Yorkshire x Hampshire x Duroc) were kept in pens measuring 3.1 m X 4.9 m and fed 2.7 kg/d of a corn-soybean based diet containing 14% CP. Daily estrous detection was initiated at approximately 6 mo of age and was facilitated with the use of mature boars. Gilts were bred at estrus during their third regularly-occurring estrous cycle by either natural service or artifical insemination of freshly-collected semen. Breeding occurred upon the detection of estrus and at 12, 24, and 36 h

thereafter. Estrous detection continued after gilts were bred to identify individuals that failed to maintain pregnancy. Thirty-six gilts remained pregnant and were assigned to be used in the experiment.

At the time of breeding, gilts were randomly assigned to be OVX or to undergo sham surgery (i.e., ovaries exposed and manipulated and corpora lutea counted, but ovaries left intact) at either d 20 or d 60 of gestation (Fig 1). Following a subsequent 10 d treatment period all gilts were bilaterally hysterectomized (HYSTX) at d 30 or 70 of gestation. Within each day of gestation group, OVX gilts received either P_4 or MPA (in a 9:1 corn oil; benzyl benzoate emulsion) and sham operated gilts received a vehicle injection of corn oil (CO, controls). Control (1 ml CO) and P_4-treated gilts (200 mg P_4 at 50 mg P_4/ml) received daily subcutaneous injections (in the neck) beginning on the day prior to OVX or sham (i.e., d 19 or d 59) and continuing until the day prior to HYSTX (i.e., d 29 or d 69). Gilts receiving MPA were given a subcutaneous injection (100 mg, 25mg/ml) on the day prior to the OVX and then a 10 mg dose (10 mg/ml) every other day (i.e., d 21, 23, 25, 27, 29, or d 61, 63, 67, 69) until HYSTX.

Surgical Procedures Initial anesthesia was induced during surgical procedures via an intravenous infusion of 5% sodium thiopental (Pentothal, Abbott Laboratories Inc., North Chicago, Ill.) into an ear vein and maintained by a

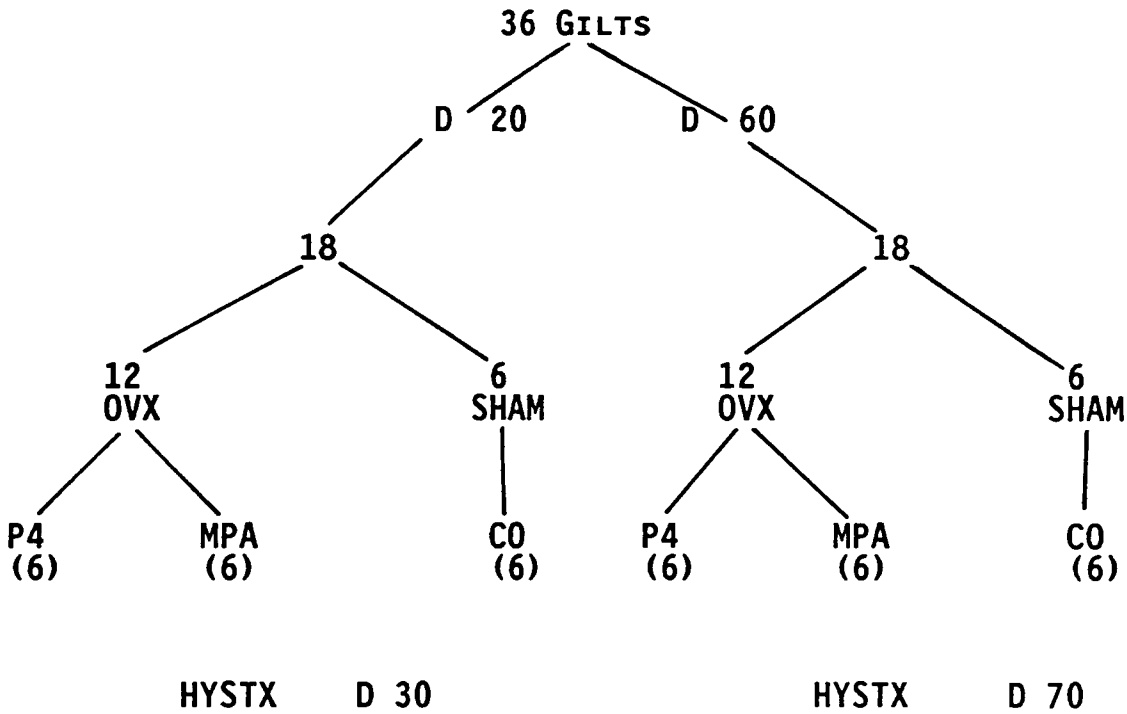

Fig 3-1: EXPERIMENTAL DESIGN

closed circuit system with a mixture of nitrous oxide and halothane (Carbine Laboratories, N.J.). Entry into the body cavity was accomplished via mid-ventral laparotomy.

At OVX the ovaries were palpated manually and exposed. Ovariectomy was accomplished by clamping the ovarian blood supply below the hilus and transfixing the vessels located within via several ligatures of cotton umbilical tape. Corpora lutea were counted following excision of both ovaries and assumed to represent ovulation rate.

Prior to HYSTX, blood samples were obtained from a uterine artery, uterine vein and anterior vena cava. Sodium citrate was added as an anticoagulant and samples were centrifuged for 10 min at 2500 x g. Plasma was harvested and stored at - 20^{o}C.

The removal of the pregnant uterus involved exposure of both uterine horns and ligation of the blood supply within the mesometrium (left and right middle uterine artery and vein), cervix and round ligament (caudal uterine artery and vein). Following excision, the entire uterus was maintained in a water bath at approximately 38^{o}C until the completion of surgery.

Data Collection Following surgery, the mesometrium was dissected from the reproductive tract to facilitate removal of the conceptus units. The cranial tip of the right uterine horn was opened at the mesometrial border to expose the end of the first placenta. The opening was extended

along this border so that each entire conceptus could be removed without damage. Uncontaminated samples of the allantoic and amniotic fluid pools were aspirated with an 18-gauge needle immediately preceding direct measurements of conceptus development as described by Knight et al.(1977). Allantoic fluid from d 30 conceptuses was pooled within each litter for subsequent hormone determination. Fetal fluids taken at d 70 of gestation were assayed for individual conceptus variation associated with intrauterine position (Suazo, 1989). For this investigation average hormone concentration within each litter will be reported.

Hormone Assay Procedures Allantoic and amniotic fluid samples as well as plasma samples were analyzed for P_4, E_1 and estrone sulfate (E_1SO_4) by radioimmunassay (RIA).

Progesterone analysis was determined by the method of Beal et al. (1980). Intra- and inter-assay coefficients of variation (CV) were 11% and 26% respectively.

Estrone and E_1SO_4 were determined using a slight modification of the procedure described by Hattersley et. al (1980). Extraction involved the addition of 5 ml diethyl ether to the sample (500 ul) and 45 s of vortexing. After a clear separation into aqueous and organic phases, extraction tubes were quick-frozen in an ethanol and solid CO_2 bath ($-70^{o}C$) and decanted. The ether extract was evaporated to

dryness under a stream of air and the process repeated for a second extraction of the sample.

A specific antibody (generously donated by R.B. Staigmiller, Miles City, Montana) was used in the determination of E_1 (Appendix A). Samples were reconstituted with 100 ul of an E_1 antibody solution followed by 100 ul ^3H-E_1. Samples were incubated for 12-18 h before separation of bound from unbound E_1 with dextran coated charcoal. A 500 ul aliquot was taken from the supernatant of each tube and mixed with 5 ml scintillation cocktail in a plastic screw-cap vial and counted for 5 min in a scintillation counter. Known samples of high and low E_1 concentration were used to determine inter- and intra-assay CV which were 25% and 13% respectively.

Following E_1 assay, the extraction tubes were allowed to thaw and excess ether evaporate. The borosilicate tubes containing the aqueous sample were then refrozen at -20°C until E_1SO_4 determination. Estrone sulfate determination involved a third extraction of the sample with 5 ml tetrahydrofuran. Due to the relatively high polarity of the tetrahydrofuran, separation of the organic from aqueous phases necessitated the addition of 400 ul of a saturated saline solution (380 g NaCl/l H_2O) prior to vortexing (45 s). Extraction tubes were quick-frozen at -85°C and the extract decanted and evaporated under a stream of air.

Samples were reconstituted with 100 ul of an antibody solution and ^3H-E_1. The antibody used for E_1SO_4

determination (Pantex, Santa Monica, Ca) had 100% and 43% (Appendix A) crossreactivity with E_1 and E_1SO_4 respectively. Samples were incubated for 12-18 h and bound and unbound hormone separated as previously described. High and low standards were again used to determine the intra- and inter-assay CV which were 13% and 31% respectively. The high and low standards as well as the solutions for the standard curves were prepared fresh and used within one week due to the rapid breakdown of E_1SO_4 to E_1 in solution.

Extraction of radiolabelled E_1 and E_1SO_4 allowed determination of percent recoveries for the two extraction procedures. The double ether extraction resulted in a 96.8% and 17.1% recovery for E_1 and E_1SO_4, respectively. Tetrahydrofuran extraction resulted in a 78% recovery for E_1SO_4 and 10.3% for E_1.

Statistical Analysis Data were analyzed using the General Linear Models procedure of the Statistical Analysis Systems (SAS, 1985). Litter variables included the number of CL, number of live and/or dead fetuses, percent fetal survival, number of ovulations (CL) not accounted for by a fetus (alive or dead), uterine length, and steroid (P_4,E_1,E_1SO_4) concentrations within the plasma and fetal fluid pools. The following analytical model was employed for litter data within each stage of gestation:

$$Y_{ij} = T_i + e_{ij}$$

where Y_{ij} represents the litter variable , T_i is the treatment to which the gilt was subjected and e_{ij} is the random error.

Conceptus variables (such as placental length and weight, fetal length and weight, and fetal fluid volumes) were studied using the following analytical model for each of the two stages of gestation:

$$Y_{ijk} = T_i + G_{ij} + e_{ijk}$$

Where Y_{ijk} represents the conceptus variable in question, T_i is the treatment, G_{ij} is the effect of gilt nested within treatment and e_{ijk} is random error.

Dunnet's test was utilized to detect differences between control and treatment groups (P_4 or MPA) when the effect of treatment was significant.

Results

Litter and Conceptus Variables Table 1 summarizes data obtained relative to litter variables and Table 2 summarizes data for conceptus measurements. Ovulation rate was not different among gilts assigned to the two gestation groups or to the three treatments within each day of

TABLE 3-1. EFFECT OF EXOGENOUS PROGESTOGEN TREATMENT ON WHOLE LITTER VARIABLES AT HYSTERECTOMY[1]

	Day of hysterectomy[2]					
	30			70		
Item	CO	P4	MPA	CO	P4	MPA
No. pregnant at HYSTX	6	6	6	6	6	6
Ovulations[3] at OVX	13.7 (.65)	15.3 (.65)	15.3 (.65)	14.5 (1.1)	16.8 (1.1)	14.8 (1.1)
Number live	12.2 (.77)	12.5 (.77)	12.8 (.77)	10.8 (1.06)	10.3 (1.06)	8.8 (1.06)
Number dead	.83 (.40)	1.50 (.40)	1.00 (.40)	1.67 (.84)	3.33 (.84)	2.50 (.84)
Corpora lutea unrepresented[4]	.83 (.54)	1.33 (.54)	2.00 (.54)	2.00 (1.13)	3.17 (1.13)	3.33 (1.13)
Fetal survival (%)	89.0 (3.96)	81.8 (3.96)	83.7 (3.96)	75.8 (8.04)	65.2 (8.04)	59.3 (8.04)
Uterine length (cm)	448.8 (34.1)	462.2 (34.1)	458.7 (34.1)	601.7 (34.1)	576.5 (34.1)	566.7 (34.1)

[1] Values expressed as least squares means, standard error of the mean in parentheses.
[2] All gilts were bilaterally ovariectomized (OVX) or underwent sham surgery ten days before hysterectomy (HYSTX).
[3] Determined by the number of corpora lutea at the time of OVX or sham.
[4] Number of corpora lutea on ovaries at OVX not represented by either a live or dead fetus.

TABLE 3-2. EFFECT OF EXOGENOUS PROGESTOGEN TREATMENT ON CONCEPTUS MEASUREMENTS AT HYSTERECTOMY[1]

	Day of hysterectomy[2]					
	30			70		
Item	CO	P4	MPA	CO	P4	MPA
Placental length (cm)	54.9 (3.00)	55.9 (2.97)	57.5 (2.93)	91.8 (5.15)	88.2 (5.50)	83.6 (5.96)
Placental weight (g)	27.2 (2.84)	27.9 (2.81)	33.9 (2.77)	263.5 (18.96)	242.2 (20.52)	241.3 (21.97)
Crown rump length (mm)	25.1 (.60)	24.6 (.60)	25.7 (.59)	173.1 (3.20)	167.6 (3.44)	177.9 (3.74)
Fetal weight (g)	1.63 (.14)	1.65 (.14)	1.69 (.14)	263.6 (13.3)	254.8 (14.3)	277.5 (15.5)
Allantoic fluid (ml)	193.1 (16.31)	165.6 (16.13)	184.2 (15.90)	233.7 (28.34)	168.0 (30.64)	160.5 (33.09)
Amniotic fluid (ml)[3]	----- -----	----- -----	----- -----	221.8 (14.5)	191.1 (15.4)	173.3 (16.6)

[1] Values expressed as least squares means, standard error of the mean in parentheses.
[2] All gilts were bilaterally ovariectomized (OVX) ten days before hysterectomy (HYSTX).
[3] Insufficient amniotic fluid volume at d 30 of gestation.

gestation. Thus, the number of potential fetuses was similar across all treatment comparisons. The number of live fetuses at HYSTX was less at d 70 vs d 30. This reduction reflects prenatal mortality which would be expected to occur during this 40-d interval. In accordance with this decrease in number of live fetuses, fetal mortality rate, number of dead and degenerating fetuses and number of unaccounted for ovulation sites were found to increase during this 40-d period as well. Ovulation sites not accounted for represent the difference between the number of CL at OVX and the total of live and dead fetuses at HYSTX. This measurment reflects both unfertilized ova and conceptuses which may have died and been reabsorbed at any point prior to HYSTX. In most cases (especially during the later stage of gestation) this variable would not be expected to reflect treatment effects due to the relatively short period between OVX and HYSTX.

There were no significant differences among treatments for any of the above-mentioned measurements at either stage of gestation. Therefore, we can conclude that in the absence of a systemic source of P_4 (in the MPA treatment) prenatal survival was equivalent to intact controls.

Measurements of conceptus variables (Table 2) at the two stages of gestation agree with expected values based on our previous studies characterizing the pattern of conceptus development under normal (untreated) conditions (Knight et al., 1977). The physiologically-important

result is that no aspect of conceptus development was adversely affected at either d 30 or d 70 in OVX gilts in which pregnancy was maintained with the nonaromatizable progestogen MPA. In fact, at d 30 of gestation all placental and fetal variables measured were numerically higher (but not statistically different) in MPA-treated than in control gilts.

Progesterone in Maternal Plasma and Fetal Fluid Pools

Plasma P_4 levels (Table 3) decreased between d 30 and d 70 in the uterine vessels of control gilts, in the uterine artery of MPA-treated gilts, and in the uterine vein of P_4-treated gilts. In addition, P_4 concentrations were significantly depressed ($P<.05$) in the uterine artery at d 70 and in the uterine vein at both days of gestation in MPA-treated gilts.

Progesterone concentrations in fetal fluids were equivalent for MPA-treated and control gilts at both d 30 and d 70 of gestation. However, P_4-treated gilts had elevated ($P<.001$) P_4 levels in both fetal fluid pools at the latter stage of pregnancy.

Estrone and Estrone Sulfate in Maternal Plasma and Fetal Fluid Pools Overall, uterine vein and allantoic fluid E_1 concentrations (Table 4) increased ($P<.001$) between d 30 and d 70 of gestation. Allantoic and amniotic fluid E_1 content was greater (up to 4000-fold) than that of the

TABLE 3-3. EFFECT OF EXOGENOUS PROGESTOGEN TREATMENT ON PROGESTERONE CONCENTRATION (NG/ML) IN PLASMA AND FETAL FLUID POOLS FROM GILTS TREATED AT TWO STAGES OF GESTATION[1]

	Day of hysterectomy[2]					
	30			70		
Item	CO	P4	MPA	CO	P4	MPA
Uterine artery	15.0 (4.7)	7.8 (4.7)	4.2 (4.7)	6.9 (1.3)	10.8 (1.3)	2.5 (1.4)
Uterine vein	17.7^{a} (4.0)	9.3^{a} (4.0)	1.6^{b} (4.0)	7.5^{a} (1.1)	6.7^{a} (1.1)	3.1^{b} (1.2)
Anterior vena cava	11.6 (3.9)	19.2 (3.9)	7.3 (3.9)	11.0 (2.9)	8.7 (2.9)	10.5 (2.9)
Allantoic fluid	.5 (.1)	.5 (.1)	.4 (.1)	1.2^{c} (.2)	4.8^{d} (1.0)	1.7^{c} (.5)
Amniotic fluid[3]	----- -----	----- -----	----- -----	$.6^{c}$ (.2)	2.2^{d} (.7)	$.6^{c}$ (.2)

a,b P_4 concentrations within day of gestation with different superscripts differ ($P<.01$).

c,d P_4 concentrations within day of gestation with different superscripts differ ($P<.001$).

1 Values expressed as least squares means, standard error of the mean in parentheses.

2 All gilts were bilaterally ovariectomized (OVX) ten days before hysterectomy (HYSTX).

3 Insufficient amniotic fluid volume at d 30 of gestation.

TABLE 3-4. EFFECT OF EXOGENOUS PROGESTOGEN TREATMENT ON ESTRONE CONCENTRATION (NG/ML) IN PLASMA AND FETAL FLUID POOLS FROM GILTS TREATED AT TWO STAGES OF GESTATION[1]

	Day of hysterectomy[2]					
	30			70		
Item	CO	P4	MPA	CO	P4	MPA
Uterine artery	.028 (.015)	.026 (.015)	.043 (.015)	.054 (.012)	.027 (.011)	.041 (.012)
Uterine vein[3]	.037 (.008)	.025 (.008)	.028 (.008)	.085 (.015)	.046 (.014)	.090 (.015)
Anterior vena cava	.033 (.009)	.032 (.009)	.039 (.009)	.044 (.015)	.041 (.014)	.042 (.014)
Allantoic fluid	43.90 (9.96)	40.70 (9.96)	36.49 (9.96)	128.91 (36.44)	148.64 (40.58)	107.44 (46.40)
Amniotic fluid[4]	----- -----	----- -----	----- -----	62.22 (13.09)	73.40 (15.44)	84.31 (25.07)

[1] Values expressed as least squares means, standard error of the mean in parentheses.
[2] All gilts were bilaterally ovariectomized (OVX) ten days before hysterectomy (HYSTX).
[3] E_1 differed as a function of day of gestation ($P<.001$)
[4] Insufficient amniotic fluid volume at d 30 of gestation.

plasma at both stages of gestation with allantoic concentrations about twice that of the amniotic fluid at d 70. In contrast to the P_4 data, progestogen replacement therapy did not significantly affect the levels of E_1 within any of the fluid pools examined or the magnitude of the change between the two d of gestation.

Plasma E_1SO_4 concentrations (Table 5) dropped significantly ($P<.001$) between d 30 and d 70 of gestation in all three vessels monitored , but were much higher than E1 concentrations overall. Therefore, total plasma estrogens (E_1 plus E_1SO_4) decreased between d 30 and d 70 of pregnancy. Allantoic fluid E_1SO_4 also fell ($P<.001$) between d 30 and d 70 of gestation and, although these levels were greater than plasma levels, the magnitude of the change was not as great as was reported for E_1.

The plasma E_1SO_4:E1 ratio (Table 6), which has been shown to decrease throughout gestation (Knight and Hopkins, 1988), fell from a high of approximately 1500:1 at d 30 to approximately 100:1 at d 70. Although the ratio declined, the magnitude of the ratio was much higher than previously reported. The reason for this discrepancy is probably due to the use of a more direct assay procedure in this study. Lastly, as seen for E_1, exogenous progestogen treatment had no affect ($P>.45$) on E_1SO_4 levels within any of the fluid pools monitored.

TABLE 3-5. EFFECT OF EXOGENOUS PROGESTOGEN TREATMENT ON ESTRONE SULFATE CONCENTRATION (NG/ML) IN PLASMA AND FETAL FLUID POOLS FROM GILTS TREATED AT TWO STAGES OF GESTATION[1]

	Day of hysterectomy[2]					
	30			70		
Item	CO	P4	MPA	CO	P4	MPA
Uterine artery[3]	22.9 (5.4)	27.0 (5.4)	24.1 (5.4)	5.9 (1.6)	4.8 (1.6)	4.2 (1.8)
Uterine vein[3]	33.5 (8.0)	41.8 (8.0)	48.2 (8.0)	7.2 (2.8)	4.4 (2.8)	7.8 (3.0)
Anterior vena cava[3]	26.6 (4.6)	19.8 (4.2)	25.4 (4.2)	6.2 (1.6)	4.3 (1.6)	4.2 (1.6)
Allantoic fluid	426.4 (108.5)	457.4 (108.5)	493.0 (108.5)	15.1 (3.1)	16.9 (5.9)	21.0 (4.4)
Amniotic fluid[4]	----- -----	----- -----	----- -----	14.1 (2.7)	25.0 (4.7)	15.2 (4.0)

[1] Values expressed as least squares means, standard error of the mean in parentheses.
[2] All gilts were bilaterally ovariectomized (OVX) ten days before hysterectomy (HYSTX).
[3] E_1SO_4 differed as a function of day of gestation ($P<.001$).
[4] Insufficient amniotic fluid volume at d 30 of gestation.

TABLE 3-6. EFFECT OF EXOGENOUS PROGESTOGEN TREATMENT ON ESTRONE SULFATE:ESTRONE RATIO IN PLASMA AND FETAL FLUID POOLS FROM GILTS TREATED AT TWO STAGES OF GESTATION[1]

	Day of hysterectomy[2]					
	30			70		
Item	CO	P4	MPA	CO	P4	MPA
Uterine artery[3]	1241.9 (522.6)	1371.2 (522.6)	1620.3 (522.6)	104.1 (117.0)	289.2 (106.8)	110.6 (117.0)
Uterine vein[3]	985.1 (471.8)	1870.6 (471.8)	2554.6 (471.8)	65.2 (49.8)	143.7 (45.5)	105.7 (49.8)
Anterior vena cava[3]	1283.9 (432.0)	710.0 (432.0)	1461.9 (432.0)	108.0 (597.1)	169.7 (545.1)	124.2 (545.1)

[1] Values expressed as least squares means, standard error of the mean in parentheses.
[2] All gilts were bilaterally ovariectomized (OVX) ten days before hysterectomy (HYSTX).
[3] E_1SO_4 differed as a function of day of gestation ($P<.001$).

Discussion

The data presented allows us to draw several conclusions. First, the litter data shows that despite the removal of the ovaries and replacement with a non-aromatizable progestogen source, the local steroid environment within the uterus is apparently suitable for normal fetal survival during d 20-30 and d 60-70 of gestation. Second, the steroidal environment is of a nature that not only allows survivability but results in normal development of the fetus and placenta. This result is particularly important at the earlier stage of gestation where placental growth and allantoic fluid accumulation are taking place at a rapid rate. Bazer et al., (1979) described a model in which increasing estrogens between d 20 and d 30 of gestation resulted in the transport of water and electrolytes across the fetal membranes. The rapid increase in allantoic fluid volume at this stage is critical for placental expansion within the uterine lumen (Knight et al., 1977). It is hypothesized that placental expansion is necessary for maximum contact between fetal and maternal tissues and that failure to do so results in secondary mortality or undersized piglets at parturition.

The reduction in plasma P_4 levels of MPA-treated gilts was expected since luteal P_4 was removed and replaced with a progestogen that does not crossreact appreciably with the antibody used in the P4 assay (<.1% crossreactivity, Appendix A). However, although depressed, the levels of P_4

measured in the plasma were appreciable and within the expected range for intact pregnant gilts (Guthrie et al., 1972; Knight et al., 1977). Despite the differences that were present in plasma P_4 concentration due to treatment, P_4 in the fetal fluids as well as E_1 and E_1SO_4 in both plasma and fetal fluids were unaffected by a change in the systemic steroid environment. Any estrogenic compounds required for fetal survival in MPA-treated gilts would have to be synthetized without the benefit of ovarian P_4 as a precursor.

It is apparent that MPA-treated gilts synthesized estrogens by utilizing steroid precusors other than those of ovarian origin. However, it is interesting to note that while our assumption is that P_4 in these gilts is of an intrauterine origin, anterior vena cava samples had considerably higher P_4 concentrations than in the uterine vessels. The possibility of an extra-uterine source of steroid synthesis (such as the maternal adrenal gland) must be considered. In addition, we would expect MPA-treated gilts to have positive uterine vein minus uterine artery P_4 levels which was not the case at d 30. Despite the fact that the uterine vein minus uterine artery difference was positive for CO and P_4-treated gilts and negative for MPA-treated gilts the differences did not vary significantly ($P>.15$).

Since normal estrogen levels were seen both in the plasma and the fetal fluid pools it can be concluded that

MPA-treated gilts are capable of in situ production of these compounds within the intrauterine tissues. Whether this in situ production takes place in the presence of luteal P_4 or is induced in the absence of a metabolizable substrate cannot be determined from this experimental protocol. However, these results provide evidence that intrauterine tissues are able to produce P_4 and other steroidal hormones in vivo as well as under in vitro conditions. The significant (albeit depressed) quantities of P_4 found in the plasma suggest that not only are these tissues producing P_4 and perhaps other C_{21} steroids for subsequent conversion to estrogens, but that a portion of the P_4 is secreted into the general circulation.

In conclusion, this investigation provided evidence for the regulation of steroid synthesis during pregnancy. The data suggest that steroid-related events within the uterus can occur independent of similar events taking place on the ovary and that intrauterine P_4 may provide a significant proportion of the pool within the systemic circulation. Furthermore, these findings indicate that estrogens are produced at normal levels in the absence of luteal P_4. It cannot be determined from these results whether intrauterine tissues were induced to synthesize above-normal quantities of steroids in the absence of luteal precursors or if de novo synthesis is the normal course of events. A study of the In vitro steroid

production of placenta and endometrium under the current treatments should further elucidate this question.

It should be kept in mind that the duration of progestogen treatment used in this investigation was relatively short and the results reported here might not apply if treatment was extended for a longer period. Lastly, fetal steroid production should be included in any discussion of steroid regulation during pregnancy. Fetal steroids are known to have an important role in other species, interacting with maternal and placental components to regulate intrauterine steroidogenesis (Diczfalusy, 1974). However, the design of the current investigation does not allow us to examine fetal steroidogenesis directly. Future investigations should attempt to include this component.

Chapter Four

PORCINE INTRAUTERINE STEROIDOGENESIS: II.RELATIVE IMPORTANCE OF THE ORIGIN OF PROGESTERONE ON IN VITRO STEROIDOGENESIS BY THE PLACENTA AND ENDOMETRIUM.

Introduction

The primary function of corpora lutea (CL) is the production of the steroid hormone progesterone (P_4). Progesterone promotes the maintenance of pregnancy by decreasing the myotonic activity of the uterus, thereby creating a quiescent environment (Henricks and Mayer, 1977), and by providing the rapidly developing conceptus with nutrition by stimulating the secretion of histotroph (Bazer et al.,1981; Geisert et al.,1982a; Bazer and Roberts, 1983). Removal of the CL via ovariectomy (OVX) or luteectomy results in the rapid termination of pregnancy in many species (Thomford and Dziuk, 1982; Hagen et al., 1984).

In several species, the placenta gains the capacity to produce large quantities of P_4. In fact, by as early as d 24 of gestation, women are able to continue pregnancy without the presence of ovaries (Jainudeen and Hafez, 1987). Domestic species such as the ovine and equine gain the capacity to maintain pregnancy following OVX later in gestation (d 50 for the ewe, d 150 for the mare; Jainudeen and Hafez, 1987). In contrast, swine rapidly terminate

pregnancy following OVX at any stage prior to the expected time of parturition (du Mesnil du Buisson and Dauzier,1957). This finding has long been considered as circumstantial evidence that the porcine placenta is not steriodogenically competent.

Alternatively, many reports show that the porcine blastocyst is steroidogenically active (Raeside, 1963; Heap et al., 1975; Perry et al.,1976; Heap et al.,1979; Gadsby et al.,1980), while several studies have provided histochemical evidence for the presence of hydroxysteroid dehydrogenase (the enzyme necessary for the synthesis of P_4) in the placenta for practically the entire duration of gestation (Christie, 1968; Dufour and Raeside, 1969). Large umbilical vein minus umbilical artery differences in P_4 concentration (Barnes et al. 1974; Hagen et al.,1983), and rapidly rebounding peripheral P_4 levels following partial luteectomy or unilateral OVX (Thomford and Dziuk, 1982; Hagen et al.,1984; Nase et al.,1985) provide additional circumstantial evidence for the production of P_4 within the pregnant uterus.

The most substantial evidence to date is provided by several studies in which the porcine placenta was capable of P_4 production under in vitro conditions (Heap, 1981; Kukoly, 1984; Jeantet and Knight, 1985). As gestation progresses, placental P_4 production increases (Kukoly, 1984) and is augmented by the addition of the steroidogenic precursor pregnenolone (P_5) to the incubation medium

(Jeantet and Knight, 1985). Moreover, these studies revealed that the porcine placenta was capable of in vitro estrogen production.

Despite the fact that P_4 is acknowledged as the hormone of pregnancy, estrogens have been implicated in many pregnancy-related events. Estrogens produced by the early blastocyst are involved in the sequence events leading to intrauterine migration (Dziuk,1985), blastocyst elongation (Geisert et al., 1982b), maternal recognition of pregnancy (Geisert et al., 1982a), and the sequestering of histotroph within the uterine lumen (Ulberg and Rampacek, 1974; Geisert et al., 1982a, Bazer and Roberts, 1983). Estrogens have also been implicated as regulators of events in the post-blastocyst conceptus such as placental expansion (Knight et al.,1977), water and electrolyte movement (Goldstein et al.,1980), cell permeability (Szego and Sloan, 1961), and uterine blood flow (Ford and Christenson, 1979).

Plasma estrone (E_1) and estrone sulfate (E_1SO_4) concentrations in the pregnant sow undergo dynamic changes (Robertson and King, 1974; Knight et al., Kukoly,1984; Jeantet., 1985) but it is not known if these compounds are: 1) created de novo by placental (and/or endometrial) tissue, 2) synthesized using ovarian P_4 as a substrate or 3) synthesized by some other mechanism. Therefore, this study was designed investigate the in vitro production of steroids by the placenta and endometrium of gilts in which

pregnancy was maintained with the synthetic progestogen, medroxyprogesterone acetate (MPA) following OVX. Because MPA cannot be aromatized to estrogens, any estrogens required for pregnancy maintenance and conceptus development are most likely of intrauterine origin. If estrogens are normally derived from ovarian P_4, the maintenance of pregnancy with MPA may induce the intrauterine tissues to compensate for the loss of endogenous estrogenic precursors by increasing steroid production above that of untreated gilts. Therefore, the objectives of this investigation are: 1) to further quantify in vitro steroid production by the placenta (PLAC) and endometrium (ENDO), and 2) to determine if steroid synthesis differs due to the systemic environment (systemic precursors either present or absent) that the tissues were subject.

Materials and Methods

Animals Thirty-six pregnant gilts were utilized for a study of in vitro steroid production by the placenta and/or endometrium taken from environments either including or devoid of luteal P_4 (see Chapter 3).

Surgical Procedures and Sample Collection Anesthesia, OVX and HYSTX were performed as described in Chapter 3. Following surgery, uncontaminated samples of the allantoic and amniotic fluid were aspirated from each placenta

immediately preceding direct measurements of conceptus development as described by Knight et al.(1977).

Concomminent with measurement procedures, random samples of the placentas of at least four fetuses were obtained. Either the entire placenta (d 30) or random samples of the allantochorion (d 70, amnion removed) were immediately placed into a sterile beaker containing medium 199 (M199, Gibco Laboratories , Grand Island, NY). Endometrial tissue was sampled from sites adjacent to the region from which placental tissue was taken. Endometrial samples were placed into a seperate sterile beaker with M199.

Tissue Incubation After completion of tissue collection, all tissue was transferred to fresh M199 and minced into 1-2 mm pieces with sterile razor blades. The incubation procedure involved placing 300 mg of either placenta (PLAC), endometrium (ENDO) or a combination (COMB, 150 mg of each tissue) in culture tubes containing 3 ml of M199 supplemented with 100,000 IU penicillin, 100,000 ug streptomycin and 250,000 ug amphotericin B per liter. Duplicate samples were prepared of the three tissue preparations for each of four incubation periods (0, .5, 1 or 2 h) either in the presence or absence of the steroid precursor pregnenolone (P_5; 56 ng/ml M199). Culture tubes were incubated in a Dubnoff shaking water bath at 37^{o}C under an atmosphere of 95% O_2: 5%CO_2. The culture tubes

were stored immediately following incubation at - 20°C until subsequent steroid, protein and DNA analyses could be undertaken.

Soluble Protein Determination To correct for potential variation of sampling and weighing, the soluble protein content of the incubated tissue was determined using a dye-binding technique described by Bradford (1976). Initially, media was separated from the tissue by centrifugtion and transfered to separate polystyrene sample tubes. Saline solution (.9%) was added to the tubes containing the tissue (2 ml PLAC and COMB, 4 ml ENDO) and the mixture homogenated with two 30 s pulses to ensure cell disruption. Duplicate samples from homogenized material were assayed and compared to a standard curve consisting of six concentrations (0 to 100 ug/mg) of bovine serum albumin (purity > 98%, Sigma, St. Louis, Mo.). Absorbance at 595 nm was measured spectrophotometrically and protein concentration per sample was calculated using a linear regression computer program.

DNA Determination The DNA content of the tissue/saline homogenate was determined as an alternative to correcting for soluble protein content. The method of determination was a highly-specific fluorochrome dye-binding assay described by Casarone et al.(1979) and Labarca and Paigen (1980). The fluorochrome dye (Hoechst 33258; Sigma Chemical, St. Louis, Mo) was specific for DNA and therefore

useful for estimating the DNA content of crude cellular extracts. Duplicate samples of the homogenate were assayed and compared to a standard curve of seven concentrations (0 to 2.4 ug) of DNA (Sigma Laboratories , St. Louis, Mo.). Fluorescence was measured with a fluorometer (Hoefer Scientific Instruments, San Francisco) calibrated to read light emitted at 460 nm. The concentration of DNA per sample was calculated using a linear regression computer program.

Hormone Assay Procedures Media samples were analyzed for P_4, E_1 and E_1SO_4 with RIA (Chapter 3).

Statistical Analysis Data were analyzed using the General Linear Models procedure of the Statistical Analysis Systems (SAS, 1985). The preliminary analyses, incorporating the protein or DNA content of the tissues as a continuous variable, indicated that P_4 tended ($P<.06$) to be affected by the soluble protein content and E_1SO_4 tended ($P<.10$) to be affected by the DNA content of the tissue. Therefore, the hormone values at each day of gestation were preadjusted for differences in protein or DNA content using the formula previously described by Kukoly (1984):

Adjusted hormone (ng/g) = hormone (ng/g) - regression coefficient (protein or DNA - mean protein or DNA).

The following statistical model was used to analyze the data:

$$Y_{ijklmn} = \mu + D_i + T_j + DT_{ij} + G(DT)_{ijk} + S_l + M_m + P_n + SM_{lm} + SP_{ln} + MP_{mn} + DS_{il} + DM_{im} + DP_{in} + TS_{jl} + TM_{jm} + TP_{jn} + DTP_{ijn} + e_{ijklmn}$$

Where μ is the overall mean for the tissue hormone concentration, D_i is the day of gestation (d 30 or 70), T_j is exogenous progestogen treatment (CO, P4 or MPA), DT_{ij} is the interaction of day of gestation and progestogen treatment, $G(DT)_{ijk}$ is the effect of gilt within DT_{ij}, S_l is tissue type (PLAC, COMB or ENDO), M_m is the time of incubation(0, .5, 1 or 2 h), P_n is the effect of the presence or absence of P_5 in the incubation medium and e_{ijklmn} is the random error. All other components of the model are interactions of the previously described effects. The effects of D_i, T_j and DT_{ij} were tested using mean squares for $G(DT)_{ijk}$ as its error term. The effects of D_i, T_j, S_l, M_m and P_n were considered to be fixed, whereas $G(DT)_{ijk}$ was considered as random.

Due to a significant interaction of tissue type with both protein and DNA content E_l concentration was re-analyzed using a reduced model within tissue subclasses. Furthermore, due to a large variability in P_4 means these data were re-analyzed using a reduced model within days of gestation.

Results

Tissue Soluble Protein and DNA Content Mean protein content (Table 1) of incubated PLAC tissue tended ($P<.10$) to be greater at d 70 than at d 30. Day of gestation did not affect ($P>.61$) the protein content of either ENDO or COMB while exogenous progestogen treatment had no affect on the composition of any of the tissues.

Mean DNA (Table 1) content of PLAC, ENDO and COMB did not differ ($P>.30$) between the two stages of pregnancy or due to exogenous progestogen treatment.

Progesterone Production by the Placenta and Endometrium Mean concentrations of P_4 present in the incubation medium following 0 toh 2 h incubation of either PLAC, ENDO or COMB are summarized in tables 2 and 3. There were no differences ($P>.65$) in P_4 concentration due to the time of incubation regardless of whether steroid was adjusted for soluble protein or DNA content. Therefore, tabular values are combined across incubation times.

Adjusting for either soluble protein or DNA content of the incubated tissue made little difference in the subsequent analysis. Overall, P_4 increased ($P<.001$) between d 30 and d 70 of gestation with media from the PLAC having significantly higher levels than the ENDO ($P<.001$) at both stages of pregnancy. The difference between PLAC and ENDO was larger at d 70 than at d 30 of gestation

Table 4-1. Soluble Protein and DNA Content of Endometrial, Placental and Mixed Tissue From Gilts Subjected to Either Endogenous or Exogenous Progestogen Treatment[1]

	PLACENTA			COMBINATION			ENDOMETRIUM		
	CO	P_4	MPA	CO	P_4	MPA	CO	P_4	MPA
D 30 of Gestation									
Soluble Protein[2]	141.20 (35.69)	118.87 (36.64)	68.24 (35.85)	552.87 (55.85)	443.69 (94.70)	358.52 (56.86)	1082.54 (124.20)	823.53 (209.27)	613.49 (123.96)
DNA	14.64 (1.75)	16.68 (1.82)	16.83 (2.08)	25.12 (3.42)	31.27 (5.48)	30.48 (3.82)	34.95 (5.44)	43.63 (7.87)	39.35 (5.47)
D 70 of Gestation									
Soluble Protein[2]	281.61 (35.44)	263.37 (38.21)	230.29 (35.87)	539.46 (56.73)	596.68 (58.50)	504.11 (58.41)	823.66 (125.58)	969.81 (124.09)	841.56 (125.48)
DNA	12.46 (2.10)	9.67 (1.89)	11.89 (1.77)	23.33 (3.36)	21.52 (3.74)	22.72 (3.28)	38.86 (4.79)	35.21 (5.01)	35.02 (4.74)

[1] Values expressed as ug of soluble protein or DNA per 100mg of tissue, standard error of the mean in parentheses

[2] Protein tended to differ as a function of day of gestation ($P<.10$)

Table 4-2. In Vitro Progesterone Production (DNA Corrected) by Intrauterine Tissues from Gilts Subjected to Either Endogenous or Exogenous Progestogen Treatment During Gestation[1]

Day of Gestation[2]	Tissue[3]	P_5	TREATMENT		
			CO	P_4	MPA
OVX D20 HYSTX D30	PLAC	Present	2.02(.31)	2.07(.38)	2.63(.31)
		Absent	1.06(.32)	1.89(.39)	1.10(.31)
	COMB	Present	1.64(.32)	2.59(.37)	2.58(.31)
		Absent	.57(.31)	1.27(.38)	.97(.31)
	ENDO	Present	1.01(.31)	1.22(.38)	.86(.31)
		Absent	1.06(.31)	.66(.37)	.42(.31)
OVX D60 HYSTX D70[4]	PLAC	Present	18.34(2.22)	18.37(2.54)	14.72(2.15)
		Absent	16.84(2.27)	14.84(2.31)	14.65(2.21)
	COMB	Present	10.73(2.18)	8.61(2.18)	8.24(2.11)
		Absent	7.93(2.12)	6.63(2.29)	5.56(2.03)
	ENDO	Present	.18(2.04)	1.04(2.08)	.69(2.03)
		Absent	-.29(2.11)	1.47(2.07)	.03(2.07)

[1] P_4 values expressed as least squares means (ng P_4/100mg Tissue); standard error of the mean in parentheses.
[2] P_4 synthesis differed as a function of day of gestation ($P<.001$).
[3] P_4 synthesis differed due to tissue type ($P<.001$).
[4] P_4 synthesis differed within d 70 of gestation, due to the presence of P_5 in the incubation medium ($P<.01$).

Table 4-3. In Vitro Progesterone Production (Protein Corrected) by Intrauterine Tissues from Gilts Subjected to Either Endogenous or Exogenous Progestogen Treatment During Gestation[1]

Day of Gestation[2]	Tissue[3]	P_5	TREATMENT		
			CO	P_4	MPA
OVX D20 HYSTX D30	PLAC	Present	2.00(.32)	2.12(.40)	2.75(.34)
		Absent	1.09(.33)	2.35(.41)	1.18(.33)
	COMB	Present	1.75(.34)	2.46(.38)	2.49(.33)
		Absent	.68(.34)	1.21(.40)	.89(.33)
	ENDO	Present	.98(.35)	1.22(.39)	.88(.33)
		Absent	1.05(.33)	.66(.38)	.45(.34)
OVX D60 HYSTX D70[4]	PLAC	Present	18.48(2.28)	19.08(2.52)	14.76(2.15)
		Absent	16.97(2.29)	15.46(2.32)	14.49(.21)
	COMB	Present	10.53(2.19)	8.65(2.18)	7.83(2.13)
		Absent	7.87(2.12)	6.83(2.38)	5.30(2.05)
	ENDO	Present	-.02(2.04)	1.60(2.09)	.43(2.03)
		Absent	-.52(2.14)	1.78(2.08)	-.46(2.09)

[1] P_4 values expressed as least squares means (ng P_4/100 mg Tissue); standard error of the mean in parentheses.
[2] P_4 synthesis differed as a function of day of gestation ($P<.001$).
[3] P_4 synthesis differed due to tissue type ($P<.001$).
[4] P_4 synthesis differed within d 70 of gestation, due to the presence of P_5 in the incubation medium ($P<.01$).

resulting in a significant day of gestation x tissue interaction (P<.001). The coincubation of the two tissues resulted in P_4 concentrations that were intermediate to PLAC and ENDO at both days of gestation.

The addition of P_5 to the incubation medium enhanced (P<.01) P_4 production at both d 30 and d 70 of gestation. Pregnenolone increased P_4 production for all three tissue preparations as indicated by a nonsignificant tissue by P_5 interaction.

Lastly, exogenous progestogen treatment had no effect on the accumulation of P_4 in the incubation medium. This was evident when the effect of progestogen treatment was examined alone or as an interaction with any of the other factors.

Estrone Production by the Placenta and Endometrium Mean concentrations of E_1 present in the incubation medium following 0 to 2 h incubations of either PLAC, ENDO or COMB are summarized in Tables 4 and 5. There were no differences (P>.15) among E_1 concentrations due to the time of incubation regardless of whether the data were adjusted for either protein or DNA . Therefore, tabular values are combined across incubation times.

As seen in regard to P_4 production, the PLAC produced significantly more (P<.001) E_1 in the incubation medium than did the ENDO. The COMB tissue preparation was

Table 4-4. In Vitro Estrone Production by Intrauterine (DNA Corrected) Tissues from Gilts Subjected to Either Endogenous or Exogenous Progestogen Treatment During Gestation[1]

Day of Gestation[2]	Tissue[3]	P5	TREATMENT CO	P4	MPA
	PLAC	Present	371.2(41.3)	272.2(42.0)	340.1(42.3)
		Absent	398.4(41.9)	315.7(42.2)	287.6(41.3)
OVX D20	COMB[4]	Present	310.4(23.8)	285.2(32.0)	343.6(23.9)
HYSTX D30		Absent	297.5(23.5)	284.0(33.9)	277.5(23.6)
	ENDO[4]	Present	174.9(19.6)	131.7(27.8)	184.2(19.4)
		Absent	266.5(19.4)	138.7(26.0)	175.1(19.4)
	PLAC	Present	647.5(41.9)	589.7(43.4)	707.4(42.1)
		Absent	689.5(41.9)	509.0(42.9)	726.8(41.9)
OVX D60	COMB	Present	368.0(23.8)	274.9(24.3)	428.3(24.4)
HYSTX D70		Absent	433.7(24.0)	358.1(23.9)	367.8(23.9)
	ENDO	Present	92.2(19.4)	78.0(19.4)	114.2(19.5)
		Absent	78.7(19.6)	69.9(19.4)	88.5(19.5)

1 E1 values expressed as least squares means (pg E1/100 mg Tissue); standard error of the mean in parentheses.
2 E1 synthesis differed as a function of day of gestation for PLAC and ENDO ($P<.01$).
3 E1 synthesis differed due to tissue type ($P<.001$).
4 E1 synthesis differed within tissue type, as a function of the interaction of P5 treatment and day of gestation ($P<.05$).

Table 4-5. In Vitro Estrone Production (Protein Corrected) by Intrauterine Tissues from Gilts Subjected to Either Endogenous or Exogenous Progestogen Treatment During Gestation[1]

Day of Gestation[2]	Tissue[3]	P_5	TREATMENT[5] CO	P_4	MPA
OVX D20 HYSTX D30	PLAC	Present	365.8(42.3)	285.6(43.2)	332.2(48.0)
		Absent	395.4(42.5)	322.8(41.8)	277.6(46.0)
	COMB[4]	Present	314.9(23.6)	287.8(31.3)	336.8(25.0)
		Absent	303.8(24.2)	284.9(33.3)	260.5(26.0)
	ENDO[4]	Present	178.0(21.5)	128.3(27.7)	183.6(20.8)
		Absent	266.1(20.4)	134.8(25.7)	173.8(21.2)
OVX D60 HYSTX D70	PLAC	Present	632.4(48.6)	601.3(43.4)	709.6(43.2)
		Absent	684.4(46.4)	515.9(45.3)	727.6(42.9)
	COMB	Present	373.2(23.6)	284.9(24.4)	435.0(23.4)
		Absent	439.3(24.0)	368.1(25.9)	372.4(23.4)
	ENDO	Present	91.3(19.4)	79.8(20.0)	115.2(19.4)
		Absent	77.6(19.9)	72.3(20.0)	88.5(19.5)

[1] E_1 values expressed as least squares means (pg E_1/100 mg Tissue); standard error of the mean in parentheses.
[2] E_1 synthesis differed as a function of day of gestation for PLAC and ENDO ($P<.05$).
[3] E_1 synthesis differed due to tissue type ($P<.001$).
[4] E_1 synthesis differed within tissue type, as a function of the interaction of P_5 treatment and day of gestation ($P<.05$).
[5] E_1 synthesis differed within tissue type, as a function of the interaction of progestogen treatment and day of gestation ($P<.001$).

intermediate in steroid production to the two tissues incubated alone.

The stage of pregnancy significantly affected E_1 production by the PLAC with d 70 concentrations being greater than those at d 30 of gestation. However, ENDO E_1 production was higher ($p<.01$) at d 30 than at d 70 of gestation. Coincubation of the tissues resulted in similar E_1 accumulation in the media for the two stages of pregnancy.

The addition of P_5 to the incubation medium had no overall effect ($P>.50$) on E_1 production for any of the three preparations. However, a significant P_5 by day of gestation interaction occurred for both ENDO and COMB. Pregnenolone enhanced E_1 production for ENDO on d 70 but not d 30 of gestation. The opposite was true for COMB tissue: E_1 production was enhanced on d 30, but not d 70 of gestation with the addition of P_5 to the media. A highly significant treatment by P_5 interaction also resulted for the COMB tissue. The presence of P_5 in the incubation medium had no effect on E_1 accumulation for COMB tissue from either CO or P_4-treated gilts, but P_5 enhanced E_1 production by MPA treated gilts.

Estrone Sulfate Production by Placenta and Endometrium

Analysis of E_1SO_4 data yielded similar results regardless as to whether the data were adjusted for soluble protein or

Table 4-6. In Vitro Estrone Sulfate Production (DNA Corrected) by Intrauterine Tissues from Gilts Subjected to Either Endogenous or Exogenous Progestogen Treatment During Gestation[1]

Day of Gestation[2]	Tissue[3,4]	P_5[5,6]	TREATMENT[7] CO	P_4	MPA
OVX D20 HYSTX D30	PLAC	Present	.94(.09)	.73(.10)	1.73(.09)
		Absent	.84(.10)	.76(.09)	.89(.09)
	COMB	Present	.76(.09)	.50(.09)	.67(.10)
		Absent	.60(.09)	.69(.09)	.52(.09)
	ENDO	Present	.56(.09)	.63(.09)	.60(.09)
		Absent	.43(.09)	.59(.09)	.59(.09)
OVX D60 HYSTX D70	PLAC	Present	.30(.10)	.42(.10)	.46(.09)
		Absent	.42(.09)	.44(.09)	.51(.09)
	COMB	Present	.36(.09)	.29(.09)	.39(.09)
		Absent	.46(.09)	.32(.09)	.32(.09)
	ENDO	Present	.24(.09)	.28(.09)	.31(.09)
		Absent	.26(.10)	.28(.09)	.40(.09)

[1] E_1SO_4 values expressed as least squares means (ng E_1SO_4/100 mg Tissue); standard error of the mean in parentheses.
[2] E_1SO_4 synthesis differed as a function of day of gestation (P<.001).
[3] E_1SO_4 synthesis differed due to tissue type (P<.001).
[4] E_1SO_4 synthesis differed as a function of the interaction of tissue type and day of gestation (P<.001).
[5] E_1SO_4 synthesis differed as a function of the interaction of P_5 treatment and day of gestation (P<.01).
[6] E_1SO_4 synthesis differed as a function of the interaction of P_5 treatment and progestogen treatment (P<.05).
[7] E_1SO_4 synthesis differed as a function of the interaction of progestogen treatment and tissue type (P<.001).

Table 4-7. In Vitro Estrone Sulfate Production (Protein Corrected) by Intrauterine Tissues from Gilts Subjected to Either Endogenous or Exogenous Progestogen Treatment During Gestation[1]

Day of Gestation[2]	Tissue[3,4]	P_5[5]	TREATMENT[6] CO	P_4	MPA
OVX D20 HYSTX D30	PLAC	Present	.96(.09)	.74(.09)	1.52(.09)
		Absent	.84(.08)	.78(.09)	.93(.09)
	COMB	Present	.76(.08)	.51(.08)	.68(.08)
		Absent	.60(.08)	.70(.08)	.52(.09)
	ENDO	Present	.56(.09)	.63(.08)	.59(.09)
		Absent	.44(.09)	.59(.08)	.58(.09)
OVX D60 HYSTX D70	PLAC	Present	.29(.09)	.45(.08)	.46(.09)
		Absent	.42(.09)	.45(.09)	.52(.09)
	COMB	Present	.37(.08)	.33(.09)	.42(.08)
		Absent	.48(.08)	.36(.09)	.34(.08)
	ENDO	Present	.21(.08)	.29(.08)	.32(.08)
		Absent	.26(.09)	.26(.08)	.41(.08)

1 E_1SO_4 values expressed as least squares means (ng E_1SO_4/100 mg Tissue); standard error of the mean in parentheses.
2 E_1SO_4 synthesis differed as a function of day of gestation ($P<.001$).
3 E_1SO_4 synthesis differed due to tissue type ($P<.001$).
4 E_1SO_4 synthesis differed as a function of the interaction of tissue type and day of gestation ($P<.001$).
5 E_1SO_4 synthesis differed as a function of the interaction of P_5 treatment and day of gestation ($P<.01$).
6 E_1SO_4 synthesis differed as a function of the interaction of progestogen treatment and tissue type ($P<.001$).

DNA content (Tables 6 and 7). The PLAC produced more ($P<.001$) E_1SO_4 than did the other tissues. Coincubation of the two tissues resulted in intermediate E_1So_4 values.

The stage of pregnancy affected the production of E_1SO_4, with greater ($P<.001$) accumulation of the conjugated steroid within the media at d 30 of gestation. The difference between PLAC and ENDO was also greater at the earlier stage of pregnancy as indicated by the signifcant ($P<.001$) day of gestation by tissue interaction.

Overall, the addition of P_5 to incubation medium did not augment ($P>.10$) E_1SO_4 accumulation in the incubation medium. However, when the interaction of P_5 treatment and stage of pregnancy was examined, tissue from d 30 HYSTX gilts was responsive ($P<.01$) to the steroid precursor while tissue from d 70 HYSTX gilts was not.

Exogenous progestogen treatment did not affect ($p>.40$) E_1SO_4 production except for the interaction of treatment and tissue type. A highly significant ($P<.001$) effect was seen with PLAC production being greater for MPA-treated gilts than the CO or P_4-treated gilts. A significant progestogen treatment by P_5 interaction ($P<.05$) was also observed, but only when the data were corrected for the DNA content of the tissue. The analysis revealed that addition of P_5 to the incubation medium augmented E_1SO_4 accumulation for tissue from CO and MPA-treated gilts, but not P_4-

treated gilts. Furthermore, a significant day of gestation by treatment by P_5 interaction ($P<.05$) showed that the previously-described effect occurred only at d 30, suggesting an abundant supply of P_4 within the tissue of P_4-treated gilts at the earlier stage of gestation.

Discussion

The results of the present study are consistent with results of previous investigations from our laboratory that have examined in vitro steroid production by intrauterine tissues during pregnancy. Prior studies have shown in vitro P_4 and E_1 synthesis by the PLAC to increase (Kukoly, 1984; Jeantet and Knight, 1985) while ENDO production remained low (Jeantet and Knight, 1985) during the time period examined in this study. The intermediate levels of steroid production for the coincubation of the two tissues indicates that PLAC production is being diluted by the less-steroidogenically-active ENDO.

A paradox exists between the expanding steroidogenic capability of the PLAC (at least under in vitro conditions) and systemic P_4 levels. While in vitro P_4 production per unit tissue continues to increase to d 100 of pregnancy (Kukoly, 1984), circulating P_4 declines from d 25 until term (Robertson and King, 1974; Baldwin and Stabenfeldt, 1975; Knight et al., 1977; Kukoly, 1984; Jeantet and Knight, 1985). It appears that the allantoic fluid pool is

the only in vivo indicator of in vitro P_4 production while in vitro E_1 and E_1SO_4 synthesis are reflected by both plasma and allantoic fluid levels (Knight et al., 1977; Kukoly, 1984; Jeantet and Knight, 1985).

The finding that P_4 production was enhanced with the addition of P_5 to the incubation medium is in agreement with previous reports (Jeantet and Knight, 1985; Beal et al., 1986). The capability of the PLAC to produce P_4 from P_5 is supported by histochemical evidence of the presence of delta^5-3ß-hydroxysteroid dehydrogenase, the enzyme (along with 3-ketosteroid isomerase) essential for this step of steroidogenesis (Christie, 1968; Dufour and Raeside, 1969). Meanwhile, the general inability of P_5 to promote E_1 and E_1SO_4 synthesis suggests a rate limiting step in the steroidogenic pathway. Aromatase activity changes little as total estrogens increase over the course of gestation (Knight and Hopkins, 1988), while extremely small quantities of androgens are present in the incubation media of incubated PLAC and/or ENDO (Kukoly, 1984). Therefore, the limiting enzymes arè probably 17 Á hydroxylase and/or 17-20 desmolase, enzymes required for the conversion of progestogens into androgens. This information suggests that any androgens produced in the in vitro system used were rapidly aromatized to estrogens and that the formation of androgens from progestogens was the limiting step.

The increase in P_4 production with the addition of exogenous P_5 to the incubation media suggests that the tissues in our in vitro system were viable and active. However, despite this increase in P_4 synthesis, concentrations in the media did not differ due to the time of incubation. Previous studies from our laboratory have revealed inconsistencies for the effect of incubation time on tissue steroid production in either the presence (Jeantet and Knight, 1985) or absence (Kukoly, 1984) of P_5.

The results of the present and previous studies appear to be contradictory. On one hand, P_5 is augmenting steroid production, while the incubation of tissue for .5, 1 or 2h has no advantage over the controls (0h). The answer to this contradiction may be due to several factors. End product inhibition may prevent further steroid synthesis due to increased incubation time in culture tubes supplemented with P_5. In culture tubes devoid of exogenous precursor, pregnenolone may be limiting, therefore preventing additional steroid production. Alternatively, the discrepency could lie in the handling of the in vitro culture tubes. In this investigation, culture tubes were removed from the incubation chamber at the appointed time and placed in an ice-water bath. The control tubes, which were prepared first, spent the entire incubation period (approximately 2.5h) in the same ice bath. Therefore, if steroidogenesis was able to continue (albeit at a decreased

rate) at the temperature in this water bath ($0^{o}C$), the difference in steroid concentration between 0 and 2h incubation times could be small. This would be especially true of tissue (such as ENDO) secreting steroids at a low rate.

In a subsequent investigation we adjusted the incubation procedure to test the hypothesis that steroidogenesis may be able to occur at near-freezing temperatures. At the end of each time period, culture tubes were centrifuged immediately to separate tissue from media. Consequently, the results of this study revealed significant increases due to time of incubation for P_4, E_1 and E_1SO_4 at 9 different stages of gestation. Therefore, in the current investigation steroidogenesis may have taken place at the low teperatures of the ice-water bath.

The fact that OVX plus exogenous MPA treatment had few detectable effects on steroid synthesis is of primary concern in this investigation. Despite finding depressed P_4 levels in the uterine vessels of MPA-treated gilts (Chapter 3), both in vitro production and allantoic fluid concentration (Chapter 3) of P_4 were equivalent to that of control gilts. Also, E_1 and E_1SO_4 concentrations in the plasma, fetal fluids (Chapter 3) and incubation media were generally unaffected by replacing luteal P_4 with exogenous MPA. Since MPA cannot be aromatized to estrogenic steroids, the data suggests that the source of these

estrogens is intrauterine P_4. One exception was that COMB tissue from MPA-treated gilts was stimulated more than COMB tissue from CO or P_4-treated gilts with the addition of P_5 to the incubation medium. Therefore, it appears that tissue from MPA-treated gilts maybe deficient in steroidogenic precursors. This is an indication that luteal P_4 might be a source of precursor for the synthesis of estrogens within the uterus.

Therefore, these results provide evidence that the intrauterine tissues are able to produce P_4 and other steroidal hormones in vivo as well as under in vitro conditions. The significant (albeit depressed) quantities of P_4 found in the plasma suggest that not only are these tissues producing progestogens for subsequent conversion into estrogens, but that a portion of the P_4 is exuded into the general circulation. The data also indicate that the P_4 measured in the plasma is not due to placental compensation in the absence of luteal P_4 since all other hormonal measurements (both in vivo and in vitro) were equivalent to that of untreated controls.

The effects of MPA treatment on fetal survival and conceptus development support these findings. The analysis of conceptus and litter variables from this (Chapter 3) and a previous study (Heap et al., 1981) revealed no detrimental effects due to the treatment regime.

In conclusion, this investigation has provided evidence for the regulation of steroid synthesis during

pregnancy. It has been shown that steroid-related events within the uterus can occur independent of similar events taking place on the ovary and that intrauterine P_4 provides a significant proportion of the pool within the systemic circulation. Furthermore, these findings indicate that estrogens are produced at completely normal levels in the absence of luteal P_4. It cannot be determined from these results whether P_4 of intrauterine origin is required for the maintenance of pregnancy or if P_4 reaches the systemic blood supply only because estrogen producing enzymes are insufficient to aromatize all of the available precursor. In addition, it cannot be assumed that once P_4 reaches the maternal circulation (whether of fetal or luteal origin) it does not reenter the uterus for subsequent aromatization.

It should be kept in mind that the duration of progestogen treatment used in this investigation was relatively short and the results reported here might not apply if treatment was extended for a longer period. Lastly, fetal steroid production should be included in any discussion of steroid regulation during pregnancy. Fetal steroids are known to have an important role in other species, interacting with maternal and placental components to regulate intrauterine steroidogenesis (Diczfalusy, 1974). However, the design of the current investigation does not allow us to directly examine fetal steroidogenesis. Future investigations should attempt to include this component.

Chapter Five

PORCINE INTRAUTERINE STEROIDOGENESIS: III. PREGNANCY MAINTENANCE AND CONCEPTUS DEVELOPMENT IN OVARIECTOMIZED GILTS ADMINISTERED PREGNENOLONE AS A PRECURSOR FOR INTRAUTERINE STEROIDOGENESIS

Introduction

Several species including the ovine, equine, and human gain the capacity at some point of gestation to continue pregnancy after the removal of their ovaries (Catchpole, 1977; Jainudeen and Hafez, 1987). The ability to maintain pregnancy in the absence of ovaries stems from the capacity of the placenta to substitute as the primary progestogen-producing tissue (Catchpole, 1977; Jainudeen and Hafez, 1987).

Alternatively, it is well established that the removal of the ovaries at any time during gestation in gilts or sows will result in the immediate termination of pregnancy (du Mesnil du Buisson and Dauzier, 1957). Thus, it has long been assumed that a luteal source of progesterone (P_4) was essential for pregnancy maintenance in swine. More specifically it has been determined by partial luteectomy techniques that sufficient luteal tissue must be present to maintain maternal plasma P_4 at 6 ng/ml or more if pregnancy is to proceed (Thomford and Dziuk,1982; Hagen et al., 1984). Circumstantial evidence such as this led researchers to hold the long-standing belief that the porcine placenta was unable to produce P_4.

Contrary to this assumption is more recent evidence indicating the porcine placenta to be quite active steroidogenically. Initially, Knight et al. (1977) reported (with ovarian-intact gilts) a positive P_4 and negative estrone (E_1) uterine artery minus uterine vein difference, suggesting that P_4 of ovarian origin is utilized within the pregnant uterus as a substrate for subsequent conversion to estogens. Also, when exogenous P_4 was administered to bilaterally-ovariectomized gilts in order to maintain pregnancy, gilts which did remain pregnant had significantly lower plasma P_4 values than their non-pregnant counterparts (Knight et al.1974; Bazer et al., 1979). Again, this evidence indicates that pregnancy has a marked affect on steroid metabolism.

Results of more recent investigations from our laboratory clearly indicate that the placenta (and to a lesser extent the endometrium) has the capacity to produce both P_4 and E_1 in vitro (Kukoly, 1984). In addition, similar studies have provided data demonstrating increased steroid production as gestation progresses from d 20-100 and that steroid synthesis is augmented by the addition of the steriod precursor pregnenolone (P_5) to the incubation medium (Jeantet and Knight, 1985). Lastly, very recent experiments (Chapters 3 and 4) indicate de novo estrogen production by the porcine placenta in vivo. In these studies gilts were ovariectomized at two different stages

of gestation and administered the non-aromatizable progestogen, medroxyprogesterone acetate (MPA). Gilts under this regimen maintained pregnancy with a normal litter sizw and normal conceptus development compared to controls. Hormone concentrations (P_4, E_1 and estrone sulfate [E_1SO_4]) in the plasma, fetal fluids and in the medium of incubated placenta and endometrium were also equivalent to those of untreated gilts. Estrogens are known to have localized effects on electrolyte and fluid movement (Goldstein et al., 1980), cell permeability (Szego and Sloan, 1961) and uterine blood flow (Ford and Christenson, 1979), functions that are important in placental events such as placental expansion and the imbibition of fetal fluids (Knight et al., 1977). Therefore, we can conclude that the estrogens required for normal placental function in gilts maintained with MPA were being produced by the intrauterine tissues.

These observations led to our hypothesis that the porcine placenta may be able to produce sufficient amounts of steroids (P_4 and E_1) to compensate for ovarian removal if adequate quantities of a steroidogenic precursor was administered. The objective of this study was to assess the effects of exogenous pregnenolone (P_5) administration on pregnancy maintenance, conceptus development, prenatal survival , and intrauterine steroidogenesis in gilts.

Materials and Methods

Animals Forty six gilts were allocated to an experiment designed to test the hypothesis that pregnancy and normal conceptus development could be maintained in gilts via intrauterine progesterone production if adequate quantities of the steroidogenic precursor P_5 (3ß-hydroxypregn-5-en-20-one) were supplied exogenously. Gilts of similar weight (100-120kg) and genetic background (Yorkshire x Hampshire x Duroc) were housed in pens measuring 3.1m X 4.9m and were fed 2.7 kg/d of a corn-soy bean ration containing 14% CP. Daily estrous detection was initiated at approximately 6 mo. of age and was facilitated with the use of mature boars. Gilts were bred at estrus during their third regularly occurring estrous cycle by either natural service or artifically inseminating freshly-collected semen. Breeding occurred at estrus and at 12, 24, and 36 h thereafter. Estrous detection continued after gilts were bred to identify individuals that failed to maintain pregnancy. Forty-one gilts remained in this study while an additional 5 gilts failed to maintain pregnancy until their assigned ovariectomy (OVX) date and were removed.

At the time of breeding gilts were randomly assigned to be OVX at either d 20, 25, 30, 35, 40, 45, 55, 60, 80 or 90 of gestation. Beginning on the day prior to OVX, all gilts were administered 1g of P_5 in a suspension of corn

oil and benzyl benzoate (4:1 ratio) via daily subcutaneous injection in the neck region. Daily injections continued through the day prior to hysterectomy (HYSTX) which occurred 10 days after OVX (i.e.; d 30, 35, 40, 45, 50, 55, 65, 70, 90 or 100of gestation).

Surgical Procedures Anesthesia was induced and maintained and OVX and HYSTX were performed as described in Chapter 3.

Data Collection Following surgery the reproductive tract was processed and examined as described in Chapter 3. Allantoic fluid from d 35 conceptuses was pooled within each litter for subsequent hormone determination. Fetal fluids recovered at d 40-100 of gestation were assayed for individual conceptus variation associated with intrauterine position (Suazo, 1989). For this investigation, average concentration of each hormone within each litter will be reported.

Hormone Assay Procedures Allantoic and amniotic fluid samples as well as plasma samples obtained from the uterine artery, uterine vein, and anterior vena cava were analyzed for P_4, E_1 and E_1SO_4 utilizing radioimmunassay (RIA). These procedures are outlined in Chapter 3.

Statistical Analysis Data were analyzed using the General Linear Models procedure of the Statistical Analysis

Systems (SAS, 1985). Litter variables included the number of ovulations (corpora lutea, CL) at OVX, number of live and/or dead fetuses, percent fetal survival, number of ovulations not accounted for by a fetus (either alive or dead), and uterine length. Conceptus variables included placental length and weight, fetal length and weight, and fetal fluid volumes. Litter and conceptus variable means were calculated within each day of gestation in which gestation was maintained.

In order to reduce the scaling effect in hormone (P_4, E_1, E_1SO_4) concentration within the plasma and fetal fluid pools logarithmic transformations were performed. The following statistical model was employed using the logarithmic scale:

$$Y_{ij} = \mu + D_i + e_{ij}$$

Where μ is the natural logarithm of the overall mean hormone concentration, D_i is the effect of day of gestation and e_{ij} is the random error. Least squares means of the data in the original scale of measurement were reported. Tukey's procedure was used to detect differences in hormone values due to day of gestation (Steel and Torrie, 1980).

Results

Pregnancy Maintenance As presented in Table 1, none of the five gilts HYSTX at d 30 and only one of the five gilts HYSTX at d 35 maintained pregnancy. Gilts HYSTX between d 40 and d 55 of gestation had pregnancy maintenance rates that ranged from 67 to 100% within each treatment period. However, gilts treated with P5 and HYSTX subsequent to d 55 had a pregnancy rate of 100% (12/12).

Litter and Conceptus Variables As presented in Table 1, CL number was equivalent within each of the periods of pregnancy examined. Thus, the number of potential fetuses was similar for each stage of gestation. The fetal survival rate, which was calculated by dividing the number of live fetuses at HYSTX by the CL number at OVX, declined (as expected) as pregnancy progressed. More importantly, all measures of whole litter survival (number live, percent fetal survival) were equivalent to those that would be expected under normal (ovarian-intact, untreated) conditions (Knight et al.,1977; Faillace and Knight, 1988). Furthermore, the measures of conceptus development (table 2) were similar to those reported in previous investigations utilizing ovarian-intact, untreated gilts at the same stages of gestation (Knight et al.,1977; Faillace and Knight, 1988). Therefore, gilts that maintained

TABLE 5-1. EFFECT OF OVARIECTOMY AND PREGNENOLONE TREATMENT AT VARIOUS STAGES OF GESTATION ON LITTER CHARACTERISTICS AT HYSTERECTOMY[1,2]

Item	Day of hysterectomy[3]								
	35	40	45	50	55	65	70	90	100
No. treated[4]	5	3	6	6	4	3	3	3	3
No. pregnant	1	3	4	4	3	3	3	3	3
Ovulations[5] at OVX	14.2 (1.60)	13.7 (.94)	12.5 (.96)	13.4 (3.37)	12.8 (1.30)	12.7 (.47)	16.0 (3.27)	13.3 (.94)	14.7 (1.70)
Number live	13.0 (0)	8.7 (3.3)	11.5 (.5)	10.8 (3.0)	10.7 (2.1)	10.7 (1.9)	9.0 (2.8)	10.3 (.9)	11.3 (1.7)
Number dead	0 (0)	3.7 (3.86)	0 (0)	1.0 (1.00)	.7 (.94)	1.7 (1.70)	2.0 (2.83)	1.3 (1.25)	1.7 (.47)
Corpora lutea unrepresented[6]	1.0 (0)	1.3 (1.89)	1.0 (.71)	1.5 (1.12)	2.3 (2.62)	.3 (.47)	5.0 (3.27)	1.7 (1.25)	1.7 (2.36)
Fetal survival (%)	92.9 (0)	62.9 (.16)	92.4 (.03)	79.5 (.06)	81.7 (.08)	83.8 (.09)	61.8 (2.0)	78.2 (.08)	78.8 (.10)
Uterine length (cm)	479.0 (0)	417.5 (11.5)	528.5 (64.9)	492.8 (20.6)	518.0 (54.8)	624.3 (49.4)	489.3 (43.9)	634.3 (98.7)	676.3 (14.7)

1 Values expressed as least squares means, standard error of the mean in parentheses.
2 0 of 5 gilts OVX at d 20 and HYSTX at d 30 maintained pregnancy
3 All gilts were bilaterally ovariectomized (OVX) ten days before hysterectomy (HYSTX).
4 All gilts received a daily subcutaneous injection of 1g of P_5 in a suspension of corn oil and benzyl benzoate beginning the day before OVX.
5 Includes data from pigs pregnant at OVX but not at the time of HYSTX.
6 Number of corpora lutea on ovaries at OVX not represented by either a live or dead fetus.

TABLE 5-2. EFFECT OF OVARIECTOMY AND PREGNENOLONE TREATMENT AT VARIOUS STAGES OF GESTATION ON CONCEPTUS CHARACTERISTICS AT HYSTERECTOMY[1]

Item	Day of hysterectomy[2]								
	35	40	45	50	55	65	70	90	100
No. pregnant	1	3	4	4	3	3	3	3	3
Placental length (cm)	80.7 (2.97)	52.8 (2.49)	73.5 (2.09)	79.8 (2.85)	87.1 (2.01)	98.1 (3.42)	77.3 (3.47)	82.9 (4.33)	91.5 (3.57)
Placental weight (g)	60.1 (2.4)	56.3 (2.6)	64.4 (3.8)	117.6 (5.7)	143.4 (8.0)	229.6 (13.6)	227.2 (20.7)	229.5 (15.9)	240.7 (15.0)
Crown rump length (mm)	33.0 (.53)	49.9 (1.01)	69.8 (.63)	86.6 (.82)	110.3 (.64)	148.4 (1.28)	159.5 (1.75)	215.0 (3.81)	249.6 (7.71)
Fetal weight (g)	3.8 (.11)	10.3 (.33)	21.4 (.55)	43.9 (.88)	78.6 (1.1)	195.7 (3.2)	207.3 (10.7)	529.2 (22.7)	879.8 (34.6)
Allantoic fluid (ml)	165.5 (16.0)	57.3 (5.1)	115.5 (13.7)	176.8 (18.0)	213.2 (23.4)	284.9 (43.3)	134.6 (22.5)	109.3 (23.0)	55.4 (10.5)
Amniotic fluid (ml)	5.1 (.2)	13.7 (.9)	22.5 (.9)	40.2 (1.9)	77.0 (3.2)	148.6 (8.2)	139.9 (8.3)	173.4 (22.3)	107.4 (11.7)

[1] All gilts received a daily subcutaneous injection of 1g of P_5 in a suspension of corn oil and benzyl benzoate beginning on the day prior to ovariectomy. Values expressed as least squares means; standard error of the mean in parentheses.

[2] All gilts were bilaterally ovariectomized (OVX) ten days prior to hysterectomy (HYSTX).

pregnancy under these conditions, did so to a degree that allowed for both normal fetal survival and normal conceptus growth.

Progesterone Plasma P_4 levels (Table 3) remain fairly steady over the course of gestation (Knight et al., 1977; Kukoly, 1984; Jeantet and Knight, 1985) decreasing slightly until about one week prior to parturition (Ash et al.,1973; Ash and Heap, 1975; Baldwin and Stabenfeldt, 1975). Plasma P_4 levels of OVX P_5-treated gilts (that maintained pregnancy) conformed to this general trend with several exceptions. Samples from the gilt that maintained pregnancy when administered P_5 between d 25 and d 34 of gestation had relatively low levels of P_4, particularly in the uterine vessels. However, although P_4 was lower than what would be expected for ovarian-intact untreated gilts, P_4 in the AVC of this gilt (10.62 ng/ml) was above the 6 ng/ml minimum reported by Thomford and Dziuk (1982). In addition, gilts HYSTX at d 45 of pregnancy had higher-than-normal P_4 concentrations in all three vessels, while gilts HYSTX on either d 55 or d 65 of gestation had elevated P_4 levels in the systemic blood supply only. However, the large variability in P_4 concentration and the small number of plasma samples from gilts maintaining pregnancy under this experimental protocol made almost all differences in steroid levels at various days of gestation nonsignificant.

TABLE 5-3. EFFECT OF OVARIECTOMY AND PREGNENOLONE TREATMENT AT VARIOUS STAGES OF GESTATION ON PROGESTERONE CONCENTRATION IN PLASMA AND FETAL FLUID POOLS AT HYSTERECTOMY[1]

	Day of hysterectomy[2]								
Item	35	40	45	50	55	65	70	90	100
Uterine artery[3]	1.47[a,b] (1.31)	9.55[a,b] (4.94)	45.20[b] (20.20)	10.38[a,b] (4.64)	5.44[a,b] (2.81)	5.66[a,b] (2.92)	7.96[a,b] (4.11)	4.10[a] (2.12)	6.64[a,b] (3.43)
Uterine vein	3.74 (3.61)	16.63 (9.26)	34.57 (16.66)	12.33 (6.87)	6.88 (3.83)	6.50 (3.62)	6.05 (3.37)	3.73 (2.08)	6.65 (3.71)
Anterior vena cava[3]	10.62[a] (11.98)	23.52[a] (15.31)	76.17[a] (42.96)	14.60[a] (9.50)	131.76[a] (85.78)	34.61[a] (22.53)	7.21[a] (4.70)	8.06[a] (5.25)	5.71[a] (3.72)
Allantoic fluid[3]	.43[4] (.0)	.49[a,b] (.35)	1.52[b] (.65)	.92[b] (.54)	2.17[b] (1.22)	.03[a] (.02)	4.87[b] (4.31)	1.44[b] (1.14)	1.40[b] (1.24)
Amniotic fluid[3]	.21[4] (.0)	.25[a,c] (.13)	.99[a,b] (.31)	2.24[b] (1.03)	1.01[a,b] (.41)	.57[a,b] (.25)	2.79[b,c] (1.82)	.18[a] (.10)	.33[a,b] (.17)

[1] All gilts received a daily subcutaneous injection of 1g of P_5 in a suspension of corn oil and benzyl benzoate beginning on the day prior to ovariectomy. Values expressed as least squares means (ng/ml); standard error of the mean in parentheses.
[2] All gilts were bilaterally ovariectomized (OVX) ten days prior to hysterectomy (HYSTX).
[3] P_4 differed as a function of day of gestation ($P<.05$)
[4] Pooled sample from the gilt HYSTX at d 35 not included in analysis of fluid P_4 levels.
[a,b] Lsmeans within a row with different superscripts differ ($P<.05$).

Allantoic fluid P_4 levels (Table 3) increased between d 35 and d 70 (except for d 65), however, the differences between days of gestation were nonsignificant. One exception were comparisons with d 65 P_4 levels in amniotic and allantoic fluid. The P_4 concentration on d 65 measured almost zero. After rebounding at d 70 progesterone concentrations failed to increase in the allantoic pool thereafter and declined in the amniotic pool during the two latter stages examined.

Estrogens Results of the analysis of plasma sampled from OVX, P_5-treated gilts indicate increases that correspond to the final rise in E_1 concentration (Table 4) reported in intact, untreated gilts (Robertson and King, 1974; Knight et al., 1977; Jeantet, 1985). However, E_1 levels in these gilts began to elevate after d 70 of gestation and did not reach the levels reported for untreated ovarian intact gilts (Knight et al., 1977; Kukoly, 1984; Jeantet and Knight, 1985).

The fluids taken from conceptuses of the P_5-treated gilts (Table 4) underwent a dramatic rise in E_1 levels during pregnancy. However, E_1 within both fluid pools began to increase much earlier than has been reported for ovarian-intact untreated gilts (Knight et al., 1977; Kukoly, 1984; Jeantet and Knight, 1985), with the first increases seen on about d 45 in the present study.

TABLE 5-4. EFFECT OF OVARIECTOMY AND PREGNENOLONE TREATMENT AT VARIOUS STAGES OF GESTATION ON ESTRONE CONCENTRATION IN PLASMA AND FETAL FLUID POOLS AT HYSTERECTOMY[1]

	Day of hysterectomy[2]								
Item	35	40	45	50	55	65	70	90	100
Uterine artery[3]	.011[a,b,c] (.039)	.010[a,b] (.022)	.016[a,b,c] (.019)	.010[a,b] (.019)	.003[a] (.022)	.005[a] (.022)	.025[a,b,c] (.022)	.211[c] (.022)	.110[b,c] (.027)
Uterine vein[3]	.018[a,b,c,d] (.100)	.013[a,b] (.058)	.015[a,b] (.050)	.028[a,c] (.050)	.003[a] (.058)	.005[a] (.058)	.059[b,c,d] (.058)	.177[c,d] (.058)	.405[d] (.058)
Anterior vena cava[3]	.007[a,b] (.268)	.010[a] (.154)	.015[a] (.134)	.005[a] (.134)	.005[a] (.154)	.004[a] (.154)	.071[a] (.189)	.341[b] (.154)	.769[b] (.154)
Allantoic fluid[3]	90.3[4] (0.0)	3.7[a] (1.3)	11.6[a,b] (2.4)	18.2[b] (5.1)	27.6[b] (7.3)	15.3[b] (4.3)	188.9[c] (78.9)	309.3[c] (115.6)	2296.7[d] (959.3)
Amniotic fluid[3]	.7[4] (.0)	2.1[a] (.8)	6.4[a,b] (1.5)	9.9[a,b] (3.3)	44.9[c,d] (13.6)	11.4[b,c] (3.6)	25.1[b,c,d] (12.0)	176.4[d,e] (75.3)	703.2[e] (273.9)

[1] All gilts received a daily subcutaneous injection of 1g of P_5 in a suspension of corn oil and benzyl benzoate beginning on the day prior to ovariectomy. Values expressed as least squares means (ng/ml); standard error of the mean in parentheses.
[2] All gilts were bilaterally ovariectomized (OVX) ten days prior to hysterectomy (HYSTX).
[3] E_1 differed as a function of day of gestation ($P<.001$)
[4] Pooled sample from gilt HYSTX at d 35 not included in analysis of fluid E_1.
[a,b,c,d,e] Means with different superscripts differ ($P<.05$).

Plasma E_1SO_4 levels (Table 5) in this study did undergo a decrease from d 35 to d 40 of pregnancy and remained constant before steadily increasing after d 65 of gestation. While the relative changes in E_1SO_4 were consistant with prior results from our laboratory the concentrations were much higher than those previously reported (Dwyer and Robertson, 1980; Knight and Hopkins, 1987). Therefore, the E_1SO_4:E_1 ratio (Table 6) was much higher than previously reported and increased rather than decreased late in gestation.

Estrone sulfate in the fetal fluids (Table 5) followed the same pattern that was recorded in the plasma. Unlike E_1, concentrations of E_1SO_4 in the plasma and fetal fluids were fairly similar thoughout gestation. Again, amniotic steroid levels were somewhat lower than those measured in the amniotic pool. The fluid E_1SO_4:E_1 ratio (Table 6) was highest for the gilt HYSTX at d 35 of pregnancy and was consistently low for the duration of the study.

Discussion

Measures of whole litter survival and conceptus development indicate that pregnancy was normal for those gilts that were able to continue gestating under the current treatment regimen. Although measures of the systemic and local steroid environment revealed some departures from what would be expected in intact-untreated

TABLE 5-5. EFFECT OF OVARIECTOMY AND PREGNENOLONE TREATMENT AT VARIOUS STAGES OF GESTATION ON ESTRONE SULFATE CONCENTRATION IN PLASMA AND FETAL FLUID POOLS AT HYSTERECTOMY[1]

	Day of hysterectomy[2]								
Item	35	40	45	50	55	65	70	90	100
Uterine artery[3]	2.80[a,b,c] (2.44)	1.76[a] (.89)	1.32[a] (.57)	1.48[a] (.64)	1.61[a] (.81)	2.65[a,b] (1.33)	4.67[a,b] (2.35)	27.91[b,c] (14.04)	97.29[c] (59.91)
Uterine vein[3]	6.70[a,b,c] (6.14)	2.28[a,b] (1.20)	1.73[a] (.79)	2.32[a] (1.06)	1.81[a] (.96)	1.74[a] (.92)	5.91[a,b] (3.13)	30.82[b,c] (16.31)	150.37[c] (79.55)
Anterior vena cava[3]	1.83[a,b] (1.39)	1.60[a] (.70)	1.05[a] (.40)	1.25[a] (.48)	1.73[a] (.76)	.66[a] (.35)	3.17[a,b] (1.70)	23.00[b,c] (10.08)	158.69[c] (69.51)
Allantoic fluid[3]	294.98[4] (0)	2.44[a] (1.33)	2.98[a] (.97)	5.98[a,b] (2.66)	14.57[a,b] (6.15)	9.76[a,b] (4.34)	47.95[b] (36.96)	11.74[a,b] (7.01)	1359.25[c] (907.30)
Amniotic fluid[3]	21.27[4] (0)	1.21[a] (.64)	2.89[a] (.91)	7.93[a,b] (3.64)	20.60[b] (8.45)	8.03[a,b] (3.48)	13.31[a,b] (8.64)	63.97[b,c] (37.13)	290.18[c] (153.74)

[1] All gilts received a daily subcutaneous injection of 1g of P_5 in a suspension of corn oil and benzyl benzoate beginning on the day prior to ovariectomy. Values expressed as least squares means (ng/ml); standard error of the mean in parentheses.

[2] All gilts were bilaterally ovariectomized (OVX) ten days prior to hysterectomy (HYSTX).

[3] E_1SO_4 differed as a function of day of gestation ($P<.001$).

[4] Pooled sample from the gilt HYSTX at d 35 not included in analysis of fluid E_1SO_4 levels.

[a,b,c] Means with different superscripts differ ($P<.05$).

TABLE 5-6. EFFECT OF OVARIECTOMY AND PREGNENOLONE TREATMENT AT VARIOUS STAGES OF GESTATION ON ESTRONE SULFATE:ESTRONE RATIO IN PLASMA AND FETAL FLUID POOLS AT HYSTERECTOMY[1]

	Day of hysterectomy[2]								
Item	35	40	45	50	55	65	70	90	100
Uterine artery	254.5	193.4	92.6	255.2	517.7	773.4	421.8	146.7	945.9
Uterine vein	372.2	225.1	134.2	165.8	5469.7	403.0	109.2	262.4	423.8
Anterior vena cava	261.4	157.2	75.9	341.5	415.9	217.1	60.9	135.4	261.0
Allantoic fluid	3.27	.67	.26	.33	.53	.64	.25	.04	.59
Amniotic fluid	29.10	.57	.45	.80	.46	.71	.53	.36	.41

[1] All gilts received a daily subcutaneous injection of 1g of P_5 in a suspension of corn oil and benzyl benzoate beginning on the day prior to ovariectomy. Values expressed as ratio of least squares means; standard error of the mean in parentheses.
[2] All gilts were bilaterally ovariectomized (OVX) ten days prior to hysterectomy (HYSTX).

gilts, these changes apparently had little effect on the viability of the growing conceptuses.

Progesterone concentrations in the maternal plasma and fetal fluids were generally similar to what has been previously reported in ovarian-intact untreated gilts (Knight et al., 1977; Kukoly, 1984; Jeantet and Knight, 1985). The relatively low levels of plasma P_4 in the gilt that maintained pregnancy in the d 25-34 treatment period, along with the fact that only 1 of 5 gilts treated at this time remained pregnant, indicates that the intrauterine tissues at this stage are marginal in their ability to synthesize steroids. The ability of exogenous P_5 to maintain pregnancy in OVX gilts improved when the treatment period coincided with a period of increased capability of P_4 production by the placenta. Besides the ability of these gilts to remain pregnant, plasma P_4 levels at d 45, d 55, and d 65 were actually greater than what would be expected in ovarian-intact untreated gilts.

The unusually high P_4 values recorded during this period also coincides with a stage reported to have relatively low plasma estrogen levels (Robertson and King, 1974; Knight et al., 1977; Knight et al. 1984). This indicates that estrogenic enzymes (aromatase) are limiting while the enzymes that promote P_4 production (3ß hydroxysteroid dehydrogenase and isomerase) were active in the presence of abundant precursor. Furthermore, since P_4 levels were high in the AVC of gilts HYSTX at d 55 and d 65

while uterine levels were relatively low, we must consider the possibility of an extrauterine source of steroids such as the maternal adrenal gland.

In contrast to plasma P_4 levels which remain fairly constant throughout gestation, plasma E_1 concentrations undergo extremely dynamic changes throughout the course of pregnancy. These changes occur in a triphasic fashion with peaks on d 10-12 (Stoner et al.,1981) d 16-30 (Robertson and King, 1974; Knight et al., 1977; Stoner et al.,1981) and on d 60 (Robertson and King, 1974; Knight et al. 1984; Jeantet,1985). Prior to d 30 of gestation, extremely dynamic, estrogen-mediated events including placental expansion and allantoic fluid imbibition are taking place within the gravid uterus. Subsequent to d 30 of pregnancy a less-active period begins as far as allantoic fluid volume and placental growth rate (length) are concerned (Knight et al., 1977). This period lasts until about d 60 when both allantoic and amniotic fluids begin to accumulate rapidly and fetal dimensions increase at an exponential rate. The relationship of relatively low estrogen values during this period and decreased allantoic volume has been discussed by Bazer et al.(1979). They proposed that the estrogen:P_4 ratio was responsible for the control of fluid influx into the placental compartments. In this model, relatively low estrogen levels between d 30 and d 60 results in decreased allantoic fluid accummulation.

Similar, but even more dramatic changes, have been reported for E_1 levels within fetal fluids of intact gilts (Knight et al.,1977; Kukoly, 1984; Jeantet and Knight, 1985). The rise of E_1 in the fluids is coincident with the rise in plasma levels (~ d 60) and continues to increase in an exponential manner until term. As stated for P_4, amniotic fluid levels are somewhat lower in E_1 concentration than allantoic fluid. Allantoic E_1 rose prematurely in the P_5-treated gilts in concert with abnormally high plasma P_4 values (Knight et al., 1977; Kukoly, 1984). It is interesting to note that while an abundance of precursor (P_4) was able to enhance E_1 production within the fetal fluids, plasma E_1 remained low. In fact, while allantoic E_1 continued to increase through d 100 of gestation, plasma E_1 started to increase later than normal and never reached the levels recorded in untreated gilts (Knight et al., 1977; Kukoly, 1984; Jeantet and Knight, 1985).

Conversely, while allantoic E_1 concentrations steadily increased in the later stages of pregnancy, P_4 failed to increase beyond d 70. Previous investigations (Knight et al.,1977; Kukoly, 1984; Jeantet,1985) have reported two general characteristics of P_4 dynamics within the fetal fluid pools: 1) an increase in allantoic fluid concentration that starts about the fifth week of gestation and continues until term and 2) relatively lower concentrations in the amniotic pool that do not change appreciably after d 45 of gestation. The treatment regime of the present study did

not allow for the large increases previously reported. This indicates that the enzymatic capacity of the intrauterine tissues during the last third of gestation are unable to maintain P_4 at normal levels in the fetal fluids, but that ample aromatase is present to convert the progestogens into estrogenic compounds. Nevertheless, while steroid metabolism may have been negatively affected during the last trimester of gestation it should be kept in mind that these changes did not adversely affect prenatal survival or conceptus development.

Conjugated estrogens such as E_1SO_4 show dynamic changes as well. Plasma concentration of E_1SO_4 are elevated at d 30 of gestation and then fall until rising again late in pregnancy (Dwyer and Robertson, 1980; Knight and Hopkins, 1988). This translates in a change in the ratio of conjugated to unconjugated estrogens from about day 30:1 on d 30 to 1:2 on d 90 (Knight and Hopkins, 1988). It is hypothesized that the importance of this ratio is that conjugated steroids serve as a physiological reservoir for their unconjugated counterparts.

The plasma E_1SO_4:E_1 ratio, which was previously shown to decline as gestation progressed (Knight and Hopkins, 1988), remained high throughout the present study. Although the treatment regimen probably had some effect on the magnitude of the ratio (as it did on plasma E_1SO_4 and E_1 concentrations), the difference in ratios between the two investigations were probably mostly a function of different

assay procedures used in measuring E_1SO_4. The RIA procedure used in the present study measured E_1SO_4 directly, whereas in the previous study E_1 was measured following the breakdown of E_1SO_4 with sulfatase enzyme. When this more direct assay procedure was used in an investigation utilizing intact-untreated gilts (Chapter 3) the E_1SO_4:E_1 ratio remained elevated at d 70 of gestation. Knight and Hopkins (1988) reported the E_1SO_4:E_1 ratio to have significantly decreased by d 60 of pregnancy.

Conclusion Despite relatively minor differences in steroid profiles that result from the administration of large quantities of the steroidogenic precursor P_5, these data indicate that the porcine placenta (and or endometrium) has the enzymatic capacity (at least after d 30) to produce an adequate quantity of P_4 to maintain pregnancy and normal conceptus development . Futhermore, these results also indicate the further aromatization of a portion of the P_4 to estrogens. Since several of the events crucial for conceptus development (e.g. electrolyte movement and fluid inbibition) are mediated by estrogens, the maintenance of normal pregnancy suggests the formation of these steroids. Plasma and fetal fluid concentrations of E_1 and E_1SO_4 further substantiate this conclusion.

The failure to maintain pregnancy in gilts following OVX and P_5 treatment at the earlier stages of gestation combined with the positive results observed at the later

stages of pregnancy, indicate that a critical capacity for P_4 production by placental tissue must be reached before a quantity can be produced and elaborated into the systemic circulation that is sufficient to maintain pregnancy. Other in vitro studies in our laboratory provide evidence to support this hypothesis. Under in vitro conditions and with P_5 added to the incubation medium as a precursor, placental P_4 production was increased at d 30, 60 and 90 of gestation for ovarian-intact gilts (Jeantet, 1985).

Additional trials should be conducted to further define the stage of gestation at which pregnancy maintenance may be achieved under the conditions described for this study. Critical to the interpretation of these results would be the hormonal study of gilts which fail to maintain pregnancy under this regimen. Dose response studies also should be conducted to identify the minimal requirement of the intrauterine tissues for adequate steroid synthesis and normal pregnancy maintenance. Furthermore, it should be determined if pregnancy can be maintained beyond the 10 d treatment period and (perhaps more importantly) if the dosage can be withdrawn following extended treatment. Lastly, verification of P_5 as an inactive precursor to P_4 with little biological activity of its own is of paramount importance.

Chapter Six

PORCINE INTRAUTERINE STEROIDOGENESIS: IV. IN VITRO STEROID PRODUCTION BY THE PLACENTA AND ENDOMETRIUM OF OVARIECTOMIZED GILTS ADMINISTERED PREGNENOLONE AS A PRECURSOR FOR INTRAUTERINE STEROIDOGENESIS

Introduction

Recent research indicates that the porcine placenta is steroidogenically active throughout gestation. A part of the evidence is derived from experiments designed to examine steroid production by the placenta (PLAC) and endometrium (ENDO) under in vitro conditions (Chapter 4; Kukoly, 1984; Jeantet and Knight, 1985). Results of these investigations have shown the PLAC (and to a lessor extent ENDO) to have increased steroidogenic capabilities as gestation progresses towards term. Other research has provided evidence for intrauterine steroid production under in vivo conditions. For example, normal gestation has been maintained when ovariectomized (OVX) pregnant gilts were administered medroxyprogesterone acetate (MPA), a biologically active, synthetic progestogen that cannot be aromatized to estrogenic steroids (Chapter 3; Heap et al., 1981). Since estrogens are hypothesized to be required for a variety of important events within the pregnant uterus (Knight et al., 1977; Bazer et al., 1979), maintaining pregnancy with MPA implies that the intrauterine tissues are synthesizing estrogens de novo and without the benefit of ovarian precursors.

Pregnenolone (P_5) is considered to be biologically inactive as a promoter of pregnancy compared to the closely related steroid, progesterone (P_4). However, P_5 is regarded to be extremely important in regard to steroid synthesis because of its role as the chief cellular intermediate in the process of steroidogenesis (Speroff, et al., 1978; Bohinski, 1987). Therefore, P_5 is an essential precursor that gonadal and other cells are able to utilize to synthesize progesterone, androgens and estrogens.

Several studies have demonstrated increased in vitro steroid production with the addition of P_5 to the incubation medium (Chapter 4; Jeantet and Knight, 1985; Beal et al., 1986). In addition, our laboratory has provided evidence for enhanced in vivo steroid production when exogenous P_5 was administered in large doses to pregnant OVX gilts (Chapter 5). Gilts treated in this manner maintained pregnancy (when treated on d 25 or later), had normal conceptus development and had plasma steroid levels similar to those of untreated-intact gilts. It is not known if the intauterine tissues already have the capacity to synthesize the necessary steroids to maintain pregnancy or if there is an induction of steroidogenic enzymes in the absence of luteal precursors. Therefore, the objective of this study is to evaluate in vitro steroid production by the placenta and endometrium of pregnant OVX gilts administered large doses of P_5.

Materials and Methods

Animals Forty one gilts were utilized for a study of in vitro steroid production by the PLAC and/or ENDO from an environment devoid of luteal P_4 but supplied with a large exogenous source of the steroidogenic precursor P_5 (see Chapter 5).

Tissue Incubation Following HYSTX and the collection of conceptus data (Chapter 3), PLAC and ENDO tissue was removed, placed in Medium 199, and incubated as described in Chapter 4, to determine the effects of OVX and exogenous P_5 on in vitro steroid production.. However, the incubation procedure was adjusted to test the hypothesis that steroidogenesis may occur within the culture tubes placed in the ice-water bath. At the end of each incubation period, culture tubes were centrifuged immediately to separate tissue from media. Consequently, the tissue and media remained in the ice-water bath less than 20 min.

Soluble Protein and DNA Determination To correct for potential errors of sampling and weighing, the soluble protein and DNA content of the incubated tissue was determined as described in Chapter 4.

Hormone Assay Procedures Media samples from the incubated tissue were analyzed for P_4, E_1 and E_1SO_4 utilizing radioimmunassay procedures as outlined in Chapter 3.

Statistical Analysis Data were analyzed using the General Linear Models procedure of the Statistical Analysis Systems (SAS, 1985). The hormone values at each day of gestation were preadjusted for differences in protein or DNA content using the formula previously described by Kukoly (1984):

Adjusted hormone (ng/g) = hormone (ng/g) - regression coefficient (protein or DNA - mean protein or DNA).

Due to significant interaction of tissue type with both protein and DNA content hormone concentration was analyzed as a reduced model within tissue subclasses. Logarithmic transformations were used in order to reduce the scaling effect in hormone (P_4, E_1, E_1SO_4) concentration within the incubation medium over the course of gestation. The following statistical model was employed using the logarithmic scale:

$$\ln(Y_{ijklm}) = \mu + D_i + G(D)_{ij} + T_k + P_l + DT_{ik} + DP_{il} + TP_{kl} + DTP_{ikl} + e_{ijklm}$$

μ is the the overall mean natural logarithm of hormone concentration, D_i is the effect of day of gestation, $G(D)_{ij}$

is the effect of gilt within D_i, T_k is the time of incubation (0, .5, 1 or 2 h), P_l is the effect of the presence or absence of P_5 in the incubation medium and e_{ijklm} is the random error. All other components of the model are interactions of the previously described effects. The effect of D_i was tested using mean squares for $G(D)_{ij}$ as its error term. The effects of D_i, T_k, and P_l were considered as a fixed effect, whereas $G(D)_{j(i)}$ was considered a random effect. Due to a large variability in hormone means data was re-analyzed as a reduced model within day of gestation subclasses.

Least-squares means for the data in the original scale of measurement were reported. Tukey's procedure (Steel and Torrie, 1980) was used to detect differences in hormone values due to day of gestation and orthogonal contrasts (0 vs. .5, 1 and 2 h; .5 vs. 1 and 2 h), and were used to detect differences due to the time of incubation.

Results

Tissue Soluble Protein and DNA Content

Mean protein content (Table 1) of PLAC tissue tended ($P<.08$) to be affected by day of gestation while the ENDO and COMB tissue were unaffected ($P>.15$) by the stage of pregnancy. Soluble protein differed ($P<.001$) for all three tissue preparations due to the effect of gilt.

Conversely, DNA content of the PLAC ($P<.01$), and ENDO ($P<.02$) tissue differed due to the stage of gestation. DNA

TABLE 1. SOLUBLE PROTEIN AND DNA CONTENT OF PLACENTA (PLAC), ENDOMETRIUM (ENDO) AND COINCUBATION (COMB) FROM OVARIECTOMIZED GILTS SUBJECT TO PREGNENOLONE TREATMENT AT VARIOUS STAGES OF GESTATION[1]

Item	Tissue	Day of hysterectomy[2] 35	40	45	50	55	65	70	90	100
PROTEIN	PLAC	431 (104)	448 (60)	463 (52)	384 (52)	433 (60)	406 (61)	241 (60)	583 (60)	447 (61)
	COMB	951 (95)	772 (55)	833 (48)	800 (47)	846 (55)	821 (55)	731 (55)	807 (58)	630 (58)
	ENDO	1538 (218)	1220 (127)	1456 (109)	1451 (109)	1480 (126)	1313 (126)	1342 (126)	1154 (126)	1278 (128)
DNA	PLAC[3]	6.1 (6.0)	7.9 (3.5)	6.6 (3.0)	7.9 (3.0)	7.9 (3.5)	6.4 (3.5)	17.3 (3.5)	28.3 (3.5)	12.8 (3.5)
	COMB[3]	10.6 (9.7)	17.7 (5.6)	18.5 (4.9)	20.9 (4.9)	21.9 (5.6)	15.7 (5.6)	47.2 (5.6)	36.0 (5.6)	21.1 (5.6)
	ENDO[3]	19.0 (15.8)	40.1 (9.1)	29.4 (7.9)	37.7 (7.9)	39.1 (9.1)	31.6 (9.1)	83.7 (9.1)	42.3 (9.1)	44.2 (9.2)

[1] All gilts received a daily subcutaneous injection of 1g of P_5 in a suspension of corn oil and benzyl benzoate beginning on the day prior to ovariectomy. Values expressed as least squares means (μg of Protein or DNA per 100mg tissue); standard error of the mean in parentheses.
[2] All gilts were bilaterally ovariectomized (OVX) ten days prior to hysterectomy (HYSTX).
[3] DNA differed due to day of gestation ($P<.02$).

in PLAC tissue was highest in the last one third of pregnancy while ENDO tissue DNA content peaked at d 70 of gestation. DNA content also differed ($P<.001$) among gilts.

Progesterone Production by the Placenta and Endometrium

Mean concentrations of P_4 present in the incubation medium following 0 to 2h incubation of either PLAC, ENDO or COMB are summarized in Tables 2-7.

Adjusting for either soluble protein or DNA content of the incubated tissue made little difference in the outcome of the analysis of the P_4 data. The level of protein or DNA did not alter ($P>.11$) P_4 synthesis for either PLAC or ENDO tissue. Progesterone production by COMB tissue differed ($P<.05$) due to the level of DNA, but not due to the concentration of soluble protein ($P>.50$). Overall, P_4 increased considerably ($P<.001$) as gestation progressed for both PLAC and COMB tissue. Endometrial P_4 production (which was considerably lower than PLAC production) was not affected ($P>.59$) by the stage of pregnancy.

The addition of P_5 to the incubation medium enhanced ($P<.001$) P_4 production for all three tissue preparations when examined across all stages of gestation. Pregnenolone stimulated P_4 production throughout all stages of gestation for ENDO tissue. However, a significant ($P<.005$) P_5 treatment by day of gestation interaction revealed that P_5 had a greater stimulatory effect at the earlier stages of pregnancy (prior to d 50) for preparations containing

TABLE 2. EFFECT OF OVARIECTOMY, PREGNENOLONE TREATMENT AND SUBSEQUENT HYSTERECTOMY AT VARIOUS DAYS OF GESTATION ON IN VITRO PROGESTERONE SYNTHESIS BY THE ENDOMETRIUM[1]

P_5	Time of incubation	Day of hysterectomy[2] 35[3,4]	40[3]	45[3]	50[3]	55[3]	65[3]	70[3]	90[3]	100
ABSENT	0h	.07 (.04)	.04 (.01)	.12 (.03)	.17 (.05)	.12 (.04)	.08 (.02)	.13 (.04)	.23 (.07)	.14 (.05)
	.5h	.17 (.09)	.06 (.02)	.14 (.04)	.14 (.04)	.13 (.04)	.10 (.03)	.12 (.04)	.19 (.06)	.18 (.06)
	1h	.12 (.06)	.12 (.04)	.10 (.03)	.31 (.10)	.07 (.02)	.09 (.03)	.11 (.04)	.18 (.05)	.09 (.03)
	2h	.11 (.07)	.12 (.04)	.18 (.05)	.17 (.05)	.10 (.03)	.13 (.04)	.15 (.04)	.206 (.06)	.05 (.02)
PRESENT	0h	.24 (.21)	.37 (.11)	.62 (.16)	.64 (.19)	.32 (.10)	.54 (.16)	.54 (.17)	.85 (.25)	.59 (.19)
	.5h	.88 (.48)	.31 (.09)	.27 (.07)	.41 (.12)	.27 (.08)	.42 (.13)	.38 (.11)	1.14 (.35)	1.42 (.47)
	1h	.64 (.48)	.28 (.08)	.47 (.12)	.43 (.12)	.28 (.09)	.69 (.21)	.43 (.14)	.77 (.23)	.71 (.22)
	2h	.47 (.25)	.24 (.08)	.32 (.09)	.37 (.10)	.22 (.07)	.35 (.11)	.30 (.09)	.44 (.14)	.81 (.24)

[1] All gilts received a daily subcutaneous injection of 1g of P_5 in a suspension of corn oil and benzyl benzoate beginning on the day prior to ovariectomy. Values are corrected for the soluble protein content of incubated tissue and expressed as least squares means (ng P_4/ 100 mg tissue); standard error of the mean in parentheses.

[2] All gilts were bilaterally ovariectomized (OVX) ten days prior to hysterectomy (HYSTX).

[3] P_4 values differed as a function of P_5 treatment overall and within day of gestation specified ($P<.001$).

[4] P_4 values differed as a function of time of incubation ($P<.05$).

TABLE 3. EFFECT OF OVARIECTOMY, PREGNENOLONE TREATMENT AND SUBSEQUENT HYSTERECTOMY AT VARIOUS DAYS OF GESTATION ON IN VITRO PROGESTERONE SYNTHESIS BY THE COINCUBATION OF ENDOMETRIUM AND PLACENTA[1]

P_5	Time of incubation	Day of hysterectomy[2] 35	40[3,4]	45[3,4]	50[3]	55[4]	65[3,4]	70[3]	90	100
	0h	.28 (.13)	.32[5] (.08)	.94 (.21)	1.51 (.33)	2.10 (.53)	1.29[5] (.31)	6.05 (1.39)	15.47 (1.84)	10.43 (2.58)
A	.5h	.47 (.19)	.61 (.14)	1.03 (.20)	2.09 (.48)	3.70 (.84)	2.10 (.48)	6.37 (1.43)	17.90 (3.62)	15.67 (4.33)
B										
S										
E	1h	.45 (.23)	.85 (.20)	1.13 (.22)	2.39 (.50)	1.39 (.35)	2.29 (.52)	3.85 (.88)	12.12 (3.51)	11.94 (2.68)
N										
T										
	2h	.59 (.28)	.89 (.20)	1.40 (.28)	1.30 (.27)	3.70 (.83)	3.16 (.71)	5.68 (1.34)	15.61 (2.79)	9.48 (2.13)
	0h	.73 (.36)	.74 (.19)	1.28 (.25)	1.98 (.42)	1.84 (.42)	2.13 (.48)	6.88 (1.54)	15.63 (4.10)	13.64 (3.59)
P	.5h	1.05 (.41)	1.56 (.35)	2.00 (.42)	4.31 (.84)	2.11 (.54)	4.08 (.99)	8.17 (1.84)	15.90 (3.76)	18.23 (4.48)
R										
E										
S	1h	1.14 (.44)	2.59 (.58)	2.76 (.54)	5.80 (1.33)	7.43 (1.67)	4.04 (.92)	7.72 (1.78)	19.61 (3.52)	11.49 (2.57)
E										
N										
T	2h	2.40 (1.05)	2.84 (.69)	3.09 (.60)	2.85 (.65)	5.39 (1.27)	6.39 (1.43)	6.36 (1.43)	16.78 (4.57)	10.29 (2.54)

[1] All gilts received a daily subcutaneous injection of 1g of P_5 in a suspension of corn oil and benzyl benzoate beginning on the day prior to ovariectomy. Values are corrected for the soluble protein content of incubated tissue and expressed as least squares means (ng P_4/ 100 mg tissue); standard error of the mean in parentheses.

[2] All gilts were bilaterally ovariectomized (OVX) ten days prior to hysterectomy (HYSTX).

[3] P_4 values differed as a function of day of gestation ($P<.001$)

[4] P_4 values differed as a function of P_5 treatment overall ($P<.001$) and within day of gestation specified ($P<.05$).

[5] P_4 values differed as a function of time of incubation overall ($P<.001$) and within day of gestation specified ($P<.05$).

P_4 values of 0h incubation time significantly ($P<.001$) less than .5, 1 or 2h.

TABLE 4. EFFECT OF OVARIECTOMY, PREGNENOLONE TREATMENT AND SUBSEQUENT HYSTERECTOMY AT VARIOUS DAYS OF GESTATION ON IN VITRO PROGESTERONE SYNTHESIS BY THE PLACENTA[1]

P_5	Time of incubation	Day of hysterectomy[2] 35	40[3,4]	45[3,4]	50[4]	55[4]	65[3,4]	70	90	100
ABSENT	0h	.34 (.15)	.36[5] (.10)	1.37 (.32)	2.65[5] (.65)	4.16[5] (1.08)	1.91[5] (.48)	11.48 (2.87)	23.10 (5.75)	19.44 (6.30)
	.5h	.51 (.22)	.95 (.24)	1.53 (.34)	4.87 (1.25)	6.42 (1.61)	3.92 (.99)	13.22 (3.42)	6.56 (1.65)	20.75 (5.73)
	1h	.87 (.48)	1.55 (.39)	2.10 (.47)	2.63 (.63)	6.83 (1.72)	6.03 (1.54)	13.70 (3.47)	24.70 (6.52)	21.02 (5.76)
	2h	.59 (.31)	2.02 (.52)	2.47 (.54)	5.60 (1.30)	8.06 (2.02)	4.50 (1.16)	12.36 (3.16)	43.48 (11.83)	14.65 (3.66)
PRESENT	0h	1.01 (.45)	.75 (.21)	1.79 (.39)	3.74 (.88)	2.88 (.72)	2.32 (.65)	16.39 (4.08)	24.73 (6.16)	22.17 (5.61)
	.5h	1.10 (.50)	1.95 (.49)	3.40 (.77)	8.27 (1.95)	6.37 (1.75)	5.61 (1.44)	17.43 (4.86)	23.63 (5.98)	30.92 (8.04)
	1h	2.20 (.95)	5.64 (1.44)	4.16 (.90)	2.76 (.70)	11.34 (3.11)	6.89 (1.72)	15.88 (3.95)	34.85 (8.68)	32.18 (8.37)
	2h	2.30 (1.02)	4.08 (1.04)	4.45 (.97)	11.14 (2.41)	14.82 (3.69)	12.31 (3.08)	19.42 (4.84)	18.66 (4.68)	17.47 (4.47)

[1] All gilts received a daily subcutaneous injection of 1g of P_5 in a suspension of corn oil and benzyl benzoate beginning on the day prior to ovariectomy. Values are corrected for the soluble protein content of incubated tissue and expressed as least squares means (ng P_4/ 100 mg tissue); standard error of the mean in parentheses.

[2] All gilts were bilaterally ovariectomized (OVX) ten days prior to hysterectomy (HYSTX).

[3] P_4 values differed as a function of day of gestation ($P<.001$)

[4] P_4 values differed as a function of P_5 treatment overall and within day of gestation specified ($P<.001$).

[5] P_4 values differed as a function of time of incubation overall ($P<.001$) and within day of gestation specified ($P<.01$).

P_4 values of 0h incubation time significantly ($P<.05$) less than .5, 1 or 2h.

TABLE 5. EFFECT OF OVARIECTOMY, PREGNENOLONE TREATMENT AND SUBSEQUENT HYSTERECTOMY AT VARIOUS DAYS OF GESTATION ON IN VITRO PROGESTERONE SYNTHESIS BY THE ENDOMETRIUM[1]

P_5	Time of incubation	Day of hysterectomy[2] 35[3]	40[3,4]	45[3]	50[3]	55[3]	65[3]	70[3]	90[3]	100[3]
	0h	.09 (.06)	.04 (.01)	.12 (.03)	.18 (.05)	.09 (.03)	.08 (.02)	.14 (.05)	.23 (.07)	.22 (.07)
A	.5h	.19 (.10)	.06 (.02)	.13 (.04)	.14 (.04)	.13 (.04)	.11 (.03)	.12 (.04)	.20 (.07)	.17 (.05)
B										
S										
E	1h	.10 (.05)	.11 (.03)	.10 (.03)	.31 (.08)	.07 (.02)	.10 (.03)	.10 (.03)	.18 (.06)	.09 (.03)
N										
T										
	2h	.09 (.05)	.13 (.04)	.17 (.05)	.17 (.05)	.11 (.03)	.11 (.04)	.14 (.06)	.19 (.06)	.05 (.02)
	0h	.42 (.26)	.35 (.11)	.67 (.19)	.65 (.17)	.33 (.10)	.54 (.16)	.61 (.24)	.85 (.26)	.62 (.20)
P	.5h	.54 (.44)	.31 (.10)	.27 (.07)	.40 (.13)	.28 (.09)	.39 (.12)	.37 (.11)	1.13 (.34)	1.44 (.49)
R										
E										
S	1h	.54 (.30)	.29 (.09)	.46 (.12)	.41 (.11)	.20 (.06)	.79 (.24)	.39 (.12)	.76 (.23)	.69 (.21)
E										
N										
T	2h	.46 (.25)	.24 (.07)	.34 (.09)	.38 (.10)	.20 (.07)	.33 (.10)	.31 (.10)	.44 (.14)	.84 (.27)

[1] All gilts received a daily subcutaneous injection of 1g of P_5 in a suspension of corn oil and benzyl benzoate beginning on the day prior to ovariectomy. Values are corrected for the DNA content of incubated tissue and expressed as least squares means (ng P_4/ 100 mg tissue); standard error of the mean in parentheses.

[2] All gilts were bilaterally ovariectomized (OVX) ten days prior to hysterectomy (HYSTX).

[3] P_4 values differed as a function of P_5 treatment overall and within day of gestation specified ($P<.001$).

[4] P_4 values differed as a function of time of incubation ($P<.05$).

TABLE 6. EFFECT OF OVARIECTOMY, PREGNENOLONE TREATMENT AND SUBSEQUENT HYSTERECTOMY AT VARIOUS DAYS OF GESTATION ON IN VITRO PROGESTERONE SYNTHESIS BY THE COINCUBATION OF ENDOMETRIUM AND PLACENTA[1]

P_5	Time of incubation	Day of hysterectomy[2] 35[3,4]	40[3,4]	45[3]	50[3]	55[4]	65[3,4]	70[3]	90	100
ABSENT	0h	.26[5] (.10)	.30[5] (.07)	1.13 (.22)	1.47 (.30)	1.07[5] (.24)	1.37 (.30)	5.79 (1.27)	17.89 (4.02)	13.13 (3.44)
	.5h	.39 (.15)	.58 (.13)	1.01 (.19)	2.16 (.49)	3.22 (.73)	2.01 (.45)	6.67 (1.48)	21.74 (5.09)	22.24 (5.38)
	1h	.46 (.17)	.88 (.20)	1.09 (.21)	2.40 (.49)	1.94 (.43)	2.19 (.48)	3.88 (.85)	11.42 (2.52)	11.61 (2.57)
	2h	.46 (.19)	.98 (.25)	1.19 (.27)	1.37 (.28)	5.68 (1.32)	3.16 (.72)	5.71 (1.26)	13.49 (3.04)	8.24 (2.22)
PRESENT	0h	.49 (.22)	.69 (.16)	1.41 (.28)	1.69 (.39)	1.33 (.30)	2.07 (.46)	6.22 (1.44)	15.26 (3.34)	15.18 (3.94)
	.5h	1.63 (.75)	1.52 (.34)	1.94 (.40)	4.24 (.81)	2.49 (.60)	4.42 (.98)	8.19 (1.79)	18.82 (4.31)	19.03 (4.19)
	1h	1.27 (.49)	2.64 (.58)	2.70 (.51)	6.32 (1.47)	7.31 (1.61)	3.90 (.86)	8.69 (2.00)	17.92 (4.00)	10.75 (2.46)
	2h	3.88 (1.85)	3.06 (.71)	2.91 (.57)	2.91 (.65)	7.59 (1.94)	6.34 (1.47)	6.18 (1.36)	13.70 (3.19)	9.26 (2.17)

[1] All gilts received a daily subcutaneous injection of 1g of P_5 in a suspension of corn oil and benzyl benzoate beginning on the day prior to ovariectomy. Values are corrected for the DNA content of incubated tissue and expressed as least squares means (ng P_4/ 100 mg tissue); standard error of the mean in parentheses.

[2] All gilts were bilaterally ovariectomized (OVX) ten days prior to hysterectomy (HYSTX).

[3] P_4 values differed as a function of day of gestation ($P<.001$)

[4] P_4 values differed as a function of P_5 treatment overall ($P<.001$) and within day of gestation specified ($P<.05$).

[5] P_4 values differed as a function of time of incubation overall ($P<.001$) and within day of gestation specified ($P<.05$).

[5] P_4 values of 0h incubation time significantly ($P<.05$) less than .5, 1 or 2h.

TABLE 7. EFFECT OF OVARIECTOMY, PREGNENOLONE TREATMENT AND SUBSEQUENT HYSTERECTOMY AT VARIOUS DAYS OF GESTATION ON IN VITRO PROGESTERONE SYNTHESIS BY THE PLACENTA[1]

P_5	Time of incubation	Day of hysterectomy[2] 35[3]	40[3,4]	45[3,4]	50[4]	55[4]	65[3,4]	70	90	100
ABSENT	0h	.31 (.14)	.43[5] (.11)	1.43[5] (.33)	2.63 (.61)	3.30[5] (.91)	1.90[5] (.47)	11.91 (3.06)	24.58 (6.39)	24.31 (6.30)
	.5h	.52 (.22)	.90 (.23)	1.55 (.34)	4.83 (1.27)	5.45 (1.45)	3.98 (1.02)	13.46 (3.35)	7.32 (1.87)	21.05 5.77
	1h	.62 (.29)	1.69 (.42)	2.05 (.45)	2.65 (.62)	7.19 (1.80)	5.99 (1.49)	13.96 (3.48)	27.41 (6.85)	21.03 (5.76)
	2h	.70 (.30)	1.80 (.46)	2.45 (.54)	5.64 (1.33)	10.75 (3.18)	4.40 (1.17)	12.13 (3.02)	36.86 (9.18)	12.48 (3.32)
PRESENT	0h	.88 (.34)	.80 (.22)	1.79 (.39)	3.71 (.89)	2.63 (.67)	2.61 (.65)	15.85 (4.09)	23.15 (5.89)	25.64 (6.56)
	.5h	1.58 (.92)	2.01 (.50)	3.43 (.75)	8.25 (1.92)	5.97 (1.65)	5.78 (1.57)	16.79 (4.65)	25.77 (6.44)	32.82 (8.40)
	1h	2.12 (.92)	4.81 (1.22)	4.06 (.92)	2.73 (.72)	12.26 (3.40)	6.95 (1.74)	16.28 (4.17)	34.71 (8.64)	29.61 (7.37)
	2h	2.48 (1.07)	3.80 (.99)	4.43 (.97)	11.29 (2.70)	18.50 (5.13)	12.03 (3.12)	19.47 (4.85)	16.35 (4.32)	14.48 (4.06)

[1] All gilts received a daily subcutaneous injection of 1g of P_5 in a suspension of corn oil and benzyl benzoate beginning on the day prior to ovariectomy. Values are corrected for the DNA content of incubated tissue and expressed as least squares means (ng P_4/ 100 mg tissue); standard error of the mean in parentheses.

[2] All gilts were bilaterally ovariectomized (OVX) ten days prior to hysterectomy (HYSTX).

[3] P_4 values differed as a function of day of gestation (P<.001)

[4] P_4 values differed as a function of P_5 treatment overall and within day of gestation specified (P<.001).

[5] P_4 values differed as a function of time of incubation overall (P<.001) and within day of gestation specified (P<.05).

P_4 values of 0h incubation time significantly (P<.05) less than .5, 1 or 2h.

placental tissue (PLAC or COMB). Nevertheless, regardless of the day of gestation, in vitro preparations containing P_5-enriched media had P_4 values which were always at least equal to (and usually higher than) preparations incubated in media devoid of added P_5.

Increasing the duration of incubation had a positive effect ($P<.001$) on P_4 production by the PLAC and COMB tissue while ENDO tissue was not affected ($P>.45$). Reanalysis of the data within each stage of gestation revealed that the time of incubation had the greatest effect on PLAC P_4 production between d 40 and d 65 of gestation. Differences in P_4 production due to the duration of incubation were less consistent for the COMB tissue with significant differences seen at d 40 and d 65 of pregnancy ($P<.001$).

Estrone Production by the Placenta and Endometrium

Mean concentrations of E_1 present in the incubation medium following 0 to 2h incubation of either PLAC, ENDO or COMB are summarized in Tables 8-13.

Adjusting for either soluble protein or DNA content made little difference in the outcome of the analysis of the E_1 data. The concentration of DNA in the incubated tissue had no effect on E_1 synthesis ($P>.12$) for any of the three tissue preparations. Soluble protein content influenced ($P<.03$) E_1 production of the ENDO tissue, tended

TABLE 8. EFFECT OF OVARIECTOMY, PREGNENOLONE TREATMENT AND SUBSEQUENT HYSTERECTOMY AT VARIOUS DAYS OF GESTATION ON IN VITRO ESTRONE SYNTHESIS BY THE ENDOMETRIUM[1]

P_5	Time of incubation	Day of hysterectomy[2] 35[3]	40	45[3]	50[3]	55	65	70	90	100
ABSENT	0h	1.1 (.5)	2.8 (.6)	3.5 (.7)	4.2 (.9)	2.1 (.5)	2.5 (.5)	13.1 (2.9)	97.1 (20.9)	1419.7 (390.4)
	.5h	2.3 (.9)	2.4 (.6)	2.7 (.5)	4.2 (.8)	3.0 (.6)	3.7 (.8)	10.2 (2.2)	81.3 (17.6)	999.4 (221.9)
	1h	3.2 (1.2)	4.7 (1.1)	3.4 (.6)	4.5 (1.0)	2.9 (.6)	3.0 (.7)	10.2 (2.3)	149.2 (32.1)	897.1 (195.6)
	2h	2.0 (.9)	2.9 (.6)	3.0 (.6)	5.6 (1.0)	2.7 (.6)	5.3 (1.1)	11.9 (2.6)	99.7 (21.7)	1005.4 (217.2)
PRESENT	0h	3.6 (2.3)	2.7 (.6)	6.6 (1.2)	5.0 (1.1)	2.6 (.6)	2.9 (.6)	11.2 (2.5)	130.2 (28.0)	1184.6 (277.2)
	.5h	3.7 (1.4)	3.5 (.8)	6.8 (1.3)	5.5 (1.1)	3.3 (.7)	3.7 (.8)	11.6 (2.5)	103.0 (22.7)	1256.6 (277.7)
	1h	3.7 (2.0)	2.7 (.6)	5.4 (1.0)	5.8 (1.1)	3.0 (.7)	6.0 (1.3)	11.4 (2.7)	122.9 (26.7)	1148.5 (251.5)
	2h	5.3 (2.0)	4.7 (1.0)	3.9 (.8)	7.0 (1.4)	2.9 (.6)	6.2 (1.4)	13.5 (3.0)	66.7 (15.1)	1392.9 (300.9)

[1] All gilts received a daily subcutaneous injection of 1g of P_5 in a suspension of corn oil and benzyl benzoate beginning on the day prior to ovariectomy. Values are corrected for the soluble protein content of incubated tissue and expressed as least squares means (pg E_1/ 100 mg tissue); standard error of the mean in parentheses.

[2] All gilts were bilaterally ovariectomized (OVX) ten days prior to hysterectomy (HYSTX).

[3] E_1 values differed as a function of day of gestation ($P<.001$)

E_1 values differed as a function of P_5 treatment overall ($P<.001$) and within day of gestation specified ($P<.05$).

TABLE 9. EFFECT OF OVARIECTOMY, PREGNENOLONE TREATMENT AND SUBSEQUENT HYSTERECTOMY AT VARIOUS DAYS OF GESTATION ON IN VITRO ESTRONE SYNTHESIS BY THE COINCUBATION OF ENDOMETRIUM AND PLACENTA[1]

P_5	Time of incubation	Day of hysterectomy[2] 35[3]	40[3,4]	45	50[3,4]	55	65[4]	70	90[4]	100[4]
ABSENT	0h	2.5 (1.2)	1.3[5] (.3)	3.3. (.7)	4.9[5] (1.1)	2.2 (.6)	3.6[5] (.9)	25.6 (5.9)	383.4 (87.4)	7010.5[5] (1745.6)
	.5h	4.5 (1.8)	3.1 (.8)	4.9 (1.0)	6.1 (1.2)	3.7 (.8)	10.2 (2.3)	29.3 (7.3)	552.9 (127.2)	12672.2 (3142.7)
	1h	4.1 (2.1)	3.4 (.8)	5.4 (1.1)	7.7 (1.5)	3.4 (.9)	11.2 (2.8)	33.2 (7.6)	623.4 (144.6)	8597.0 (1942.9)
	2h	3.6 (1.7)	2.9 (.7)	5.0 (1.0)	8.8 (1.7)	3.4 (.8)	19.3 (4.4)	40.3 (9.5)	771.4 (180.5)	12748.5 (2893.9)
PRESENT	0h	7.0 (3.5)	2.1 (.5)	6.8 (1.4)	6.8 (1.3)	2.5 (.6)	4.8 (1.1)	36.4 (8.2)	381.1 (86.5)	5867.4 (1554.9)
	.5h	6.1 (2.4)	4.1 (.9)	5.0 (1.1)	14.2 (2.8)	5.4 (1.2)	15.0 (3.7)	33.9 (7.7)	428.0 (96.7)	9635.1 (2389.5)
	1h	4.9 (1.9)	7.0 (1.6)	6.9 (1.4)	9.6 (1.9)	4.1 (.9)	13.6 (3.1)	40.7 (9.4)	1045.4 (244.6)	8992.7 (2032.3)
	2h	7.9 (3.5)	5.7 (1.4)	5.9 (1.2)	10.4 (2.1)	4.1 (1.0)	21.0 (4.7)	48.1 (10.9)	624.6 (141.2)	19933.8 (4963.5)

[1] All gilts received a daily subcutaneous injection of 1g of P_5 in a suspension of corn oil and benzyl benzoate beginning on the day prior to ovariectomy. Values are corrected for the soluble protein content of incubated tissue and expressed as least squares means (pg E_1/ 100 mg tissue); standard error of the mean in parentheses.

[2] All gilts were bilaterally ovariectomized (OVX) ten days prior to hysterectomy (HYSTX).

[3] E_1 values differed as a function of day of gestation ($P<.001$)

[4] E_1 values differed as a function of P_5 treatment overall ($P<.001$) and within day of gestation specified ($P<.01$).

[5] E_1 values differed as a function of time of incubation overall ($P<.001$) and within day of gestation specified ($P<.05$).

[6] E_1 values of 0h incubation time significantly ($P<.05$) less than .5, 1 or 2h.

TABLE 10. EFFECT OF OVARIECTOMY PREGNENOLONE, TREATMENT AND SUBSEQUENT HYSTERECTOMY AT VARIOUS DAYS OF GESTATION ON IN VITRO ESTRONE SYNTHESIS BY THE PLACENTA[1]

P_5	Time of incubation	Day of hysterectomy[2] 35	40[4]	45[3,4]	50[3,4]	55[4]	65[4]	70	90	100
ABSENT	0h	2.8 (1.1)	1.8 (.4)	3.3[5] (.7)	6.0[5] (1.2)	2.7 (.6)	6.5[5] (1.4)	83.6 (18.4)	874.0 (191.4)	14117.5 (4023.5)
	.5h	3.1 (1.2)	2.5 (.5)	6.0 (1.2)	9.3 (1.8)	3.0 (.7)	15.6 (3.8)	55.9 (12.8)	1008.5 (246.1)	19597.8 (4389.9)
	1h	6.4 (3.1)	5.5 (1.2)	4.4 (.9)	7.9 (1.5)	4.5 (1.0)	17.9 (4.4)	45.0 (10.0)	1241.7 (288.1)	15020.5 (3289.5)
	2h	7.8 (3.6)	3.3 (.8)	7.5 (1.4)	10.3 (2.0)	5.6 (1.2)	18.3 (4.2)	70.2 (19.0)	1036.0 (248.7)	10863.6 (2390.0)
PRESENT	0h	6.2 (2.4)	3.0 (.7)	5.5 (1.0)	6.7 (1.3)	2.7 (.6)	4.9 (1.2)	64.7 (14.2)	1242.9 (273.4)	10149.4 (2263.3)
	.5h	3.9 (1.5)	3.4 (.7)	7.4 (1.5)	12.4 (2.4)	3.0 (.7)	19.4 (4.4)	125.1 (30.9)	1217.0 (270.2)	24080.8 (5514.5)
	1h	7.2 (2.7)	4.0 (9.0)	8.5 (1.6)	11.9 (2.3)	5.0 (1.1)	19.0 (4.6)	63.1 (13.9)	1268.0 (279.0)	14330.9 (3281.8)
	2h	8.0 (3.1)	4.4 (1.0)	7.5 (1.4)	17.2 (3.3)	5.9 (1.3)	28.4 (6.3)	76.3 (21.1)	1394.3 (308.1)	25264.9 (5684.6)

[1] All gilts received a daily subcutaneous injection of 1g of P_5 in a suspension of corn oil and benzyl benzoate beginning on the day prior to ovariectomy. Values are corrected for the soluble protein content of incubated tissue and expressed as least squares means (pg E_1/ 100 mg tissue); standard error of the mean in parentheses.

[2] All gilts were bilaterally ovariectomized (OVX) ten days prior to hysterectomy (HYSTX).

[3] E_1 values differed as a function of day of gestation ($P<.001$)

[4] E_1 values differed as a function of P_5 treatment overall ($P<.001$) and within day of gestation specified ($P<.01$).

[5] E_1 values differed as a function of time of incubation overall ($P<.001$) and within day of gestation specified ($P<.05$).

E_1 values of 0h incubation time significantly ($P<.05$) less than .5, 1 or 2h.

TABLE 11. EFFECT OF OVARIECTOMY, PREGNENOLONE TREATMENT AND SUBSEQUENT HYSTERECTOMY AT VARIOUS DAYS OF GESTATION ON IN VITRO ESTRONE SYNTHESIS BY THE ENDOMETRIUM[1]

P_5	Time of incubation	Day of hysterectomy[2] 35[3,4]	40	45[3]	50[3]	55	65	70	90	100
ABSENT	0h	1.0[5] (.4)	2.9 (.7)	3.3 (.7)	4.2 (.8)	2.0 (.5)	2.7 (.6)	11.7 (3.2)	97.6 (21.2)	1505.9 (353.9)
	.5h	2.0 (.8)	2.4 (.5)	2.6 (.5)	4.2 (.8)	3.1 (.7)	4.1 (.9)	10.4 (2.3)	82.7 (19.4)	1074.0 (233.1)
	1h	3.5 (1.3)	4.4 (1.0)	3.5 (.7)	4.5 (.8)	2.9 (.6)	3.4 (.7)	11.4 (2.6)	149.2 (32.2)	942.2 (203.5)
	2h	2.5 (.9)	2.9 (.6)	2.8 (.6)	5.6 (1.1)	2.8 (.6)	4.3 (1.0)	12.0 (3.4)	98.8 (22.2)	979.6 (211.6)
PRESENT	0h	2.3 (1.0)	2.8 (.6)	6.7 (1.3)	5.0 (.9)	2.8 (.6)	2.9 (.6)	9.9 (2.3)	130.6 (28.3)	1057.0 (237.8)
	.5h	4.5 (2.6)	3.5 (.8)	6.6 (1.2)	5.5 (1.0)	3.4 (.7)	3.3 (.7)	11.9 (2.6)	102.3 (22.1)	1454.1 (340.5)
	1h	4.9 (1.9)	2.7 (.6)	5.5 (1.0)	5.9 (1.1)	3.0 (.7)	6.9 (1.5)	12.9 (2.8)	122.0 (26.5)	1209.8 (262.5)
	2h	5.1 (2.0)	4.6 (1.0)	4.5 (.9)	6.9 (1.3)	2.7 (.63)	5.8 (1.3)	12.7 (3.1)	66.4 (14.8)	1342.3 (304.7)

[1] All gilts received a daily subcutaneous injection of 1g of P_5 in a suspension of corn oil and benzyl benzoate beginning on the day prior to ovariectomy. Values are corrected for the DNA content of incubated tissue and expressed as least squares means (pg E_1/ 100 mg tissue); standard error of the mean in parentheses.

[2] All gilts were bilaterally ovariectomized (OVX) ten days prior to hysterectomy (HYSTX).

[3] E_1 values differed as a function of day of gestation ($P<.001$)

[4] E_1 values differed as a function of P_5 treatment overall ($P<.001$) and within day of gestation specified ($P<.05$).

[5] E_1 differed due to the time of incubation ($P<.001$).

[5] E_1 values of 0h incubation time significantly ($P<.01$) less than .5, 1 or 2h.

TABLE 12. EFFECT OF OVARIECTOMY, PREGNENOLONE TREATMENT AND SUBSEQUENT HYSTERECTOMY AT VARIOUS DAYS OF GESTATION ON IN VITRO ESTRONE SYNTHESIS BY THE COINCUBATION OF ENDOMETRIUM AND PLACENTA[1]

P_5	Time of incubation	Day of hysterectomy[2] 35[3]	40[3,4]	45	50[3,4]	55[4]	65[4]	70	90[4]	100[4]
	0h	1.9 (.8)	1.6[5] (.4)	3.5 (.7)	4.4[5] (.9)	2.0[5] (.5)	3.6[5] (.8)	26.7 (6.0)	339.4 (78.7)	6100.8 (1653.3)
A	.5h	4.5	3.1	5.0	6.2	3.8	11.0	28.5	518.1	11593.2
B		(1.9)	(.8)	(1.0)	(1.2)	(.9)	(2.5)	(7.3)	(124.9)	(2654.8)
S										
E	1h	5.5	3.1	5.3	8.0	3.8	11.1	32.4	611.0	8520.0
N		(2.1)	(.7)	(1.0)	(1.6)	(.9)	(2.8)	(7.3)	(138.7)	(1942.6)
T										
	2h	3.9	2.7	4.6	9.0	3.5	17.4	41.9	891.7	13163.1
		(1.7)	(.7)	(1.1)	(1.8)	(.8)	(4.1)	(9.5)	(206.0)	(3619.8)
	0h	4.5	2.5	7.1	6.3	2.4	5.2	37.3	395.9	7031.6
		(2.1)	(.6)	(1.5)	(1.4)	(.6)	(1.9)	(8.9)	(89.1)	(1849.3)
P	.5h	7.3	4.1	5.0	14.1	5.6	15.1	33.4	394.7	8247.5
R		(3.4)	(.9)	(1.1)	(2.8)	(1.3)	(3.5)	(7.5)	(93.6)	(1864.0)
E										
S	1h	5.1	6.8	6.9	10.2	4.2	14.5	38.3	1029.9	9378.5
E		(2.0)	(1.5)	(1.3)	(2.0)	(1.0)	(3.3)	(9.1)	(235.8)	(2203.9)
N										
T	2h	8.3	4.9	5.8	11.3	4.0	18.7	48.1	707.8	20643.8
		(4.0)	(1.2)	(1.1)	(2.3)	(1.0)	(4.5)	(10.9)	(169.2)	(4954.5)

[1] All gilts received a daily subcutaneous injection of 1g of P_5 in a suspension of corn oil and benzyl benzoate beginning on the day prior to ovariectomy. Values are corrected for the DNA content of incubated tissue and expressed as least squares means (pg E_1/ 100 mg tissue); standard error of the mean in parentheses.

[2] All gilts were bilaterally ovariectomized (OVX) ten days prior to hysterectomy (HYSTX).

[3] E_1 values differed as a function of day of gestation ($P<.001$)

[4] E_1 values differed as a function of P_5 treatment overall ($P<.001$) and within day of gestation specified ($P<.01$).

[5] E_1 values of 0h incubation time significantly ($P<.05$) less than .5, 1 or 2h.

TABLE 13. EFFECT OF OVARIECTOMY PREGNENOLONE, TREATMENT AND SUBSEQUENT HYSTERECTOMY AT VARIOUS DAYS OF GESTATION ON IN VITRO ESTRONE SYNTHESIS BY THE PLACENTA[1]

P_5	Time of incubation	Day of hysterectomy[2] 35	40	45[3,4]	50[3,4]	55[4]	65[4]	70	90	100
	0h	2.6 (1.0)	1.9 (.4)	3.7[5] (.8)	5.3[5] (1.0)	2.2 (.5)	6.6[5] (1.5)	82.0 (18.7)	789.3 (182.3)	16125.7 (3723.9)
ABSENT	.5h	3.0 (1.2)	2.4 (.5)	6.4 (1.2)	8.9 (1.7)	2.6 (.6)	14.6 (3.6)	68.7 (15.2)	943.1 (233.9)	19228.9 (4268.8)
	1h	4.4 (1.8)	5.7 (1.3)	4.1 (.8)	8.6 (1.7)	4.7 (1.0)	17.4 (4.2)	49.6 (11.0)	1311.8 (291.2)	15095.8 (3361.7)
	2h	10.7 (4.1)	3.3 (.7)	7.2 (1.4)	10.7 (2.1)	6.9 (1.8)	20.4 (4.8)	66.5 (18.1)	1009.5 (233.1)	10574.2 (2484.9)
	0h	5.4 (2.1)	3.1 (.7)	5.5 (1.1)	6.7 (1.3)	2.5 (.6)	6.0 (1.3)	67.0 (15.4)	1326.3 (298.4)	10333.9 (2357.3)
PRESENT	.5h	4.6 (2.4)	3.5 (.8)	7.7 (1.5)	12.5 (2.4)	2.7 (.6)	17.3 (4.2)	112.6 (27.7)	1192.5 (264.7)	24591.9 (5606.9)
	1h	7.0 (2.7)	3.5 (.8)	7.8 (1.6)	11.4 (2.3)	5.2 (1.2)	18.3 (4.5)	58.3 (13.2)	1244.1 (275.0)	14287.9 (3157.6)
	2h	9.0 (3.4)	4.5 (1.0)	7.5 (1.4)	18.5 (3.8)	7.0 (1.7)	30.0 (6.9)	82.7 (22.9)	1547.2 (361.4)	24322.8 (6007.7)

[1] All gilts received a daily subcutaneous injection of 1g of P_5 in a suspension of corn oil and benzyl benzoate beginning on the day prior to ovariectomy. Values are corrected for the DNA content of incubated tissue and expressed as least squares means (pg E_1/ 100 mg tissue); standard error of the mean in parentheses.

[2] All gilts were bilaterally ovariectomized (OVX) ten days prior to hysterectomy (HYSTX).

[3] E_1 values differed as a function of day of gestation (P<.001)

[4] E_1 values differed as a function of P_5 treatment overall (P<.001) and within day of gestation specified (P<.01).

[5] E_1 values differed as a function of time of incubation overall (P<.001) and within day of gestation specified (P<.05).

E_1 values of 0h incubation time significantly (P<.05) less than .5, 1 or 2h.

(P<.09) to affect PLAC tissue and had no affect (P>.52) on COMB tissue.

The stage of pregnancy significantly (P<.001) affected E_1 production for all three tissue types. Estrone production remained low through d 65 and then continued to increase through d 100 of gestation. Overall, the addition of exogenous P_5 to the incubation medium enhanced E_1 production for all three tissue types. However, reanalysis of the E_1 data within each day of gestation revealed that PLAC tissue was most responsive to exogenous P_5 at d 45 and d 50 of gestation. ENDO tissue was able to respond to exogenous P_5 at d 35, 45 and 50 of pregnancy while COMB tissue had increased E_1 production with P_5 enriched media at d 35, 40 and 50 of gestation. Nevertheless, within each stage of gestation, tissue incubated in media containing P_5 synthesized E_1 at a rate at least equal to tissue incubated in control medium.

The duration of incubation significantly affected E_1 production when the data was analyzed across all stages of gestation for both PLAC and COMB tissue types. Estrone production by ENDO tissue did not differ (P>.55) due to time of incubation. Reanalysis of the data within each stage of pregnancy revealed that time of incubation altered E_1 production by the PLAC tissue removed between d 50 and d 65 of gestation. E_1 production was enhanced in COMB tissue due to longer incubation times for tissue obtained between d 40 and d 65 (except d 45) of pregnancy. Despite the fact

that ENDO tissue E_1 synthesis did not differ due to time of incubation overall, ENDO tissue from the gilt that maintained pregnancy at d 35 of pregnancy was responsive to increased incubation time.

Estrone Sulfate Production by the Placenta and Endometrium

Mean concentrations of E_1SO_4 in the incubation medium following 0 to 2h incubation of either PLAC, ENDO or COMB tissue are summarized in Tables 14-19.

Adjusting for either soluble protein or DNA content of the incubated tissue made little difference in the outcome of the E_1SO_4 data. The level of protein or DNA did not alter ($P>.60$) E_1SO_4 synthesis for PLAC, ENDO or COMB tissue. The stage of gestation affected ($P<.002$) E_1SO_4 production for all three tissue types. Overall, PLAC E_1SO_4 synthesis declined between d 35 and d 40, remained constant through d 65 and increased to d 100 of gestation. ENDO tissue also increased E_1SO_4 producton in the last trimester of pregnancy (d 70-100), but did not have elevated levels at d 35 of gestation.

Exogenous P_5 had no effect ($P>.16$) on E_1SO_4 synthesis for any of the three tissue types. Reanalysis of the E_1SO_4 data within each stage of gestation failed to show significant P_5 effects within any of the periods of pregnancy examined.

The duration of incubation did affect ($P<.03$) E_1SO_4 production by the PLAC and COMB tissue across the stages of

TABLE 14. EFFECT OF OVARIECTOMY, PREGNENOLONE TREATMENT AND SUBSEQUENT HYSTERECTOMY AT VARIOUS DAYS OF GESTATION ON IN VITRO ESTRONE SULFATE SYNTHESIS BY THE ENDOMETRIUM[1]

P_5	Time of incubation	Day of hysterectomy[2] 35	40	45[3]	50	55[4]	65	70	90	100
ABSENT	0h	.27 (.13)	.17 (.04)	.23 (.05)	.17 (.04)	.22 (.07)	.17 (.04)	.22 (.14)	.68 (.17)	2.07 (.67)
	.5h	.30 (.14)	.18 (.05)	.49 (.11)	.22 (.05)	.16 (.04)	.26 (.07)	.19 (.05)	.57 (.15)	1.53 (.40)
	1h	.44 (.20)	.28 (.08)	.24 (.05)	.22 (.06)	.22 (.06)	.55 (.15)	.67 (.18)	.59 (.15)	1.60 (.41)
	2h	.52 (.29)	.41 (.11)	.35 (.08)	.21 (.05)	.15 (.04)	.31 (.08)	.22 (.06)	.53 (.14)	1.67 (.43)
PRESENT	0h	.17 (.12)	.33 (.09)	.31 (.07)	.27 (.07)	.21 (.05)	.29 (.07)	.25 (.07)	.80 (.20)	2.12 (.59)
	.5h	.38 (.17)	.28 (.07)	.40 (.09)	.19 (.04)	.26 (.07)	.24 (.06)	.44 (.11)	.59 (.15)	2.03 (.53)
	1h	.44 (.28)	.30 (.08)	.28 (.06)	.32 (.07)	.22 (.06)	.28 (.07)	.35 (.10)	.98 (.25)	2.10 (.54)
	2h	.41 (.19)	.34 (.09)	.30 (.07)	.28 (.08)	.33 (.08)	.23 (.06)	.22 (.06)	.29 (.08)	1.76 (.45)

[1] All gilts received a daily subcutaneous injection of 1g of P_5 in a suspension of corn oil and benzyl benzoate beginning on the day prior to ovariectomy. Values are corrected for the soluble protein content of incubated tissue and expressed as least squares means (ng E_1SO_4/ 100 mg tissue); standard error of the mean in parentheses.

[2] All gilts were bilaterally ovariectomized (OVX) ten days prior to hysterectomy (HYSTX).

[3] E_1SO_4 values differed as a function of day of gestation ($P<.001$)

[4] E_1SO_4 values differed as a function of time of incubation ($P<.05$).

E_1SO_4 values differed as a function of P_5 treatment ($P<.05$).

TABLE 15. EFFECT OF OVARIECTOMY, PREGNENOLONE TREATMENT AND SUBSEQUENT HYSTERECTOMY AT VARIOUS DAYS OF GESTATION ON IN VITRO ESTRONE SULFATE SYNTHESIS BY THE COINCUBATION OF ENDOMETRIUM AND PLACENTA[1]

P_5	Time of incubation	Day of hysterectomy[2] 35	40	45	50	55	65	70	90	100
ABSENT	0h	.36 (.18)	.20 (.05)	.18 (.04)	.22 (.05)	.20 (.05)	.37 (.09)	.40 (.10)	1.06 (.28)	4.99 (1.32)
	.5h	.60 (.26)	.24 (.06)	.35 (.07)	.19 (.04)	.21 (.05)	.29 (.07)	.34 (.08)	1.67 (.41)	5.40 (1.43)
	1h	.56 (.30)	.31 (.08)	.20 (.04)	.21 (.04)	.28 (.08)	.39 (.10)	.32 (.08)	1.45 (.36)	6.35 (1.53)
	2h	.87 (.44)	.37 (.09)	.26 (.06)	.19 (.04)	.29 (.07)	.43 (.10)	.41 (.11)	1.27 (.35)	5.97 (1.44)
PRESENT	0h	.26 (.14)	.24 (.07)	.22 (.05)	.23 (.05)	.17 (.04)	.32 (.08)	.20 (.05)	2.10 (.51)	5.43 (1.53)
	.5h	.49 (.21)	.19 (.05)	.19 (.04)	.26 (.05)	.26 (.06)	.46 (.12)	.26 (.06)	1.29 (.31)	5.52 (1.46)
	1h	.42 (.17)	.17 (.04)	.29 (.06)	.24 (.05)	.31 (.08)	.50 (.12)	.68 (.07)	2.50 (.63)	7.53 (1.81)
	2h	.72 (.34)	.23 (.06)	.28 (.06)	.38 (.08)	.13 (.03)	.27 (.07)	.27 (.07)	1.98 (.52)	8.06 (2.14)

[1] All gilts received a daily subcutaneous injection of 1g of P_5 in a suspension of corn oil and benzyl benzoate beginning on the day prior to ovariectomy. Values are corrected for the soluble protein content of incubated tissue and expressed as least squares means (ng E_1SO_4/ 100 mg tissue); standard error of the mean in parentheses.

[2] All gilts were bilaterally ovariectomized (OVX) ten days prior to hysterectomy (HYSTX).

[3] E_1SO_4 values differed as a function of day of gestation ($P<.001$)

[3] E_1SO_4 values differed as a function of time of incubation overall ($P<.05$).

TABLE 16. EFFECT OF OVARIECTOMY, PREGNENOLONE TREATMENT AND SUBSEQUENT HYSTERECTOMY AT VARIOUS DAYS OF GESTATION ON IN VITRO ESTRONE SULFATE SYNTHESIS BY THE PLACENTA[1]

P_5	Time of incubation	Day of hysterectomy[2] 35	40	45	50	55[3]	65	70	90	100
	0h	.59 (.25)	.20 (.05)	.19 (.04)	.13 (.03)	.27 (.07)	.38 (.09)	.51 (.12)	2.14 (.57)	11.97 (3.76)
A	.5h	.33 (.14)	.17 (.04)	.22 (.05)	.13 (.03)	.16 (.04)	.47 (.11)	.27 (.07)	1.79 (.55)	11.55 (2.85)
B										
S										
E	1h	.75 (.40)	.14 (.03)	.20 (.04)	.24 (.05)	.32 (.08)	.24 (.06)	.52 (.13)	2.64 (.75)	12.55 (3.34)
N										
T										
	2h	.33 (.17)	.26 (.07)	.22 (.05)	.19 (.04)	.29 (.07)	.53 (.13)	.62 (.19)	4.33 (1.21)	8.20 (1.98)
	0h	.45 (.20)	.17 (.04)	.22 (.05)	.17 (.04)	.23 (.06)	.22 (.06)	.28 (.07)	2.41 (.64)	11.62 (2.86)
P	.5h	.47 (.21)	.20 (.05)	.19 (.04)	.13 (.03)	.17 (.04)	.34 (.08)	.39 (.11)	4.20 (1.31)	10.39 (2.63)
R										
E										
S	1h	.59 (.25)	.27 (.07)	.22 (.05)	.17 (.04)	.25 (.06)	.48 (.12)	.42 (.10)	3.04 (.74)	11.35 (2.87)
E										
N										
T	2h	.54 (.24)	.22 (.05)	.23 (.05)	.25 (.05)	.34 (.08)	.48 (.12)	.19 (.05)	3.80 (.93)	10.26 (2.54)

[1] All gilts received a daily subcutaneous injection of 1g of P_5 in a suspension of corn oil and benzyl benzoate beginning on the day prior to ovariectomy. Values are corrected for the soluble protein content of incubated tissue and expressed as least squares means (ng E_1SO_4/ 100 mg tissue); standard error of the mean in parentheses.

[2] All gilts were bilaterally ovariectomized (OVX) ten days prior to hysterectomy (HYSTX).

[3] E_1SO_4 values differed as a function of day of gestation ($P<.001$)

E_1SO_4 values differed as a function of time of incubation overall and within d 55 ($P<.05$).

TABLE 17. EFFECT OF OVARIECTOMY, PREGNENOLONE TREATMENT AND SUBSEQUENT HYSTERECTOMY AT VARIOUS DAYS OF GESTATION ON IN VITRO ESTRONE SULFATE SYNTHESIS BY THE ENDOMETRIUM[1]

P_5	Time of incubation	Day of hysterectomy[2] 35	40	45[3]	50	55[4]	65	70	90	100
	0h	.33 (.17)	.16 (.04)	.25 (.06)	.18 (.04)	.23 (.06)	.16 (.04)	.21 (.07)	.66 (.17)	1.95 (.54)
A	.5h	.33 (.14)	.19 (.05)	.49 (.11)	.21 (.05)	.18 (.05)	.26 (.07)	.19 (.05)	.48 (.13)	1.69 (.43)
B										
S										
E	1h	.40 (.18)	.30 (.08)	.23 (.05)	.22 (.05)	.22 (.06)	.58 (.15)	.66 (.18)	.58 (.15)	1.71 (.43)
N										
T										
	2h	.45 (.20)	.40 (.10)	.32 (.08)	.21 (.05)	.15 (.04)	.31 (.08)	.26 (.09)	.61 (.16)	1.62 (.41)
	0h	.24 (.12)	.33 (.09)	.34 (.08)	.28 (.06)	.23 (.06)	.29 (.07)	.23 (.08)	.79 (.20)	1.76 (.46)
P	.5h	.28 (.19)	.28 (.07)	.39 (.09)	.18 (.04)	.27 (.07)	.24 (.06)	.42 (.11)	.56 (.14)	2.27 (.64)
R										
E										
S	1h	.40 (.19)	.30 (.08)	.27 (.06)	.32 (.07)	.20 (.05)	.29 (.07)	.31 (.08)	.96 (.24)	2.27 (.58)
E										
N										
T	2h	.41 (.18)	.33 (.08)	.30 (.07)	.28 (.06)	.28 (.08)	.23 (.06)	.26 (.07)	.34 (.09)	1.78 (.47)

[1] All gilts received a daily subcutaneous injection of 1g of P_5 in a suspension of corn oil and benzyl benzoate beginning on the day prior to ovariectomy. Values are corrected for the DNA content of incubated tissue and expressed as least squares means (ng E_1SO_4/ 100 mg tissue); standard error of the mean in parentheses.

[2] All gilts were bilaterally ovariectomized (OVX) ten days prior to hysterectomy (HYSTX).

[3] E_1SO_4 values differed as a function of day of gestation ($P<.001$)

[4] E_1SO_4 values differed as a function of time of incubation ($P<.05$).

E_1SO_4 values differed as a function of P_5 treatment ($P<.05$).

TABLE 18. EFFECT OF OVARIECTOMY, PREGNENOLONE TREATMENT AND SUBSEQUENT HYSTERECTOMY AT VARIOUS DAYS OF GESTATION ON IN VITRO ESTRONE SULFATE SYNTHESIS BY THE COINCUBATION OF ENDOMETRIUM AND PLACENTA[1]

P_5	Time of incubation	Day of hysterectomy[2] 35	40	45	50	55	65	70	90	100[3]
ABSENT	0h	.40 (.17)	.16 (.04)	.21 (.04)	.21 (.05)	.23 (.06)	.37 (.09)	.46 (.11)	1.03 (.28)	4.25 (1.22)
	.5h	.60 (.26)	.23 (.06)	.34 (.06)	.19 (.04)	.21 (.05)	.29 (.07)	.33 (.08)	1.72 (.45)	5.09 (1.25)
	1h	.50 (.21)	.36 (.09)	.19 (.04)	.22 (.05)	.25 (.06)	.38 (.10)	.29 (.07)	1.40 (.34)	6.52 (1.59)
	2h	.86 (.39)	.41 (.12)	.24 (.06)	.20 (.04)	.28 (.07)	.42 (.11)	.48 (.13)	1.31 (.36)	6.89 (2.04)
PRESENT	0h	.32 (.16)	.19 (.05)	.24 (.05)	.20 (.05)	.18 (.04)	.33 (.08)	.20 (.05)	2.13 (.51)	5.07 (1.41)
	.5h	.44 (.23)	.19 (.05)	.18 (.04)	.25 (.05)	.25 (.06)	.47 (.11)	.24 (.06)	1.31 (.33)	5.18 (1.25)
	1h	.40 (.17)	.18 (.04)	.28 (.06)	.25 (.05)	.30 (.07)	.51 (.12)	.59 (.15)	2.40 (.59)	8.09 (2.05)
	2h	.67 (.36)	.28 (.07)	.27 (.06)	.41 (.09)	.14 (.04)	.26 (.07)	.27 (.07)	1.98 (.56)	8.65 (2.23)

[1] All gilts received a daily subcutaneous injection of 1g of P_5 in a suspension of corn oil and benzyl benzoate beginning on the day prior to ovariectomy. Values are corrected for the DNA content of incubated tissue and expressed as least squares means (ng E_1SO_4/ 100 mg tissue); standard error of the mean in parentheses.

[2] All gilts were bilaterally ovariectomized (OVX) ten days prior to hysterectomy (HYSTX).

[3] E_1SO_4 values differed as a function of day of gestation ($P<.001$)

E_1SO_4 values differed as a function of time of incubation overall ($P<.05$) and within d 100 ($P<.05$).

TABLE 19. EFFECT OF OVARIECTOMY, PREGNENOLONE TREATMENT AND SUBSEQUENT HYSTERECTOMY AT VARIOUS DAYS OF GESTATION ON IN VITRO ESTRONE SULFATE SYNTHESIS BY THE PLACENTA[1]

P_5	Time of incubation	Day of hysterectomy[2] 35	40	45	50	55[3]	65	70	90	100
ABSENT	0h	.60 (.26)	.22 (.06)	.19 (.04)	.13 (.03)	.25 (.07)	.39 (.10)	.46 (.12)	2.29 (.63)	11.34 (2.90)
	.5h	.32 (.13)	.17 (.04)	.21 (.05)	.13 (.03)	.15 (.04)	.40 (.10)	.24 (.06)	1.99 (.63)	11.07 (2.71)
	1h	.65 (.30)	.14 (.03)	.20 (.04)	.24 (.05)	.32 (.08)	.22 (.05)	.48 (.12)	3.11 (.84)	12.38 (3.32)
	2h	.42 (.18)	.24 (.06)	.22 (.05)	.19 (.04)	.31 (.09)	.65 (.17)	.70 (.21)	3.58 (.96)	8.56 (2.21)
PRESENT	0h	.45 (.20)	.18 (.05)	.22 (.05)	.17 (.04)	.22 (.06)	.26 (.06)	.31 (.08)	2.29 (.66)	11.60 (2.92)
	.5h	.41 (.23)	.20 (.05)	.18 (.04)	.13 (.03)	.16 (.04)	.25 (.07)	.45 (.12)	4.77 (1.47)	9.64 (2.43)
	1h	.60 (.25)	.28 (.07)	.22 (.05)	.17 (.04)	.25 (.06)	.45 (.11)	.40 (.10)	3.00 (.73)	10.88 (2.66)
	2h	.59 (.25)	.20 (.05)	.24 (.05)	.24 (.05)	.35 (.09)	.54 (.14)	.19 (.05)	3.27 (.86)	11.18 (3.03)

[1] All gilts received a daily subcutaneous injection of 1g of P_5 in a suspension of corn oil and benzyl benzoate beginning on the day prior to ovariectomy. Values are corrected for the DNA content of incubated tissue and expressed as least squares means (ng E_1SO_4/ 100 mg tissue); standard error of the mean in parentheses.

[2] All gilts were bilaterally ovariectomized (OVX) ten days prior to hysterectomy (HYSTX).

[3] E_1SO_4 values differed as a function of day of gestation ($P<.001$)

E_1SO_4 values differed as a function of time of incubation overall ($P<.05$) and within d 55 ($P<.06$).

gestation. Estrone sulfate synthesis did not differ (P>.16) due to time of incubation for ENDO tissue. However, reanalysis of the data within each day of gestation showed significant time effects for PLAC tissue from d 55 and 65 (DNA adjusted data only) of pregnancy. Coincubation of the two tissues was only affected by time of incubation when attained from gilts HYSTX at d 100.

Discussion

The results of the present study are consistent with previous investigations from our laboratory that have examined in vitro steroid production by intrauterine tissues during pregnancy. Prior studies have shown in vitro P_4 and E_1 synthesis by the PLAC to increase (Kukoly, 1984; Jeantet and Knight, 1985) while synthesis by ENDO increases to a lesser extent (Jeantet and Knight, 1985) during the time period examined in this study. The intermediate levels of steroid production for the coincubation of the two tissues suggests that PLAC production is being diluted by the less-steroidogenically-active ENDO.

A paradox exists between the expanding steroidogenic capability of the PLAC (at least under in vitro conditions) and systemic P_4 levels. While in vitro P_4 production per unit tissue continues to increase to d 100 of pregnancy (Kukoly, 1984), circulating P_4 declines until term

(Robertson and King, 1974; Baldwin and Stabenfeldt, 1975; Knight et al., 1977; Kukoly, 1984; Jeantet and Knight, 1985). It appears that the allantoic fluid pool is the only in vivo indicator of in vitro P_4 production while in vitro E_1 and E_1SO_4 synthesis are reflected by both plasma and allantoic fluid levels (Chapters 3 and 4; Knight et al., 1977; Kukoly, 1984; Jeantet and Knight, 1985).

The finding that P_4 production was enhanced with the addition of P_5 to the incubation medium is also in agreement with previous reports (Chapter 4; Jeantet and Knight, 1985; Beal et al., 1986). The capability of the PLAC to produce P_4 from P_5 is supported by histochemical evidence of the presence of delta^5-3-hydroxysteroid dehydrogenase, the enzyme (along with 3-ketosteroid isomerase) essential for this step in steroidogenesis (Christie, 1968; Dufour and Raeside, 1969). Meanwhile, the general inability of P_5 to promote E_1SO_4 synthesis and the limited ability of P_5 to promote E_1 production suggests a rate-limiting step in the steroidogenic pathway. Previous studies from our laboratory have also found significantly enhanced P_4 synthesis and limited E_1 response with the presence of P_5 in the incubation medium (Chapter 4; Jeantet and Knight, 1985). Aromatase activity changes little as total estrogens increase over the course of gestation (Knight and Spradlin, 1988), while small quantities of androgens are present in the incubation media of incubated

PLAC and/or ENDO (Kukoly, 1984). Therefore, the limiting enzymes are probably 17 Á hydroxylase and/or 17-20 desmolase, enzymes required for the conversion of progestogens into androgens. This information suggests that any androgens produced in our in vitro system were rapidly aromatized to estrogens and that the formation of androgens from progestogens was a limiting step.

The increase in P_4 and E_1 production with the addition of exogenous P_5 to the incubation medium indicates that the tissues in our in vitro system were viable and active. Further evidence for the viability of the tissues in this system is the increases in P_4, E_1 and E_1SO_4 synthesis associated with the time of incubation. Previous studies from our laboratory have failed to find consistent increases in steroid production with increased incubation time in either the presence (Chapter 4; Jeantet and Knight, 1985) or absence (Kukoly, 1984) of P_5.

The results of the previous studies appear to be contradictory. On one hand, P_5 is augmenting steroid production, while the incubation of tissue for .5, 1 or 2h has no advantage over the controls (0h). However, in the previous investigations, culture tubes were removed from the incubation chamber at the appointed time and placed in an ice-water bath. The control tubes, which were prepared first, spent the entire incubation period (approximately 2.5h) in the same ice bath. Therefore, if steroidogenesis

was able to continue (albeit at a decreased rate) at the temperature of the ice-water bath (0°C), the difference in steroid concentation between 0 and 2h incubation times could be small. This would be especially true of tissue (such as ENDO) secreting steroids at a low rate.

In the present investigation we adjusted the incubation procedure to test this hypothesis. At the end of each time period, culture tubes were centrifuged immediately to separate tissue from media. Consequently, the results of this study revealed significant increases due to time of incubation for P_4, E_1 and E_1SO_4. Therefore, it can be concluded that steroidogenesis may be able to occur at the low teperatures of an ice-water bath. However, despite overall increases in steroid concentration with increased incubation time, analysis of the data within each day of gestation showed that the duration of incubation did not affect steroid synthesis at all stages of pregnancy. In fact, during the later stages of pregnancy, when steroid production was at its highest, effects due to the time of incubation were all but absent. Furthermore, concentrations of P_4, E_1 and E_1SO_4 in the 0 h culture tubes increased as gestation progressed, just as in the tubes incubated for .5, 1 or 2 h. This suggests that our in vitro culture system is measuring both preformed steroids that are being released regardless of incubation time and newly synthesized hormone produced in increasing

amounts during longer incubation periods. Unfortunately, during periods of high steroid production (late gestation), control tubes have steroid levels that are so high that we are not able to measure steroid synthesis that is actually taking place in vitro.

The results of the conceptus, litter (presented in Chapter 5) and in vitro data provide additional evidence that the intrauterine tissues are able to produce P_4 and other steroidal hormones in vivo as well as under in vitro conditions. The relatively normal quantities of P_4 found in the plasma suggest that not only are these tissues producing progestogens for subsequent conversion into estrogens but that a portion of the P_4 is exuded into the general circulation. The presence of sufficient plasma P_4 for the maintenance of pregnancy is probably not due to placental compensation in the absence of luteal P_4 since previous studies have failed to show placental compensation in the absence of a systemic substrate (Chapter 4). A comparison of in vitro steroid production by tissue from P_5-treated gilts with that of control gilts (d 70, Chapter 4) supports the conclusion that the intrauterine tissues are synthesizing steroids at similar rates. However, caution must be used in the interpretation of these results since we are unable to directly compare all of the stages of gestation examined in this study.

The effects of P_5 treatment on fetal survival and conceptus development support these findings. The analysis

of conceptus and litter variables (Chapter 5) revealed no detrimental effects due to the treatment regimen in gilts that were able to maintain pregnancy. However, a only 1 of 10 gilts treated between d 20 and d 34 of gestation maintained pregnancy under the conditions of this experiment. In all likelihood, the intrauterine tissues of gilts at this stage of pregnancy were unable to produce steroids in sufficient quantities for pregnancy maintenance. OVX gilts administered MPA at a similar same stage of gestation (Chapter 3) maintained a normal pregnancy and had normal estrogen levels conmpared to intact-controls. Therefore, estrogen metabolism was unchanged in the absence of systemic steroidogenic precursors. In the case of P_5-treated gilts, P_4 was probably being produced at too low a rate to maintain plasma concentrations at an adequate level.

Conclusion This investigation has provided additional evidence for the regulation of steroid synthesis during pregnancy. The data give further credence to the hypothesis that steroid-related events within the uterus can occur independent of similar events taking place on the ovary and that intrauterine P_4 provides a significant portion of the pool within the systemic circulation. Furthermore, these findings clearly indicate that estrogens can be produced at normal levels in the absence of luteal P_4. It cannot be determined from these results whether P_4 of intrauterine

origin is normallly required for the maintenance of pregnancy or if P_4 reaches the systemic blood supply only because estrogen producing enzymes are insufficient to aromatize all of the available precursor. In addition, it cannot be assumed that once P_4 reaches the maternal circulation (whether of fetal or luteal origin) it does not later reenter the uterus for subsequent aromatization.

It should be kept in mind that the duration of P_5 treatment used in this investigation was relatively short and the results reported here might not apply if treatment was extended for a longer period. Critical to the interpretation of these results would be a study of the enzymatic properties of the tissues in question, particularly at the earlier stages of gestation. Dose response studies should also be conducted to quantify the minimal P_5 requirement of these tissues. Lastly, future investigations should attempt to modify the in vitro culture system so that steroid synthesis rather than steroid release can be more accurately elucidated.

Chapter Seven

CONCLUSION

This series of investigations has further elucidated the role of intrauterine steroidogenesis in the maintenance of pregnancy, fetal survival and conceptus development. Furthermore, the results have allowed for a clearer understanding of the relationship of the pregnant uterus and the ovary in the synthesis of steroid hormones.

The relatively normal fetal survival rate, conceptus measurements and plasma estrogen concentrations indicated by the data outlined in Chapter 3 imply that steroid-related events within the pregnant uterus can occur independent of similar events taking place within the ovary. In addition, plasma P_4 data indicate that intrauterine P_4 may provide a significant proportion of the P_4 pool within the systemic circulation. Furthermore, the results of Chapter 4 indicate that in vitro tissue steroid production was unaffected by OVX and MPA treatment, suggesting that the enzymatic activity within the PLAC and ENDO was not altered by an absence of systemic precursor.

The data from Chapter 5 reveals that the porcine placenta (and or endometrium) has the enzymatic capacity (at least after d 30) to produce an adequate quantity of P_4 to maintain pregnancy and support normal conceptus development. However, this happened only when a large quantity of the

steroidogenic precursor P_5 was administered daily. As in Chapter 3, these results also indicate the further aromatization of a portion of the P_4 to estrogens. Since several of the events crucial for conceptus development (e.g. electrolyte movement and fluid inbibition) are mediated by estrogens, the maintenance of normal pregnancy suggests the formation of these steroids. Plasma and fetal fluid concentrations of E_1 and E_1SO_4 further substantiate this conclusion.

The failure to maintain pregnancy in gilts following OVX and P_5 treatment at the earlier stages of gestation combined with the positive results observed at the later stages of pregnancy, indicate that a critical capacity for P_4 production by placental tissue must be reached before a quantity can be produced and elaborated into the systemic circulation that is sufficient to maintain pregnancy. The data presented in Chapter 6 suggest that the presence of sufficient plasma P_4 for the maintenance of pregnancy is probably not due to placental compensation in the absence of luteal P_4.

It should be kept in mind that the duration of treatments used in this series of investigations was relatively short and the results reported here might not apply if the treatments were extended for a longer period. Critical to the interpretation of these results would be a study of the enzymatic properties of the tissues in question, particularly at the earlier stages of gestation.

Dose response studies should also be conducted to quantify the minimal P_5 requirement of these tissues. Lastly, future investigations should attempt to modify the in vitro culture system so that steroid synthesis rather than steroid release can be more accurately elucidated.

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APPENDIX A

CROSSREACTIVITIES OF SOME COMMON STEROIDS WITH ANTIBODIES FROM DESCRIBED ASSAYS

Steroid	P4 Ab[a]	E1 Ab	E1SO4 Ab
Estrone	<.01	100	100
Estrone Sulfate		11.2	46.3
DHEA	<.01	<.1	.22
DHEA Sulfate			.166
17α-hydroxypregnenolone			.004
Estriol	<.01		
Corticosterone	.96		
Androstenedione	.01	<.1	.038
17α-hydroxyprogesterone	3.22	<.1	.002
Dihydroprogesterone	<.01	<.1	.017
Pregnenolone	.09	<.1	.23
Cholesterol	<.01		
Cortisol	.01		
Aldosterone			.003
11-deoxycorticosterone			.003
Cortisone		.01	
5-androstenediol			.009
17β-estradiol	<.01	<.1	2.9
17α-estradiol	<.01		.066
Testosterone	.03		.055
Progesterone	100		.008

[a] Beal et al., 1980

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