ENDOMETRIAL GENE EXPRESSION DURING THE ESTROUS CYCLE AND EARLY PREGNANCY IN SWINE

BY

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CHAPTER I

INTRODUCTION

Recognition of the dam to the presence of the conceptus in the uterus, termed "maternal recognition of pregnancy," occurs through many different mechanisms in mammals (Geisert et al., 1994; Roberts et al., 1996). Establishment of pregnancy involves conceptus-endometrial relationships which inhibit luteolysis. In swine, the critical period for maintenance of pregnancy is between Day 11 to 12 of pregnancy (see Geisert et al., 1990). During this period, porcine conceptuses undergo a dramatic transformation from a spherical (10mm) morphology to a long filamentous thread-like structure by Day 12 of pregnancy (Anderson, 1978; Geisert et al., 1982). At the time of trophoblast elongation, conceptus estrogen release redirects the secretion of endometrial $PGF_{2\alpha}$ into the uterine lumen rather than into the maternal vasculature (see Bazer et al., 1984). Thus, uterine secretion of $PGF_{2\alpha}$ is shifted from an endocrine to exocrine secretion between Day 12 and 15 of gestation (Bazer et al., 1984) which permits maintenance of corpora lutea throughout pregnancy in the pig (Bazer & Thatcher, 1977).

During the first 30 days of pregnancy, average loss of porcine embryos is approximately 20 to 30 % (Pope, 1994). However, few porcine embryos are lost (5 to 10%) before Day 12 of gestation (Polge, 1982; Pusateri et al., 1990; Anderson et al.,

1993) or from Day 18 to Day 30 of pregnancy (Spies et al., 1959; Perry & Rowlands, 1962). Therefore, one critical period of embryonic mortality occurs immediately following the time of maternal recognition of pregnancy between Days 12 through 18 of early gestation in swine.

The endometrial epithelium is the primary-limiting barrier for blood-derived molecules that are released into the uterine lumen (McCrae, 1988). In swine, proteins secreted from the uterine epithelial cells may play an essential role in providing proper components for the conceptus development during early pregnancy. Moreover, release and synthesis of porcine uterine secretory proteins is regulated by morphological changes of the endometrial epithelial cells, induced by the early conceptus.

Conceptus alteration of the maternal endometrial cellular ultrastructure and function during early pregnancy is not completely defined. Conceptus effects on endometrial gene expression would provide information concerning endometrial changes necessary for establishment of pregnancy. The present thesis will describe changes of endometrial gene expression during the estrous cycle and early pregnancy of the pig. The following review of literature covers the previously published information concerning conceptus development, endometrial secretion and gene expression during the estrous cycle and early pregnancy of the pig.

CHAPTER II

REVIEW OF THE LITERATURE

Endocrinology of the Estrous Cycle

The length of the estrous cycle in swine, defined as number of days from estrus to initiation of the next estrus period, is approximately 21 days. The estrous cycle consists of the follicular and luteal phases which are two distinct stages of the cycle. The follicular phase is further divided into proestrus and estrus, while the luteal phase can be subdivided into metestrus and diestrus.

Overall endocrine profiles for the estrous cycle in the pig are presented in Figure 1. Hansel et al. (1973) described the endocrine changes which occur during the follicular phase of the estrous cycle. Production of estradiol by graafian follicles from Day 15 of the estrous cycle results in increased plasma estrogen concentration in the presence of low serum progesterone concentrations. The increased estradiol:progesterone ratio triggers the ovulatory surge of luteinizing hormone (LH) at the onset of estrus (Hansel et al., 1973). Estradiol-17β has a positive feedback on pulsatile gonadotrophin releasing hormone (GnRH) release from hypothalamic neurons which increases LH secretion to surge levels from anterior pituitary (Van de Wiel et al., 1981). Inhibin release from the

developing ovarian follicles feeds back to regulate basal concentrations of FSH from the anterior pituitary gland (see review by Taya et al, 1996).

The increased pulsatile release of GnRH stimulates increase release of LH which peaks about 40 to 42 hour prior to ovulation of the graafian follicles (Liptrap & Raeside, 1966). The surge concentrations of LH attain an apex about 12 hours from the beginning of the LH surge (Van de Wiel et al., 1981) with ovulation of mature graafian follicles initiated approximately 38 to 42 hour after the onset of estrus (Du Mesnil du Buisson et al., 1970). Following ovulation, the concentration of LH remains low, while the concentration of FSH increases a few days after ovulation with the decrease in follicular inhibin release (Van de Wiel, 1981). Ovulation of the graafian follicles is followed by development of functional corpora lutea, resulting in increased concentration of plasma progesterone gradually over the first 10 days of the estrous cycle (Hansel et al., 1973). Plasma progesterone remains at peak levels until it rapidly declines on Day 15 to 16 of the estrous cycle (Hansel et al., 1973).

Luteal regression during the estrous cycle of the pig results from endometrial release of $PGF_{2\alpha}$ after Day 12 of the estrous cycle (Bazer & Thatcher, 1977; Krzymowski et al., 1978; Bazer et al., 1984). Utero-ovarian venous prostaglandin $F_{2\alpha}$ concentration rises and is associated with a decline in plasma progesterone concentration on Day 14 and 15 in the cyclic pig (Moeljono et al., 1977). With regression of the corpora lutea (CL) and development of the next wave of follicles, concentration of estradiol-17β increases between Day 16 and 20 of the estrous cycle, which is associated with declining follicle stimulating hormone (FSH) levels (Van de Wiel et al., 1981).

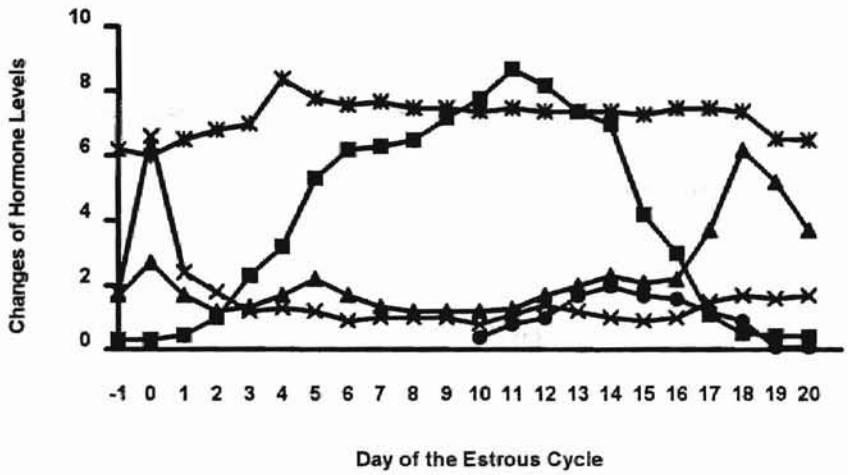


Figure 1. A model depicting relative hormone changes during the estrous cycle: progesterone (■), estrogen (▲), luteinizing hormone (×), follicle stimulating hormone (*) in the peripheral plasma, and prostaglandin $F_{2\alpha}$ (●) in the utero-ovarian vein (adapted from Hansel et al., 1973; Moeljono et al., 1977; Anderson, 1993).

Endocrinology of Early Pregnancy

The corpus luteum is composed of two types of steroidogenic cells: small and large luteal cells (Niswender & Nett, 1988;Wiesak et al., 1992). After ovulation, large luteal cells, which originate from the granulosa cells, contribute the major amount of progesterone synthesis (Niswender & Nett, 1988). Both, large and small luteal cells have 3β-hydroxysteroid dehydrogenase that plays an essential role in the synthesis and secretion of progesterone (Lemon & Mauleon, 1982; Yuan et al., 1993). Small luteal cells, which are of thecal cell origin, increase progesterone synthesis in response to luteinizing hormone (Lemon & Loir, 1977). Large luteal cells are responsible for increasing progesterone synthesis through their interaction with small luteal cells in cell culture (Lemon & Mauleon, 1982).

Pregnancy is terminated following removal of the ovaries during any stage of gestation in the pig as plasma progesterone concentration rapidly declines after removal of the ovaries (Nara et al., 1981). Progesterone is required for maintenance of pregnancy in the pig; therefore, the pig is totally dependent upon the corpora lutea (CL) as the primary source of progesterone during the entire gestation (Nara et al., 1981). The minimal basal concentration of plasma progesterone necessary to maintain pregnancy in the pig is 6.0 ng/ml (Ellicott & Dziuk, 1973). Plasma progesterone concentrations decline on Day 12 to 15 of pregnancy followed by small increase on Day 27 to 28 (Robertson & King, 1974). Plasma progesterone is maintained at approximately 13 to 19 ng/ml throughout the 114 day gestation (Robertson & King, 1974).

Luteinizing hormone (LH) plays an important role as a luteotropic hormone in most species (Niswender & Nett, 1988). Hypophysectomy or treatment with antiovine LH rabbit serum does not disturb subsequent porcine corpus luteum function and development until after Day 12 of gestation (Anderson & Melamphy, 1967; Spies et al., 1967). Therefore, LH is important for maintaining CL function in mated or hysterectomized pigs after Day 12 of gestation (Anderson & Melamphy, 1967). Concentration of basal LH does increase between Days 13 and 18 of gestation (Parlow et al., 1964).

In contrast to the estrous cycle, concentration of $PGF_{2\alpha}$ does not increase in the utero-ovarian vein during early pregnancy (Moeljono et al., 1977). Basal concentration of $PGF_{2\alpha}$ is low, and the frequency and concentration of $PGF_{2\alpha}$ peaks are less in pregnant compared to cyclic gilts (Moeljono et al., 1977). The mechanism for regulating the endometrial movement of $PGF_{2\alpha}$ secretion in the pig will be described later.

Migration of Embryos

Hunter (1974) indicated that fertilized eggs are present in the ampullary-isthmic junction within a few hours of ovulation. Embryos continue to cleave in this region of oviduct for approximately 36 hours (Hunter, 1974). Following increased progesterone concentration in plasma, embryos move rapidly from the oviduct into the tips of the uterine horns approximately 48 hours after ovulation (Dhindsa et al., 1967; Hunter, 1974). The embryos remain near the tip of the uterine horn until Day 6 (Hunter, 1974). Porcine

blastocysts gradually migrate between the uterine horns through the adjoining uterine body between Day 7 and 12 of pregnancy (Dziuk et al., 1964; Dhindsa et al., 1967; Anderson, 1978). The rate of embryo migration is not influenced by the number of porcine embryos (Dziuk, 1964), as a high number of embryos migrate at the same rate as a few embryos (Dziuk, 1985). Treatment of gilts with progesterone greatly accelerates the rate of embryo migration through the oviduct (Day & Polge, 1968), which indicates that progesterone may control embryo migration through the isthmus in the pig (Dziuk, 1985).

The activity of embryo migration throughout the uterus may involve rhythmic myometrial contractions (Perry et al., 1973). However, intrauterine migration of porcine embryos between Days 6 and 12 of pregnancy is not totally passive or random (Perry et al., 1973). Increased conceptus estradiol-17β production is temporally related to the embryo migration and increased myometrial activity in the pig (Pope et al., 1982). Silastic beads impregnated with estradiol-17β stimulate intrauterine migration, while silastic beads containing cholesterol do not affect intrauterine migration or spacing in the pig (Pope et al., 1982a). Cholesterol-impregnated beads that are surgically placed into the uterine lumen on Day 7 of estrous cycle do not migrate as far as estradiol-containing beads when they are placed in opposite uterine horn (Pope et al., 1982). In estradiol-treated pigs, cholesterol-impregnated beads introduced at the base of the uterine horn migrate less than the beads introduced at the tip of the uterine horn (Pope et al., 1986). Therefore, there is an association between estradiol and position within the uterine horn for embryo migration.

Intrauterine migration of embryos from one uterine horn to the opposite uterine horn is complete by Day 12 of pregnancy (Dhindsa et al., 1967), when myometrial

contraction decreases (King et al., 1982). Another reason uterine migration is completed by Day 12 is trophoblast elongation prevents uterine movement (Perry, 1981). Porcine conceptuses elongate by a rapid morphological change from a spherical to a filamentous form between Day 11 and 12 of pregnancy (Anderson, 1978; Geisert et al., 1982b). During elongation, the uterine epithelium and trophoblast come in close contact with each other and conceptuses are positioned in a fold on the mesometrial border of the uterus (Geisert et al., 1982a). In addition, conceptuses do not overlap one another (Perry et al., 1981). Therefore, the filamentous blastocysts do not have enough space to migrate any further after Day 12 of pregnancy.

Conceptus secretion of prostaglandin may play an essential role in stimulating local myometrial contraction during the migration process (Perry et al., 1973). However, conceptuses from Day 12 gilts can overcome the inhibitory effects of indomethacin, a prostaglandin synthetase inhibitor on uterine migration, a result which indicates that estradiol-17β can have a direct effect on myometrial function independent of uterine prostaglandin release (Pope et al., 1982). Alternatively, estradiol-17β has an indirect effect on myometrial function through histamine secretion (Pope et al., 1982). Endometrial histamine release increases uterine blood flow (Harvey & Owen, 1979), enhances myometrial activity (Wislocki & Guttamacher, 1924; Pope et al., 1982), and initiates embryo intrauterine migration (Pope et al., 1982).

The entire uterus is occupied by conceptuses on Day 12 of pregnancy (Dhindsa et al., 1967). Porcine conceptuses space equidistantly from each other and rarely overlap the adjacent conceptus in the entire uterus on Day 12 of pregnancy (Perry et al., 1981). To maintain the CL, porcine embryos must occupy as much uterine surface area as possible as

at least four embryos, two in each horn, is necessary to maintain porcine CL before Day 18 of pregnancy (Polge et al., 1966; Dhindsa & Dziuk, 1968a). Embryo distribution within the majority of the uterus is a critical for establishment of pregnancy and is essential for the efficient utilization of uterine space for embryo survival (see review by Bazer et al., 1982).

Establishment and Maintenance of Pregnancy

Carbohydrates, lipids, proteins, water, electrolytes, and minerals are secreted from the uterine endometrium to enable such cell functions as basic metabolism, uterine proliferation, and establishment and maintenance of conceptus development in the pig. Uterine function is maintained through continued secretion of progesterone from the corpora lutea.

The main function of the corpora lutea is to synthesize and secrete progesterone, which is essential for pregnancy throughout gestation in the pig (Ellicott et al., 1973). Basal secretion of luteinizing hormone is important for maintaining CL function in mated pigs after Day 12 of pregnancy (Anderson & Melamphy, 1967). However, there is an interaction of LH with prostaglandins on porcine luteal cell production of progesterone (Wiesak et al., 1992). Other hormones, such as prolactin, may also regulate luteal function and development in pregnant pigs (Young et al., 1990).

Hysterectomy of the gilt before Day 12 of the estrous cycle extends the functional lifetime of corpora lutea (Spies et al., 1959; Flowers et al., 1987). Thus, the porcine

uterus secretes a luteolytic substance that plays an essential role in regulation of CL function. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has been demonstrated to be the luteolytic factor in the pig, the source of $PGF_{2\alpha}$ is the uterine endometrium (Bazer, 1992). In addition, a low quantity of $PGF_{2\alpha}$ is also secreted from the ovary in the pig (Jones & Gadsby, 1993; Olofesson & Leung, 1994). The concentration of $PGF_{2\alpha}$ in the utero-ovarian vein increases prior to and during luteolysis in the pig (Moeljono et al., 1977). Timing of $PGF_{2\alpha}$ release into the maternal vasculature is timed with the first sensitivity of the CL to luteolysis. The porcine CL is insensitive to treatment with $PGF_{2\alpha}$ for the first 12 days of the estrous cycle in vivo (Diehl & Day, 1974). The reason for the insensitivity of porcine CL to $PGF_{2\alpha}$ before Day 12 of the estrous cycle is thought to occur through maintenance of LH binding to its receptors until Day 10 (see Bazer et al., 1982). However, the pig is susceptible to the exogenous $PGF_{2\alpha}$ before Day 12 if gilts received multiple injections of $PGF_{2\alpha}$ from Day 5 through 10 of the estrous cycle (Estill et al., 1993). Shortening the length of the estrous cycle occurs through sustained reductions in serum progesterone by $PGF_{2\alpha}$-induced luteolysis (Estill et al., 1993). High-affinity binding sites to $PGF_{2\alpha}$ are located on large luteal cells, with low affinity sites located on small luteal cells (Gadsby et al., 1990). Therefore, repeated administration of $PGF_{2\alpha}$ before Day 12 of the estrous cycle results in an increase of $PGF_{2\alpha}$-sensitive large luteal cells in the pig (Estill et al., 1993). However, porcine CL is sensitive to multiple administration of $PGF_{2\alpha}$ before the first 12 days of the estrous cycle does not mediate through $PGF_{2\alpha}$-induced receptor up-regulation (Estill et al., 1995). The exact mechanism(s) that involved in the development

of porcine CL sensitivity to multiple administration with $PGF_{2\alpha}$ before Day 12 remains to be investigated.

Decreased uterine endometrial $PGF_{2\alpha}$ secretion into the uterine-ovarian vein during early pregnancy is not mainly due to increase metabolism of $PGF_{2\alpha}$ by luteal cells or uterine endometrium (Maule Walker et al., 1977), because luteal and endometrial cells only perform a slightly higher metabolism for $PGF_{2\alpha}$ and PGE_2 during early pregnancy compared to the estrous cycle in the pig (Maule Walker et al., 1977). Therefore, porcine conceptuses must induce changes to either inhibit $PGF_{2\alpha}$ synthesis or movement in the uterine endometrium for maternal recognition of pregnancy.

The plasma concentration of estradiol-17β in the utero-ovarian vein is greater in pregnant compared to cyclic gilts between Days 12 and 17 (Moeljono et al., 1977). Porcine conceptuses synthesize estrogen by Day 12 of pregnancy (Perry et al., 1976; Fischer et al., 1985), and the concentrations of estrogens in porcine uterine luminal flushings increase during rapid conceptus elongation on Day 11 and 12 of pregnancy (Geisert et al., 1982al; Ford et al., 1991). The importance of estrogen in maintaining the CL in the pig was demonstrated through daily treatment of gilts with exogenous estradiol valerate (5 mg/day) from Day 11 to 15 of the estrous cycle (Frank et al., 1977). Estrogen administration decreased the concentration of $PGF_{2\alpha}$ in the utero-ovarian vein between Days 12 and 20 and maintained luteal function for greater than 100 days in gilts (Frank et al., 1977). Prolonged luteal function through Day 60 can be achieved if gilts are administered estradiol benzoate on Day 11 and from Day 14 to 18 of the estrous cycle (Geisert et al., 1987). These data indicate that at least two periods of estrogen stimulation, Days 11 and 14 to 18, are necessary for prolonged CL maintenance (Geisert

et al., 1987). Estrogen synthesis and secretion by porcine uterine endometrium itself is low during early pregnancy (Fischer et al., 1985), therefore conceptus estrogen synthesis and secretion acts as the major signal to protect the CL in the pig (Bazer et al., 1977).

Porcine conceptuses transform from a spherical to a long filamentous thread-like structure by Day 12 of pregnancy (Anderson, 1978; Geisert et al., 1982). It is during trophoblast elongation that conceptus estrogen release induces the secretion of endometrial $PGF_{2\alpha}$ into the uterine lumen rather than into the maternal vasculature (Bazer et al., 1984). Thus, uterine secretion of $PGF_{2\alpha}$ is shifted from an endocrine (into maternal vasculature) to exocrine (into uterine lumen) secretion on Day 12 and 15 of gestation (Bazer et al., 1984), which permits maintenance of CL function throughout pregnancy in the pig (Bazer et al., 1977). Therefore, estrogens produced by porcine conceptus are luteostatic rather than luteotropic in early pregnancy as there is a change in direction of endometrial $PGF_{2\alpha}$ movement in the uterus (Bazer et al., 1984).

Estrogens may also directly affect luteal cells or stimulate luteotropic substances to stimulate CL function and development in the pig (Christenson et al., 1994). Although porcine conceptus elongation occurs normally, treatment of pregnant gilts with indomethacin interferes with pregnancy between Days 10 and 25 (Kraeling et al., 1985; Geisert et al., 1986). These results indicate that prostaglandin synthesis is essential for conceptus survival in the pig. During initial placental attachment, uterine luminal concentration of prostaglandin E_2 (PGE_2) is greater than that of $PGF_{2\alpha}$ (Geisert et al., 1982a). Treatment of cyclic gilts with estrogen on Day 11 to 13 increases the ratio of PGE_2 to $PGF_{2\alpha}$ in the uterine lumen (Geisert et al., 1982), and intrauterine infusion of PGE_2 on Day 7 maintains progesterone secretion and CL function in cyclic gilts

(Akinlosotu et al., 1986, 1988). PGE_2 has been shown to be a luteotropic substance as it protects the porcine CL from intra-luteal administration $PGF_{2\alpha}$ (Ford & Christenson, 1991). Conceptus PGE_2 production markedly increases between Day 11 to 12 of gestation (Davis et al., 1983; Geisert et al., 1990), suggesting it plays an important antiluteolytic role during the period of maternal recognition of pregnancy in the pig (Akinlosotu et al., 1986; Geisert et al., 1987). Pulsatile secretion of $PGF_{2\alpha}$ and PGE_2 also increases from Day 13 to 16 in utero-ovarian vein in cyclic gilts (Christenson et al., 1994); however, pulsatile $PGF_{2\alpha}$ secretion does not occur in pregnant gilts (Moeljono et al., 1977). In contrast, prostaglandin secretion peaks earlier, and PGE_2 is the predominant uterine prostaglandin that is secreted into utero-ovarian vein on Day 11 to 12 of pregnancy (Christenson et al., 1994). Secretion of PGE_2 and PGI_2 into the maternal vasculature may regulate early CL development and function in the pig (Jones & Gadsby, 1993). Additionally, Kennedy et al. (1986) indicated that PGE_2 receptors are present in porcine uterine endometrium, suggesting a direct effect on uterine function as well.

The trophectoderm secretes and expresses interferons, IFN-γ (Type II) and a novel Type I interferon, which has antiviral activity during the peri-attachment in the pig (La Bonnardiere et al., 1991). Interferon antiviral activity increases in the uterine lumen between Day 11 and 17 of gestation in the pig (Cross & Roberts, 1989). However, interferon antiviral activity is absent in uterine flushings before Day 11 in pregnant gilts and from Day 12 to 17 in cyclic gilts (Cross & Roberts, 1989). In uterine flushings, interferon antiviral activity is not detected in cyclic gilts (Short et al., 1992). However, interferon antiviral activity attains a maximum level on Day 16 of gestation in porcine uterine flushings (Short et al., 1992). Conceptus protein synthesis increases in co-cultures

of endometrium and conceptus (Ashworth et al., 1996). However, porcine conceptus antiviral activity secretion decreases in the co-culture system, suggesting metabolism of the interferon by endometrium (Ashworth et al., 1996). Porcine conceptus-derived interferons do not function as antiluteolytic hormones as occurs in ruminant species (Harney & Bazer, 1989). In addition, conceptus-derived interferons have a paracrine function in the pig (D'Andrea et al., 1994).

Oxytocin stimulates uterine $PGF_{2\alpha}$ secretion in the sow (Cross et al., 1988; Kieborz et al., 1991; Printz et al., 1994; Whiteaker et al., 1994, 1995). Thus, oxytocin may regulate endometrial $PGF_{2\alpha}$ secretion during luteolysis in the pig as treatment of sows with oxytocin in late diestrous increased uterine $PGF_{2\alpha}$ release (Kiebora et al., 1991). Whiteaker et al. (1994) demonstrated that OTR is present in the endometrium of gilts on Day 15 of the estrous cycle. Endometrial oxytocin binding affinity and OTR concentrations are high during luteolysis in cyclic gilts in contrast to the low OTR concentrations observed during early pregnancy of the pig (Okano et al., 1996). Data suggest that porcine conceptuses may suppress endometrial OTR expression during pregnancy (Okano et al., 1996). Interestingly, high quantities of oxytocin are produced by porcine endometrium at the time of maternal recognition of pregnancy in the pig (Trout et al., 1995). However, the action of oxytocin during early pregnancy in the pig is not mainly mediated through endometrial OTR population density (Ludwig et al., 1997).

Embryonic Mortality

In the pig, fertilization rate is usually high as many studies have indicated that 95 to 100% of the oocytes ovulated are fertilized (see Pope & First, 1985). Throughout a great number of studies, the average number of viable embryos present at the beginning of pregnancy in the pig is approximately 14 to 16 (see Pope & First, 1985). However, only 9 to 10 of these embryos usually survive to the time of parturition (Pope & First, 1985).

Embryonic mortality simply refers to the death of embryos throughout the first 30 days of gestation. Moreover, embryonic mortality covers the death of fertilized ova and embryos by the end of placentation on Day 30 of gestation. Average loss of embryos during the first 30 days of pregnancy is approximately 20 to 30 % (see Pope 1994). However, few embryos (5 to 10%) are lost before Day 12 of gestation (Polge, 1982; Pusateri et al., 1990; Anderson et al., 1993) or from Day 18 to 30 of pregnancy (Spies et al., 1959; Perry & Rowlands, 1962). Therefore, the critical period of embryonic mortality occurs between Day 12 and 18 of gestation which is the time of maternal recognition of pregnancy and placental attachment in swine.

Length of the uterine horns may play an important role in the number of embryos which can develop and survive. The average length of porcine uterine horns significantly increases from Day 3 to 18 of pregnancy (Perry & Rowlands, 1962). When the amount of uterine space per CL is between 5 to 25 cm, prenatal survival is significantly correlated with uterine space (Chen & Dziuk, 1993). However, when space per CL is above 25 cm, prenantal survival not correlated with uterine space (Chen & Dziuk, 1993). Therefore, it appears that the conceptus needs approximately 25 cm of space within the uterine horn to

survive. Length of the uterus maybe important as porcine embryos compete for limited space and nutritional resources (Pope, 1994) as conflict may arise when uterine space becomes a limiting factor in the pig (Roberts et al., 1993).

Stage of conceptus development can greatly effect survival of embryos in a litter. Embryonic mortality is higher in Day 6 embryos transferred into the uterus of a Day 7 pregnant sow compared to the reciprocal transfer (Pope & First, 1985), suggesting embryos that are advanced in development can effect the survival of lesser developed embryos (Pope et al., 1982). It is clear that more advanced conceptuses have a greater chance of survival than their lesser developed littermates (Morgan et al., 1987).

The Chinese Meishan pig is a model for embryonic survival as it is noted for its high prolificacy. Meishan sows have similar ovulation rate compared with Large White females (Bolet et al., 1986; Bidanel et al., 1989), but Bazer et al. (1988) indicated that ovulation rate is lower for Meishan sows than European breeds. By contrast, Meishan sows have higher ovulation rate in comparison with Large Whites when they are compared at similar age (Anderson et al., 1993; Haley & Lee, 1995). Additionally, another study indicated that ovulation rate at puberty is similar in Meishan gilts compared to crossbred gilts (Christenson, 1993). However, following the onset of puberty, ovulation rate is higher in Meishan gilts than in the crossbred gilts (Christenson, 1993). Data would indicate that Meishan females have a higher ovulation rate and are capable of maintaining a higher level of embryonic survival when compared at a similar age.

Meishan embryos grow at a slower rate than Western breeds of swine (Ashworth et al., 1990; Anderson et al., 1993; Youngs et al., 1993). Additionally, uterine placental space between the attached embryos are significantly smaller in Meishan than in Large

White pigs (Lee et al., 1995). Therefore, Meishan embryos grow slowly and have greater organization of embryonic spacing, which is responsible for high litter size of Chinese Meishan pigs (Lee et al., 1995). Transfer of Yorkshire embryos into a Meishan recipient on Day 2, results in decrease conceptus diameter, content of DNA, protein, and estrogen on Day 12 of gestation (Youngs et al., 1994). These results suggest that it is the environment of the uterus in a Meishan sow rather than genotype of donor embryos which affects embryonic characteristics on Day 12 (Youngs et al., 1994). Concentration of serum estrogen and progesterone do not significantly differ between Meishan and Large White gilts; however, the concentration of serum prolactin is higher in cyclic and pregnant Meishan gilts (see Bazer et al., 1992). In addition, the accumulation of uterine secretions in the uterine lumen is greater in pregnant Meishan gilts, which may relate to low embryonic mortality in Meishan gilts (Bazer et al., 1992).

Embryonic survival can also be affected by dietary and steroid treatment. There is a high correlation between embryonic survival and the concentration of plasma progesterone in the gilts on a low plane of feeding, indicating that holding plasma progesterone below a threshold concentration decreases porcine embryonic survival (Jindal et al., 1996). The timing of change in feed after mating is critical on embryonic survival in the pig (Jindal et al., 1996). However, treatment of pigs with progesterone does not improve conceptus development (Schultz et al, 1966; Wildt et al., 1976). Administration with estradiol-17β does not cause embryonic death between Day 11 and 12 of gestation in the pig (Pope et al., 1987). However, exogenous estradiol-17β results in the demise of the porcine embryos when administered on Day 9 and 10 of gestation (Blair et al., 1991). Administration of exogenous estradiol-17β before the critical time for

maternal recognition of pregnancy has been proposed to remove the uterine epithelial surface glycocalyx which results in the failure of embryos to attach to the uterus (Blair et al., 1991).

Porcine embryonic mortality is based on an inherent variation of conceptuses in survival ability at the critical time for maternal recognition of pregnancy. Information relating to the mechanism by which the Meishan breed has greater survival compare to commercial breeds of swine would have a tremendous impact on swine production. Studies are now suggesting that the increased survival is related to slow growth of Meishan embryos and slow released conceptus-derived estrogen production (Ashworth et al., 1990; Anderson et al., 1993; Youngs et al., 1993). The reason for the difference in embryonic survival could be related to the rate of conceptus development and non-competition for uterine space available to each embryo.

Morphology of Porcine Endometrium during the Estrous Cycle

The uterine wall of the pig is composed of serosa, myometrium, and emdometrium (Corner, 1921). There are three different cell types in endometrial tissue: glandular epithelium, luminal epithelium, and connective tissue stroma (Corner, 1921; Banks, 1986; Stroband et al., 1986). The columnar uterine epithelium lining the lumen of the uterus has a thickness of 25 to 30 μm just after ovulation, reaching 35 to 50 μm during the first week of the estrous cycle in the pig (Corner, 1921). Just after ovulation, numerous mitotic divisions occur in the luminal epithelium, but are not observed in deep glandular

epithelium (Corner, 1921). When the ova pass into the uterus on Day 4 to 5 of gestation, mitotic activity in uterine glandular epithelium is greatly diminished (Corner, 1921). The luminal epithelial cells attain maximum height between Day 4 and 8 of the estrous cycle (see review Sider et al., 1986). Electron-dense secretory granules that are located at the apical border of luminal epithelium fuse with the apical membrane, resulting in merocrine secretion (see Stroband et al., 1986). Furthermore, these electron-dense secretory granules release their contents and disappear between Day 8 and 10 in the cyclic pig (Stroband & Van der Lende, 1990). Luminal epithelium increases synthetic activity during the estrous cycle in the pig (Geisert et al., 1982a; Stroband et al., 1986; Keys & King, 1989). In contrast to luminal epithelium, glandular epithelial cells attain maximum height between Day 9 and 12 of the estrous cycle in the pig (Sider et al., 1986). The height of luminal epithelium ranges between 17 and 25 μm on Day 10 in cyclic gilts and then increases from Day 16 to 19 following regression of CL (Keys & King, 1989). Therefore, increasing luminal epithelial cell height corresponds to an increase in synthetic and secretory function between Day 16 and 19 of the estrous cycle (Keys & King, 1989). Furthermore, uterine luminal epithelial cells decrease their activity after Day 19 in cyclic pigs (Keys & King, 1989). On Day 21 of the estrous cycle, a few mitotic divisions and numerous cell death of porcine epithelium are observed (Corner, 1921).

Morphology of Porcine Endometrium during Early Pregnancy

Comparing the endometrial morphology of pregnant and non-pregnant pigs, several differences are apparent (Geisert et al., 1982). In pregnant pigs, the numbers of electron-dense secretory granules increase close to the basement membrane in porcine luminal epithelium on Day 11 (Stroband & Van del Lende, 1990). In contrast, cyclic pigs do not exhibit a significant change to the number and positioning of secretory granules (Stroband et al., 1986). Moreover, the apical surface of uterine epithelium flattens in the pregnant gilt on Day 11 and 12 of gestation, but not during the estrous cycle (Geisert et al., 1982; King et al., 1982). Estrogen that derived from porcine conceptus leads to release the contents of the secretory vesicles in porcine glandular epithelium on approximately Day 11 of gestation (Geisert et al., 1982a). The uterine epithelial cells and the trophoblast become in close contact with one another when conceptus elongation is completed.

Polysaccharides that stained by fluorescent lectins change on the porcine blastocyst surface just before attachment (Whyte & Robson, 1984), suggesting that changes in the carbohydrate composition of the blastocyst surface may facilitate the attachment process. In addition, the trophoblast attaches to the glycocalyx covering uterine epithelial cells, suggesting that reduction in glycocalyx on the surface coat is involved with initial embryonic attachment in pregnant pigs (Dantzer, 1985). Furthermore, the porcine uterine glycocalyx is thicker, denser, and more fibrous between Days 13 and 19 in pregnant gilts compared to Day 13 in the cyclic gilt or Day 10 in pregnant gilts (Keys & King, 1992). It

is clear that the uterine glycocalyx in the extracellular coat facilitates embryonic attachment in the pig (Dantzer, 1985; Blair et al., 1991).

Initial attachment of the conceptus to the endometrium, occurs on Day 13 of pregnancy in the pig (Keys et al., 1988). During attachment, protuberances are developed on the apical surface of the porcine uterine epithelium (King et al., 1982; Dantzer, 1985). The phenomenon involves of interlocking of the conceptus trophectoderm and uterine luminal surface epithelial microvilli (Dantzer, 1985; Keys & King, 1990). The porcine conceptus is positioned and held in the uterine horn through the endometrial protrusions and trophoblastic caps on Day 13 of pregnancy (Dantzer et al., 1985). Conceptus-derived estrogen plays an essential role for the attachment process in the pig (Geisert et al., 1982). Additionally, conceptus-derived estrogen increases the permeability of uterine subepithelial capillaries in the porcine conceptus on Day 13 (Keys & King, 1990). Attachment is completed by intermingling of porcine uterine and trophoblastic microvilli after Day 18 of gestation (Dantzer, 1985). The attachment between uterine endometrium and blastocyst remains superficial throughout pregnancy (Dantzer, 1985).

Endometrial Secretion

The fetal and maternal circulatory systems are completely separated by fetal chorionic tissue and uterine epithelium in the pig. Porcine conceptuses rely upon an adequate supply of nutrient and gas exchange from the uterine epithelium to the chorioallantois throughout the gestation period (Roberts & Bazer, 1980). As a result,

endometrial products are released into the uterine lumen of the uterus, which provides an adequate environment for conceptus development. The required nutrients are transported by diffusion or by facilitated active transport into conceptuses (see Vallet et al., 1994).

There is a marked quantitative and qualitative change in the uterine lumen protein secretion during the luteal phase of the estrous cycle (Murray et al., 1971). Uterine protein secretion remains stable from Day 2 to 9 of the estrous cycle (Murray et al., 1972). However, protein concentration within the uterine lumen increases on Day 12, reaching a maximum on Day 15 of the estrous cycle (Murray et al., 1972). On the subsequent days of the estrous cycle, uterine luminal protein concentration decreases until the next estrus (Murray et al., 1972). Synthesis of the major uterine secretory proteins is regulated by progesterone (Catchpole, 1973; Knight et al., 1973b) while secretion of a number of uterine proteins can be modified by conceptus estrogen production during pregnancy (Geisert et al., 1982a,b). The following will briefly review the vast number of factors secreted by the porcine uterus during early pregnancy.

Uteroferrin (UF), a progesterone-modified glycoprotein, is synthesized by the glandular epithelium of porcine uterine endometrium (Chen et al., 1975; Roberts & Bazer, 1980, 1984). In addition, UF is detected in fetal liver and yolk sac in the pig, most likely as a target and storage site for endometrial UF secretion (Roberts et al., 1986). Porcine uterine UF secretion increases at the time of maternal recognition of pregnancy (Geisert et al., 1982; Zavy et al., 1984). Low concentrations of UF can be detected during early pregnancy with a second peak secretion of UF occurring after Day 30 in pregnant gilts (Basha et al., 1979; Simmen et al., 1988b). High concentrations of UF can be detected during mid-pregnancy and late-pregnancy in the pig (Simmen et al., 1990). In

ovariectomized gilts, UF secretion into the uterine lumen increases under progesterone administration (Basha et al., 1980a, b). Treatment with low doses of exogenous estrogen alone without progesterone does not influence UF synthesis and secretion in the pig (Basha et al., 1980). However, administration of exogenous estrogen and progesterone increase both total endometrial secretory proteins and UF release in the pig (Basha et al., 1980). Uteroferrin gene expression is not effected by prolactin (Fliss et al., 1991), but exogenous progesterone increases while exogenous estrogen decreases UF mRNA concentrations in immature and ovariectomized mature gilts (Fliss et al., 1991). Uteroferrin acts as a major iron carrier, which transports iron from the uterus to the developing fetus (Buhi et al., 1979; Roberts et al., 1986). In addition, Fliss et al. (1989) indicated that UF functions as a hematopoietic growth factor.

Retinol-binding protein (RBP) is another major porcine secretory protein that is produced by endometrial luminal and glandular epithelium during the luteal phase of the estrous cycle and pregnancy (Adams et al., 1981). RBP is present in porcine endometrial tissues on Day 15 of pregnancy (Harney et al, 1994). Additionally, RBP is present in serum, follicular fluid, uterine flushings, allantoic fluid, and endometrial culture medium (Vallet, 1994). RBP is a major porcine conceptus secretory protein, which is detected on Day 10 of gestation (Harney et al., 1990, 1994). There is a marked increase of RBP in the uterine lumen when conceptus elongation occurs in the pig (Trout et al., 1992). RBP within the uterine flushing is enhanced 7 to 8 fold when filamentous conceptuses are present (Trout et al., 1992). Furthermore, RBP mRNA dramatically increases in the filamentous blastocyst of the pig (Yelich et al., 1997).

Porcine endometrial RBP gene expression is low on Days 0, 5, and 10 of the estrous cycle and on Day 10 of pregnancy (Harney et al., 1993). However, endometrial RBP mRNA is enhanced from Day 10 to 12 in cyclic and pregnant pigs (Harney et al., 1993). On Day 18, endometrial RBP mRNA concentrations decrease in cyclic pigs but remain elevated in pregnant pigs (Harney et al., 1993). Retinol, the alcohol form of vitamin A, is an important nutrient for the porcine fetus (Thompson et al., 1964). RBP acts as a retinol carrier, which transports retinol from the uterine endometrium to conceptus in early pregnant pigs (Adams et al., 1981; Harney et al., 1990, 1994). Uncontrolled lipid peroxidation results in cell damage or death (see Vallet, 1995). However, UF induces lipid peroxidation in porcine endometrial tissues from Day 13 to 30 (Vallet, 1995). The reaction of lipid peroxidation can be prevented by retinol-binding protein in vitro (Vallet, 1995). Therefore, RBP may also play a role in protecting uterine and conceptus tissues from the activity of lipid peroxidation (Vallet, 1995).

Conversion of retinol to retinoic acid allows interactions with retinoic acid receptors (RARs and RXRs), which are the members of steroid/thyroid receptor family, and cellular binding proteins (CRBP I and II) which bind to retinoic acid (De Luca, 1991). Gene expression for RAR-α and RAR-γ has been detected in Day 15 porcine conceptus tissue (Harney et al., 1994a). Furthermore, RAR-α, β, and γ mRNA are expressed by spherical, early tubular, late tubular, and filamentous conceptuses in the pig (Yelich et al., 1997). Conceptus RAR-γ mRNA increases from the spherical conceptus, slightly decreases at the tubular morphology, and markedly increases in filamentous (Yelich et al., 1997). However, RAR-β gene expression is low and shows no dramatic change on all stages of conceptus development in the pig (Yelich et al., 1997). The main biological

function of RBP is to transport retinol to the conceptus for activation of RAR which may play an essential role during porcine embryo development.

The porcine endometrium secretes a protein that has plasmin/trypsin inhibitory activity (Mullins et al., 1980). The plasmin/trypsin inhibitor (PI) is present in the luminal epithelium (Fazleabas et al., 1983), but not in the glandular epithelium (Fazleabas et al., 1982; Zhang et al., 1991). The synthesis and secretion of PI is influenced by either progesterone stimulation only or a combination of progesterone and estrogen in the pig (Fazleabas et al., 1982). The porcine endometrial PI is not detected in ovariectomized gilts treated with estrogen only over a period of time (Fazleabas et al., 1983). Concentrations of PI released into the porcine uterine lumen attain a maximal level on Day 12 of pregnancy (Fazleabas et al., 1983), which suggests that conceptus-derived estrogen triggers endometrial PI release (Fazleabas et al., 1983). Endometrial PI mRNA has not been investigated during periattachment in the pig. However, gene expression for PI has been detected high on Day 30 of gestation with a dramatic decrease thereafter (Stallings-Mann et al., 1994). A biological function of PI is to protect the invasive porcine trophoblast from eroding uterine epithelium (Mullins et al., 1980; Fazleabas et al., 1982) which is highly invasive when placed in ectopic sites outside the uterine lumen of the pig (Samuel & Perry, 1972).

Epidermal growth factor (EGF) is produced by the porcine endometrium and proposed to enhance DNA synthesis (DiAugustaine et al., 1988). The glandular epithelium has been demonstrated to express EGF on Day 15 through 18 of the estrous cycle and early pregnancy in the pig (Kennedy et al., 1994). EGF receptors have been detected in the glandular epithelium on Day 13 in pregnant sows (Zhang et al., 1992).

Additionally, porcine endometrial gene expression for EGF receptor is detected on Day 4, 13, 15, and 20 of the estrous cycle and Day 10, 12, 14, 18, and 22 of pregnancy (Kennedy et al., 1994). Gene expression of endometrial EGF and EGF receptor shows no marked change during both the estrous cycle and early pregnancy (Kennedy et al., 1994). Heparin-binding epidermal growth factor-like growth factor (HB-EGF) is another member of the EGF family. In cyclic pigs, the major source of HB-EGF is the uterine luminal epithelium, although uterine glandular epithelial and stromal cells also secrete HB-EGF (Kim et al., 1995). HB-EGF mRNA has been detected in a wide range of porcine body tissues (Vaughan et al., 1992). HB-EGF mRNA is greater on Day 4 than either on Day 10 or 20 of the estrous cycle in porcine CL (Kenndy et al., 1993). However, gene expression for HB-EGF does not been detected in oviduct and endometrium from Day 15 to 18 in cyclic and pregnant gilts (Kenndy et al., 1994). Overall, HB-EGF precursors may facilitate cell-to-cell interactions for developing porcine conceptus during early pregnancy (Kim et al., 1995).

Transforming growth factors (TGF) are polypeptide growth hormones that have extensive sequence homology to EGF (see review by Petraglia et al., 1996). In addition, EGF receptor can bind EGF, HB-EGF, and TGFα (Fisher & Lakshmanan, 1990). TGFα, TGFβ, and their gene expression are present in human uterine endometrium, which may play important roles for human embryo development (see review by Petraglia et al., 1996). TGFα was originally recognized as a product of tumor tissues; therefore, the biological functions of TGFα in normal cells need more study. TGFα stimulates regeneration of epithelium in human (Schultz et al., 1987). However, the exact biological functions and

mechanisms of TGFα in porcine conceptus and endometrium remains to be further determined.

Conceptus $TGF\beta_1$,$TGF\beta_2$, $TGF\beta_3$, and their receptors are present from Day 11 to 14 in pregnant gilts (Gupta et al., 1996); however, they remain constant in porcine spherical and tubular conceptuses on Day 11 of gestation (Gupta et al., 1996). TGFβ isoforms are present in porcine embryonic disc and visceral endoderm on Day 10 and 11 of gestation with a decline from Day 12 to 14 (Gupta et al., 1996). In swine, $TGF\beta_2$ mRNA is not detected in porcine conceptuses from Day 10 and 12 of gestation (Yelich et al., 1995). Gene expression of $TGF\beta_3$ increases in spherical conceptus from Day 10 to 12 of pregnancy with expression peaking in tubular to filamentous forms (Yelich et al., 1995). These data indicate that TGFβs may play an essential role in conceptus differentiation and attachment process in the pig.

Acidic FGF (aFGF), basic FGF (bFGF), and keratinocyte growth factor (kGF) are members of fibroblast growth factor (FGF) family (Reich-Slotky et al., 1994). The aFGF and bFGF share a 55% amino acid sequence homology (see review by Petraglia et al., 1996). Both aFGF and bFGF are present in uterine flushing from Day 15 to 19 pregnant gilts (Brigstock et al., 1989). Gene expression for bFGF shows no significant change during the estrous cycle and early pregnancy in the pig (Katsahambas & Hearn, 1996). Therefore, the exact biological function of FGF during early pregnancy is unknown of this time.

Keratinocyte growth factor (KGF), a member of the FGF family, is produced by fibroblasts and is mitogenic in human epithelial cells (Marchese et al., 1990). Endometrial KGF mRNA is dramatically higher in the luteal phase than follicular phase in the monkey

(Koji et al., 1994). Gene expression for endometrial KGF is progesterone dependent (Koji et al., 1994; Siegfried et al., 1995). Moreover, endometrial KGF mRNA is present higher for cyclic gilts that are ovariectomized on Day 4 treated with progesterone from Day 4 to 14 compared to gilts treated with estrogen (Tuo et al., 1994). However, gene expression for KGF receptor is more dependent on estrogen than progesterone in human endometrium (Siegfried et al., 1995). Gene expression for endometrial KGF markedly increases from Day 9 to 13 and then decreases from Day 13 to 30 in pregnant gilts (Tuo et al., 1994). These data suggest that KGF may play important roles of endometrial epithelial function and/or activity during early pregnancy.

Insulin-like growth factors (IGFs) are single-chain polypeptides that are structurally related to insulin (see review by Petraglia et al., 1996). IGF-I and IGF-II are two major forms of IGFs, which share 62% sequence homology (see review by Petraglia et al., 1996). Synthesis of IGF-I by the uterine endometrium is greatest on Day 10 and 12 of gestation (Letcher et al., 1989), which is associated with maximal secretion of conceptus-derived estrogen at the time of maternal recognition of pregnancy in the pig (Letcher et al., 1989). Endometrial IGF-I gene expression peaks on Day 12 in cyclic and pregnant gilts (Simmen et al., 1992). In swine, endometrial IGF-binding protein-2 (IGFBP-2) mRNA is low on Day 10 (Simmen et al., 1992) and endometrial IGF-I receptor mRNA shows no change during early pregnancy (Simmen et al., 1992). Present data suggest that a developmental change in endometrial IGF gene expression occurs in the pig. Endometrial secretion of IGF-I during early pregnancy is correlated with conceptus elongation and estrogen synthesis. IGF-I stimulates P450 aromatase activity in vitro and enhances DNA synthesis of Day 12 and 13 conceptuses (Estrada et al., 1991;

Hofig et al., 1991). Endometrial IGF-I appears to be involved with endometrial function as endometrial IGF-I receptors are detected through Day 20 of gestation in the pig (Chastant et al., 1994). However, IGF-I receptors are not present in early Day 4 to 10 conceptuses nor are detected in porcine placenta on Day 20 of gestation (Chastant et al., 1994). Therefore, there is some question as to the direct effect of IGF-I on conceptus function. The gene expression for IGF-II and IGF-II binding proteins (IGFBP2) is detected in luminal and glandular epithelium in the pig (Simmen et al., 1990). However, the regulation of endometrial IGFBP2 mRNA is not associated with the regulation of IGF-I mRNA and IGF-II mRNA (Simmen et al., 1990). IGF-I, IGF-II, and their receptors may play important roles during porcine conceptus development (Simmen & Simmen, 1993).

Interleukins (ILs) are cytokines for which fourteen isoforms have been reported, IL-1 to IL-14 (see review by Petraglia et al., 1996). IL-1 and IL-6 are two distinct inflammatory cytokines which have been identified in human placenta (see review Petraglia et al., 1996). Gene and protein expression for IL-1β are high in pig conceptuses on Days 11, 12, and 13 of gestation (Tuo et al., 1996), suggesting that increased porcine conceptus IL-1β production might be related to conceptus development during periattachment process in the pig.

The members of IL-6 cytokine family include IL-6, leukemia inhibitory factor (LIF), oncostatin M (OSM), ciliary neurotrophic factor (CNTF), IL-11, and cardiotrophin-1 (CT-1) (see review by Hibi et al., 1996). In swine, IL-6 activity is present during the estrous cycle and early pregnancy (Anegon et al., 1995). Gene expression of IL-6 has been detected only on Day 6 and 11 in porcine endometrium (Anegon et al.,

1994). However, the exact biological function of IL-6 during early pregnancy in the pig requires more investigation.

Leukemia inhibitory factor (LIF) inhibits mouse embryonic stem cell differentiation in vitro, stimulates hepatic acute-phase protein synthesis, induces neurotransmitter phenotype from adrenergic to cholinergic, inhibits adipocyte lipoprotein lipase activity, and increases bone resorption activity (see review Hilton & Gough, 1991). The uterine concentrations of LIF are markedly elevated on Day 12 in pregnant gilts (Anegon et al., 1994). Endometrial LIF mRNA is highly expressed on Day 11 in the pig (Aneogon et al., 1994). These data indicate that LIF may play an important role of porcine conceptus development at the time of maternal recognition of pregnancy.

Colony-stimulating factor-1 (CSF-1), a hematopoietic growth factor, is produced by human endometrium (Sherr, 1990). In the mouse, endometrial CSF-1 is regulated by estrogen and progesterone (Pollard et al., 1987). Additionally, receptors for CSF-1 are present in mouse blastocysts during implantation (Arceci et al., 1989), suggesting that CSF-1 may play an important role for mouse conceptus development. In swine, immunoreactive CSF-1 is present in endometrial glandular epithelium but not surface epithelium on Day 0 (Tuo et al., 1995). Immunoreactive CSF-1 is located in porcine luminal epithelium but not glandular epithelium on Day 3 of gestation (Tuo et al., 1995). On Day 10 through 15 pregnancy, immunoreactive CSF-1 is located in both the luminal and glandular epithelial cells in pregnant gilts (Tuo et al, 1995). Endometrial CSF-1 mRNA is not influenced by daily exogenous estrogen or progesterone treatment from 4 to 14 in Day 4 ovariectomized gilts (Tuo et al., 1995) as well as in the mouse (Pollard et al., 1987). Porcine endometrial CSF-1 mRNA level is low but increases from Day 10 to 112

of pregnancy (Tuo et al., 1995). However, endometrial CSF-1 gene expression during early pregnant gilts is still remain to be determined.

Uterine-Conceptus Interactions

Successful placentation in the pig requires close interaction(s) between the developing conceptus and the uterine endometrium. The porcine uterine lumen closes to bring the uterine epithelium in close contact with itself on approximately Day 11 and 12 of gestation (Geisert et al., 1982b). Porcine conceptuses progress from a 10mm spherical to elongated filamentous form (100mm) within 4 to 6 hours between Day 10 and 12 of gestation (Geisert et al., 1982b). Following trophoblast elongation, attachment of the conceptus to the uterine endometrium is initiated on Day 13 of pregnancy (Corner, 1921; Keys & King, 1988). The superficial adhesion of uterine epithelium and trophectoderm forming the epitheliochorial type placentation remains throughout entire gestation period in the pig (King, 1993). Ultrastructual changes occur in the subepithelial capillaries of the uterus during initial placenta development, as a result endothelial transport increases facilitating nutrient exchange at the time of trophoblastic attachment in the pig (Keys & King, 1988). In the pig, maternal and placental capillaries come within a few microns of each other by late pregnancy (Strodband & Van del Lende, 1990).

Ultrastructure changes of uterine epithelium indicate metabolic activity increases and epithelial surface changes for attachment in the pig. Porcine conceptus-derived estrogen stimulates proliferation of uterine epithelial and glandular cells (Cathpole, 1973).

Initial conceptus synthesis and release of estrogen stimulate glandular epithelial cells to release materials into the lumen at the same time (Geisert et al., 1982a; Fazleabas et al., 1983). Conceptus estrogen may either directly or through other mediating substances regulate the uterine endometrium to facilitate embryonic attachment in the pig. Endometrium produces many stimulatory and/or inhibitory factors to provide the proper environment for conceptus development. Our laboratory has demonstrated conceptus gene expression for retinoic acid receptors, retinol-binding protein, and transforming growth factors on Day 10 and 12 of gestation (Yelich et al., 1997). Endometrial gene expression for a number of cytokines and receptors is relatively unknown at this time. This thesis will utilize reverse transcription polymerase chain reaction (RT-PCR) to identify endometrial gene expression of selected cytokines and receptors during the estrous cycle and early pregnancy in the pig. Identification of gene expression changes during the estrous cycle and early pregnancy will allow a more focused approach to determine gene products involved with establishment of pregnancy in the pig.

CHAPTER III

ENDOMETRIAL GENE EXPRESSION DURING THE ESTROUS CYCLE AND EARLY PREGNANCY IN SWINE

Introduction

Recognition of pregnancy depends upon endometrial responsiveness to the signals from the dam and conceptus, which occurs through many different mechanisms in mammals (Geisert et al., 1994; Roberts et al., 1996). In the pig, critical period for maternal recognition of pregnancy is between Day 11 and 12 (see Geisert et al., 1990), which proceeds a time of high early embryonic mortality from Day 12 to 18 of gestation (Pope, 1994). Successful establishment of pregnancy involves conceptus-endometrial relationships to inhibit luteolysis.

The major porcine luteolytic factor, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), which is synthesized by uterine endometrial cells (Bazer et al., 1984; Bazer, 1989, 1992), causes regression of porcine corpora lutea. The direction of endometrial $PGF_{2\alpha}$ release from the uterus is shifted during early pregnancy in the pig (Moeljono et al., 1977). The plasma concentration of $PGF_{2\alpha}$ increases after Day 12 of the estrous cycle as a result of

endometrial activity (Hallford et al., 1974), which is associated with the decline in plasma progesterone concentration from Days 14 to 17 of the estrous cycle (Guthrie & Lewis, 1986). Conceptus estradiol secretion increases on Day 12 in pregnant gilts (see Geisert et al., 1990). Porcine conceptus-derived estrogens induce endometrial $PGF_{2\alpha}$ secretion into the uterine lumen instead of the maternal vasculature between Days 12 and 15 of gestation (Bazer et al., 1984), thereby maintaining luteal function in the pig (Bazer et al., 1992). Administration of exogenous oxytocin stimulates uterine $PGF_{2\alpha}$ secretion in the sow (Kieborz et al., 1991), and increases endometrial $PGF_{2\alpha}$ in vitro (Cross et al., 1988). Furthermore, high quantities of oxytocin are produced by porcine endometrium at the time of maternal recognition of pregnancy (Trout et al., 1995). Endometrial oxytocin receptors have been detected in cyclic gilts (Whiteaker et al., 1994; Okano et al., 1996) and early pregnant gilts (Okano et al., 1996).

Porcine endometrial estrogen receptor (ER) increases in cyclic and pregnant pigs, which coincides with increasing conceptus estrogen during the period of maternal recognition of pregnancy (Geisert et al., 1993). Therefore, conceptus estrogen secretion can greatly influence endometrial function and secretion. Porcine uterine secretions are essential for conceptus development during early pregnancy because the pig has a non-invasive type of placentation. Retinol binding protein (RBP), which is stimulated by progesterone, is produced by the uterine endometrium, to transport retinol to the porcine conceptus during early pregnancy (Adams et al., 1981; Harney et al., 1990, 1994). Porcine endometrial RBP gene expression is low on Days 0, 5, and 10 of the estrous cycle and on Day 10 of pregnancy (Harney et al., 1993). However, endometrial RBP mRNA is enhanced from Day 10 to 12 in cyclic and pregnant pigs (Harney et al., 1993). On Day

18, endometrial RBP mRNA concentrations decrease in cyclic pigs but remain elevated in pregnant pigs (Harney et al., 1993).

Retinoic acid binds retinoic acid receptors (RARs) which are the members of steroid/thyroid receptor family (De Luca, 1991). During early pregnancy, gene expression for RBP, RAR-α, and RAR-γ has been detected in Day 15 porcine conceptus tissue (Harney et al., 1994). Yelich et al. (1997) has indicated that RAR are present in Day 10 to 12 conceptuses. Retinoic acid can induce transforming growth factor β_3 ($TGF\beta_3$) synthesis (Sporn et al., 1986; Roberts & Sporn, 1988). TGFβs may play an essential role in porcine conceptus differentiation and attachment to the endometrial surface (Gupta et al., 1996; Yelich et al., 1997).

Conceptus effects on endometrial gene expression would provide information concerning endometrial changes necessary for establishment of pregnancy. Conceptus secretion of many cytokines has been established (see review by Geisert et al., 1997). Evaluation of receptors and cytokine production and their receptors is needed to begin to understand conceptus-uterine interactions which occurs in the pig. Reverse-transcription polymerase chain reaction (RT-PCR) has been utilized to detection low gene expression of growth factors and receptors (Rappolee et al., 1989). The objective of the present paper is to utilize previous optimal RT-PCR conditions (Yelich et al., 1997) to detect gene expression for glyceraldehyde-3-phosphate dehydrogenase (G3PDH), β-Actin, retinol-binding protein (RBP), estrogen receptor (ER), retinoic acid receptors (RAR-α, β, γ), transforming growth factor β_3 ($TGF\beta_3$), progesterone receptor (PR), and oxytocin receptor (OTR) in uterine endometrium from the cyclic and early pregnant gilts. Additionally, PCR conditions were developed to determine gene expression for

prostaglandin $F_{2\alpha}$ receptor ($PGF_{2\alpha}R$) in porcine endometrium during the cyclic and early pregnant gilts.

Materials and Methods

Endometrial collection

Postpubertal crossbred gilts at similar age (7-8 months), weight (100-130 kg) and having expressed two complete estrous cycles were randomly assigned to either cyclic and pregnant treatment groups. Gilts were observed for estrus behavior twice daily. Gilts assigned to the pregnant group were mated to fertile boars at the onset of estrus (Day 0) and 12 and 24 h later. Cyclic gilts were hysterectomized (two/day) on either Days 0, 5, 10, 12, 15, and 18 of the estrous cycle, while pregnant gilts were hysterectomized (two/day) on either Days 10, 12, 15, and 18 of gestation (Harney et al., 1993). Gilts were hysterectomized following induction of anesthesia with 5% solution of thiopentone sodium (Abbott Laboratories, North Chicago, IL) and maintenance on a closed-circuit system of halothane (2 to 5% Fluothane, Aveco Co., Fort Dodge, IA) and oxygen (1.0 liters/min). Uterine horns and ovaries were exposed by midventral laparotomy and surgically removed. The incision site was closed and the gilt treated with antibiotic procaine penicillin (20000IU/Kg BW) postsurgery. The uterine horns were trimmed free of the broad ligament and ovaries. Uterine horns of pregnant gilts were flushed with 20 ml of sterile saline to recover conceptuses. Endometrial tissue was dissected from the

underlying myometrium and snap-frozen in liquid nitrogen. Endometrial samples were store at -80°C until of extraction of RNA.

Endometrial RNA Isolation

Total RNA was obtained through guanidium/thiocynate phenol extraction (Puissant & Houdebire, 1990). Guanidium thiocyanate (10 ml) containing 2-mercaptoethanol (78 µl/10 ml) was added to 1 g of endometrial tissue in a 50 ml sterile tube. Tissue was homogenized on ice in a tissue homogenizer (Vir Tishear). Following homogenization, 1 ml of 2M sodium acetate, 10 ml water-saturated phenol and 2 ml chloroform was added and the tube gently vortexed. Following centrifugation at 4000 rpm for 15 min, the upper phase was removed with disposal sterile pipette and placed in a new 50 ml sterile tube. Ten ml of isopropanol was added to precipitate RNA and tube was then placed in -80°C ultralow freezer for 1 h. After sample was thawed and centrifuged at 3000 g for 10 min, the upper phase was poured off. The pellet was resuspended in 2 ml of 4 M LiCl and centrifuged at 4000 g for 15 min to repellet. The aqueous phase was removed and the tube blotted on a clean paper towel. The resulting pellet was resuspended in 2 ml of 10 mM Tris (pH=7.5), 1 mM EDTA, 0.5 % SDS, and placed in a clean 15 ml sterile conical tube. Chloroform (2 ml) was added, tube gently vortexed, and centrifuged at 3000 g for 10 min. The upper aqueous phase was transferred into a new 15 ml sterile conical tube and enough 3 M sodium acetate (pH=5.0) added to upper phase to give 0.2 M solution. Sample was mixed with 2 ml isopropanol and the

tube was stored in an ultralow freezer -80°C overnight. Following thawing, tubes were centrifuged at 3000 g for 30 min. Supernatant was poured off, tube blotted, 1 ml of 70% ethanol was added and tubes were centrifuged for 10 min at 3000 g. The aqueous phase was poured off and each tube was blotted on clean absorbent papers. After each pellet dried for 15 min, the pellet was resuspended in 200 µl TE buffer (10 mM Tris (pH=7.5), 1 mM EDTA). Pellets were allowed to equilibrate at room temperature for 10 min, vortexed gently, and transferred to 1.6 ml sterile tubes. Total RNA was quantified by absorbance at 260nm/280nm.

Removal of DNA contamination from RNA

Endometrial samples of 50 µg total RNA were diluted in 100 µl of TE buffer. Then, 10 µl DNase (1U/µl in 10 mM Tris-Cl, pH=8.3, 50 mM KCl, 1.5 mM $MgCl_2$) was added to the 100 µl RNA sample. Following vortexing, each tube was incubated at 37°C for 30 min. Again, 10 µl DNase was added to each tube and incubated at 37°C for 30 min. The following reagents were added to each tube which was placed on ice: 12 µl of cold 2M sodium acetate (pH=4.5), 132 µl of cold, water saturated phenol (pH=5), and 52.8 µl of cold chloroform/isoamyl alcohol (49:1). After addition of reagents, tubes were briefly vortexed. Tubes were incubated on ice for 15 min and centrifuged at 12000 rpm for 15 min. The upper aqueous phase was carefully removed with a microcapillary pasteur pipette and transferred into a fresh tube. One hundred microliters of cold isopropanol was

added to precipitate RNA overnight at -20°C. Following thawing and centrifugation at 12000 rpm for 15 min, the aqueous phase was discarded and the remaining pellet was washed with 100 µl of a cold 75% ethanol. Tubes were incubated on ice for 15 min and centrifuged at 12000 rpm for 10 min. Ethanol was removed with a capillary pasteur pipette, all droplets from the tube wall were removed. The pellet was dried for 30 min at room temperature. The pellet was redissolved in 70 µl of DEPC water at room temperature for 10 min. Following vortexing, 10 µl of the sample was placed in 990 µl sterile water for spectrophotometric analysis. Purity of RNA was determined by calculations of 260/280 ratios.

cDNA Preparation

Total RNA was reverse transcribed to cDNA in vitro in 20 µl of a solution which contained 200U of Moloney murine leukemia virus reverse transcriptase-RNase H (M-MLV-RT) following addition of 2.0 µl containing 15 mer oligo-dT primer (Promega Corporation, Madison, WI), 2.0 µl of dNTP, 50 mM Tris-HCl (pH=8.3), 75 mM KCl, 3 nM $MgCl_2$, 10 mM dithiothreitol, 20U of RNasin, 0.5 µg total RNA (10 µl vol), and brought to volume with ddH_2O and placed in a Perkin Elmer Cetus (Norwalk, CT) DNA Thermal Cycle Model 480. The sample was incubated at 22°C for 15 min, followed by incubation at 42°C for 30 min. Sample reaction was terminated by denaturing 95°C for 5

min and quickly cooling to 4°C. The cDNA sample was diluted with 20 µl of ddH_20 and was stored at 4°C.

PCR Primers

Table 1 shows the PCR primer sequences and size of the amplified products. Optimal conditions for PCR which were developed by Yelich et al. (1997) are presented in Table 2. The primers for glyceraldehyde 3-phosphate dehydrogenase (G3PHD) were developed based on sequence homology with human (Arcari et al., 1984) and rat (Tso et al., 1985) G3PDH cDNA sequences. β-Actin primers were designed based on the cDNA sequence of pig β-Actin (GenBank Accession U07786). RBP primers were developed from the porcine RBP cDNA sequence (Trout et al., 1991). ER primers were based on human ER cDNA sequence (Greene et al., 1986). RAR-α primer sequences are based on homology with murine (Heiermann et al., 1993) and human (Giguere et al., 1987) RAR-α cDNA sequences. The primers for RAR-β were designed based on the homology with murine (Heiermann et al., 1993) and human RAR-β (Benbrook et al., 1988), and primers for RAR-γ were based on homology with murine (Zelent et al., 1989) and human RAR-γ (Krust et al., 1989) cDNA sequences. The primers for $TGF\beta_3$ were based $TGF\beta_3$ cDNA sequence of pig (Mulheron et al., 1992). PR and OTR primers were adapted from the porcine cDNA sequences (Iwai et al., 1991; Gorbulev et al., 1993, respectively). The

primers for $PGF_{2\alpha}$ were developed from sequence homology between bovine (Sakamoto et al., 1994) and ovine (Graves et al., 1995) $PGF_{2\alpha}$ cDNA sequences.

PCR Optimization and Optimal Amplification Conditions

PCR reactions were carried out in a total volume of 25 µl that were covered with 25 µl of mineral oil. Amplification was conducted in a Perkin Elmer Cetus DNA Thermal Cycler Model 480 (Norwalk, CT). Optimal conditions for amplification for $PGF_{2\alpha}R$ gene marker was determined through utilization of a pooled sample of porcine endometrial cDNA. Porcine endometrial cDNA was amplified with Taq DNA polymerase (Promega; Madison, WI) and a 3×2×3 factorial combination of primer (100, 200, or 500 nM of each primer), deoxynucleotidetriphosphates (dNTPs; 100 or 200 µM each), and $MgCl_2$ (0.75, 1.5, or 2.25 mM). All samples were kept on ice and then placed into the 95°C heat block to minimize time required for samples to attain denaturation temperature. First PCR cycle consisted of denaturation at 95 °C for 2 min, annealing at 55 °C for 1 min, and 2 min extension at 72 °C. All optimal PCR conditions for amplification are presented in Table 2. All PCR products were resolved in a 3% agarose gel at 82 V for 1.5 h. Following 30 min staining in ethidium bromide (0.5 g/mL) and destaining in deionized water for 30 min, agarose gels were exposed to Foto Prep I ultraviolet light source and were photographed with a MP4 Instant Camera System (Fotodyne, Inc. Hartland, WI). The strongest single band of PCR product of the predicted bp size determined as the optimal PCR condition.

If extra bands were detected with the expected band, the annealing temperature of the optimization was increased. Amplification of porcine genomic DNA was also utilized with each optimization to determine if the primers amplified a similar size product for both genomic DNA and cDNA. All PCR results in this study yielded genomic products that were larger than PCR products amplified from porcine endometrial cDNA. Moreover, individual endometrial RNA samples were detected for genomic DNA contamination with porcine microsattellite marker PCR primers (SW-419; Rohrer et al., 1994) and no visible PCR product was observed for all samples. In addition, all isolated total RNA utilized spectrophotometer readings with 260/280 ratios close to 2.0 indicated little or no genomic DNA contamination.

Verification of PCR products

The identity of amplified PCR products was verified by restriction enzyme digest or direct sequence analysis as previously described by Yelich et al. (1997a,b). The identity of progesterone receptor (PR) amplified product was verified by Drd I and Hinf I restriction enzyme digest. The Drd I digest yielded the two predicted fragments of approximately 77 and 223 bp while the second digest with Hinf I yielded the two predicted fragments of approximately 134 and 156 bp. The amplified PCR product for oxytocin receptor (OTR) was identified by cutting with the restriction enzymes Pvu II and Bst YI. The Pvu II digest yielded the two expected fragments of 136 and 616 bp. The Bst YI digest yielded the three predicted fragments of 538, 153, and 61 bp.

Densitometric Analysis

Following PCR amplification, samples were loaded in a volume of 15 µl and separated on a 3 % agarose gel using 1 x TBE running buffer. After products were stained with ethidium bromide, bands of cDNA were photographed with illumination by ultraviolet light. Optical density was obtained by densitometric analysis of one dimensional electrophoretic (1-D) gels using the NIH Image 1.60 processing and analysis program (National Institutes of Health). Data were analyzed as relative amounts of mRNA transcript/β-actin mRNA.

Statistical Analysis

Bands within each photograph of all PCR products were measured by optimal density analysis and then divided by the corresponding optimal density of its corresponding β-Actin expression. Data were analyzed covariance by using the General Linear Models (GLM) of the Personal Computer Statistical Analysis System (1995) to determine effect of status (cyclic v. pregnant), day of the estrous cycle or pregnancy, as well as status and day interactions. Relative gene expression for each photograph in cyclic status were compared with pregnant status from Day 10 to 18. Data were analyzed to check the effect of day for each PCR product from Day 0 to 18 in cyclic status, as well as from Day 10 to 18 in pregnant status. The alpha level used to determine statistical

significance was $P < 0.05$. Status means where separated using least significant difference (LSD) (Steel & Torrie, 1980).

Results

The sensitive technique of RT-PCR was applied to total RNA samples isolated from cyclic and early pregnant endometrial tissues in the pig. In this manner we have searched for detectable levels of the transcripts for G3PDH, β-Actin, RBP, ER, RAR-α, β , γ, TGFβ$_3$, PR, OTR, and PGF$_{2\alpha}$R. Each reverse-transcribed endometrial cDNA sample was tested with the G3PDH and β-Actin primers to determine loading errors of RNA or cDNA across status and day.

Results of endometrial gene expression in cyclic and pregnant gilts are presented in Figures 2 to 12. Levels of β-Actin gene expression in porcine endometrium is presented in Figure 2. Gene expression was relatively equal across days of the estrous cycle and pregnancy. However, β-Actin gene expression was greater ($P < 0.05$) in pregnant compared to cyclic gilts. Relative level of endometrial G3PDH gene expression (Figure 3) is constant across all days of the estrous cycle and early pregnancy; however, a significant day by status interaction was detected ($P < 0.05$). G3PDH gene expression decreased on Day 10 to 18 of the estrous cycle while remaining unchanged in pregnant gilts.

Endometrial RBP mRNA expression increased from Day 10 to 12 ($P < 0.01$) and remains high between Day 12 and 18 during the estrous cycle and pregnancy (Figure 4). A significant day by status effect was detected for RBP gene expression ($P < 0.01$).

Additionally, gene expression for endometrial RBP is greater between Day 10 through 18 of the early pregnancy compared to the estrous cycle in the pig ($P < 0.05$).

Relative gene expression for endometrial ER is presented in Figure 5. Gene expression for ER remains constant in porcine endometrium across all stages of the estrous cycle and early pregnancy, but endometrial ER gene expression is greater during pregnancy than the estrous cycle ($P < 0.05$).

A significant status by day interaction ($P < 0.05$) was detected indicating day trends in RAR-α mRNA in porcine endometrium varied between the estrous cycle and early pregnancy (Figure 6). Endometrial RAR-α mRNA in porcine endometrium was detected on all days of the estrous cycle and early pregnancy, but was greater during early pregnancy compared to the estrous cycle ($P < 0.05$). Porcine endometrial RAR-β mRNA increased on Day 15 of pregnancy (Figure 7). In cyclic gilts, content of endometrial mRNA for RAR-β slightly increased from Day 0 to 12 and then was stable thereafter ($P < 0.01$). Gene expression for endometrial RAR-β was greater in early pregnant compared to cyclic gilts ($P < 0.01$). Endometrial RAR-γ gene expression was not different and varied across all days of the estrous cycle and early pregnancy (Figure 8).

Endometrial TGFβ_3 gene expression is greater during early pregnancy (Figure 9) than in the estrous cycle in the pig ($P < 0.05$). Gene expression for endometrial TGFβ_3 is increased from Day 12 to 15 of the estrous cycle, but the change was not statistically significant. Endometrial PR mRNA content (Figure 10) is greater in pregnant than in the cyclic gilts from Day 10 through 18 ($P < 0.05$). Endometrial PR gene expression is greater on Day 0 and 5 followed by a decrease on Day 10 ($P < 0.05$) through 18 of the estrous cycle.

Relative endometrial OTR gene expression is presented in Figure 11. A day by status interaction was detected ($P < 0.05$). Endometrial OTR gene expression increased from Day 10 through 18 in cyclic gilts, but was similar across days of pregnancy. Gene expression for endometrial OTR in pregnant gilts was higher than in cyclic gilts ($P < 0.05$). Gene expression for OTR changed across the days of the estrous cycle ($P < 0.05$). Endometrial OTR mRNA is high on Day 0, greatly decreasing by Day 5, followed by a marked increase between Day 10 and 12 were it remained high to Day 18 of the estrous cycle.

Figure 12 shows that endometrial gene expression for $PGF_{2\alpha}R$. There was a day by status interaction for porcine endometrial $PGF_{2\alpha}R$ gene expression ($P < 0.01$). Compared with cyclic porcine endometrium, gene expression for $PGF_{2\alpha}R$ was significantly greater from Day 10 to 18 during early pregnancy ($P < 0.05$). Endometrial $PGF_{2\alpha}R$ mRNA increased from Day 0 to 18 in cyclic gilts ($P < 0.05$).

Discussion

We have demonstrated that detectable gene expression for eleven different selected enzymes, proteins, cytokines, and receptors occurred during the estrous cycle and early pregnancy in the pig. These included G3PDH, β-Actin, RBP, ER, RAR-α, β, γ, $TGF\beta_3$, PR, OTR, and $PGF_{2\alpha}R$, which may be required and play important roles for porcine conceptus development. The critical period for maternal recognition of pregnancy in the pig is between Day 11 and 12 of gestation (see Geisert et al., 1990). Oxytocin stimulates

porcine endometrial $PGF_{2\alpha}$ secretion (Cross et al., 1988; Kieborz et al., 1991; Printz et al., 1994; Whiteaker et al., 1994, 1995). In addition, high quantities of oxytocin are produced by porcine endometrium at the time of maternal recognition of pregnancy (Trout et al., 1995). Conceptus-derived estrogens alter uterine endometrial $PGF_{2\alpha}$ secretion, which inhibits luteal regression in the pig (Bazer et al., 1984, 1992). The pig has a non-invasive type of placentation; therefore, uterine secretions are essential for the porcine conceptus development during early pregnancy. In addition, conceptus-derived estrogen is associated with protein synthesis and secretion in uterine lumen during early pregnancy in the pig (Godkin et al., 1982; Geisert et al., 1982, 1990).

Endometrial G3PHD and β-Actin were analyzed to determine equal loading of cDNA and served as controls to demonstrate level expression in each sample. Relative levels of gene expression for G3PDH and β-Actin in porcine endometrium were constant throughout days of the estrous cycle and early pregnancy. Relative levels of endometrial G3PDH and levels of β-Actin mRNAs were higher during early pregnancy between Day 10 through 18 compared to estrous cycle. Endometrial β-Actin mRNA served as the adjustment for expression of other genes. Relative levels of endometrial G3PDH mRNA were higher in pregnant than in cyclic gilts between Day 12 and 18, which may indicate a change in uterine metabolism induced by the early conceptuses. The present results show that RBP mRNA in porcine endometrium is low on Days 0, 5, and 10 of the estrous cycle. However, endometrial RBP mRNA is increased from Day 10 to 12 in cyclic and pregnant pigs. Higher RBP gene expression in porcine endometrium plays an important role in protecting uterine and conceptus tissues from lipid peroxidation that induced by uteroferrin (Vallet, 1995). Additionally, production of endometrial RBP suggests that

transport of retinol from the dam into conceptus in important for establishment and maintenance of early pregnancy in the pig (Adams et al., 1981; Harney et al., 1990, 1994). Relative levels of ER gene expression in porcine endometrium showed no significant changes between Day 10 and 18 during the estrous cycle and early pregnancy. The gene expression patterns for endometrial RBP and ER in cyclic and pregnant gilts of the present results are similar to previous published results by Northern blot analysis (Harney et al., 1993; Geisert et al., 1993). Therefore, the present results of RBP and ER mRNAs show high reliability to support the other results we obtained by PCR.

The results indicate that RAR-α and RAR-β mRNAs are detected throughout the estrous cycle and early pregnancy in the pig. Moreover, higher gene expression for RAR-α and RAR-β in porcine endometrium during early pregnancy indicates that RAR-α and RAR-β mRNAs may be regulated by the presence of porcine conceptus. Gene expression for RAR-γ is detected in the porcine endometrium, but showed no distinct change during the estrous cycle and early pregnancy. Endometrial RAR-γ gene expression is not affected by the developing porcine conceptus, although conceptus RAR-γ mRNA increased in the spherical conceptus, slightly decreased at the tubular morphology, and markedly increases in filamentous embryos (Yelich et al., 1997).

Retinoic acid induces $TGF\beta_3$ synthesis (Sporn et al., 1986; Roberts & Sporn, 1988). Endometrial $TGF\beta_3$ gene expression in porcine endometrium is higher during early pregnancy than the estrous cycle, suggesting $TGF\beta_3$ may play essential roles in porcine conceptus differentiation and attachment process (Gupta et al., 1996; Yelich et al., 1997) as well as in porcine uterus growth and development.

Progesterone, a primary product from corpora lutea, is essential for maintenance of pregnancy in the pig (Ellicott et al., 1973). Most secretory activities of uterine proteins are regulated by progesterone while some are modified by estrogen (Geisert et al., 1982a, b). Endometrial PR gene expression is high on Day 0 and 5, decreases on Day 10, and remains low thereafter during the estrous cycle.

Endometrial OTR are present in cyclic gilts (Whiteaker et al., 1994; Okano et al., 1996) and early pregnant gilts (Okano et al., 1996). In the present study, endometrial OTR mRNA levels are highest on Day 0, markedly decline on Day 5, greatly increase between Day 10 and 12, and then remain high throughout the estrous cycle. Therefore, the pattern of endometrial OTR mRNA decreased on Day 5 of the estrous cycle maybe the result of increased progesterone which down-regulates oxytocin receptor. The mechanism of higher endometrial gene expression for OTR on Day 10 and 12 of gestation results from down-regulation of progesterone receptors that are present in the uterine epithelium (Geisert et al., 1994b). Porcine endometrial OTR gene expression is constant but higher between Day 12 through 18 of early pregnancy than during the estrous cycle. Moreover, the concentrations of OTR in the porcine endometrium are lower during early pregnancy compared to during luteolysis in cyclic gilts (Okano et al., 1996). Therefore, endometrial OTR gene expression may be modified by progesterone synthesis and/or secretion.

Gene expression for $PGF_{2\alpha}R$ in endometrium of pigs is increased from Day 0 through 18 during the estrous cycle, and is constantly expressed across all days of early pregnancy. Results indicate that progesterone may induce $PGF_{2\alpha}$ gene expression much like RBP. Alternatively, the loss of PR in the uterine epithelium may allow the increase in $PGF_{2\alpha}$ as is observed with OTR. Endometrial $PGF_{2\alpha}R$ mRNA is present higher in early

pregnant than cyclic gilts, which indicates that endometrial $PGF_{2\alpha}R$ gene expression may play an essential role for maintenance and establishment of early pregnancy in the pig. Additionally, Kennedy et al. (1986) indicated that PGE_2 receptors are present in porcine uterine endometrium, which implies that a direct effect on porcine uterine function.

Porcine conceptuses effect on endometrial gene expression would provide information concerning endometrial changes necessary for maintenance and establishment of pregnancy.

CHAPTER IV

GENERAL DISCUSSION

The critical period for maintenance of pregnancy is between Days 11 and 12 of gestation in the pig (see Geisert et al., 1990). The increase in porcine conceptus-derived estrogen secretion coincides with blastocyst elongation that begins at approximately 10 mm spherical morphology on Day 12 of gestation (Geisert et al., 1982). Moreover, conceptus-derived estrogens alter uterine endometrial $PGF_{2\alpha}$ secretion, which inhibits porcine CL regression (Bazer et al., 1984, 1992). Additionally, release of conceptus estrogen is related with increased protein synthesis and secretion in the porcine uterine lumen during early pregnancy (Godkin et al., 1982; Geisert et al., 1982, 1990). In swine, proteins secreted from the uterine epithelium may play essential roles in providing proper components for the conceptus development during early pregnancy. However, changes of uterine endometrial ultrastructure and function by the presence of porcine conceptuses during early pregnancy are not completely defined.

Our results indicate that G3PDH, β-Actin, RBP, ER, RAR-α, β, γ, $TGF\beta_3$, PR, OTR, and $PGF_{2\alpha}R$ mRNAs are detected by Day 18 in porcine endometrium during the estrous cycle and pregnancy. Therefore, ER, RAR-α, β, γ, PR, OTR, and $PGF_{2\alpha}R$ are

major receptors of porcine uterine endometrium and may facilitate maternal recognition of pregnancy in the pig.

Endometrial G3PDH and β-Actin serve as controls to show the constant expression in each sample. Higher endometrial gene expression for β-Actin in pregnant than cyclic gilts are utilized as the adjustment of the other present results. However, endometrial G3PDH shows higher gene expression for pregnant than for cyclic gilts between Day 12 and 18, and may indicate that a change in uterine metabolism induced by the early porcine conceptuses. Porcine endometrial RBP mRNA in cyclic and pregnant pigs has been previously reported by Harney et al. (1993), as well as endometrial ER gene expression by Geisert et al. (1990). Endometrial RBP gene expression is low on Days 0, 5, and 10 in cyclic gilts and on Day 10 in pregnant gilts. However, RBP mRNA in porcine endometrium is increased from Day 10 to 12 of the estrous cycle and pregnancy. Endometrial ER gene expression remains constant between Day 10 and 15 in both statuses in the pig. RBP and ER gene expression were utilized and compared for the reliability of the present results.

Progesterone is critical for maintenance of pregnancy in the pig (Ellicott et al., 1973) and regulates the activity of uterine endometrial secretions in the pig (Catchpole, 1973). Progesterone receptors are present in porcine uterine endometrium, which are stimulated by progesterone followed by estrogen priming. On Day 5, increased PR receptor and progesterone from CL may inhibit endometrial OTR and $PGF_{2\alpha}R$ mRNAs but stimulate gene expression for RBP and RAR-β in porcine uterine endometrium. On Day 10, endometrial PR mRNA decreased by down regulation of progesterone, which may allow gene expression for ER, RAR-β, OTR, and $PGF_{2\alpha}R$ increased in the porcine

endometrium. Between Day 10 and 12 in cyclic gilts, endometrial gene expression for OTR and RBP markedly increased. At the same time, gene expression for RAR-β and $PGF_{2\alpha}R$ in porcine endometrium slightly increased. Although RBP mRNA in porcine endometrium markedly increased from Day 10 to 12 of gestation, gene expression for ER, RAR-α, β, γ, $TGF\beta_3$, PR, OTR, and $PGF_{2\alpha}R$ were constant in porcine endometrium from Day 10 to 12 of pregnancy. The present results indicate that gene expression for ER, RAR-α, β, γ, $TGF\beta_3$, PR, OTR, and $PGF_{2\alpha}R$ in porcine endometrium may directly and/or indirectly play roles for maintenance and establishment of pregnancy in the pig. Moreover, endometrial ER, RBP, PR, RAR-β, OTR, and $PGF_{2\alpha}R$ gene expression are modified by estrogen and progesterone.

Conceptus-derived estrogens play important roles for the attachment process in the pig (Geisert et al., 1982). However, ER gene expression in porcine endometrium is present during the process of embryo attachment. $PGF_{2\alpha}$ may not play important roles in porcine conceptus development and elongation, but plays essential roles in placental attachment after Day 13 in pregnant gilts (Geisert et al., 1986). In addition, metabolic activity of uterine endometrial $PGF_{2\alpha}$ is slightly higher in early pregnant pigs (Maule Walker et al., 1977). Endometrial $PGF_{2\alpha}R$ mRNA remains constant during early pregnancy by Day 18 in the pig, which indicates that endometrial gene expression for $PGF_{2\alpha}R$ may play an essential role for maintenance and establishment of pregnancy in the pig.

The presence of porcine conceptuses in the uterus, which effect gene expression in the uterine endometrium would provide information concerning endometrial changes necessary for maintenance and establishment of pregnancy. However, many functions and

regulations remain to be investigated about porcine endometrial secretions. Future studies in porcine endometrial gene expression might be directed towards the relationships among uterine protein secretions, gene expression, and receptors.

Table 1. PCR primer sequences and the expected product size in base pairs (bp)

Gene marker	PCR primer sequences 5' upstream/3' downstream	Product size bp
Glyceraldehyde-3-phosphate-dehydrogenase	5' ACCACAGTCCATGCCATCAC 3' 5' TCCACCACCCTGTTGCTGTA 3'	452
β-Actin	5' ATCTTGATCTTCATGGTGCTGGGC 3' 5' ACCACTGGCATTGTCATGGACTCT 3'	545
Retinol binding protein	5' TTCCGAGTCAAAGAGAACTTCG 3' 5' TCATAGTCCGTGTCGATGATCC 3'	311
Estrogen Receptor	5' GAGATCCTGATGATTGGTCT 3' 5' TACTGGACGACGACCTCTAC 3'	477
Retinoic acid receptor α	5' GCATCCAGAAGAACATGGTGT 3' 5' CTGCTTGGCGAACTCCACAGT 3'	392
Retinoic acid receptor β	5' GCAGGAATGCACAGAGAGCTAT 3' 5' GAAGGCCTGTTTCTGTGTCAT 3'	473
Retinoic acid receptor γ	5' GGCATGTCCAAGGAAGCTGT 3' 5' GTTCTCCAGCATCTCTCGGAT 3'	795
Progesterone Receptor	5' CTGGAAATTCAACACTCAGTG 3' 5' TGAGAAGAACCGACTGGACTTCG 3'	290
Transforming Growth Factorβ_3	5' ACCAACTACTGCTTCCGCAAT 3' 5' TGTTAGAGAGCTGCTCCACCT 3'	(317)
Oxytocin Receptor	5' GCTGCGTCCCTATGTGTATAAGGT 3' 5' TACCCGAGGTTTCGGATCACAA 3'	752
Prostaglandin $F_{2\alpha}$ Receptor	5' TTCACTGGGAAGATAGGTT3' 5' TCAGAAATAGCAGCAGCAACCTT 3'	446

Table 2. Optimal PCR conditions for amplification of glyceraldehyde-3-phosphate dehydrogenase (G3PDH), β-actin, retinol-binding protein (RBP), estrogen receptor (ER), retinoic acid receptors (RAR-α, β, γ), transforming growth factor β_3 ($TGF\beta_3$), progesterone receptor (PR), oxytocin receptor (OTR), and prostaglandin $F_{2\alpha}$ receptor ($PGF_{2\alpha}R$)

Optimal PCR conditions

Marker	$MgCl_2$ mM	dNTP's μM	Primer μM	Annealing Temp °c	Cycle Number	cDNA μL
G3PDH	2.5	0.5	1.0	60	30	0.5
β-Actin	3.75	0.5	2.5	65	30	0.5
RBP	2.5	0.5	2.0	55	30	0.5
ER	2.5	0.25	1.5	60	30	0.5
RAR-α	1.25	0.25	1.5	55	35	1.0
RAR-β	2.5	0.5	1.5	60	30	1.0
RAR-γ	1.25	0.25	1.5	60	35	1.0
$TGF\beta_3$	2.5	0.5	1.5	60	30	0.5
PR	2.5	0.5	1.5	65	35	0.5
OTR	2.5	0.5	1.5	65	35	0.5
$PGF_{2\alpha}R$	3.75	0.5	0.5	58	35	3.0

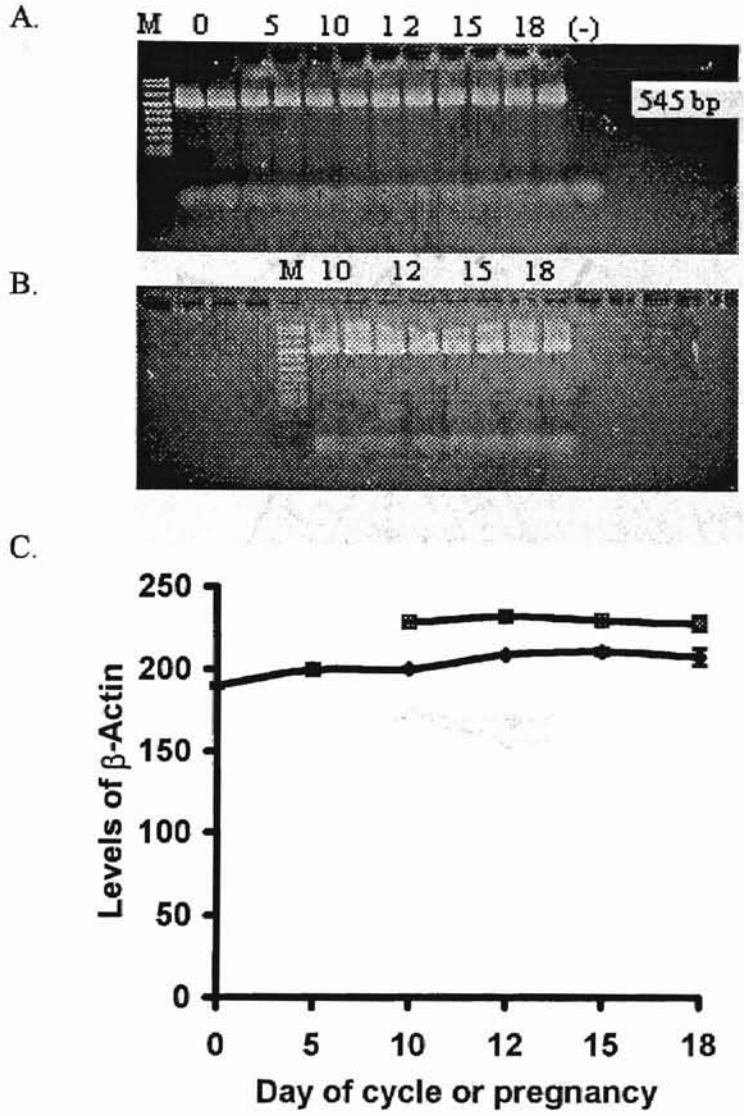


Figure 2. Ethidium bromide-stained gels for β-Actin cDNA in porcine endometrium on Days 0, 5, 10, 12, 15, and 18 (n=2/day) of the estrous cycle (A) and Days 10, 12, 15, and 18 (n=2/day) of pregnancy (B). The M lane contains the molecular markers: 1114, 900, 692, 501, 489, 404, 320, 242, 190, 147, 124, 110, and 67 base pairs, from the top to the bottom of the gel. Last lane in A is negative control (-). Corresponding graph (C) represents the calculated means from scanning densitometry data of the estrous cycle (♦) (MSE=34.68157) and pregnancy (■) (MSE=13.4239).

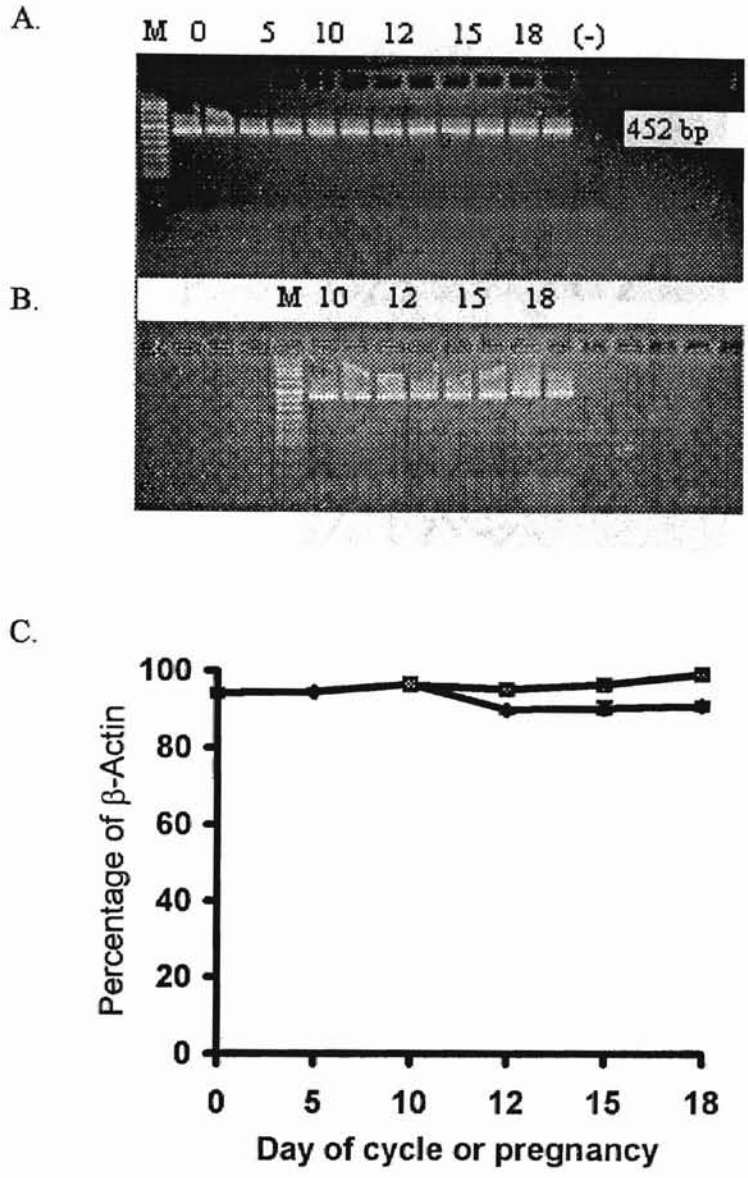


Figure 3. Ethidium bromide-stained gels for G3PDH cDNA in porcine endometrium on Days 0, 5, 10, 12, 15, and 18 (n=2/day) of the estrous cycle (A) and Days 10, 12, 15, and 18 (n=2/day) of pregnancy (B). The M lane contains the molecular markers: 1114, 900, 692, 501, 489, 404, 320, 242, 190, 147, 124, 110, and 67 base pairs, from the top to the bottom of the gel. Last lane in A is negative control (-). Corresponding graph (C) represents the calculated means from scanning densitometry data of the estrous cycle (♦) (MSE=2.2) and pregnancy (■) (MSE=2.25). Sample means are expressed as a percentage of β-Actin expression.

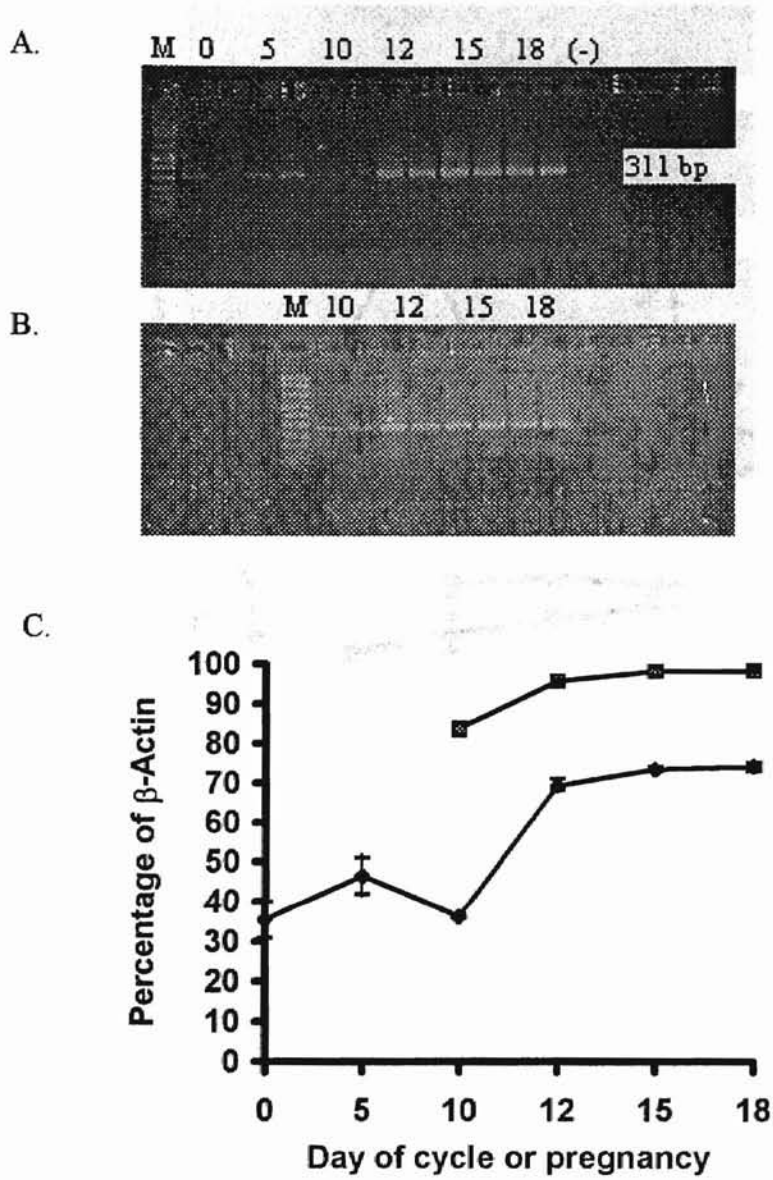


Figure 4. Ethidium bromide-stained gels for RBP cDNA in porcine endometrium on Days 0, 5, 10, 12, 15, and 18 (n=2/day) of the estrous cycle (A) and Days 10, 12, 15, and 18 (n=2/day) of pregnancy (B). The M lane contains the molecular markers: 1114, 900, 692, 501, 489, 404, 320, 242, 190, 147, 124, 110, and 67 base pairs, from the top to the bottom of the gel. Last lane in A is negative control (-). Corresponding graph (C) represents the calculated means from scanning densitometry data of the estrous cycle (♦) (MSE=14.75) and pregnancy (■) (MSE=1.875). Sample means are expressed as a percentage of β-Actin expression.

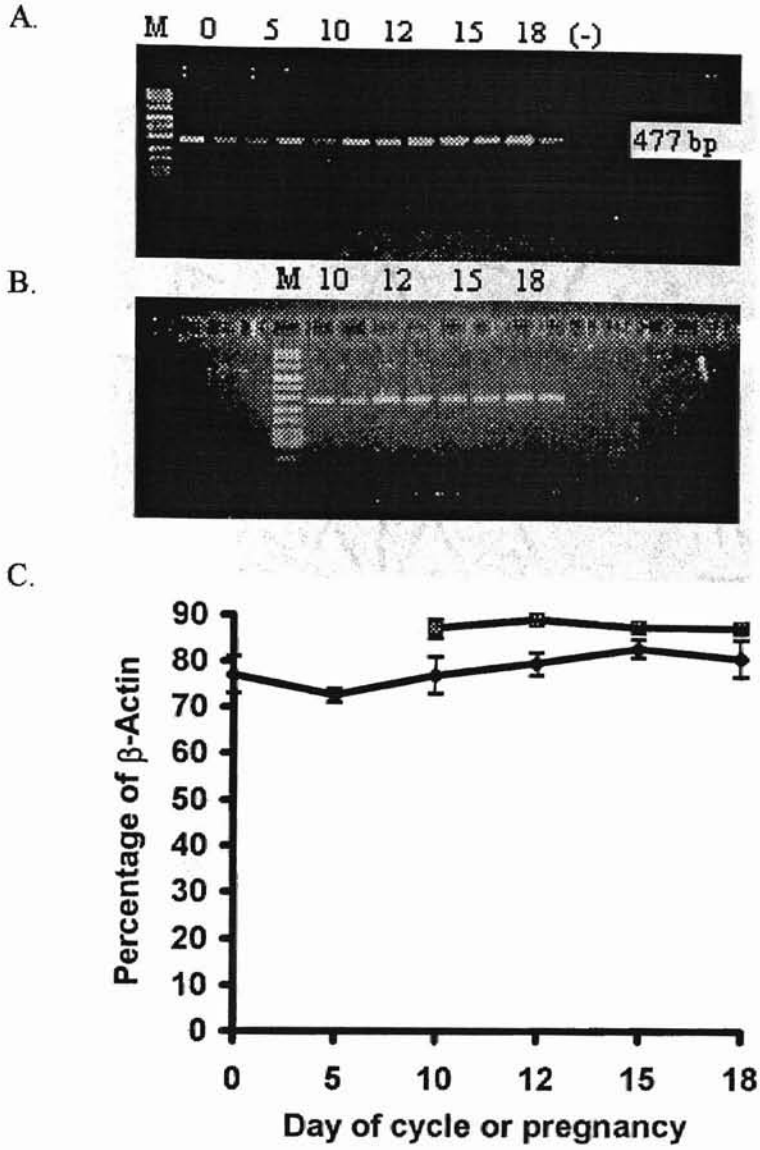


Figure 5. Ethidium bromide-stained gels for ER cDNA in porcine endometrium on Days 0, 5, 10, 12, 15, and 18 (n=2/day) of the estrous cycle (A) and Days 10, 12, 15, and 18 (n=2/day) of pregnancy (B). The M lane contains the molecular markers: 1114, 900, 692, 501, 489, 404, 320, 242, 190, 147, 124, 110, and 67 base pairs, from the top to the bottom of the gel. Last lane in A is negative control (-). Corresponding graph (C) represents the calculated means from scanning densitometry data of the estrous cycle (♦) (MSE=20.16667) and pregnancy (■) (MSE=2.25). Sample means are expressed as a percentage of β-Actin expression.

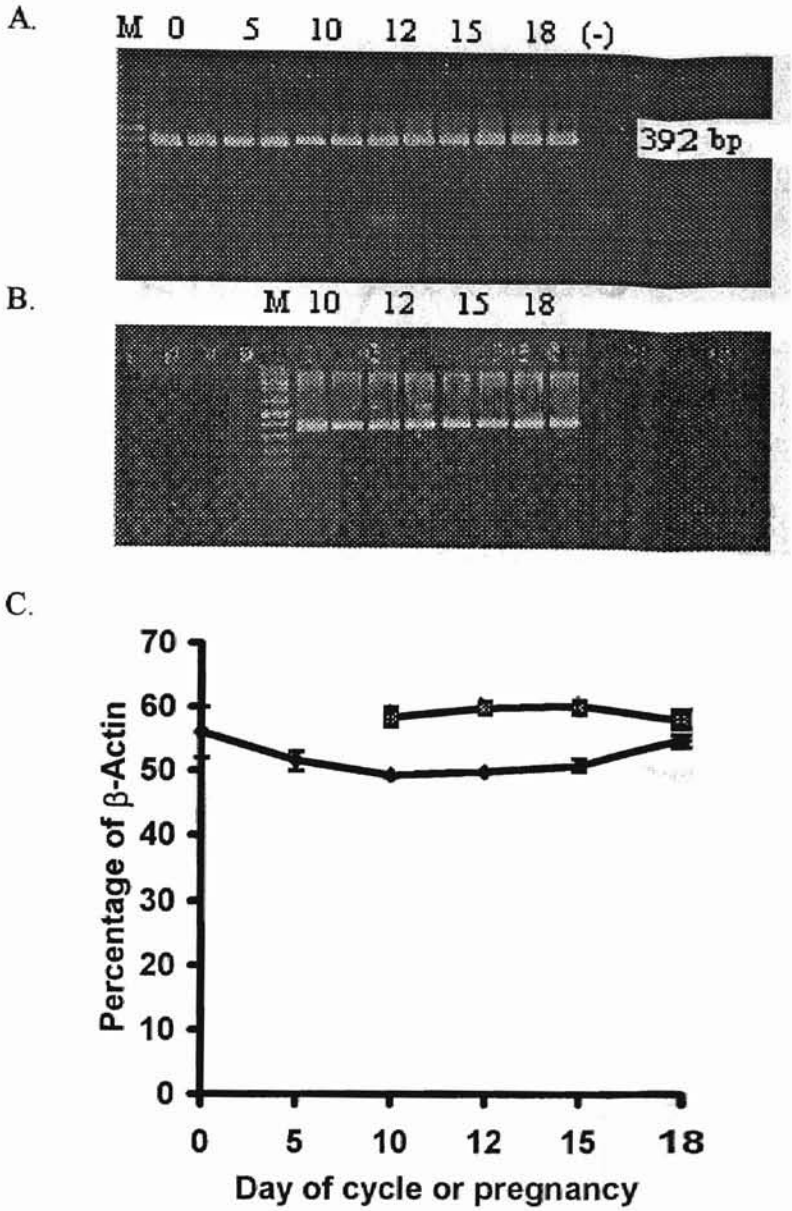


Figure 6. Ethidium bromide-stained gels for RAR-α cDNA in porcine endometrium on Days 0, 5, 10, 12, 15, and 18 (n=2/day) of the estrous cycle (A) and Days 10, 12, 15, and 18 (n=2/day) of pregnancy (B). The M lane contains the molecular markers: 1114, 900, 692, 501, 489, 404, 320, 242, 190, 147, 124, 110, and 67 base pairs, from the top to the bottom of the gel. Last lane in A is negative control (-). Corresponding graph (C) represents the calculated means from scanning densitometry data of the estrous cycle (♦) (MSE=6.75) and pregnancy (■) (MSE=3.25). Sample means are expressed as a percentage of β-Actin expression.

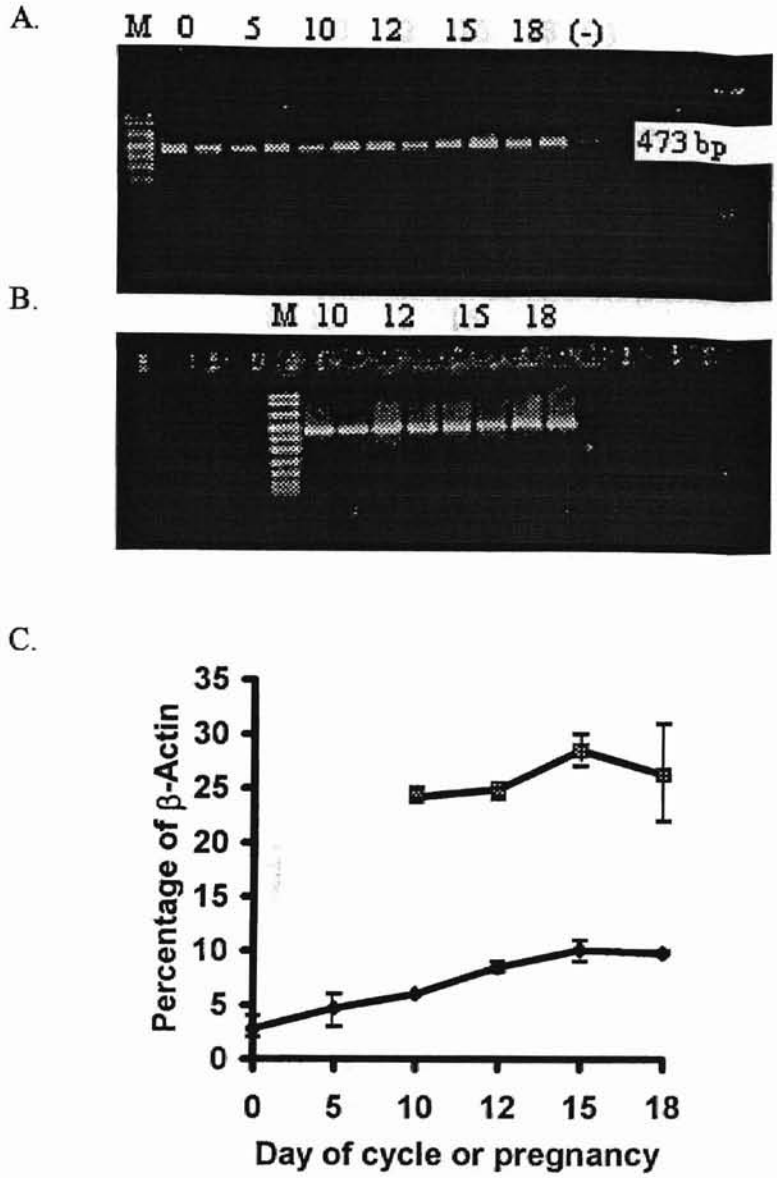
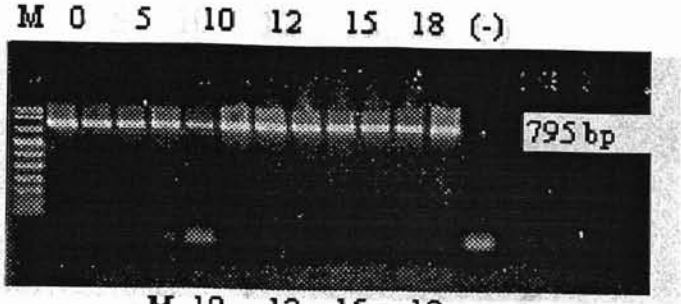
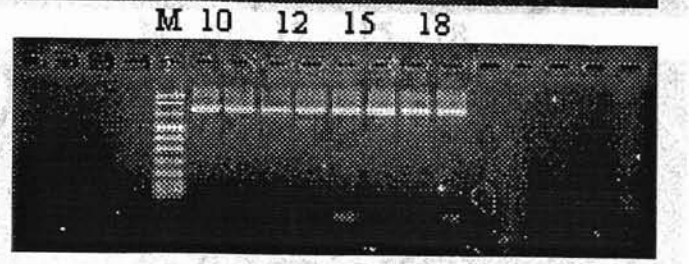


Figure 7. Ethidium bromide-stained gels for RAR-β cDNA in porcine endometrium on Days 0, 5, 10, 12, 15, and 18 (n=2/day) of the estrous cycle (A) and Days 10, 12, 15, and 18 (n=2/day) of pregnancy (B). The M lane contains the molecular markers: 1114, 900, 692, 501, 489, 404, 320, 242, 190, 147, 124, 110, and 67 base pairs, from the top to the bottom of the gel. Last lane in A is negative control (-). Corresponding graph (C) represents the calculated means from scanning densitometry data of the estrous cycle (♦) (MSE=1.5) and pregnancy (■) (MSE=11.5). Sample means are expressed as a percentage of β-Actin expression.

A.



B.



C.

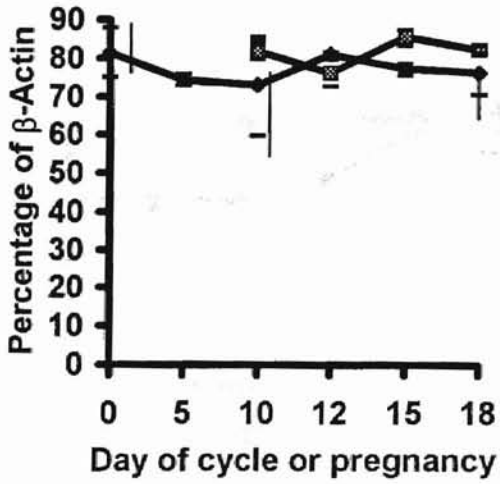


Figure 8. Ethidium bromide-stained gels for RAR-γ cDNA in porcine endometrium on Days 0, 5, 10, 12, 15, and 18 (n=2/day) of the estrous cycle (A) and Days 10, 12, 15, and 18 (n=2/day) of pregnancy (B). The M lane contains the molecular markers: 1114, 900, 692, 501, 489, 404, 320, 242, 190, 147, 124, 110, and 67 base pairs, from the top to the bottom of the gel. Last lane in A is negative control (-). Corresponding graph (C) represents the calculated means from scanning densitometry data of the estrous cycle (♦) (MSE=82.08333) and pregnancy (■) (MSE=11.75). Sample means are expressed as a percentage of β-Actin expression.

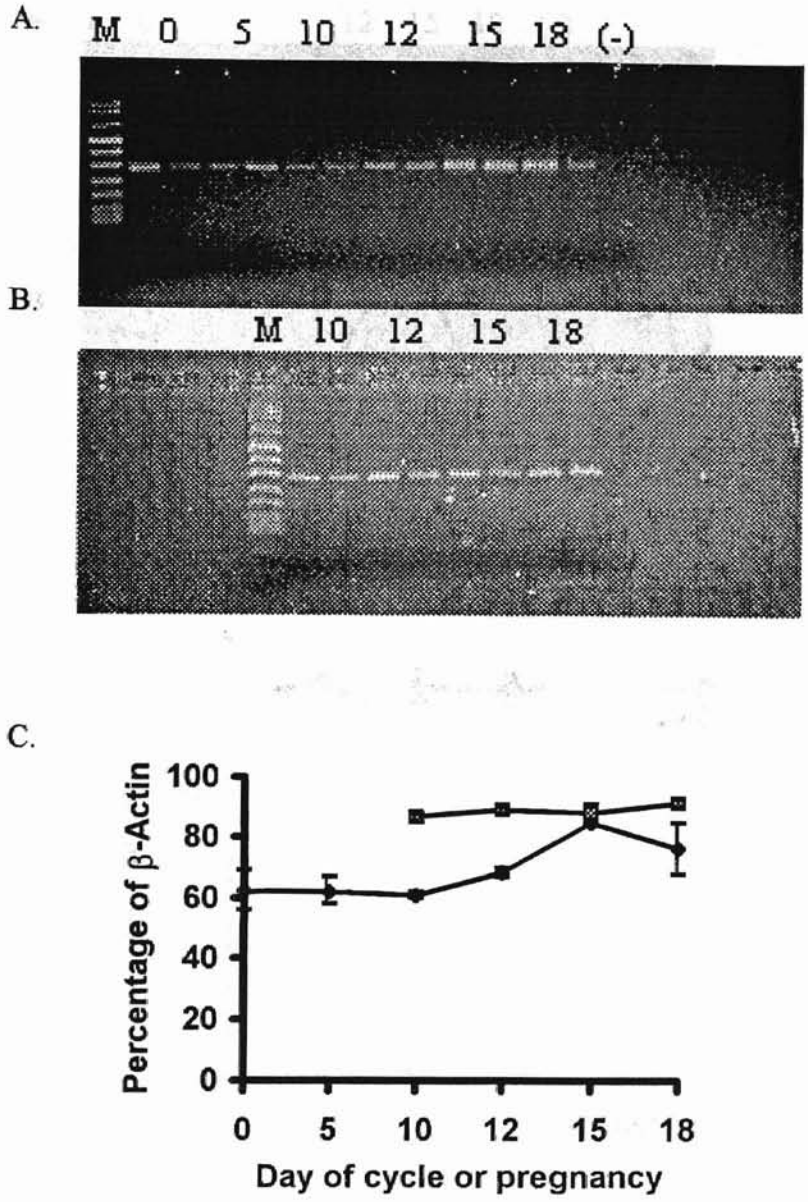


Figure 9. Ethidium bromide-stained gels for TGFβ_3 cDNA in porcine endometrium on Days 0, 5, 10, 12, 15, and 18 (n=2/day) of the estrous cycle (A) and Days 10, 12, 15, and 18 (n=2/day) of pregnancy (B). The M lane contains the molecular markers: 1114, 900, 692, 501, 489, 404, 320, 242, 190, 147, 124, 110, and 67 base pairs, from the top to the bottom of the gel. Last lane in A is negative control (-). Corresponding graph (C) represents the calculated means from scanning densitometry data of the estrous cycle (♦) (MSE=46.0833) and pregnancy (■) (MSE=5.875). Sample means are expressed as a percentage of β-Actin expression.

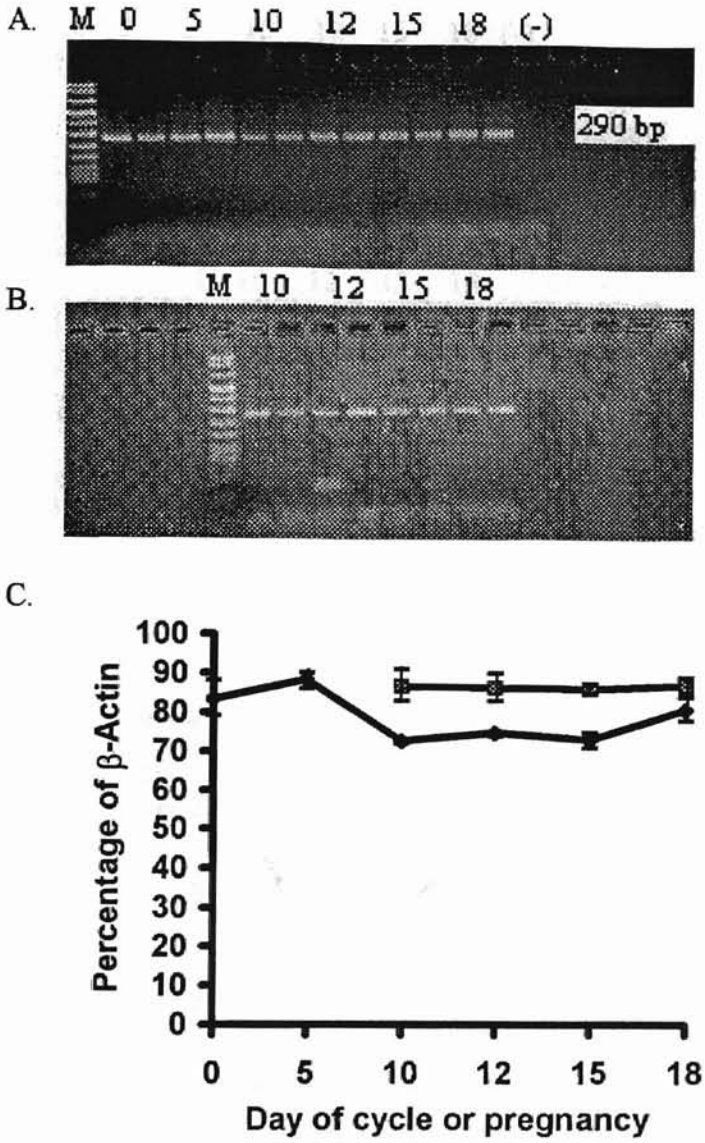


Figure 10. Ethidium bromide-stained gels for PR cDNA in porcine endometrium on Days 0, 5, 10, 12, 15, and 18 (n=2/day) of the estrous cycle (A) and Days 10, 12, 15, and 18 (n=2/day) of pregnancy (B). The M lane contains the molecular markers: 1114, 900, 692, 501, 489, 404, 320, 242, 190, 147, 124, 110, and 67 base pairs, from the top to the bottom of the gel. Last lane in A is negative control (-). Corresponding graph (C) represents the calculated means from scanning densitometry data of the estrous cycle (♦) (MSE=12.58333) and pregnancy (■) (MSE=16.625). Sample means are expressed as a percentage of β-Actin expression.

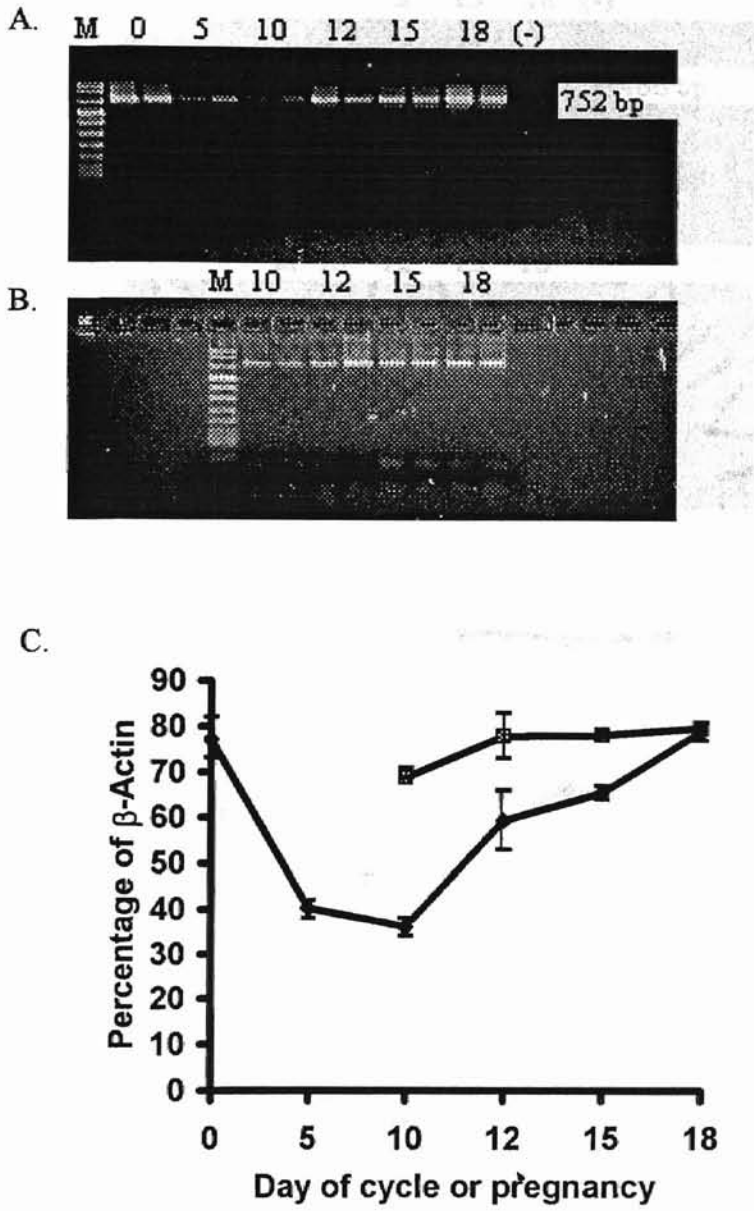
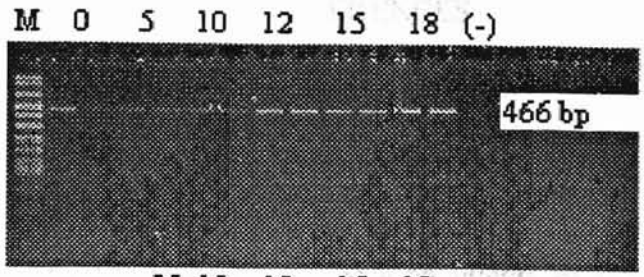
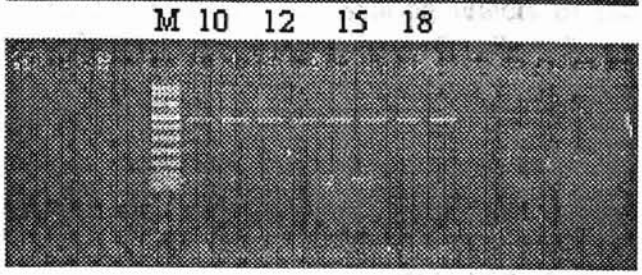


Figure 11. Ethidium bromide-stained gels for OTR cDNA in porcine endometrium on Days 0, 5, 10, 12, 15, and 18 (n=2/day) of the estrous cycle (A) and Days 10, 12, 15, and 18 (n=2/day) of pregnancy (B). The M lane contains the molecular markers: 1114, 900, 692, 501, 489, 404, 320, 242, 190, 147, 124, 110, and 67 base pairs, from the top to the bottom of the gel. Last lane in A is negative control (-). Corresponding graph (C) represents the calculated means from scanning densitometry data of the estrous cycle (♦) (MSE=25.58333) and pregnancy (■) (MSE=13.25). Sample means are expressed as a percentage of β-Actin expression.

A.



B.



C.

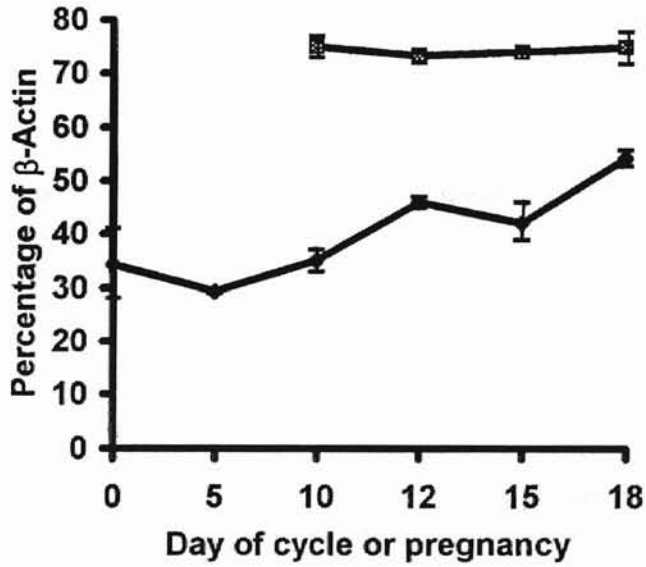


Figure 12. Ethidium bromide-stained gels for $PGF_{2\alpha}R$ cDNA in porcine endometrium on Days 0, 5, 10, 12, 15, and 18 (n=2/day) of the estrous cycle (A) and Days 10, 12, 15, and 18 (n=2/day) of pregnancy (B). The M lane contains the molecular markers: 1114, 900, 692, 501, 489, 404, 320, 242, 190, 147, 124, 110, and 67 base pairs, from the top to the bottom of the gel. Last lane in A is negative control (-). Corresponding graph (C) represents the calculated means from scanning densitometry data of the estrous cycle (♦) (MSE=20.58333) and pregnancy (■) (MSE=7.125). Sample means are expressed as a percentage of β-Actin expression.

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