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PHYSIOLOGICAL MECHANISMS MEDIATING NUTRITIONAL EFFECTS ON EMBRYONIC SURVIVAL IN THE PIG

by

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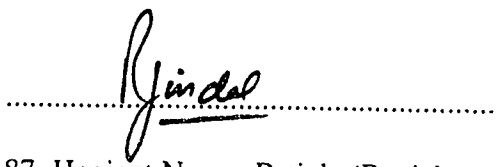

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The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **PHYSIOLOGICAL MECHANISMS MEDIATING NUTRITIONAL EFFECTS ON EMBRYONIC SURVIVAL IN THE PIG** submitted by RAJESH JINDAL in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Animal Physiology**.

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Dated 26/9/96

Dedicated to my family

Abstract

Effects of dietary intake on embryonic survival (ES) in the pig were studied in a series of experiments. In Experiment 1 (82 gilts), ES (84.7 v 64.5%) and plasma progesterone concentrations on d3 (10.5 v 4.5 ng/ml) were greater (P<.05) in gilts on restricted (1.5 x maintenance) feed intake from d1 of pregnancy compared to gilts continued on high plane of nutrition. Intermediate results were seen when feed restrictions were applied from d3. Irrespective of treatment, ES was greater and variance in ES lower with increased progesterone concentrations on d3. Thus, timing of nutritional changes after mating is critical, and progesterone could mediate nutritional effects on ES.

In Experiment 2 (52 gilts), a 10-h delay in the post-ovulatory rise in plasma progesterone was observed in gilts on 2 x (H) compared to 1.5 x (N) maintenance from d1 of pregnancy. Preliminary data suggested that nutritional effects on ES could occur even before embryos enter the uterus. Also, on d11-12 uterine plasmin/trypsin inhibitor (UPTI) activity was higher in H gilts, but no difference in insulin like growth factor-1 (IGF-1) concentrations in uterine flushings were seen between N and H gilts. In Experiment 3 (54 gilts), the hypothesis that exogenous progesterone administration can reverse the detrimental effects of high feed intake on ES during early pregnancy was tested. ES was higher (84.8 v 70.0%; P=.004) and less variable (P<.05) in gilts on a high feed intake after mating but given progesterone treatment (6 x 75 mg injections, 12-h intervals) from 24h after estrus onset than in control gilts.

A similar role of progesterone in mediating effects of lactational catabolism on ES in weaned primiparous sows was examined in Experiment 4. A significant relationship between ES and plasma progesterone on d3 and 4 (r=.59, .74, respectively; $P<0.05$) of pregnancy indicated an involvement of progesterone.

It is concluded that progesterone may mediate nutritionally-induced effects on ES in the pig by altering the oviductal and (or) uterine environment, and the timing of changes in feed allowance in gilts after mating is critical.

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Chapter I

General Introduction

Introduction

Success of a commercial swine breeding unit depends upon the reproductive efficiency of the breeding herd. Embryonic mortality in swine represents a major loss of productivity in that nearly 30% of eggs ovulated fail to materialize as live born piglets. This loss of valuable genetic material significantly slows down the rate of genetic progress and reduces number of piglets per sow, resulting in a major loss to the pork industry. In the pig, although embryonic mortality appears to be a normal process, it is not a biological inevitability, as in prolific breeds like the Chinese Meishan embryonic survival is higher than in the breeds commonly used in western countries. Also even a proportion of European sows have 100% embryonic survival. Therefore, a possibility of improving the reproductive performance of the pig has been recognized for many decades and has initiated several studies to determine the factors influencing the extent of embryonic mortality. Though various attempts to improve embryonic survival in the pig have been consistently unsuccessful and the mechanisms mediating effects on embryonic survival have not been clearly identified, these studies have largely contributed to present day knowledge of the regulation of early pregnancy. The underlying causes of early embryonic mortality are complex and may be attributed to a number of factors like genetic abnormalities, genetic variations in embryonic development, uterine infections, feeding practices, hormonal imbalance, etc., which by acting autonomously or in combination can alter the follicular development, the process of ovulation or fertilization, embryonic development and the oviductal/uterine environment, thus, causing an asynchrony between the embryo and uterus.

The fertilization of ova takes place in the ampulla of the oviduct. The zygote enters the uterus nearly 48h after ovulation, when it is at the 4-cell stage. The oviductal environment and passage rate of embryos, and possibly early embryonic development may be affected by gonadal steroids. After hatching from the zona pellucida, a blastocyst undergoes morphological changes from spherical to elongated and then to a filamentous shape. The process of attachment in the pig is superficial, and is completed by d26 of pregnancy. The pig trophoblast is able to produce large quantities of estrogen at d11-12 of pregnancy and these estrogens have a major role in the maintenance of pregnancy. Acting locally or systemically, these estrogens can prevent luteolysis (Bazer et al., 1982) and modify the uterine environment (Geisert et al., 1982, 1987), and the timing of uterine exposure to estradiol is critical for embryonic survival. At all times the embryo shows dynamic changes in its morphological and metabolic activities. The uterus in turn must be dynamic to recognize the presence and accommodate the changing needs of the embryo (Dziuk, 1987). A continual biochemical dialogue goes on between the uterus and developing embryo which is mediated through hormones, enzymes and other compounds (Heap et al., 1981; Ford and Stice, 1985). An embryo that fails to send or respond to a signal will not survive. Thus, any of the intrinsic or extrinsic factors that can slow down or enhance the embryonic, oviductal and (or) uterine development, or can modify the oviductal/uterine environment, can lead to an asynchrony between the embryo and oviduct/uterus. The asynchrony between the embryo and uterus is considered to be a major cause of embryonic mortality (Pope, 1988).

Recently, the role of dietary intake in gilts and sows as a factor influencing embryonic mortality and (or) litter size has received a great deal of attention, though the literature shows controversial results. Therefore, in a series of experiments reported in this thesis the effect of feeding levels during early pregnancy in the gilt, and during lactation in the primiparous sow on embryonic survival was studied, and possible mechanisms mediating these effects have been examined.

In this chapter, available literature is reviewed in order to have an understanding of the physiological changes occurring in the embryo, oviduct and uterus in relation to embryonic viability. The first section deals with the extent and timing of embryonic mortality. In the second section, literature dealing with early pregnancy has been reviewed to have some idea of the developmental process of an embryo during early pregnancy, changes taking place in the oviductal and uterine environment, asynchrony among embryos and (or) between the embryo and uterus, and thereby understand the potential causes of embryonic mortality in the pig. Finally, in the third section the effect of pre- and post-mating nutritional levels in the gilt, and dietary intake and metabolic body condition during lactation in the primiparous sow, on embryonic viability is reviewed, so as to develop hypotheses regarding possible mechanisms for these nutritional effects on embryonic mortality.

The first experiment (Chapter II) of the present series of studies deals with the effect of nutritional changes during the immediate period after mating on embryonic survival and plasma progesterone concentration during early pregnancy. It indicated that the timing of the change in feed allowance after mating is crucial to demonstrate nutritionally-induced effects on embryonic survival, and plasma progesterone

concentrations may mediate these effects. Having this information, Experiment 2 (Chapter III) further explored the role of plasma progesterone as a potential mediator of these effects on embryonic survival. The stage of pregnancy when nutritionally-induced effects on embryonic survival take place was investigated. Changes in uterine secretions (uterine environment) as a result of differences in dietary intake during early pregnancy were also studied in order to understand the mechanism by which feed-intake can affect embryonic survival. The third experiment (Chapter IV) determined the possibility of reversing the detrimental effects of high nutritional levels during early pregnancy on embryonic survival, by progesterone supplementation. Finally, Experiment 4 of the study (Chapter V) tested the hypothesis that in primiparous sows effects of dietary intake and the body condition during lactation on subsequent reproductive performance are also mediated by plasma progesterone concentration during early pregnancy.

The experiment presented in Chapter II is the extended form of the paper published as Jindal et al., 1996a. The data in Chapters III and IV (Experiments 2 and 3) have been submitted to JAS for publication. Results of the Experiments 2 and 4 (Chapters III and V) were presented at the 13th International Congress on Animal Reproduction, held in Sydney, Australia (Jindal et al., 1996b, Abst. P16-9), and the data from Experiment 4 (Chapter IV) were presented at the 29th annual meeting of The Society for the Study of Reproduction, held in London, Canada, (Jindal and Foxcroft, 1996, Abst. 327).

Literature Review

It is well documented that in the pig more than 30% of the eggs ovulated fail to materialize as live born piglets. This embryonic mortality reduces sow productivity, and the number of piglets born per year, thus representing a major loss to the pork industry. This embryonic loss is not inevitable, as a proportion of the pig population has 100% embryonic survival. Most embryonic deaths occur during early pregnancy, and can be attributed to a number of possible factors which can influence the viability of an embryo through various known/unknown mechanisms. Recently, pre- and post-mating nutritional status of the gilt has received much attention as one of the important factors. Therefore, in this section, the available literature on the extent and timing of embryonic loss and various events taking place during the oviductal and uterine phase of pregnancy which might influence embryonic loss is reviewed. Nutrition as an important factor affecting embryonic survival, and possible mechanisms mediating nutritionally induced changes in embryonic survival will also be discussed. The pig will be used as the main species for this discussion, though relevant literature from other species may be quoted occasionally.

(A) Extent and Timing of embryonic mortality

Litter size in any species mainly depends upon ovulation rate, fertilization rate and prenatal mortality. In pigs the fertilization rate is assumed to be 90-100% under normal conditions (Perry and Rowlands, 1962, Lambert et al., 1991), thus, prolificacy

is mainly determined by the number of ovulations and prenatal mortality. The number of ovulations in a female is the maximum potential litter size. As the fertilization rate is very high, any reduction in litter size below the number of eggs shed is mainly due to prenatal loss. Within a breed, prenatal mortality is 1.7 times as important as ovulation rate in determining the number of piglets born (Leymaster, et al., 1986). Van der Lende (1989) reported that 60% of the total variation in the number of embryos on d35 of gestation could be explained by differences in embryonic mortality. The extent of pre-natal mortality within and among pig populations varies from zero to 100%. It appears that embryonic loss is not a requirement for each litter, and in general is not influenced by the number of embryos. In various studies the number of embryos smaller than normal, transfer of super number of embryos or adding embryos to the existing litter could not eliminate this embryonic loss (Dziuk, 1968; Pope et al., 1972; Webel and Dziuk, 1974), though in 20% of litters embryonic survival (ES) is 100% (Dziuk, 1987). Recently, Van der Lende and Schoenmaker (1990) reviewed the data from 139 groups of gilts and sows obtained from 78 publications, which clearly indicated the extent and the variation in embryonic mortality among pig populations. The data related to studies with western breeds were later summarized (Van der Lende et al., 1994; Table I.1). In previous (Pharazyn et al., 1991a; Pharazyn,1992) as well as the present studies in our laboratory, included in this thesis, embryonic survival in gilts varied from 0 to 100%.

Prenatal mortality in swine occurs in two periods: embryonic (before d30 of pregnancy) and fetal (after d30), both determining the number of piglets farrowed. Previous studies by Scofield et al. (1974) and other reviews (Pope and First, 1985;

Table I.1. The means, standard deviations, and ranges for the number of corpora lutea, number of embryos, embryonic mortality and stage of pregnancy at slaughter for groups of gilts and sows of Western European breeds.

	Number of corpora lutea	Number of embryos	Embryo mortality	Stage of pregnancy, days
Gilts studies (n=52)				
Mean ± SD	13.5 ± 3.4	10.7 ± 3.1	20.9 ±14.9	27.6 ± 8.1
Range	10.2 - 16.4	8.0 - 13.9	9.5 - 38.3	20 - 35
Sows studies (n=15)				
Mean ± SD	16.4 ± 7.6	12.0 ± 4.8	26.5 ± 9.0	26.7 ± 7.9
Range	10.7 - 23.6	8.6 - 17.2	19.6 - 36.8	20 - 34

Reproduced from Van der Lende et al., 1994

Van der Lende and Schoenmaker, 1990) indicated that the major part of the embryonic mortality occurs before d18 of pregnancy; and that one third of the embryonic mortalities occur between d6 and 9, and two-thirds between d9 and 18. Recently, embryonic in crossbred pigs has been examined by Lambert et al. (1991) who reported that most of the mortalities (21%) occurred between d3 and 10 of gestation, nearly 13% between d10 and 30, and 3.2% after d30. These results are in agreement with the earlier data of Perry and Rowlands (1962) which demonstrated that nearly 22% of recovered embryos showed signs of degeneration before d10 of pregnancy, i.e., before or at the time of establishment of pregnancy and attachment of the embryo to the endometrium. As this initial period of pregnancy is crucial for embryonic survival, a clear understanding of the events taking place during early pregnancy is important.

(B) Early pregnancy in the pig

Mammalian oocytes have limited nutrients stored in the yolk which are sufficient to provide the energy and other materials required for the first few cleavage divisions (King and Thatcher, 1993). As cell numbers increase through the morula and blastocyst stages, the zygote must be able to absorb and metabolize maternal secretions in the oviductal and uterine fluid. A consideration of early pregnancy and embryonic development can therefore be divided into an oviductal and a uterine phase:

1. Oviductal phase

i) Early embryonic development:

Fertilization of porcine ova takes place in the ampulla of the oviduct, near the ampullary-isthmic junction (Hunter, 1977a). The embryo moves to the ampullary-isthmic junction within a few hours of fertilization and remains there for about 36h (Dziuk, 1985). Cleaving embryos have been found from 17-19 h after ovulation (Hunter, 1974). The 2-cell stage lasts for 6-8 h; however, the 4-cell stage normally lasts for about 20-24 h. The embryos are mainly in the 4-cell stage when they first enter into the uterus approximately 48h after ovulation (Hunter, 1974) or nearly 72h after estrus onset, however, the entry of the oocyte/zygote can extend over a 24-h period. Unlike species like the horse, even unfertilized eggs can enter the uterus along with fertilized ones. The time spent in the oviduct before entry into the uterus not only gives sufficient time to the embryo to develop and interact with the oviductal environment, but also to the uterus to undergo morphological and physiological changes to be receptive for the embryo.

In general the passage rate of the embryo through the oviduct depends upon the peristaltic contractions, beat of the cilia, flow of secretions, local prostaglandins and gonadal steroids (Hunter, 1977b). The passage rate may be greatly accelerated by administration of progesterone before ovulation (Day and Polge, 1968), whereas estrogen has been found to trap eggs in the oviduct for period of up to 21d (Dziuk, 1985) indicating the influence of ovarian hormones on the oviduct. In general, progesterone facilitates embryonic migration through the isthmus, by enhancing β-

adrenergic activity, whereas estrogen tends to restrict this movement by enhancing α-adrenergic activity in the smooth musculature of the oviduct, as reviewed in detail by Pharazyn (1992). Therefore, it can be expected that any changes in estrogen and (or) progesterone concentrations, or the ratio between the two will affect the time spent by an embryo in the oviduct. The physiological aspects of this phenomenon are discussed later in Chapters III and IV.

ii) Oviductal environment:

The oviduct and its secretions provide a biochemical environment which is essential for normal gametic and embryonic transport, capacitation of sperm, fertilization, and early development of the embryo. In addition, the oviductal fluid keeps the surface epithelium moist and may be bacteriostatic (Leese, 1988).

Progress in culturing embryos throughout their preimplantation stages at physiological rates is hampered by species specific 'blocks' to development. For example, the development of embryos from random-bred pigs is arrested at the 4-cell stage (Leese, 1988), suggesting that the oviduct contributes something which is missing in vitro. Bavister and Minami (1986) overcome the '2-cell block' in hamster zygotes for the first time by culturing them in the mouse ampullae, and Papaioannou and Ebert (1986) successfully used the immature mouse oviduct as a surrogate environment for rabbit and pig embryos.

Active secretion by the oviduct was first demonstrated by Bishop (1956) who reported that the volume and pressure of oviductal secretions in ovariectomized (OVX) rabbits were restored to those in the estrous rabbit oviduct by estradiol

injection. Both secretory volume and pressure were reduced during pregnancy. An increase in oviductal fluid formation was also seen on d1 after mating, which then declined. Mass et al. (1979) reported a pH value of 7.1-7.3 during the follicular phase with a sudden increase to 7.5-8.0 at ovulation and during the luteal phase in rhesus monkeys. The epithelial lining of the oviduct is also sensitive to the ovarian hormonal fluctuations that occur during the estrous or menstrual cycle and pregnancy (Verhage and Jaffe, 1988). Likewise, in the rabbit oviduct, the epithelial cell height and secretory activity increase during estrogen dominance, with the release of secretory granules occurring after coitus or progesterone administration (Leese, 1988). A morphological gradient of ovarian steroid responsiveness in the fimbria, ampulla and isthmus region of the primate oviduct has been reported by Brenner and Maslar (1988). All these studies indicate that ovarian estrogen and progesterone influence/control the morphological and secretory activity of the oviduct; thus, any change in the concentrations or timing of the release of these hormones can be expected to bring about changes in oviductal functions.

Several studies have established that oviductal secretory proteins (OSP) are synthesized de-novo and released. These OSP have been identified, defined and described in cattle (Malayer et al., 1988), sheep (Buhi et al., 1989a), swine (Buhi et al., 1989b), baboons (Verhage and Fazleabas, 1988) and humans (Verhage et al., 1988). It is suggested that OSP are produced in response to endogenous estrogen at estrus and during the follicular phase. Verhage and Fazleabas (1988) showed some specific protein in OVX baboon oviduct which appeared to be enhanced by estrogen treatment compared to control and progesterone treated baboons. Further, a differential synthesis

and release of 'estrus-associated proteins' (EAP) in the ampulla and isthmus at the time of fertilization and early cleavage-stage embryonic development has been reported in pigs (Buhi et al., 1990) and sheep (Murray, 1992, 1993). An antiestrogenic effect of nidatory progesterone modifying the activity of the enzyme involved in the glycosylation of steroid-regulated OSP was suggested by Murray (1993).

Although the functional significance of OSP has not been elucidated, they contribute to the extracellular matrix of the embryo by associating with the zona pellucida and to the exclusive microenvironment of the embryo by entering the perivitelline space, and in culture appear to be involved in a selection mechanism before first cleavage and regulate cell division and rates of blastocyst formation (Murray, 1993; Nancarrow and Hill, 1995).

The expression of mRNAs and proteins for growth factors and their receptors in oviductal tissue has also been indicated in the recent literature. The expression of IGF-1 mRNA in isolated porcine oviductal epithelial cells, and secretion of IGF-1 protein into their culture-conditioned media and oviductal fluid has been reported (Lee et al., 1992; Wiseman et al., 1992). Also, as reviewed by Chegini et al. (1994), the expression of EGF, TGF-α, EGF receptors, IGF-1, IGF-1 receptor and IGF-1 binding protein mRNAs, and the presence of immunoreactive EGF, TGF-α, TGF-β, IGF-1 and IGF-BPs have been reported. Considering the importance of various growth factors in other female reproductive tissues, an important role for these oviductal growth factors can also be expected, and any changes in their concentrations during early pregnancy can modify the oviductal environment, and possibly the development and viability of an embryo.

2. Uterine Phase

i) Embryonic development during the pre-attachment period:

After their entry to the uterus, the embryos remain in the vicinity of the tip of the uterine horn for up to d6 of pregnancy. By d6 the embryos are at the early blastocyst stage when they hatch from the zona-pellucida (Perry and Rowlands, 1962; Hunter, 1977a). Though the exact mechanism causing zona lysis is not clear, embryonic/uterine factors and mechanical mechanisms may be involved (Stroband and Van der Lende, 1990). The newly hatched pig blastocyst is about 0.2 mm in diameter and has 65-120 cells, 25% of which belong to the inner cell mass (Stroband and Van der Lende, 1990). A rapid expansion of this blastocyst to a diameter of up to 10 mm occurs between hatching and d11 (Stroband et al., 1984). From d11-12, they start elongating, initially mainly due to cell reorganization, and later on by hyperplasia, and a reduction in their diameter results in up to 100 cm long filamentous structures. Blastocyst growth may be as fast as 30-45 mm/h, but due to variation in the developmental stage, they can be seen in spherical, tubular or filamentous stages during this phase (Stroband and Van der Lende, 1990). Almost at the time of onset of elongation, the embryos develop aromatase activity and start to synthesize and secrete estrogens (Geisert et al., 1990). These embryonic estrogens have a very important role in the establishment and maintenance of pregnancy, as discussed later. Between d7-12, the embryos migrate through the uterine horns and redistribute themselves over the full length of both horns (Dhindsa et al., 1967). Both estradiol-17β and histamine are

involved in the intra-uterine migration process (Pope et al., 1982a) which seems to be a random process, even if the number of ovulations on both the ovaries are equal (Dziuk et al., 1964). By d13, the majority of the filamentous embryos possess an embryonic disc. Embryogenesis is initiated in some embryos by d15, and by d18 a primitive vasculature can be seen (Anderson, 1978).

The process of implantation is superficial and starts as a gradual attachment between the trophectoderm and uterine endometrium during or shortly after embryonic elongation (King et al., 1982). Small knob like protrusions of the uterine lumen, produced in response to the presence of a conceptus may aid their anchorage rather than invasion of maternal tissue (Dantzer, 1985). The elongated blastocysts become aligned along the mesometrial aspect of uterus, occupying almost the entire length of each horn, usually with no overlap. The process of attachment is completed over the entire surface of the conceptus by d26 of pregnancy (Amoroso, 1952).

ii) Establishment and maintenance of pregnancy:

Short (1969) first described the mechanism of the establishment of pregnancy as the 'maternal recognition of pregnancy'. The initiation of the maternal recognition events start soon after fertilization. The ability to differentiate between zygotes and unfertilized oocytes, with only the former being transported down the oviduct in the mare is a good example of the early maternal recognition of pregnancy (Betteridge and Mitchel, 1974). In several species, a platelet activating factor (PAF) can be extracted from recently fertilized oocytes (O'Neill, 1987), representing one of the first products of the conceptus which may be the initial signal responsible for 'maternal recognition

of pregnancy'. A pregnancy-dependent protein complex designated as 'Early Pregnancy Factor' (EPF) is detected in the plasma of pregnant pigs and other species during the first or second day of gestation (Koch, 1986). The fertilized egg is thought to release a small molecular weight compound that stimulates production of EPF-A by the oviduct which in turn is transported to the ovary, where it activates EPF-B production, and the two sub-units combine into a final complex (see review, King and Thatcher, 1993).This process represents the earliest interaction between the conceptus and dam, and possibly the true 'maternal recognition of pregnancy'. However, in one of the studies (Koch and Ellendorff, 1985) this activity was measured in 6 of 10 sows 24 h after mating with a vasectomized boar, though the tests were negative at 48 and 72 h post-mating. Thus, a simple and more practical interpretation, 'maternal recognition of pregnancy' can be defined as the method by which the conceptus signals its presence to the uterus, resulting in prolongation of the functional life span of the corpus luteum and maintenance of pregnancy. In cattle and sheep the conceptus factor responsible for inducing an antiluteolytic-anti $PGF_{2\alpha}$ effect has been defined as bovine trophoblastic protein-1 (bTP-1) and ovine trophoblastic protein-1 (oTP-1), respectively (Godkin et al., 1984; Helmer et al., 1987), which belong to an interferon family (see Roberts, 1989). The trophoblast interferons α and gamma have also been found and stratified in the pig but their specific roles as a luteotrophic factor is not yet certain (La Bonnardiere, 1993). Although, $PGF_{2\alpha}$ is the natural luteolysin in pigs also, the regulatory mechanism for maintenance of pregnancy is the estrogen produced by the early conceptus (Perry et al., 1973; Robertson and King, 1974), and this estrogen is

believed to initiate the events which result in the maintenance of the corpus luteum (CL).

The role of endometrial $PGF_{2\alpha}$ synthesis and release in the luteolytic process has been reviewed extensively (Bazer et al., 1982, 1984). Administration of $PGF_{2\alpha}$ 12 days after onset of estrus in cyclic or pregnant gilts initiates CL regression; however, before d11 of the cycle the CLs appear to be refrectory to $PGF_{2\alpha}$. After d11, an increase in the number of $PGF_{2\alpha}$ receptors in the luteal cells can result in increased CL sensitivity to $PGF_{2\alpha}$ (Gadsby et al., 1988). Release of $PGF_{2\alpha}$ from a non-pregnant uterine horn affects CL function on the ipsilateral (local effect) as well as contralateral ovary (systemic effect) (Anderson et al., 1966) and embryos must be present in both uterine horns between d10-12 to prevent luteolysis (Polge et al., 1966).

iii) Production and role of conceptus estrogens:

By d11 of pregnancy, spherical blastocysts, 5-7 mm in diameter, can synthesize estrogen, with maximal output at the time of early elongation (Heap et al., 1979). Estradiol concentrations in conceptus tissues are nearly fifty-fold higher at d12 than d14 of development (Conley et al., 1992). Within the filamentous blastocysts, regional differences exist in the synthesis of estrogen, with the tissue adjacent to the embryonic disc having the greatest synthetic capability (Bate and King, 1988). A physiological role for estrogen in the maintenance of pregnancy was originally shown by Perry et al. (1973). It has been since shown that maintenance of prolonged CL function requires two phases of estrogen stimulation, the first at d11 and the second more prolonged phase between d14-18 (Geisert et al., 1987). The uterine content of estrogen increases

on d11-12, declines on d13-14, and then shows a sustained increase after d14 (Zavy et al., 1980; Stone and Seamark, 1985). The conceptus estrogens have a systemic, as well as local endometrial, effects (Ford et al., 1982a; Bazer et al., 1986, 1989). The timing of trophectodermal estrogen production coincident with the rapid elongation of the trophoblastic membrane allows the conceptus to stimulate locally a large surface area of endometrium (Geisert et al., 1990).

iv) Mechanism of action:

The initial increase in conceptus estrogens at the time of the trophoblast elongation stimulates a rapid release of calcium into the uterine lumen; this is associated with release of glandular secretory vesicles, an increase in total uterine protein, uteroferrin and plasmin inhibitors and thus alters the uterine environment (Geisert et al., 1982). A similar increase of calcium, protein and uteroferrin (Geisert et al., 1982), and maintenance of the CL (Geisert et al., 1987) can be achieved in cyclic gilts by administering exogenous estrogen on d11 of the estrous cycle. Initially, the conceptus estradiol creates an advanced microenvironment in the blastocyst's immediate surroundings: Later as the quantity of estradiol synthesized increases, the advanced environment becomes more generalized. There is a transient increase in the blood flow to the gravid uterus (Ford and Christenson, 1979; Ford et al., 1982b) and ovaries (see Thatcher et al., 1986), and also an increase in uterine vascular permeability through increased fenestration of the subepithelial capillaries of the endometrium (Keys and King, 1988) at the time of the blastocyst elongation and coincident with the second phase of estrogen release. These effects may in fact be

mediated through conversion of estradiol to catechol-estrogen, a short lived estrogen metabolite (Mondschein et al., 1985) and possibly by local stimulation of endometrial PGE production which would facilitate transcapillary transport of nutrients required for conceptus development (Geisert et al., 1990). The timing of uterine exposure to estradiol is critical and has differential effects on embryonic survival (Long and Diekman, 1986). Premature exposure to estradiol alters the uterine endometrial surface, thereby preventing the normal attachment of the embryos (Blair et al., 1991). Although estrogens can have a direct embryocidal effect on early embryos before the time they elongate, it seems more probable that the effect is due to acute changes in the uterine milieu. As will be discussed later, the less developed embryos can not tolerate an advanced uterine environment, and this is considered to be the major cause of embryonic mortality (Pope, 1988). After comparing the embryonic estradiol-17β ($E_2$17β) synthesis by Meishan with Yorkshire breed Anderson et al. (1993) proposed that lower $E_2$17β production by Meishan embryos may result in more gradual alterations in the uterine environment and an increase in the survival of less developed embryos.

As has been extensively reviewed (see Bazer et al., 1982, 1986, 1989), endometrial $PGF_{2\alpha}$ is responsible for the luteal regression in the pig. It has been proposed (Bazer and Thatcher, 1977) that the conceptus estrogens interact with the uterine endometrium to alter the pattern of $PGF_{2\alpha}$ secretion from an endocrine to an exocrine pathway. Thus, the amount of $PGF_{2\alpha}$ entering the uterine venous drainage and reaching the CL is not sufficient to cause luteolysis. In the non-pregnant pig, luteal progesterone production enhances PGF synthesis by the uterine endometrium and its

release is mainly endocrine. In contrast, in the pregnant pig, although progesterone-induced PGF synthesis is similar as in non-pregnant animals, secretion is controlled by conceptus estrogen which alters the direction of PGF release to an exocrine direction, i.e., towards uterine lumen, thus preventing PGF entering the uterine venous drainage (Fig. I.1) and exerting a luteolytic effect on the CL (Bazer et al. 1982). The precise mechanism of this change in the direction of $PGF_{2\alpha}$ by conceptus estrogens is not clear. An involvement of estrogen-stimulated release of endometrial calcium is proposed, as treatment with calcium ionophores shifted orientation of $PGF_{2\alpha}$ secretion to the luminal side during in-vitro perifusion (Mirando et al., 1988). Further, orientation of prostaglandin secretion into the uterine lumen involves an interaction between estrogen and prolactin (Young and Bazer, 1988). Neither estrogen nor prolactin alone alters $PGF_{2\alpha}$ secretion (Mirando et al., 1988). A supportive, direct luteotrophic effect of the conceptus estrogens on the CL (Ball and Day, 1982; Conley et al., 1989) is also possible.

v) Uterine environment:

During early gestation, the uterine environment undergoes rapid changes in response to maternal steroids and secretory products of the conceptus in order to provide nutrients, protection, and other factors required for normal development of the embryo and maintenance of a successful pregnancy. For this purpose a continuous exchange of information between the uterus and the developing embryo is essential. A conceptus which is out of phase with the uterine environment, may not be able to develop normally and may be lost. At least some of the factors affecting embryonic

survival may exert their effects by changing the uterine mileu and (or) a proper synchrony between the embryo and uterus. Fig. I.2 shows tissues contributing to the uterine luminal environment and the potential interactions among them. Major secretory products in the histotrophe and their functions are reviewed in the following section.

vi) Endometrial secretory products:

a. Uteroferrin: Uteroferrin, Mr 35000, is one of the most abundent glycoprotein, synthesized and secreted by epithelial cells of uterine glands. With its high binding affinity for iron (two atoms per molecule) uteroferrin acts as major iron carrier from the uterus to the developing fetus for its haemoglobin synthesis (Roberts et al., 1986, 1993). It is taken up by the specialized absorptive cells of the placental areolae and after entering the fetal circulation via the umblical vein, is distributed in sites of iron metabolism such as liver and spleen (Renegar et al., 1982). Biosynthesis and secretion of uteroferrin is mainly under the control of progesterone but at low doses estrogen acts synergistically (Roberts and Bazer, 1988). The highest concentrations of uteroferrin mRNA were observed at mid and late pregnancy, with detectable but very low levels during early pregnancy. Uteroferrin protein in the uterine content follows the same pattern as the mRNA from early to mid pregnancy, but levels are low during late pregnancy (Simmen et al., 1988).

b. Growth factors: Insulin like growth factor-I (IGF-I) is a potent mitogen which can promote cell division, cell differentiation and tissue morphogenesis by endocrine, autocrine and paracrine mechanisms (Zapf and Froesch, 1986). High concentrations of IGF-1 mRNA are reported in pig uterine tissue around d12, with low but detectable levels in mid and late pregnancy (Tavakkol et al., 1988). IGF-I synthesis and secretion into the uterine lumen may be influenced by blastocyst derived estrogens as maximal concentrations of IGF-I in uterine luminal fluid are seen at d10 and 12 of pregnancy, temporally coinciding with blastocyst elongation and estrogen synthesis (Simmen and Simmen, 1990; Simmen et al., 1993). Pre-implantation stage pig embryos display type-I IGF-receptors. Keeping in view its role in conceptus development and timing of secretion, in the present studies we estimated IGF-I content in uterine flushings on d11 or 12 of pregnancy as one of the parameters in the Experiment 2, Phase 2 (Chapter III).

IGF-II originates from the luminal and glandular epithelium and stromal cells, and is considered as a fetal growth factor. Uterine IGF-II mRNA concentrations are very low during the pre-implantation phase, with a more than a 10-fold rise by d30, and then the level declines at d90-110 (Simmen and Simmen, 1990). Binding of IGF-I and II to their plasma membrane receptors is modulated by the binding proteins (Rutanen et al., 1988). Expression of IGFBP-2, but not IGF-II, is induced by progesterone (Davis and Blair, 1993). On d12, a small, transient rise in IGFBP-3A mRNA in the uterus was seen to be coincident with increased IGF-I mRNA expression (Simmen and Simmen, 1990).

Uterine luminal fluid mitogen (ULFM), Mr 4800, is another growth factor present in cyclic and early pregnant sows (Simmen et al., 1989), with highest activity

at d8, which then declines at d11-14. The decline in the activity of ULFM during the elongation phase of the embryos is proposed to be due to its re-routing from lumen to stromal cells under the influence of conceptus derived estrogens (Simmen and Simmen, 1990).

Epidermal growth factor-like growth factor, Mr <10,000, has been identified from d12 pregnant sows. This growth factor may be utilized for conceptus developmental needs (Simmen and Simmen, 1990), and also induces endometrial growth and differentiation. Receptors for EGF are present on both stromal and glandular epithelial cells (Zhang et al., 1992).

c. Uterine plasmin/trypsin inhibitor (UPTI): UPTI, one of the Kunitz class of protease inhibitors, Mr 14,000, has been purified from the pig uterine secretions (see review Roberts and Bazer, 1988). Like uteroferrin, UPTI synthesis and secretion is also progesterone-responsive, however, estrogen may act synergistically to modulate the events (Roberts et al., 1993). Injection of estradiol valerate on d12-14 increased the release of these inhibitors into the uterine lumen (Fazleabas et al., 1982; Young et al., 1987), indicating the role of blastocyst-estrogens as a stimulus for UPTI release into the lumen. Between d10-16 of pregnancy, pig blastocysts release high amounts of plasminogen activator (Fazleabas et al., 1983). Plasminogen activator activates plasminogen to plasmin which is proteolytic in nature and is involved in cellular remodelling and morphogenesis of the blastocyst (Bode and Dziadek, 1979). Increased activity of UPTI during this period maintains uterine epithelial integrity by controlling the proteolytic activity of plasmin and preventing invasion of the otherwise potentially

invasive pig trophoblast. As UPTI synthesis is mainly regulated by progesterone, in Experiment 2 of the present studies luminal UPTI was measured in gilts with nutritionally-induced differences in plasma progesterone concentrations during early pregnancy (Chapter III).

d. Other contents: Antileukoproteinase (Simmen and Simmen, 1990), uterine-associated basic proteins (Baumbach et al., 1986), lysozyme (Roberts et al., 1976) and retinol binding proteins (Adam et al., 1981; Harney et al., 1993) are some other progesterone dependent products characterized in the pig uterus. In addition, all cell types of the uterus secrete prostaglandins. Several other components like glucose, fructose, ascorbic acid, riboflavin, calcium and acid phosphatase have also been isolated from the pregnant pig uterus (Zavy et al., 1982)

vii) Secretory products of the conceptus:

In addition to its steroidogenic activity, the pig embryo can synthesize and release various proteins whose nature changes with the age and morphological development of the blastocyst. It has been demonstrated that the pig conceptus secretes both type I (IFN-α)- and type II (IFN-γ)-like interferons during the early elongation phase of development (see La Bonnardiere, 1993). In ruminants, trophoblastic IFN or trophoblastic protein-1 (oTP-1 in sheep and bTP-1 in cattle) exert hormone-like effects through receptors present on the endometrium, and produce an anti-luteolytic effect by suppressing the synthesis and release of $PGF_{2\alpha}$ (Bazer and Johnson, 1991). However, in pigs these cytokines have not been implicated in protecting the CL but in addition to

their antiviral activity (Mirando et al., 1990), exert multiple effects on cells of immune system in a paracrine manner (see La Bonnardiere, 1993).

A major protein released by the pig conceptus during early pregnancy (d10-12) is retinol binding protein (Roberts et al., 1993) which is involved in the transport of retinol to the embryo (Adam et al., 1981). The synthesis of retinol binding protein by the conceptus appears to precede uterine retinol binding protein-mRNA expression (Trout, 1992). The conceptus appears to signal uterine secretion of retinol binding protein, suggesting a high demand for retinol at this stage of development (Roberts et al, 1993) for extensive tissue remodelling and growth of extra embryonic membranes. Retinoic acid is known to control genes involved in embryonic organization in mice (Simeone et al., 1990). Further, supplementary vitamin A and β-carotene have been reported to improve embryonic survival in gilts (Brief and Chew, 1985) and sows (Coffey and Britt, 1993).

The pig conceptus also produces prostaglandins whose concentration in the uterine luminal fluid increases by 10-fold during the attachment period (Davis and Blair, 1993). In addition, as indicated by in-vitro studies, a number of other hormonally-regulated proteins are also produced by the pig conceptus which remain to be identified.

There is a continual interaction between the developing embryos and the uterus, whereby they modify each others morphological and secretory activities (Geisert et al., 1990, 1992). A simplified schematic representation of the complex relationships is presented Fig. I.3. Though there is lack of a very clear understanding of the role of individual factors, all of them may be assumed to contribute to

maintenance of a proper synchrony between the developing embryo and the uterus, and thus, a successful pregnancy.

3. Asynchrony between embryo and the uterus and within litter diversity in relation to embryonic survival

As already mentioned, the uterus undergoes a number of morphological and physiological changes to provide a proper environment for normal development of an embryo and successful maintenance of pregnancy. At the same time the conceptus products, together with the ovarian steroids, not only prolong life span of the CL, but also increase uterine arterial blood flow and vasculature permeability, and modify uterine secretory activites. Thus a continual biochemical dialogue goes on between the two. Therefore, for a successful pregnancy, it is very essential that the endocrine and conceptus products are released in appropriate amounts and at an appropriate time.

Various studies in different species have shown that for successful embryo transfer procedures, estrus should be synchronized in the donor and the recipient animal, indicating the need for a proper synchrony between the uterus and embryo. In sheep transfer of d4 embryos to a d7 uterus resulted in an accelerated growth rate until d12, but failure of implantation thereafter (Lawson et al., 1983). In pigs advancement of the uterus by estradiol injection on d9 and 10 of pregnancy resulted in failure in embryonic survival beyond d14 (Gries et al., 1989). In pigs, there is considerable developmental variation among littermates during the pre-implantation period (Anderson, 1978; Papaioannou and Ebert, 1988), and the embryos at a more mature stage of development have a higher survival rate than these less developed embryos

(Pope et al., 1982b). In experiments involving transfer of d5 and d7 embryos to d6 recipients, the more developed embryos had a better embryonic survival (Pope et al, 1982b); however, in the absence of older embryos, the less developed embryos survived and developed normally (Pope et al., 1986). Thus it seems that the uterus will not wait for an embryo to become synchronous, however, the reverse is possible (Pope, 1988).

Relatively advanced embryos secrete greater amounts of estradiol than their retarded litter-mates (Pope, 1988), thus inducing a uterine environment which might be embryocidal for the slower growing embryos. The indirect embryocidal effect of these estrogens is clear from experiments in which exogenous administration of estrogens around d10 resulted in mortality of less developed embryos at the onset of implantation (Pope et al., 1986; Geisert et al., 1991).

An alternative suggestion is that the embryos compete for some critical biochemical substance(s) that is necessary for their growth, and the more developed embryos sequester that more efficiently than the retarded ones (Bazer, 1968). Recently Roberts et al. (1993) proposed that retinol could be the required substance, and the competition for retinol may be one of the causes of embryonic loss. Retinol seems to be required for normal cell division, proper organ development and for growth of the placenta (Thompson and Pitt, 1964), and according to the hypothesis, the advanced embryos are competent to signal their needs by synthesizing estradiol at an earlier stage. Also, the more developed conceptuses secrete large quantities of apo-retinol-binding-protein (apo-RBP), possibly as a result of an autocrine effect of estradiol, thus they are able to protect themselves from exposure to the high concentrations of retinol

in the uterus at that time. In contrast, the less developed embryos, secreting insufficient apo-RBP, can die owing to premature and inappropriate gene expression resulting from intracellular conversion of retinol to retinoic acid and its derivatives.

It appears that relatively delayed embryos may not be necessarily inherently defective (less viable). Rather they interact with a uterine environment that is not favorable for their survival. Thus, anything that would advance or retard the development of either the uterus or the embryo potentially influences the proportion of embryos that survive. Based upon the embryo transfer experiments quoted above, and also some investigations with highly prolific Meishan pigs (Bazer et al., 1988), under normal conditions greater uniformity among the litter-mates seems to improve embryonic survival, and possibly the litter size.

Causes of the asynchrony among littermates:

The physiological significance of follicular heterogeneity was first discussed by Foxcroft and Hunter (1985). In a series of studies in the cyclic gilt and lactating and weaned sow (Foxcroft et al, 1987; Grant et al., 1989; Hunter et al, 1989) evidence was presented for considerable within animal variation in the development of preovulatory follicles. Considerable variability exists in the development of porcine follicles at the time of recruitment and it was suggested that this is carried over into the pattern of ovulation and embryonic development. Subsequently, follicular development and the pattern of oocyte maturation within gilts was reported to be skewed, so that the majority (76%) of the follicles and the oocytes is more developed than a lesser developed minority of 24% (Xie et al., 1987, 1990a). Basically all the follicles have

the same intrinsic viability, but this skewed follicular development and oocyte maturation may result in differences in the pattern of ovulation (Fig. I.4). Electrocautery of the late maturing follicles reduced the number of lesser developed embryos (Pope et al., 1988). In earlier studies, Hunter (1972) observed a similar pattern of ovulation in gilts injected with human chorionic gonadotropin (hCG) and suggested that a majority of the follicles ovulated over a short period of time, while remaining minority of follicles ovulated over a more protracted interval. Later, in the experiments by Xie et al. (1990b), the first or last ovulating oocytes were removed from hCG-treated sows, stained and then placed in the oviduct. After 75 h, the first ovulating oocytes produced the most developed and the last ovulating oocytes were the less developed embryos. In other gilts, the effect was carried over into embryonic development to d12 (Xie et al., 1990b). However, the duration of ovulation, as observed by transrectal ovarian ultrasonography, was not found to affect embryo diversity (Soede et al., 1992)

As previously mentioned, gamete and zygote transport in the oviduct is influenced by estrogen and progesterone concentrations. So, as discussed in subsequent chapters, any factor, affecting the concentrations of these hormones can potentially affect the embryonic stay in the oviduct, and possibly the rate of development. Also, small amount of follicular $PGF_{2\alpha}$ can enter the oviduct at the time of ovulation and increase oviductal motility, and hence oocyte transportation.

Time to fertilization seems not to be affecting embryonic diversity in gilts mated shortly before ovulation (Xie et al., 1990c). However, as suggested by Soede and Kemp (1993), extended duration of fertilization as a result of a low accessory

sperm count can be associated with increased embryonic diversity. A shorter duration of the ovulatory process has been reported in Meishan pigs, known for lower embryonic mortality as compared to European breeds.

Although, within litter embryonic uniformity was suggested as a determinant of high prolificacy of Meishan pigs (Bazer et al., 1988), later studies did not support this hypothesis (Anderson et al., 1991; Wilmut et al., 1992). More recently, advanced follicular (Biggs et al., 1993) and oocyte maturation in the follicles (Faillace and Hunter, 1994) and slower blastocyst growth (Youngs et al., 1993), in Meishan as compared to other European breeds, have been suggested as contributing factors to greater embryonic survival.

(C) Nutrition as an important factor influencing embryonic survival in pigs

The nutritional status of the pig is one of the important factors seen to affect the viability of an embryo. Available literature on the relationship between feeding levels of the gilt and lactating sow and embryonic survival will be discussed in this section.

1. Feeding level and embryonic survival in the gilt

The role of nutrition during early pregnancy has received a great deal of attention, but with inconsistent results. Historical data indicate apparent conception

failure or low conception rates in gilts or sows on low feed and protein levels at the time of mating (King and Young, 1957; Baker et al., 1969; Hawton and Meade, 1971). In some of the studies, fasting of gilts for 1,2 or 3 days post-mating had no effect on embryonic survival (Ray and McCarty, 1965). Similarly, fasting of sows from 10 days before to 14 days or even up to 30 days after mating had no effect on embryonic survival (Anderson, 1975). However, in other studies feeding management during the 3-week period before mating resulted in an increase in embryonic survival (Dyck, 1974). The nutritional status of a gilt during both the pre- and post-mating period can influence both the ovulation rate and survival of pre-implantation stage embryos. A high level of dietary intake for at least 4-5 days before mating ("Flush-feeding") maximizes ovulation rate (den Hartog and van Kempen, 1980; Aherne and Kirkwood, 1985; Beltranena et al., 1991). However, continuation of this high level of feeding after mating may have a detrimental effect on embryonic survival (Gossett and Sorenson, 1959; Dyck et al., 1980, Dyck and Strain., 1983; Ashworth, 1991; Pharazyn, 1992). In some of the studies where high feed intake was maintained from the pre-mating through post-mating period (Sorenson et al., 1961; Frobish, 1970), the reported lower percent embryonic survival may be associated with an increased ovulation rate as an inverse relationship between very high ovulation rates and embryonic survival has been reported (Wrathall, 1971). den Hartog and van Kempen (1980) evaluated the previous literature and concluded that gilts fed ad libitum prior to mating and switched to a restrict feed intake after mating had a greater number of embryos present at d30 than gilts continued on ad libitum feed intake. In subsequent studies (Dyck and Strain, 1983) , as high feed intake from d1 to 10, but not from d11 to 20, after mating reduced

embryonic survival to d30 in gilts, the critical window for nutritional effects on embryonic survival seemed to be the early post-coitum period. However, the same author in another study (Dyck, 1991) failed to substantiate the effect of manipulation of diet intake after mating on embryonic and fetal survival in gilts. In another recent study, Cassar et al. (1994) did not find any significant difference in embryonic survival between gilts fed a recommended gestation ration after mating compared to gilts fed a high energy diet containing additional energy in the form of corn starch.

Further evidence for an effect of nutrition during early pregnancy, and the critical window for nutritional changes to show an effect on embryonic survival comes from a series of experiments done in our laboratory. In an initial study, the effect of a change in dietary energy and/or protein intake on early embryonal survival was examined (Pharazyn et al., 1991a). Starting from d3 of gestation, the gilts were fed different combinations of low/high energy and (or) protein. The lack of any response on embryonic survival was partially attributed to the delay in changing energy and protein intake on d3 of pregnancy, rather than at the time of mating, thus possibly missing the critical period in which nutritional changes may be important for embryonic survival. This suggestion was supported by a further study in which feeding 1.8 vs 2.5 kg/day from the day after mating resulted in a significant improvement in embryonal survival from 70.0 to 87.7%, thus, indicating that only a change in feed intake in the immediate post-mating period may affect subsequent embryonic survival. The need for more convincing data on time of changes in feed intake was the motivation for the studies reported in Chapter II. Evidence that the timing of changes in feed intake is crucial for demonstrating effects of nutrition on embryonic survival,

probably indicates the reason for the inconsistencies in the previous literature, and this is discussed in detail in relation to the results reported in this thesis.

2) Effect of feed intake during lactation/early pregnancy on embryonic survival in sows:

The lactating sow has a higher requirement for energy and protein as approximately 75% of the energy required goes to milk production (Aherne and Kirkwood, 1985). Feed intake during pregnancy is directly related to loss of appetite and body weight loss during lactation. Any reduction in feed intake during this period is compensated by an increased mobilization of body reserves so as to maintain the milk production, and also the sow's own growth (Mullen and Williams, 1990). Loss of body weight and back fat thickness are good determinants of the rate of mobilization of fat depots during lactation in order to buffer the nutritional stress through low feed-intake. Gross changes in body weight and body condition have been associated with subsequent reproductive performance of the sow. Since the reproductive tissues have a low priority for nutrients the decrease in feed intake or the availability of nutrients will have more profound effects on the reproductive system as compared to other physiological functions. Excessive weight loss in lactation can affect weaning to estrus interval, the proportion of sows returning to estrus, pregnancy rate and embryonic survival (Mullan and Williams, 1989; Kirkwood et al., 1987, 1990; Baidoo, 1989).

The effect of dietary intake during lactation on embryonic survival has been contested. Greater embryonic mortality in sows as a result of low feed intake during lactation as compared to high plane of nutrition has been reported by King and Williams (1984), Hughes et al. (1984), Kirkwood et al. (1987, 1990) whereas no effects on litter size were found by others (Reese et al., 1982; King and Dunkin, 1986). No effect of feed intake during lactation on ovulation rates has been reported, except for a trend for lower ovulation rate in sows losing over 25 kg body weight (Rojkittikhun et al., 1992). The inverse relationship between lactational food intake and weaning to estrus interval is mainly seen in primiparous sows, whereas effects on embryonic survival is more pronounced in multiparous females (see Hughes, 1989). In the studies by Kirkwood et al. (1990) feed intake during early pregnancy had no effect on embryonic survival. However, sows with low dietary intake in both pregnancy and lactation had a high embryonic mortality. In a recent study in this laboratory (Zak et al., 1996), the pattern of catabolism in lactation as a consequence of differences in feeding regimen has been associated with subsequent reproductive performance of the sow. In that study sows were fed to appetite from d1 to 28 of lactation (group AA), or restrict fed to 50% from d22 to 28 (group AR) or d1 to 21 (group RA). Weaning to estrus interval increased in AR and RA sows and ovulation rate was lower compared to AA sows. Ovulation rate was significantly lower in the RA and AR sows as compared to ad libitum feed group. Embryonic survival did not differ between RA and AA sows, but was lower in AR sows. Thus, in sows not only the feeding level, but also the pattern of tissue loss is important for regulating the reproductive performance.

An association between sow feed intake (and metabolic changes in the body of a sow) and the endocrine status during lactation and pregnancy has been studied/reviewed in detail (see Kirkwood and Aherne, 1985; Baidoo et al., 1992; Foxcroft, 1992; Einarsson and Rojkittikhun, 1993). The role of progesterone as a mediator of embryonic survival in sows has been suggested by Aherne and Kirkwood (1985). This hypothesis was tested in the present studies (Chapter V).

3 . Plasma progesterone as a mediator of nutritionally induced effects on embryonic survival in gilts

An inverse relationship between dietary levels and circulatory progesterone concentrations has been demonstrated in sheep (Cumming et al., 1971; Parr et al., 1982, 1987) and pigs (Dyck et al., 1980; Pharazyn, 1992). In cattle, an increase in dietary protein intake during early pregnancy resulted in lower plasma progesterone concentrations (Jorden and Swanson, 1979). In the study by Dyck et al. (1980) feeding 3.5 vs 1.5 kg/d resulted in a significant depression in progesterone concentration, whereas feeding 2.25 kg/d gave intermediate concentrations. Further, lower plasma progesterone concentrations during early pregnancy have been associated with increased embryonic mortality in the sheep (Ashworth et al., 1989), cattle (Maurer and Echternkemp, 1982) and pigs (Pharazyn, 1992).

Plasma progesterone concentrations reflect a balance between luteal synthesis and metabolic clearance by the liver and kidney. The exact mechanism by which plasma progesterone concentration mediates the nutritionally induced effects on

embryonic survival is not clearly understood. Based on various reports, dietary intake during the periovulatory period can either affect the metabolic clearance rate of progesterone, or can bring about changes in its secretory pattern.

i) Metabolic clearance of progesterone:

Increased feed intake in gilts has been associated with an increase in the metabolic clearance of progesterone in gilts (Symonds and Prime, 1989) and sheep (Parr et al., 1993a,b). Increasing feed intake from 1 to 3 kg/d in ovariectomized gilts resulted in an increased metabolic clearance rate of progesterone from 39.7 to 57.0 ml/min/kg (Symonds and Prime, 1989). Progesterone metabolism and clearance from the blood is mainly under the control of hepatic mixed function oxidases (MFO). The MFO system is a series of enzymes involved in the synthesis of steroid hormones, and has a primary role in metabolism and excretion of drugs and other xenobiotics which find their way into the body. Hepatic metabolism of steroids has been discussed in detail by Pharazyn (1992). To summarize, progesterone catabolism usually involves reduction at C-20. By a series of reactions with 5α-pregnane-3,20α-diol there is formation of up to six pregnanediols. To increase their solubility in water they get complexed with a number of compounds and are subsequently excreted into the bile (Adlercreutz and Martin, 1980). In association with bile, these metabolites enter the intestinal lumen where they are subjected to deconjugation by microbes and reabsorbed in the mucosa. In the intestinal mucosa there is again reconjugation and the metabolites are taken up into the blood, and finally get excreted into the urine.

Changes in dietary intake can alter the level of mixed function oxidase activity. Increased dietary protein intake enhanced the microsomal levels of cytochrome P_{450} in the liver (Campbell and Hays, 1974), which is the terminal oxidase of the MFO system, resulting in a faster steroid clearance. Hepatic metabolic activity and progesterone metabolism have been associated with increased food intake or the associated increase in growth rate (Argyris, 1971). In rats, food restriction to 75% ad libitum changed the hepatic microsomal levels of cytochrome P_{450} though differential effects were seen between male and female rats (Hashmi et al., 1986). The enhanced activity of the MFO system in the liver as a result of higher nutritional levels would increase progesterone catabolism, thus causing lower circulatory concentrations of progesterone. Also, as discussed earlier, lower progesterone concentrations during early pregnancy can result in reduced embryonic survival. Further, blood flow rates in the hepatic portal vein in gilts (Symonds and Prime, 1989) and sheep (Parr, 1993b) were directly related to the level of feed intake. In ewes on ad libitum feed intake, there was a marked decline (70%) in the peripheral concentration of progesterone within 24h of feeding (Parr, 1992).

These studies suggest that the nutritionally-induced effects on progesterone concentration and embryonic survival can at least partly be explained by feed associated changes in the hepatic portal blood flow and progesterone clearance rate..

ii) Nutritionally-induced changes in progesterone secretion:

In previous studies in our laboratory (Pharazyn, 1992), irrespective of the nutritional regimen, embryonic survival was related to the plasma progesterone

concentrations during early pregnancy. Also, there was substantial variation in plasma progesterone concentrations among gilts (1.3 to 25.5 ng/ml) measured on d3 after onset of estrus (Pharazyn, 1992). This possibility that variation in plasma progesterone concentration might be related to differences in the pattern of luteinization and development of corpora lutea, as a consequence of differences in the maturational state of preovulatory follicles, has been discussed by Hunter and Weisak (1990). As already discussed, periovulatory plasma progesterone concentrations have been associated with embryonic survival in ewes (Ashworth et al., 1989), cattle (Maurer and Echternkemp, 1982) and gilts (Pharazyn, 1992). In sheep, there is a strong correlation between embryonic survival and the time after ovulation when plasma progesterone concentration starts to rise, and the subsequent rate of increase in plasma progesterone concentration (Ashworth et al., 1989). Confirming and extending the previous suggestion by Ashworth et al. (1994), Hunter et al. (1996) recently reported that the time interval from the onset of the post-ovulatory LH surge until the rise in plasma progesterone in highly prolific Meishan gilts was shorter (54.5 h) than in Large White gilts (74.3 h), indicating the possibility of an association between the early rise in plasma progesterone and embryonic survival and (or) litter size.

In the preliminary studies in our laboratory, Pharazyn (1992) tried to establish a link between changes in feed intake during early pregnancy and changes in plasma progesterone concentrations and embryo survival. Feeding 1.8 vs 2.5 kg/day from d1 of pregnancy resulted in higher embryonic survival and nearly a 10-h difference in the time when plasma progesterone concentration started rising after onset of estrus between the two groups. Though the differences were not statistically significant, the

experiment gave an indication of the possible role of plasma progesterone during early pregnancy as a mediator of nutritionally-induced effects on embryonic survival. This hypothesis was tested in a better controlled experiment as part of our present studies (Chapter III) in which gilts were individually fed on the basis of their metabolic body weight during pre- and post-mating periods, and progesterone concentrations were standardized to the time of LH peak rather than to the time of estrus onset. Further evidence for the role of plasma progesterone as a mediator of these effects comes from studies by Parr et al. (1987, in which the possibility of improving embryonic survival by exogenous progesterone administration was demonstrated in sheep. Also, progesterone supplementation to ewes during the first three days of pregnancy induced changes in embryonic development and enhanced growth of surviving fetuses (Kleemann et al., 1994). Data from historical studies on the effect of exogenous progesterone on embryonic survival in the gilt are equivocal (Sammelwitz et al., 1956; Reddy et al., 1958; Davis and Sorensen, 1959; Morissette et al., 1963). In these studies, normal luteal phase endogenous progesterone concentrations were used for progesterone supplementation. In the only recent experiment assessing the role of progesterone as a mediator of nutritional effects on embryonic survival (Ashworth, 1991), post-mating progesterone supplementation in gilts with high embryonic mortality as a consequence of ad libitum feeding improved embryonic survival from 66.4 to 82.8%, though the results were based on a relatively small number of gilts. In that study progesterone was administered from d4 to 30 of pregnancy, whereas based on previous studies (Dyck and Strain, 1983; Pharazyn et al., 1991a; Pharazyn, 1992), and also results of our Experiment 1 (Jindal et al., 1996a), the critical window for

nutritional changes to demonstrate an effect on embryonic survival in gilts is immediately post-mating period. This was confirmed in the Experiment 3 (Chapter IV) of the present studies.

As already discussed, the literature supports two main hypotheses by which dietary intake during early pregnancy can influence circulatory levels of progesterone:

a) The changes in feed intake can affect the metabolic clearance rate of progesterone, thus changing its circulatory concentrations. Also reduced feed intake is associated with reduced blood flow to the liver via the hepatic portal circulation, and also with reduced sequestration of steroids into the gut contents and entero-hepatic recirculation. These effects would result in higher plasma concentrations at a fixed rate of progesterone secretion.

b) The level of feed-intake can bring about changes in production and (or) pattern of secretion of progesterone from corpora lutea. Differences in plasma progesterone profiles among gilts may be related to differences in the pattern of luteinization. Variation in the time of onset of the post-ovulatory progesterone rise could be due to earlier production of progesterone by more developed corpora lutea. As in the previous gilt studies in this laboratory (Pharazyn, 1992), and also the present studies (Jindal et al., 1996a; Chapter II, III) nutritional treatments were imposed very close to the time of ovulation, they could have little or no influence on the maturation of preovulatory follicles, rather an effect on the process of luteinization itself is possible. Thus, lower dietary intake (normal recommended levels) during immediate post-mating period resulted in an earlier rise in plasma progesterone concentrations.

The ovarian arteries and veins are present in close apposition to each other and a local counter-current transfer of steroid hormones has been demonstrated within the ovarian pedicle from the ovarian vein to the artery (Krzymowski et al., 1982). Thus progesterone dynamics within the vasculature of ovaries, oviducts and uterus may differ from that of the systemic circulation. A local utero-ovarian pathway is involved in uterine induced luteolysis in species like swine, cattle, sheep, hamster, rat, etc. A greater concentration of progesterone in the arterioles supplying the oviducts than the systemic circulation during pre-ovulatory period has been reported in gilts (Hunter et al., 1983). Later, Pharazyn et al. (1991b) observed a greater plasma progesterone concentration in the oviductal venous blood than jugular or uterine veins during early pregnancy in gilts, thus substantiating a local counter-current steroid transfer of progesterone from ovarian vein to oviductal artery. Therefore, any differences in progesterone production can also affect the oviductal or uterine activities directly. Recently, Ashworth et al. (1995) demonstrated that the nutritional changes in circulatory progesterone were mediated through changes in both ovarian production and hepatic metabolism.

The ovum, after its release, stays in the oviduct for about 3 days before entering the uterus. The oviduct, uterus and their secretions provide a biochemical environment essential for normal development of an embryo, and thus a successful establishment and maintenance of pregnancy. As already reviewed, the oviductal (Murray, 1993) and endometrial (Roberts et al., 1993) development and secretory activities are regulated by ovarian hormones. Either due to differences in the metabolic clearance rate or production rate, any changes in progesterone concentration during

early pregnancy can modify the oviductal and (or) uterine environment, possibly changing the synchrony between the embryo and uterus as suggested by Pope (1988), thus affecting the viability of an early embryo. Therefore, continuation of increase feed intake during the post-mating period can increase the embryonic mortality by delaying post-ovulatory rise in plasma progesterone, and bringing about changes in the oviductal/uterine secretions.

4. Plasma progesterone as a mediator of nutritional effects on embryonic survival in sows

In contrast to studies in gilts, in studies with lactating sows, the dietary treatments are imposed during lactation, and lactational catabolism may therefore influence progesterone secretion by two ways:

a) Some of ongoing differences in metabolic status of a sow due to serious catabolism during lactation may persist during the periovulatory period. For example, variations in metabolic regulators like IGF-1 (Zak et al., 1996) can affect gonadotrophins and (or) gonadotrophin releasing hormone, thus influencing the maturation and luteinization of the follicles. As discussed earlier, the follicular heterogeneity could result in differences in progesterone production during early pregnancy, thus affecting the viability of an embryo.

b) The follicles that are recruited into the ovulatory population at the first post-weaning estrus, undergo their initial stages of development during lactation, and may be adversely affected by the catabolic status of the sow at this time. These imprinting effects may influence the maturation of the follicles and process of luteinization, and

thus the amount and (or) pattern of progesterone secretion which can in turn affect the embryonic survival, possibly in the same way as in gilts.

In the present studies we have further tested the hypothesis that plasma progesterone concentrations during early pregnancy mediates the nutritionally-induced effects on embryonic survival.

Figure I.1. Theory of maternal recognition of pregnancy in the pig: Direction of PGF release in pregnant and non-pregnant pigs. (Reproduced from Bazer et al., 1982).

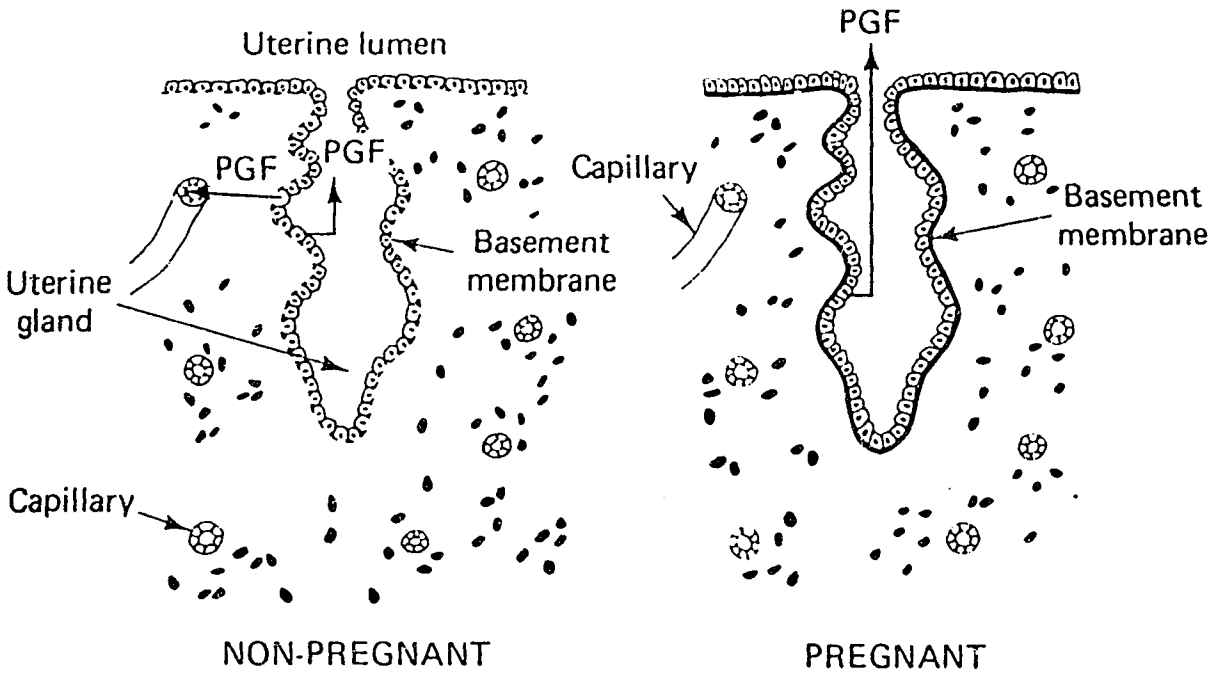

Uterine lumen
PGF
PGF
Basement membrane
Uterine gland
Capillary
NON-PREGNANT
PGF
Capillary
Basement membrane
PREGNANT

Fig. I.2. The tissues contributing the uterine luminal environment and the potential interactions among the tissues.

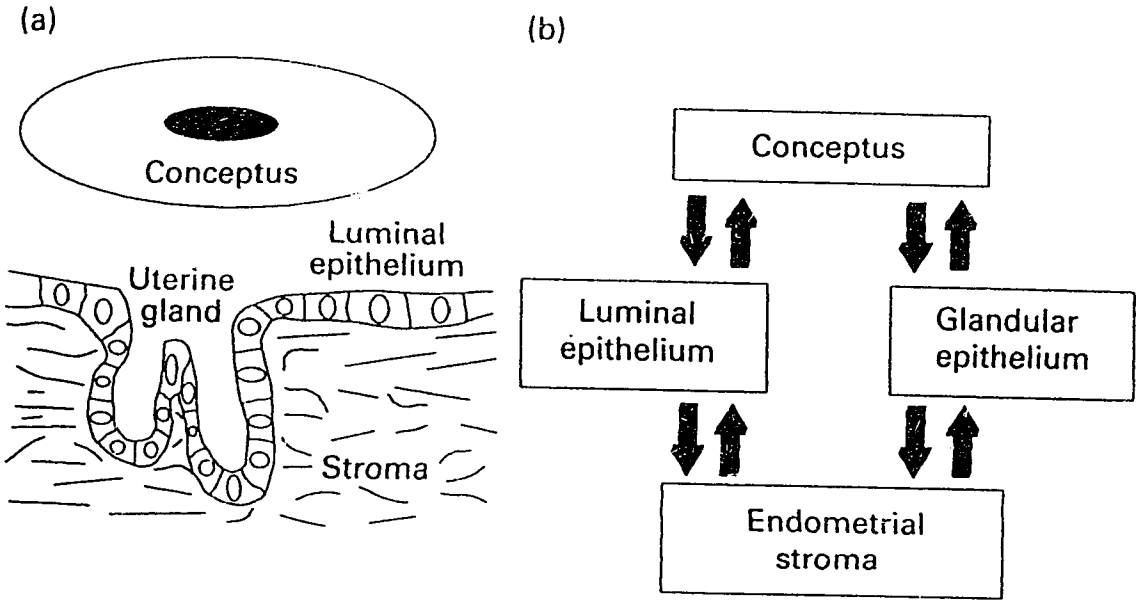

(a)
Conceptus
Luminal epithelium
Uterine gland
Stroma
(b)
Conceptus
Luminal epithelium
Glandular epithelium
Endometrial stroma

Figure I.3. Interactions between the uterus and preimplantation pig embryo. C-E = catechol estrogens; P = progesterone; E = estrogens; GFs = growth factors, e.g. Insulin-like growth factor I and II, Epidermal growth factor, etc.; PGF and PGE = prostaglandins F and E; PA = protease plasminogen activator; PI = protease plasminogen inhibitor. Arrows indicate direction of secretion. (Reproduced from Foxcroft et al., 1994).

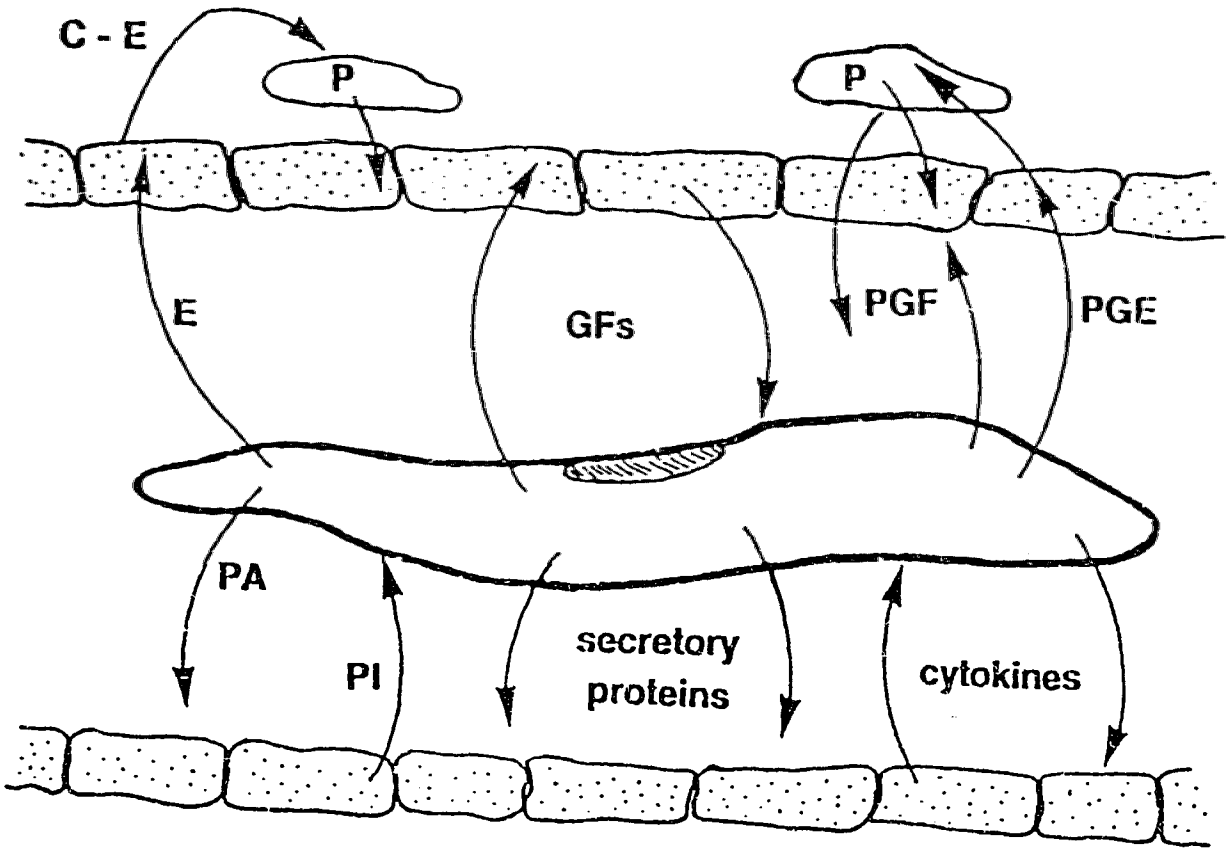
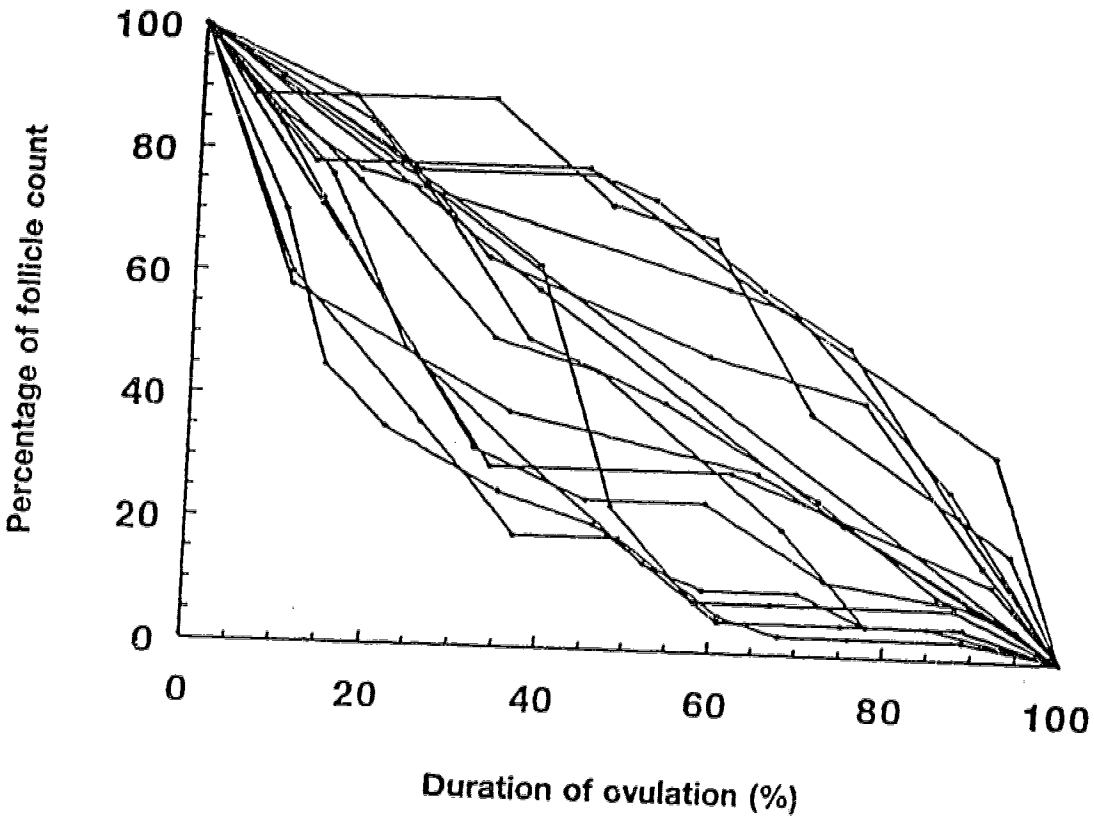

C - E
P
P
E
GFs
PGF
PGE
PA
PI
secretory
proteins
cytokines

Figure I.4. The course of ovulation in sows. The progression of ovulation in time is expressed as percentage of the total duration per sow. The follicle count is expressed as percentage of the maximum follicle count per sow. Each line represents an individual sow. (Reproduced from Van der Lende et al., 1994).


100
80
60
40
20
0
Percentage of follicle count
0
20
40
60
80
100
Duration of ovulation (%)

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Chapter II

Embryonic mortality in gilts: Dependence on nutrition and progesterone

Introduction

In gilts 30-35% prenatal mortality is well established and continues to represent a major loss to the pig industry. This loss is not a biological inevitability, as a proportion of sows can have 100% embryonic survival. Attempts to improve embryonic survival in the pig have produced inconsistent results and the mechanisms mediating effects on embryonic survival have not been clearly identified. Although, embryonic loss may be attributed to several factors, the role of nutrition during the pre- and post-mating period has received considerable attention. A high dietary intake for at least 4-5 days before mating ("flush feeding") maximizes ovulation rate (den Hartog and van Kempen, 1980; Aherne and Kirkwood, 1985; Beltranena et al., 1991). However, continuation of this high level of feeding after mating may have a detrimental effect on embryonic survival. den Hartog and van Kempen (1980) reviewed the previous literature and concluded that gilts fed ad libitum before mating and switched to a restricted feed intake after mating had a greater number of embryos present at day 30 than gilts continuing on ad libitum feed intake. Subsequently, Dyck and Strain (1983) reported that high feed intakes before mating, followed by a 10-d period of restricted feeding after mating, maximized both ovulation rate and embryonic survival. The critical window affecting embryonic survival therefore, seemed to be the early post-coitum period. However, in another study, Dyck (1991) failed to find any effect of manipulation of diet intake after mating on embryonic and fetal survival in gilts. Similarly, in a recent study, Cassar et al. (1994) did not find any significant differences in embryonic survival between gilts fed a recommended gestation diet after mating, compared to gilts fed a high energy diet

containing additional corn starch. Explanation for the lack of a consistent experimental evidence for a detrimental effect of high planes of nutrition immediately after mating on embryonic survival is needed.

An inverse relationship between level of nutrition and circulating progesterone concentration has been demonstrated in pigs (Dyck et al., 1980; Prime et al., 1988). Such nutritionally-induced changes in circulating progesterone may modify the magnitude and composition of endometrial secretions (Roberts and Bazer, 1988) and allantoic fluid protein content (Van der Lende, 1989), thus affecting the growth, development and ultimately the survival rate of embryos. An inverse relationship between plasma progesterone during the periovulatory period and embryonic mortality has also been reported for cattle (Maurer and Echternkemp, 1982) and sheep (Ashworth et al., 1989). In both sheep (Parr et al., 1987) and in gilts (Ashworth, 1991) progesterone supplementation after mating has been reported to reduce the high embryonic mortality resulting from high levels of feed intake. Though the exact mechanism mediating this effect is not known, an increased feed and/or protein intake may lead to enhanced metabolic clearance rate of progesterone through an increase in hepatic blood flow and (or) synthesis of hepatic mixed function oxidases which are responsible for steroid metabolism in the liver (Dziuk, 1982).

In our laboratory, further evidence for an effect of nutrition during early pregnancy on embryonic survival in the gilt, and the possible involvement of periovulatory plasma progesterone concentrations, was obtained in a series of experiments. In the initial study, effects of a change in dietary energy and/or protein intake on early embryonic survival was examined (Pharazyn et al., 1991). The lack of

response to low/high energy and /or protein on embryonic survival was partially attributed to the delay in changing energy and protein intake to day 3 of pregnancy, rather than at the time of mating, thus possibly missing the critical period in which the nutritional changes may be important for embryonic survival. This suggestion was supported by a further study in which Pharazyn (1992) reported that feeding 1.8 vs 2.5 kg feed/day from the day after mating resulted in a significant improvement in embryonic survival rate from 70.0 to 87.7 %, thus indicating that only a change in feed intake in the immediate post-mating period may affect subsequent embryonic survival. The effect of feed intake during early pregnancy on periovulatory plasma progesterone concentrations was also studied in these experiments. The mean time intervals (30.8 and 37.6 h) from the periovulatory LH peak to the beginning of a rise in plasma progesterone in gilts provided 2.0 and 2.8 kg feed/d, respectively, were significantly different (Pharazyn, 1992). Furthermore, the effect of high or normal feed intake on embryonic survival in another experiment of Pharazyn (1992) was associated with differences in plasma progesterone concentration. Gilts with lower progesterone concentrations 72h after estrus had lower mean embryonic survival rates and greater variability in embryonic survival, compared to gilts with high progesterone concentrations.

Therefore, in the light of 1) conflicting information in the literature, and 2) data from our own laboratory suggesting possible reasons for previous inconsistent results, the present study was undertaken with the following objectives:

a) To convincingly test the hypothesis that the timing of changes in feed intake in the immediate post-mating period is crucial for demonstrating nutritional effects on early embryonic mortality.

b) To provide further evidence that differences in plasma progesterone in the immediate postovulatory period might mediate nutritional effects on embryonic survival.

Materials and Methods

The experiment was conducted with Pig Improvement (Canada) Ltd. Camborough gilts. During the late pre-pubertal period gilts were fed a standard barley-wheat-soybean grower diet containing 12.5 MJ DE/kg and 14% crude protein, ad libitum. Gilts were checked for estrus once daily until puberty during periods of direct exposure to a rotation of mature vasectomized boars. The experimental plan is shown in Fig. II.1. When the gilts attained puberty, they were individually fed a total of 2.5 kg feed per day, split between morning and afternoon feeds, for one complete estrous cycle.

Starting two days before the expected date of the next heat, gilts were checked for the onset of estrus three times daily (7.30 a.m., 3.30 p.m. and 11.30 p.m.) using direct contact with a vasectomized boar, but not permitting mating. The 24-h period from first occurrence of estrus was designated d0. A total of 82 gilts were artificially inseminated twice, once 16h after the first observation of standing heat and again 8h later, using fresh pooled semen from three fertile boars specifically designated for this experiment and supplied by Alberta Swine Genetics Corp., Leduc, Alberta. All inseminations were done by the same trained person and the same batches of semen were used across treatments. Gilts that returned to estrus after 21 days were removed from the experiment, leaving 72 presumed pregnant animals in the study.

After insemination, gilts were randomly, and as for as possible evenly, allocated within replicate groups to one of the three feeding regimens. Gilts were changed from the earlier allowance of 2.5 kg/d to either the normal NRC (1988) recommended feed allowance for pregnant gilts of 1.5 x maintenance per day on d1 (Group N1) or d3 (Group N3), or to 2 x maintenance from d1 (Group H). In contrast to earlier experiments, maintenance requirements were calculated for individual gilts on the basis of their metabolic body weight (Body wt.$kg^{.75}$), within a 10 kg range, to provide a maintenance energy allowance of 461 kJ DE/kg metabolic body wt.

Single blood samples (2.5 ml) were collected by acute venepuncture into heparinized tubes from a peripheral ear vein 72h after first detection of standing heat, using short-term nose-snare restraint of the animal. Plasma was harvested by centrifugation immediately after collection and stored at -30° C until analyzed for progesterone concentration using the double antibody radioimmunoassay (RIA) described by Beltranena et al. (1991). The sensitivity of the assay, defined as 85% of total binding, was 0.01 ng/tube. All samples were run in a single assay with an intra-assay coefficient of variation (CV) of 5.6% and an extraction efficiency of 81%, with no correction for recovery.

From d15 onwards all gilts were fed a standard allowance of 1.8 kg feed/day until slaughter on d28±3 of pregnancy at a local abattoir. Gilts were weighed on d0, d15 and d28±3 of pregnancy. Immediately after slaughter the reproductive tract was recovered and both ovaries examined to determine the number of corpora lutea and thus ovulation rate. Several cuts into the ovarian tissue in different planes were made to determine the presence of any hidden corpora lutea. Each uterine horn was opened by

blunt dissection along the antimesometrial axis and gentle eversion of the uterus was used to recover embryos within their trophoblastic vesicles. Embryos were initially subjectively classified as being viable or non-viable on the basis of appearance within the amnion. The crown-rump length of each embryo within the amnion was recorded. For final analysis a crown-rump length of more than 2 SD less than the mean for that gilt was used as an objective measure of abnormal development. Two measures of embryonic survival were determined: 1) Total embryonic survival (total ES), expressed as the percentage of corpora lutea represented by an embryo and 2) viable embryonic survival (viable ES) expressed as the percentage of corpora lutea represented by a normally developed embryo at d28±3 of pregnancy. At slaughter, five gilts were found to have cystic ovaries, and data from these animals were not included in the final analysis. Results were therefore based on a total of 67 gilts with 21, 24 and 22 gilts in groups H, N1 and N3, respectively.

Statistical Analyses

The effects of treatment (dietary regimens) on body weight gain from d0 to d15 of pregnancy, ovulation rate (total number of corpora lutea), total number of embryos, total ES, viable ES, average crown-rump length of embryos, and gilt plasma progesterone concentration were assessed using the general linear models (GLM) procedures of SAS (1985). The normality in the ES data was confirmed using Proc Univarate (SAS, 1985) and thus, no transformation of the data was done. When treatment effects were significant ($P<0.05$), multiple comparisons were performed using Student-Neuman-Keuls (SNK) test (Snedecor and Cochran, 1980). As the gilts were

slaughtered on d28±3, the average crown-rump length of all embryos from each gilt was adjusted to d28 using day of slaughter as a covariate before performing analysis of variance. To establish relationships between embryonic survival and progesterone concentrations, data from each of the three treatment groups as well as combined data (irrespective of dietary treatment) was sorted by ascending plasma progesterone concentrations and split into three equal parts. Within treatments, the variance in viable embryonic survival in the least and the highest progesterone concentration groups were then compared by Students t-test (SAS, 1985). The mean viable embryonic survival was also compared across treatments in each of the three progesterone categories (low, 1.52±0.14; medium, 5.19±0.30 and high, 12.17±0.81 ng/ml, respectively). The data were also subjected to correlation analysis to establish associations between plasma progesterone and ES both within dietary group and across dietary treatment groups.

Results

Despite the differences in feed intake imposed, differences in mean weight gain for the first 15d of pregnancy in the various groups of gilts (Table II.1) were not significant (P=0.24).

There were no differences (P=0.62) in ovulation rate based on total number of corpora lutea on both ovaries, among group N1 (14.50±0.38), N3 (14.95±0.42) and H (14.95±0.38) (Table II.1). Although the total number of embryos was not statistically different among the three groups (P=0.13), total ES was affected by the dietary treatment (P=0.044). Multiple comparisons indicated that the total ES in group N1 was higher than

the pooled results of groups N3 and H (85.93% vs 72.15%, respectively; P=0.045) and that in group H was lower than the combined estimates of groups N1 and N3 (66.96% vs 81.64%, respectively; P=0.021). There was no significant effect of dietary treatment detected for total number of viable embryos or crown rump length of embryos (Table II.1). However, the mean viable ES was affected (P=0.027) by treatment and results of multiple comparisons using SNK are shown in Fig. II.2. The viable ES in group N1 was higher (P<0.05) than that in group H (84.67% vs 64.45%, respectively) and group N3 (74.01%) was intermediate. Viable ES in group N1 was also higher than that in groups N3 + H combined (P=0.023) and lower (P=0.016) in group H when compared to groups N1 + N3.

Mean plasma progesterone concentration at 72h following onset of estrus was affected (P<0.001) by the dietary treatment (10.5±1.0, 3.7±0.8 and 4.5±0.7 ng/ml in groups N1, N3 and H, respectively, Fig. II.2), and the concentration in group N1 was higher (P<0.05) than for groups N3 and H. Within groups, a positive correlation existed between plasma progesterone and viable ES in group N1 only (r=0.44, P=0.03). Relationships between plasma progesterone concentrations 72h after onset of estrus and embryonic survival in the three treatment groups of gilts are shown in Fig. II.3. Irrespective of the dietary regimen, the mean viable ES differed (P=0.004) among high (91.12±1.81%), intermediate (64.76±7.31) and low (69.08±6.71%) plasma progesterone groups. Also, irrespective of the dietary regimen, the variability in viable ES was different (P<0.001) between low and high progesterone concentration groups. When the data were analyzed within dietary treatments this difference in variability between low

and high progesterone concentration animals was only detected ($P<0.001$) in the N1 gilts.

Discussion

As high feed intake from d1 to d10 of pregnancy, but not from d11 to d20 after mating, reduces embryonic survival in gilts on d30, Dyck and Strain (1983) suggested that the critical window for nutritional effects on embryonic survival was the early post coitum period. Although Pharazyn et al. (1991) could not detect any effect on embryonic survival to d28 of feeding two levels of energy and protein to pregnant gilts from d3 to d15 of gestation, in a later experiment he (Pharazyn, 1992) fed either normal (1.8 kg/day) or high (2.5 kg/day) levels of feed starting from d1 until d15 post-mating and found higher embryonic survival (87.7 vs 70.0%) in the normal feed group. These results indicated that the critical window for nutritional effects on embryonic survival in gilts could be limited to the immediate post-mating period. In the present study, therefore, feed intake was reduced on either d1 or d3 of pregnancy to have a clear understanding of the critical period during which nutrition can affect embryonic survival. The results indicate that both measures of embryonic survival, i.e.. the total ES and the viable ES, were significantly affected by treatment. The highly significant difference in viable ES between groups N1 and H (85 vs 64%, respectively), and an intermediate embryonic-survival in group N3, substantiates the detrimental effect of a high level of feed intake during the immediate post-mating period. Thus, in relation to the first objective of our study it appears that the critical period during which a reduction in

dietary intake has a positive effect on early embryonic survival is the day after onset of estrus. A comparison of the data on total and viable ES indicates that most of the embryonic loss occurred during the pre-implantation period and only a few embryos, represented by non-viable embryos at slaughter on d28, had died in the post-implantation stages. These results are in agreement with previous reports indicating that the major part of all embryonic mortality occurs before d18 of pregnancy (see, Van der Lende et al., 1994).

Cassar et al. (1994) did not find any significant effect of high vs normal energy (36.0 vs 22.2 MJ DE/d) diets during early pregnancy on embryonic survival on d25 in gilts, but ES was relatively higher in the normal energy group. These differences could be explained by the experimental model they used. In their study gilts were group-fed 1.5 to 2 kg feed/day before insemination and their individual feed intakes could not be estimated (Glen Cassar. Personal communication.). After insemination, gilts on the NRC recommended energy allowance were given 1.5 kg of the basic diet/day, whereas those on high energy received 1.0 kg corn starch in addition to the basic diet. Therefore, the precise pattern of feed intake resulting from the change to either the normal or high energy diets on the day after mating is uncertain and could preclude determination of clear effects on embryonic survival. In the present study, gilts were individually fed 2.5 kg of a commercial finisher diet before mating. Starting from d1 or d3, N1 and N3 gilts were then brought down to normal NRC (1988) recommended dietary levels, in contrast to the high plane of feeding that continued in H gilts. The change in feed intake in these gilts was, therefore, individually controlled and consistent for all gilts within a treatment group.

Average plasma progesterone concentration at 72h after onset of estrus was significantly higher ($P<0.05$) in N1 gilts as compared to the gilts on the high plane of feed (10.47±1.0 vs 4.49±0.70 ng/ml). This suggests that the inverse relationship between plane of nutrition and circulating progesterone concentrations, also demonstrated in pigs by Dyck et al. (1980) and Prime et al. (1988), may be of functional significance.

In the only experiment published to date to directly assess the role of progesterone as a mediator of embryonic survival in gilts, Ashworth (1991) investigated embryonic survival and conceptus growth to d30 in gilts subjected to different dietary combinations and to post-mating progesterone supplementation. In gilts with high ovulation rates and high embryonic loss as a consequence of continuous ad libitum feeding, supplementary progesterone treatment after mating was reported to increase viable ES on d30 from 66.4 to 82.8%, although the estimates were based on relatively limited numbers of gilts per treatment.

To provide further indirect evidence in support of a functional relationship between differences in plasma progesterone and embryonic survival the ES data were sorted on the basis of plasma progesterone concentration. The group with the highest average plasma progesterone concentrations, irrespective of the dietary regimen, had higher ($P<0.05$) embryonic survival compared to the low and medium plasma progesterone groups (91.12% vs 69.08 and 64.76%, respectively). Further, irrespective of dietary regimen, the variance in ES was higher ($P<0.001$) in the low plasma progesterone group than the high progesterone group (see Fig. II.3) . Both these observations support the hypothesis that differences in plasma progesterone

concentrations in the immediate post-ovulatory period may mediate nutritional effects on embryonic survival. An interesting finding in the present study was a higher correlation between viable ES and plasma progesterone concentrations in group N1 compared to the other two groups (r=0.44 vs 0.02 and 0.08, respectively) This suggests that below a certain threshold level of plasma progesterone, ES is low and may not be further influenced by plasma progesterone concentrations. However, above this threshold, ES may increase with increase in plasma progesterone.

The physiological basis for a relationship between nutritional status and plasma progesterone concentrations needs further consideration. Plasma progesterone concentrations reflect a balance between synthesis by the corpora lutea and metabolic clearance by the liver and kidney. Symonds and Prime (1989) have shown an increase in hepatic blood flow and progesterone turnover in response to an increase in food intake in the gilt. Altered blood flow to the liver as a result of changes in feed intake may also alter the level of hepatic mixed function oxidase activity, resulting in a change in catabolism of progesterone. An association between increased feed intake, depressed plasma progesterone, and increased metabolic clearance rate for progesterone has been shown by Parr (1982). Another possibility is increased synthesis of progesterone and an earlier rise in relation to ovulation. Pharazyn (1992) reported an average difference of 10h in the time to the rise in plasma progesterone after the preovulatory LH peak between gilts on normal and high feed intakes from the day after mating. Whether due to altered synthesis or metabolism, such variability in the timing to the increase in plasma progesterone may alter the degree of synchrony between the developing embryo and the uterine environment, thus affecting embryonic viability (Pope, 1988). As suggested by

Knight et al. (1974) and Roberts and Bazer (1988), changes in circulating progesterone, due to an indirect factor like nutritional status or following progesterone administration, can modify the magnitude of polypeptides secreted by the epithelial cells of the porcine uterus. Oviductal secretory proteins have also been identified in pigs (Buhi et al., 1990) and it has been suggested that these proteins might have some effect on early cleavage-stage preimplantation development. Thus, there is a possibility that any changes in post-ovulatory progesterone concentrations may affect early embryonic survival by modification of the oviductal environment.

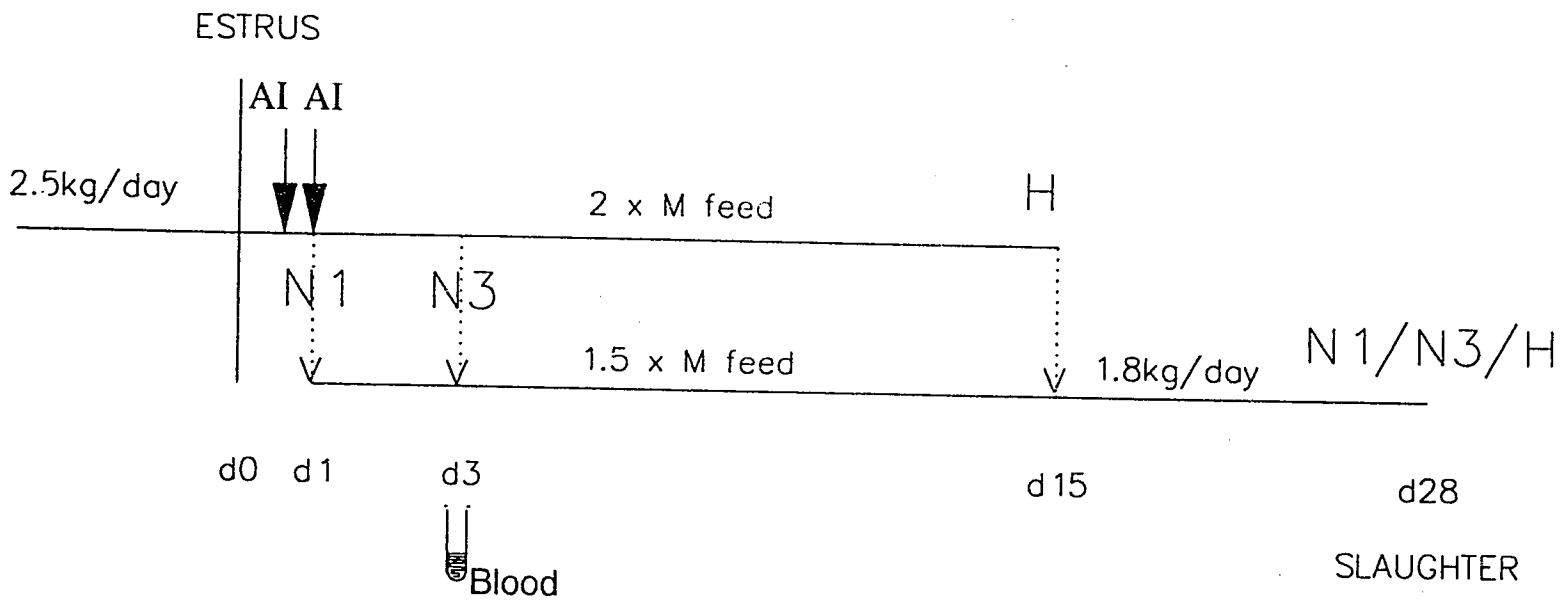
Table II.1. Average weight (Avg. wt.) gain, ovulation rate , total number of embryos, total embryonic survival, total viable embryos, and crown-rump-length of embryos in various dietary groups (Mean ± SEM)

Group	Avg. wt. Gain, d0 to 15, kg	Ovulation rate	Total embryos	Total embryonic survival, %[a]	Viable embryos	Crown-rump-length, mm
N1[b]	9.85 ±.78	14.50 ±.38	12.41 ±.69	85.93 ±4.45	12.25 ±.70	22.46 ±.37
N3	11.40 ±1.35	14.95 ±.42	11.54 ±.95	77.35 ±6.12	11.04 ±.95	22.35 ±.50
H	12.58 ±1.82	14.95 ±.38	10.19 ±1.23	66.96 ±8.02	9.80 ±1.17	22.69 ±.31

[a] Overall significant effect of treatment; $P < .044$.

[b] N1, N3: gilts with feed intake reduced to 1.5 x maintenance from d 1 and 3 of pregnancy, respectively. H: gilts receiving 2 x maintenance from d 1 of pregnancy.

Fig. II.1. Experimental Plan



EXPERIMENTAL SCHEME

Figure II.2. Embryonic survival (ES) at d28 of pregnancy and plasma progesterone (P4) concentration on d3 in groups of gilts fed either 1.5 x maintenance from d1 (N1) or d3 (N3) or 2 x maintenance from d1 (H) of pregnancy.

Bars with unlike superscripts differ ($P<0.05$).

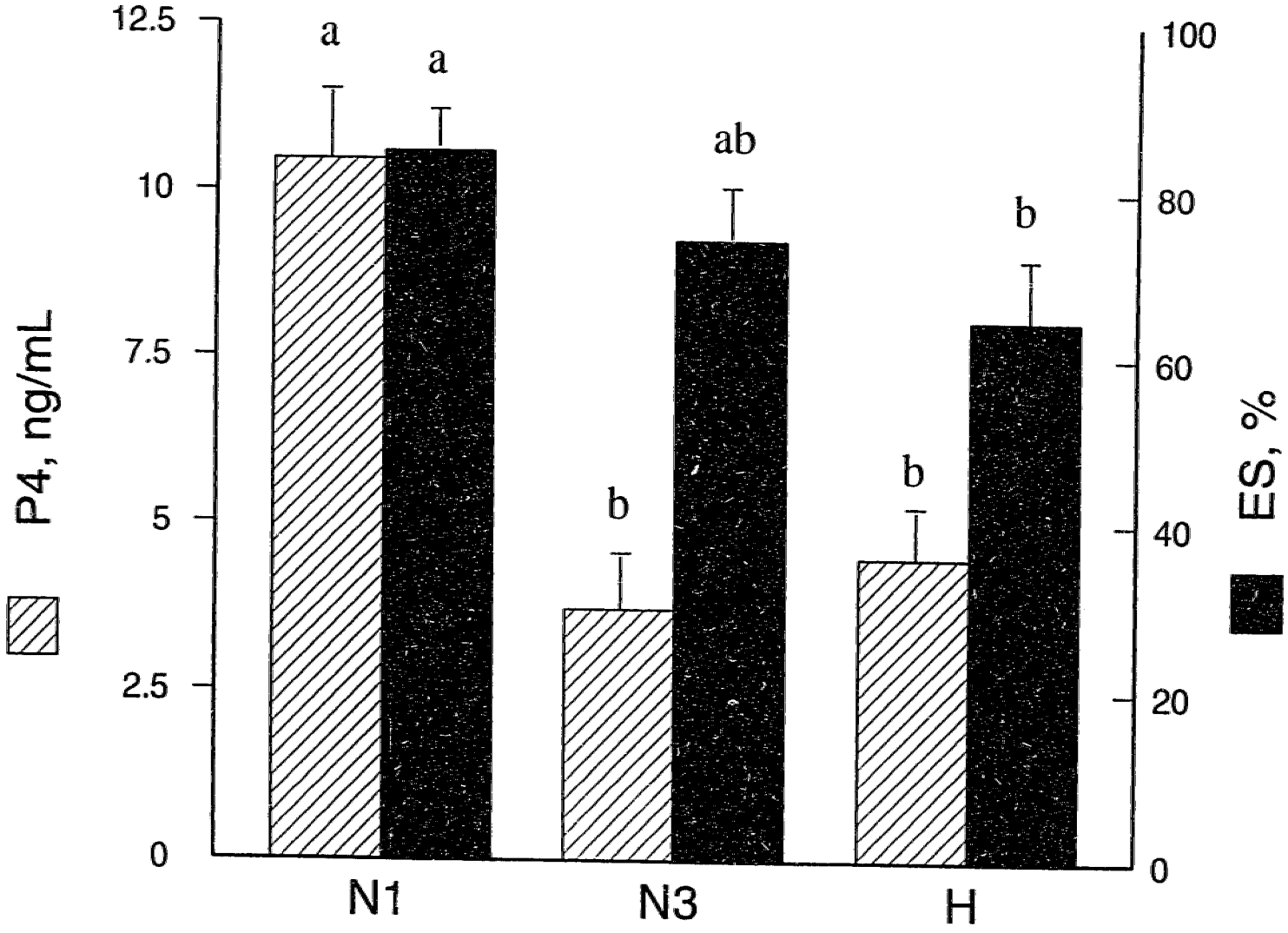
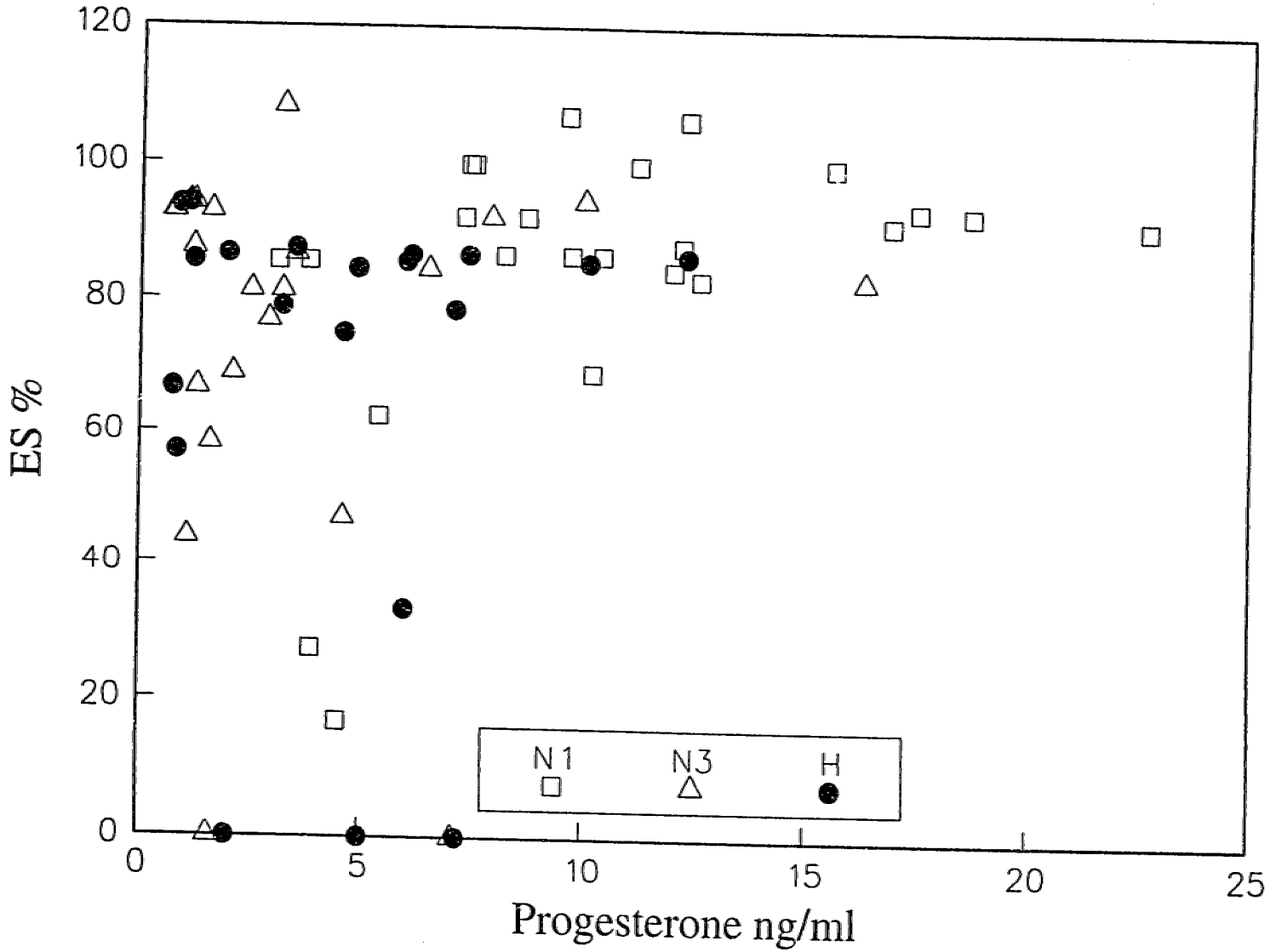

12.5
10
7.5
5
2.5
0
P4, ng/mL
100
80
60
40
20
0
ES, %
a
a
b
ab
b
b
N1
N3
H

Figure II.3. Relationship between plasma progesterone concentration 72h after onset of estrus and embryonic survival in gilts fed either 1.5 x maintenance from d1 (N1) or 3 (N3) or 2 x maintenance from d1 (H) of pregnancy.


120
100
80
60
40
20
0
ES %
0
5
10
15
20
25
Progesterone ng/ml
N1
N3
H

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Chapter III

Progesterone: A mediator of nutritionally induced effects on embryonic mortality in gilts

Introduction

Previous literature reviews (den Hartog and van Kempen, 1980) and more recent results, including Experiment 1 of the present studies, indicate that continuation of a high plane of nutrition during the post-mating period (periovulatory period) can result in lower embryonic survival and lower plasma progesterone concentrations during early pregnancy (Dyck and Strain, 1983; Pharazyn, 1992; Jindal et al., 1996). Also, irrespective of the nutritional regimen, embryonic survival was correlated with plasma progesterone concentrations during early pregnancy (Pharazyn, 1992; Jindal et al., 1996). Substantial variation in plasma progesterone concentration among gilts (1.3 to 25.5 ng/ml) measured on d3 after onset of estrus has been reported by Pharazyn (1992). Such variation in plasma progesterone concentrations during early pregnancy might be due to heterogeneity in the development of corpora lutea (Hunter and Wiesak, 1990) and thus in progesterone synthesis, and/or differences in hepatic metabolism of progesterone, that could be functionally related to variations in embryonic survival.

The oviduct and its secretions provide a biochemical environment that is essential for the establishment of pregnancy and appears to be controlled by ovarian estrogens and progesterone (Buhi et al., 1990). Also, the rate of uterine development depends on the development of corpora lutea and their ability to produce progesterone (Dziuk, 1987). It is known that progesterone modifies the secretion of several endometrial proteins (Ashworth and Bazer, 1989; Roberts et al., 1993) and such changes in the uterine environment can affect embryonic survival. In pigs, uterine

synthesis of insulin-like growth factor-1 (IGF-1) increases during early pregnancy with the peak of uterine IGF-1 secretion occurring on d12 of pregnancy (Letcher et al., 1989), coincident with blastocyst elongation. IGF-1 promotes cell division, cell differentiation and tissue morphogenesis by endocrine, autocrine and paracrine mechanisms (Zapf and Froesch, 1986). The ability of IGF-1 to mediate secretion of other proteins in reproductive tissues is known (Thrailkill et al., 1988). Also, IGF-1 may stimulate conceptus aromatase activity leading to increased estradiol biosynthesis (Hofig et al., 1991), which in turn can modify temporal aspects of the secretion of uterine proteins. Uterine plasmin/trypsin inhibitor (UPTI) is one of the Kunitz class of protease inhibitors (Laskowski and Kato, 1980) and is proposed to control the activity of the protease plasminogen activator (PA), thus helping to maintain the integrity of the uterine epithelium despite the invasive potential of the trophoblast (Samuel and Perry, 1972). UPTI synthesis and secretion are influenced by progesterone (Fazleabas et al., 1982). As the secretion of these and other uterine proteins can be modified by plasma progesterone concentrations during early pregnancy, variability in progesterone synthesis (or metabolic clearance) may lead to asynchrony between the embryo and the uterus (Pope, 1988). The timing of the rise in plasma progesterone may, therefore, be an important factor in determining such asynchrony and the likelihood of an embryo remaining viable. Significant correlations between embryonic survival and the time after ovulation at which plasma progesterone concentrations begins to rise, and the subsequent rate of increase in plasma progesterone concentrations, exist in the sheep (Ashworth et al., 1989). In preliminary studies Pharazyn (1992) fed 1.8 kg/day or 2.5 kg/day; the lower feeding level resulted in higher embryonic survival, while the high

feed intake resulted in nearly a 10-h delay in the time at which progesterone concentrations started rising after onset of estrus. Though these differences were not statistically significant, the results suggested that the pattern of progesterone secretion during early pregnancy could be an important mediating factor of nutritionally-induced effects on embryonic survival in gilts.

The present study was undertaken: 1) to substantiate the hypothesis that nutritional effects on embryonic survival may be mediated by differences in progesterone concentration during the periovulatory period; 2) to determine the stage of pregnancy at which the nutritionally induced effects on embryonic survival take place; and 3), to understand the mechanism by which feed intake can affect embryonic survival.

Materials and Methods

The study was conducted with 52 Pig Improvement (Canada) Ltd. Camborough gilts. During their first or second estrous cycle gilts were individually fed twice daily a total of 2.5 kg of a standard commercial, barley-wheat-soybean grower feed, containing 12.5 MJ of DE/kg and 14% crude protein. Gilts had ad libitum access to water. The study was conducted in two phases:

Phase 1. Four days before the expected date of next estrus, jugular cannulae were fitted surgically in 21 gilts. The cannulae were maintained aseptically and were flushed with heparinized saline solution twice daily. Starting two days before the

expected date of estrus the gilts were heat checked four times a day at 6-hour intervals using fence-line contact with a mature vasectomized boar. The gilts were artificially inseminated twice, 12 and then 24h after the onset of estrus, using pooled semen from three boars specifically designated for the experiment to minimize boar effects. All inseminations were done by the same trained person. The 24-h period from first observed standing heat was designated d0. Starting from d1 gilts were individually fed either 1.5 x maintenance (group N) or 2 x maintenance (group H) per day split between morning and afternoon meals. The maintenance requirements were calculated for individual gilts on the basis of their metabolic body weight ($B.Wt.kg^{.75}$) to provide a maintenance energy allowance of 461 kJ DE/kg of metabolic body weight. Blood samples (2.5 ml) were collected from indwelling catheters at 6-h intervals on d -1, 0 and 2, and at hourly intervals on d1. The plasma was harvested immediately after collection of samples and stored at -30°C for later analysis for luteinizing hormone (LH) and progesterone concentrations.

All gilts were slaughtered at local abattoir on d3-5 of pregnancy. Immediately after slaughter, genitalia were removed from each gilt. Corpora lutea were counted on both ovaries as a measure of ovulation rate as described in chapter II. The oviduct of each side was cleared from the mesentary by blunt dissection. Each of the uterine horns was cut at about 2.5-3.5 cm from the uterotubal junction and clamped tightly. Ova/embryos were collected by flushing the oviducts and the attached portion of the uterine horn four to six times with 15ml physiological saline. For flushing, saline was infused from the infundibular end of the oviduct with the help of a syringe with a blunt-edged needle and the ova/embryos were collected in a clean petri-dish. If

embryonic recovery was not complete, small portion (10-12 cm) of the uterine horns were flushed three to four times to recover maximum number of embryos. These ova and embryos were examined microscopically and catagorized according to their developmental stage. Embryonic recovery, and presumably embryonic survival was calculated as percentage of number of corpora lutea represented by an embryo.

The plasma samples were analysed for progesterone concentrations using the double antibody RIA method described by Beltranena et al. (1991). The sensitivity of the assay, defined as 84 ± 0.50% of total binding was 0.01 ng/tube. The intra- and inter-assay coefficients of variation were 6 and 12%, respectively, with an extraction efficiency of 80%, and no correction for recovery. The plasma LH concentration was estimated to determine the time of the preovulatory LH peak concentration, by the homologous double antibody RIA method as described by Cosgrove et al. (1991). All samples were evaluated in a single assay with an intra-assay variation of 8.5%. The sensitivity of the assay, defined as 85% of total binding, was 0.01 ng/tube. Plasma progesterone concentrations at 0, 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66, 72 and 78 h from the LH peak were calculated in both the groups. The time from the LH peak at which progesterone began to rise and the subsequent rate of increase was determined by using a two phase regression of plasma progesterone concentration as reported by Pharazyn (1992) and is described in the following equations:

$$Y = a + b_1 (x-t) + b_2 * Abs (x-t)$$

$b_a = b_1 - b_2$

$b_b = b_1 + b_2$

where

Abs = absolute value

a = y coordinate of the inflection point

t = x coordinate of the inflection point

b_a = slope of line left of inflection point

b_b = slope of line right of inflection point

The x-coordinate of the inflection point was defined as the time at which progesterone concentrations began to rise in the peripheral plasma. The rate of increase in plasma progesterone was defined as the slope of the second regression line and peri-ovulatory concentrations were defined as the average progesterone concentration prior to the inflection point.

Phase 2. Gilts were individually fed 2.5 kg feed per day and heat checked starting two days before the expected date of onset of estrus as in Phase 1. After double insemination using pooled semen, these gilts were allocated randomly to group N or H and fed as described for Phase 1. Blood samples (2.5 ml) were collected by acute venepuncture at 48 and 72 h after detection of estrus. Plasma samples were analysed for progesterone using the RIA described above, in a single assay with an intraassay coefficient of variation of 6.2%. The gilts were slaughtered on d11 or 12 of pregnancy. Genitalia were collected and corpora lutea were counted on both ovaries.

The uteri were cleared of their ligaments and weighed on a scale. Both uterine horns were clamped tightly and the uterus was hung vertically on a stand. To collect uterine flushings, each uterine horn was infused with 15 ml of physiological saline and the secretions were collected into a clean culture tube by releasing the clamp. After transfering the embryos, if any, into a watch glass, these secretions were centrifuged for 10 minutes, and the supernatent was stored at -30° C until analysed for IGF-1 and uterine plasmin/trypsin inhibitor (UPTI). In order to flush out the remaining embryos, each horn was flushed twice with 100 ml of physiological saline and the flushings were collected into a clean beaker. These flushings were examined under a dissecting microscope for the presence and developmental stage of embryos, catagorized as either spherical, elongated or filamentous blastocysts. Embryonic survival was calculated as the percentage of corpora lutea for a particular gilt, represented by an embryo. IGF-1 in uterine flushings was quantified as described by Cosgrove et al. (1992) in a single RIA procedure with an intraassay coefficient of variation of 3.19%. The detection limit of the assay was 0.08 ng/tube, at a binding of 89.8%. UPTI activity was estimated by the procedure described by Stallings-Mann et al. (1994), with modification that 2-fold dilutions of the trypsin standard in HEPES buffer were run to obtain a regression for calculating the mass of UPTI in unknown assay tubes.

Statistical Analyses

In Phase I, ovulation rates and embryonic survival were compared between the two dietary treatment groups by the GLM procedure of SAS (1988). Plasma progesterone concentrations were analysed with respect to time of pre-ovulatory LH

peak. The mean periovulatory (pre-rise) progesterone concentrations, time intervals from LH-peak to the rise in plasma progesterone concentrations, and rate of rise in plasma progesterone concentrations were compared between the two dietary treatments using the Student's t-test procedure (SAS, 1988).

In Phase-2, the effect of the dietary regimens on ovulation rate (total number of corpora lutea), total number of embryos, embryonic survival, plasma progesterone concentrations at 48 and 72h and wet uterine weights were assessed by the GLM procedures of SAS (1988). Effect of treatment. and also treatment and day of slaughter interaction on uterine IGF-1, UPTI were compared using GLM procedures of SAS (1988). Correlation analysis (SAS, 1988) was used to examine associations among various parameters. The variance (standard deviation) in embryonic survival between the two treatment groups in both the phases were compared using the Student's t-test procedure (SAS, 1988). In both phases data from gilts with zero embryonic recovery were removed from the analysis.

Results

The peri-ovulatory progesterone concentrations, post-LH-peak time of progesterone rise and rate of increase in progesterone concentrations for Phase 1 are given in Table III.1. The pre-ovulatory LH peak occurred on average 9h after observed onset of estrus. The mean interval from LH-peak to the beginning of the post-ovulatory progesterone rise of 28.75 ± 2.27 h in group N was shorter than that of 38.55 ± 3.19 h in group H gilts (P=0.02) (Table III.1, Fig. III.1). Periovulatory

progesterone concentrations of 0.87 ± 0.30 and 1.0 ± 0.21 ng/ml in group N and H gilts, respectively were not different (P=0.74). Also, there was no difference (P=0.82) in the rate of rise in plasma progesterone (0.24 ± 0.03 and 0.23 ± 0.04 ng/ml/h in N and H gilts, respectively). Mean progesterone concentrations at various intervals from LH peak in the two treatment groups of gilts are shown in Fig III.1. Mean number of corpora lutea (ovulation rates) were 15.33 ± 0.76 and 14.67 ± 0.71 in groups H and N, respectively. The observed percentage recovery of embryos on d3 to 5 was 86.50 ± 2.10 and 74.20 ± 6.24 in N and H groups, respectively , and there was a difference in variability in embryonic survival (P<0.05) between the two groups. The data on embryonic development and recovery in the two groups are shown in Tables III.2, III.3 and III.4. Due to unavoidable variation in the time of slaughter and concerns about the reliability of the technique for recovery of embryos, it was not possible to determine conclusively whether nutritional treatments affected the rate of oviductal transport and the development of embryos. Therefore, data in Tables III.3, and III.4 suggest treatment effects, but results of statistical analyses are not presented.

Mean plasma progesterone concentration data obtained from group N and H gilts in Phase 2 of the study are presented in Table III.5. Mean plasma progesterone concentration 72h after onset of estrus was higher (P=0.02) in group N than in group H. The ovulation rates of N and H groups were 15.75 ± 0.40 and 15.33 ± 0.49, and the observed recovery rates of embryos from the uterus after slaughter on d11 or 12 were 80.5 ± 3.4 and 71.8 ± 4.4 %, respectively (P=0.14). The mean total wet weight of the dissected uterus plus conceptus before flushing was 820 ± 42 and 729 ± 42 g, in N and H gilts, respectively (P=0.11).

The results of the analysis of uterine flushings are also shown in Table III.5. There was no difference (P=0.37) IGF-1 concentrations in the uterine flushings between the two treatment groups; however, UTPI concentrations were higher in group H than group N gilts (P=0.03). The interactions between treatment and the day of slaughter were also significant for UPTI only (P=0.06).

There was a positive correlation between progesterone concentrations at 72 h and embryonic survival in both group N (r=0.62; P=0.03) and H (r=0.54; P=0.06) gilts. Uterine weight was positively related to plasma progesterone concentrations at 72 h in group N (r=0.72; P=0.007) and to uterine IGF-1 concentrations (r=0.79; P=0.002) in group H gilts. A positive correlation was also seen between embryonic survival and uterine weight in group N (r=0.62; P=0.03) gilts only.

The data on embryonic recovery and the developmental stage of embryos are shown in Tables III.6 and III.7. Again due to unavoidable variation in the slaughter time and thus time of embryonic recovery, and problems in separating the entangled masses of embryos in the filamentous stage, it was difficult to determine with confidence, whether the nutritional treatment affected the rate of development. Therefore, data in Tables III.6 and III.7 suggest treatment effects, but results of statistical analyses of these data are not presented.

Discussion

In this study the pre-rise progesterone concentrations were low and not different between the two treatment groups of gilts. Low levels of progesterone in gilts

until 50h (Pharazyn, 1992) or 2-3 days (Gutherie et al., 1972) after onset of estrus have been reported previously. The timing of the plasma progesterone rise after the LH-peak was delayed by 10h in gilts that were continued on high plane of feeding after mating, indicating an effect of nutrition during the periovulatory period on plasma progesterone concentrations. Preliminary observations made by Pharazyn (1992), indicated that feeding 1.8 vs 2.5 kg feed per day resulted in a difference in the timing of the rise in progesterone concentrations, though the differences were not significant. In the present study gilts were fed individually on the basis of their metabolic body wt., and estrus was detected at 6-h intervals, thus providing a better comparison of the effects of feed intake before and after mating. Also, in this study, because progesterone concentrations were normalized to the time of the peak concentration of LH, rather than to the time of onset of estrus (Pharazyn, 1992), the comparisons made are likely to be more reliable. The difference in timings of events indicate that high nutrition during the periovulatory stage can affect the time at which progesterone starts to rise. This could be a crucial factor in timing changes in the uterine environment, which may affect the synchrony between the uterus and the developing embryos and thus their viability at this stage. Also, there is a possibility of nutritional effects on the earlier increase of local oviductal concentrations of progesterone (Pharazyn et al., 1991) which may affect early embryonic development by changing the oviductal environment.

Variation in the time at which progesterone started rising within groups and between treatments could be due to increased production of progesterone by advancing development of the corpora lutea. However, as the nutritional treatments were imposed

very close to the time of ovulation, they could have little or no influence on the maturation of preovulatory follicles. Thus, if anything, the nutritional changes might affect the process of luteinization itself. Variation in luteal development and subsequent progesterone production by the developing corpora lutea may be reflected in differences in peri-ovulatory progesterone concentrations and the time required for progesterone concentrations to increase in the peripheral circulation. A remote possibility is that differences in plasma progesterone profiles between gilts may be related to luteal heterogeniety. Heterogeniety in corpora lutea has been reported in the cow (Estergreen et al., 1968), sheep (Hunter and Southee, 1983) and pig (Ottobre et al., 1984).

An alternative hypothesis, supported by results reported in the literature, is that changes in feed intake imposed may affect the metabolic clearance of progesterone and hence circulating concentrations of progesterone. Reduced feed intakes may be associated with reduced blood flow to the liver via the hepatic portal circulation (Symonds and Prime, 1989) and also reduced sequestration of steroids into the gut contents and entero-hepatic recirculation. These effects would create higher plasma concentrations at a fixed rate of progesterone secretion. As the rate of rise in progesterone concentrations was not different in the two groups (Table III.1), the lower concentrations of plasma progesterone in H gilts were only due to a delayed increase in progesterone concentration in relation to the LH peak. This could result in differences in the timing of changes in the oviductal and/or uterine environment and hence an asynchrony between embryo and reproductive tract which may cause embryonic mortality.

In pigs the first cleavage occurs between 60 and 108 h after estrus onset when the embryo is in the oviduct (Stroband and Van der Lende, 1990). Embryos enter the uterus at 48h (with an extension of up to 24h) after ovulation when they are at the 4-8 cell stage, after which they remain near the uterotubal junction until d5 or 6 of gestation. The present study was conducted in two phases to determine whether the nutritionally induced effects on embryonic survival and/or development take place during very early pregnancy, i.e., before or during transfer of embryos from oviduct to the uterus, or at a later stage, i.e., during the expansion of embryos in the uterus before attachment. In Phase 2, mean progesterone concentration at 72h was again different between the two groups, thus confirming the results from Experiment 1 (Jindal et al., 1996). Though the data on the rate of embryonic development are not conclusive, the dietary treatment appeared to affect variation in recovery of embryos (presumed to be an estimate of embryonic survival). The reduced variation in embryonic survival in group N in both phases of the study indicate that the impact of nutritional changes on embryonic survival could occur at an early stage of pregnancy, thus emphasizing the need for careful nutritional management of gilts during early pregnancy.

Pharazyn et al. (1991) reported similar levels of plasma progesterone in the jugular vein and in veins draining the left and right uterine horns, but progesterone concentrations in the ovarian and oviductal veins were many times higher. Further, a significant elevation in plasma progesterone in the oviductal vein was observed within 24h of the onset of estrus. Since oviductal (Buhi et al., 1990; Murray, 1993) and endometrial (Ashworth and Bazer, 1989; Roberts et al., 1993) secretions are modified by ovarian hormones, such changes in the plasma progesterone concentrations during

early pregnancy could affect embryonic survival by modifying the secretion of oviductal and/or endometrial proteins. Phase 1 of the study was carried out at the stage when the embryos were in the oviduct or had just entered the uterus. A difference in the variations in embryonic recovery, as indicated by a SD of 6.69 vs 19.67 ($P<0.05$) in group N and H, respectively at this stage indicates the importance of oviductal environment, and at least some effect on cleaving/developing embryos during tubal transport.

A changing uterine environment will influence the timing of early development and the viability of embryos (Stroband and Van der Lende, 1990). As there was no difference between uterine IGF-1 concentrations between the two groups of gilts, secretion of uterine IGF-1 may not be dependent on nutrition during early pregnancy. Differential regulation of IGF-1 gene in various tissues has been reported (Charlton et al., 1993). In this phase of the study the gilts were slaughtered on d11 or 12, at which time uterine IGF-1 reaches a peak level (Simmen and Simmen, 1990). It is also possible that the nutritional treatment might have affected the time of initial rise in IGF-1 and once it reached peak levels, no differences were detectable. Further studies are required to confirm this hypothesis.

The difference in UPTI concentrations between the two groups indicates an effect of nuritional regimens and the rise in post-ovulatory progesterone concentrations on this important uterine protein. Statistically significant interaction between treatment and the day of slaughter for UPTI concentrations further suggests the modification of uterine environment by dietary treatments. As the gilts were slaughtered on d11-12, it is possible that due to the early rise in progesterone, the UPTI concentrations started

rising, and reached peak concentrations, earlier than the day of slaughter. Further studies are required to confirm these hypotheses. A significant correlation between embryonic survival and plasma progesterone concentrations at 72 h after onset of estrus confirms our previous findings (Jindal et al., 1996).

Table III.1. Periovulatory plasma progesterone concentrations, post-LH-peak time of rise in progesterone, and rate of rise in progesterone in two dietary treatment groups of gilts (Mean ± S.E.)

Group	Periovulatory progesterone concentration, ng/mL	Post-LH-peak time of rise in progesterone, h	Rate of rise in progesterone, ng·mL·h
N	.87 ± .03	28.75 ± 2.27[a]	.24 ± .03
H	1.00 ± .21	38.55 ± 3.19[b]	.23 ± .04

N = gilts receiving 1.5 x maintenance from d 1 of pregnancy.

H = gilts receiving 2 x maintenance from d 1 of pregnancy.

[a,b] Means differ significantly between the two groups (P = .02).

Table III.2. Mean Ovulation rate (Ovu. Rate), Recovery of unfertilized oocytes (Unfert.), or embryos at various stages of development, Total embryos and Embryonic survival (ES) in gilts belonging to dietary treatment groups N and H.

Gilt No.	Group	Ovu. rate	Unfert.	1-cell	2-cell	3-cell	4-cell	5-cell	6-cell	7-cell	Total Embryos	ES
129	N	13	0	0	0	0	11	0	0	0	11	84.61
214	N	17	0	0	4	0	11	0	0	0	15	88.23
106	N	15	0	0	0	0	0	0	0	12	12	80.00
133	N	17	1	0	0	0	13	1	0	0	14	82.35
136	N	12	0	0	8	0	2	0	0	0	10	83.33
201	N	14	0	0	0	0	6	5	1	2	14	100.00
203	N	17	0	0	0	2	2	9	2	0	15	88.23
140	N	19	0	0	0	0	15	0	0	0	15	78.95
127	N	14	0	0	6	0	7	0	0	0	13	92.86
143	H	16	0	0	3	0	8	0	0	0	11	68.75
219	H	13	0	0	0	0	1	4	4	0	9	69.23
131	H	16	0	0	0	0	12	0	0	0	12	75.00
205	H	16	0	0	7	0	3	0	0	0	10	62.50
139	H	15	0	0	0	0	12	0	1	0	13	86.66
223	H	10	0	0	0	0	7	0	0	0	7	70.00
117	H	14	3	3	2	0	0	0	0	0	5	35.71
138	H	15	0	0	0	0	15	0	0	0	15	100.00
135	H	17	0	0	0	0	16	0	1	0	17	100.00

Table III.3. Embryonic recovery up to 66h, from 68 to 78h, 79 to 90h and 91 to 120h of pregnancy in gilts belonging to dietary treatment groups N and H.

Recovery time	Group	Unfert.	1 cell	2 cell	3 cell	4 cell	5 cell	6 cell	7 cell	Total recovery
Up to 66h	N	0	0	18	0	37	5	1	2	63
	H	3	3	5	0	8	0	0	0	19
From 67 to 78h	N	1	0	0	2	15	10	0	0	28
	H	0	0	0	0	38	0	1	0	39
From 79 to 90h	N	0	0	0	0	15	0	0	0	15
	H	0	0	0	0	24	0	1	0	25
From 91 to 120h	N	0	0	0	0	0	0	0	12	12
	H	0	0	7	0	4	4	4	0	19

Table III.4. Site of embryonic recovery in gilts belonging to dietary treatment groups N and H.

Treatment group	Site of embryonic recovery		
	Oviduct	Oviduct + Uterus	Uterus
	Number of gilts	Number of gilts	Number of gilts
N	3	4	2
H	6	2	1

Table III.5. Plasma progesterone concentrations and insulin-like growth factor-1 (IGF-1) and uterine plasmin/trypsin inhibitor (UPTI) concentrations in uterine flushings, in dietary treatment groups N and H (mean ± S.E.).

Group	Progesterone 48h after estrus onset, ng/ml	Progesterone 72h after estrus onset ng/ml	Uterine IGF-1 ng/ml		UPTI* μg/mL	
			d11	d12	d11	d12
N	6.78 ± .68	14.65 ± 1.17[a]	18.30 ± 2.68	11.63 ± 4.38	335.52 ± 119.32	101.02 ± 62.17[a]
H	7.69 ± .56	10.85 ± 1.00[b]	21.35 ± 7.06	17.81 ± 3.40	384.00 ± 85.07	552.35 ± 74.30[b]

N = gilts receiving 1.5 x maintenance from d 1 of pregnancy.

H = gilts receiving 2 x maintenance from d 1 of pregnancy.

[a,b] Means differ significantly between two groups ($P < .05$).

*Significant interaction between treatment and the day of slaughter ($P < .06$).

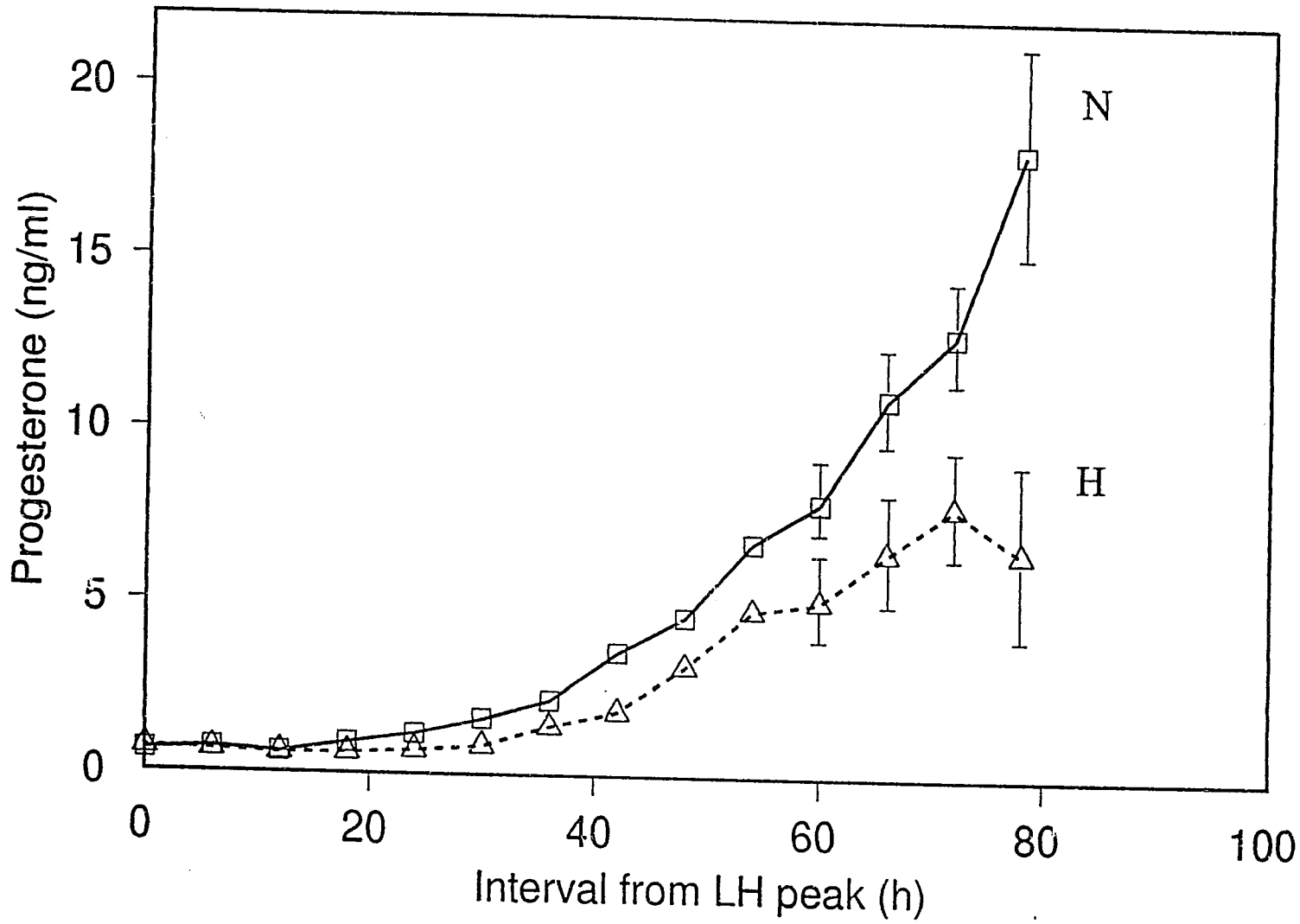
Table III.6. Ovulation rate (Ovu. rate), recovery of embryos at different stages of development, total embryos and embryonic survival (ES) in gilts belonging to group N slaughtered on d11-12.

Gilt No.	Ovu. rate	Spherical	Elongated	Filamentous	Total embryos	ES
305	14	0	9	3	12	85.71
324	15	12	0	0	12	80.00
359	16	0	10	2	12	75.00
364	18	11	0	0	11	61.11
312	17	0	2	12	14	82.35
309	15	10	1	0	11	73.33
365	14	12	0	0	12	85.71
221	16	2	14	0	16	100.00
323	13	8	0	0	8	61.53
319	18	12	3	0	15	83.33
302	18	14	0	0	14	77.77
353	15	0	0	15	15	100
Total		**81**	**37**	**32**	**152**	
%		**53.28**	**24.34**	**21.05**		

Table III.7. Ovulation rate (Ovu. rate), recovery of embryos at different stages of development, total embryos and embryonic survival (ES) in gilts belonging to group H slaughtered on d11-12.

Gilt #	Ovu. rate	Spherical	Elongated	Filamentous	Total embryos	ES
314	16	11	0	0	11	68.75
360	15	10	2	0	12	80.00
315	15	8	1	0	9	60.00
358	16	8	4	0	12	75.00
350	15	4	6	0	10	66.66
301	19	14	4	0	18	94.73
209	15	0	0	6	6	40.00
311	16	2	12	0	14	87.50
322	13	2	4	2	8	61.54
304	16	15	0	0	15	93.75
313	16	0	4	8	12	75.00
208	12	7	0	0	7	58.33
Total		**81**	**28**	**12**	**121**	
%		**66.90**	**23.14**	**9.92**		

Figure III.1. Plasma progesterone concentrations (Mean ± S.E.) in N (fed 1.5 x maintenance from d1 of pregnancy) and H (fed 2 x maintenance from d1 of pregnancy) gilts at different time intervals from the postovulatory LH-peak.


Progesterone (ng/ml)
20
15
10
5
0
0
20
40
60
80
100
Interval from LH peak (h)
N
H

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Chapter IV

Supplementary progesterone reverses nutritionally-induced detrimental effects on embryonic survival in gilts

Introduction

In various attempts to improve embryonic survival in the pig the role of nutrition before and after mating has received much attention, but the mechanisms mediating such effects have not been identified clearly. Previous literature (den Hartog and van Kempen, 1980) and recent studies (Dyck and Strain, 1983; Pharazyn, 1992; Jindal et al., 1996) have demonstrated positive effects of flush feeding during the pre-mating period on ovulation rates, but continuation of these high levels of nutrition during the immediate post-mating period could be detrimental to embryonic survival. An inverse relationship between level of nutrition and circulating progesterone concentration has been reported in pigs (Dyck et al., 1980; Prime et al., 1988; Ashworth, 1991; Jindal et al., 1996). Also, irrespective of the nutritional regimen, embryonic survival was related to plasma progesterone concentrations during early pregnancy (Pharazyn, 1992; Jindal et al., 1996) and the variability in viable embryonic survival was higher in gilts with lower plasma progesterone (Jindal et al., 1996). Substantial variation in plasma progesterone concentrations among gilts (1.3 to 25.5 ng/ml) measured on d3 after onset of estrus was reported by Pharazyn (1992).

It is known that the ovarian hormones can control the development and secretions of the oviduct (Buhi et al., 1990; Murray, 1993) and endometrium (Dziuk, 1987; Ashworth and Bazer, 1989; Simmen and Simmen, 1990; Roberts et al., 1993), thus modifying the oviductal and the uterine environment by bringing about physiological and biochemical changes which can affect the normal development and survival of early embryos. Several major progesterone-responsive polypeptides have

been purified from porcine uterine secretions , and their cDNA cloned (Roberts et al., 1993). In Experiment 2 (Phase 2) of the present studies, the plane of feed intake during early pregnancy affected uterine protease/trypsin inhibitor (UPTI) concentrations in the gilt (Chapter III). Though the swine trophoblast is potentially invasive (Samuel and Perry, 1972), the integrity of the uterine epithelium is proposed to be maintained by UPTI. In a preliminary study by Pharazyn (1992), and also in our controlled experiment (Chapter III), high intake of feed immediately post-mating resulted in a delay of nearly 10h in the time at which the progesterone concentrations started rising after the preovulatory LH-peak. The onset of the rise in plasma progesterone has also been reported to be 20h earlier in highly prolific Meishan compared to Large White gilts (Hunter et al., 1996). As the secretion of several uterine proteins can be modified by plasma progesterone concentrations during early pregnancy, the timing of the rise in progesterone may be an important factor in determining synchrony in the rate of uterine and embryonic development and therefore the likelihood of an embryo remaining viable.

For a successful pregnancy, there should be proper synchrony between the developing embryo and the uterine environment. The conceptus synthesizes various proteins, prostaglandins and steroids which together with ovarian steroids modify uterine morphology and endometrial secretory activity. Differences in the protein composition of uterine secretions between cyclic and pregnant females is well established. Also these conceptus proteins may be important for remodelling and preparation of the uterus for establishment and completion of pregnancy, by stimulating increased endometrial folding and formation of the glycocalyx on the

uterine microvilli required for conceptus attachment. Conceptus estrogens secreted on d11-12 are considered to be the principal antiluteolytic signal in the pig to prevent luteal regression by redirecting $PGF_{2\alpha}$ into the uterine lumen (Bazer, et al., 1989). Also, these estrogens have been shown to increase uterine arterial blood flow and uterine vasculature permeability (Ford and Stice, 1985). On the other hand, various endometrial proteins can also affect the development, secretory activity and survival of the embryos. In vitro studies (Rice et al., 1981) have shown an increase in protein secretions by porcine blastocysts, in the presence of endometrial tissue. In swine, IGF-1 concentrations in uterine luminal fluid change during early pregnancy with maximal concentrations at d10 and 12, coincident with blastocyst elongation (Simmen and Simmen, 1990). Also, as mentioned earlier, UPTI prevents the embryo from invading the endometrium. Therefore, for successful maintenance of pregnancy, secretion of the endometrial proteins and the conceptus products in appropriate amounts, and at an appropriate time, is essential.

In pigs, considerable variation exists in the morphological stage of development among littermates and proper synchrony within a litter appears important for the successful maintenance of pregnancy. The first experimental evidence for a relationship between embryonic diversity and embryonic mortality comes from the work of Pope et al. (1982) in which d5 and d7 embryos were transferred together into d6 recipients. The results indicated that between d60-70 more fetuses survived from d7 embryos than d5 and it was concluded that an increase in embryonic diversity resulted in the loss of smaller embryos after d11 of the pregnancy. These observations were substantiated by results from another experiment (Pope et al., 1986) in which d6

embryos were transferred into pregnant d7 recipients, and d7 embryos into d6 recipients. Higher variation in development and greater embryonic mortality was observed when d6 embryos were transferred to d7 recipients than the reverse. Relatively advanced embryos secrete greater amounts of estradiol than their less developed littermates (Pope, 1988). The changes in the uterine environment elicited by enhanced estrogen secretion from more advanced embryos may be detrimental to lesser developed embryos. The indirect embryocidal effect of these estrogens is clear from experiments in which administration of exogenous estrogens around d10 led to the mortality of less developed embryos at the onset of implantation (Pope et al., 1986; Morgan et al., 1987a,b; Giesert et al., 1991). Those embryos that do not survive, may not be inherently defective (non-viable), but interact with a uterine environment that may not be favourable for their survival. Thus anything that would advance or retard the development of either the uterus or the embryo potentially influences the proportion of embryos that survive.

As progesterone concentrations during early pregnancy can bring about major changes in the oviductal and uterine environments, any changes in plasma progesterone concentrations, either due to an indirect factor like nutrition (Ashworth, 1991; Pharazyn, 1992; Jindal et al., 1996), or exogenous progesterone treatment, can influence the timing of early development and the viability of the embryos. Normally, embryos are retained within the oviduct for about two days and secretions in the oviduct may affect cleavage rate or embryonic viability (Fukui et al., 1988; Gandolfi and Moor, 1987). In pigs, co-culture of one-cell embryos with oviduct epithelial cells allowed for a greater transition from single cell to morulae, than if cultured with

fibroblast cells (White et al., 1989). Additionally, the time spent in the oviduct may also be necessary to give the uterus time to prepare to nurture the embryo. Eventually the rising progesterone concentrations seems to cause dilation of the oviduct and as a result, transport of the embryos to the uterus (Dziuk, 1985). After leaving the oviduct, the embryo remains stationary in the vicinity of the utero-tubal junction from d3-6 of gestation. As embryos mature from d7-12 of gestation, they migrate from the oviductal junction, through the bifurcation of the uterus and become intermixed with the embryos from the other side (Dziuk, et al., 1964; Dhindsa et al., 1967). As we know, progesterone dominance of the uterus is required during oviductal transport of the embryos and their early existence in the uterus (Dziuk, 1985), and any changes in progesterone concentrations during early pregnancy can affect the length of time spent by the embryos in the oviduct.

In studies of the highly prolific Meishan breed of pigs, a slower embryonic developmental rate (Youngs et al., 1993) and lower estradiol production (Anderson et al., 1993) was observed than for embryos from Yorkshire pigs. This difference in developmental pattern may be related to the increased embryonic survival reported for the miniature swine (Ford et al., 1988; Conley et al., 1988). Temporal differences in uterine histotroph composition have also been observed between highly prolific Meishan and the Large white pigs (Bazer et al., 1988, 1991; Simmen et al., 1989). Kleemann et al. (1994) indicated that progesterone supplementation to ewes during the first 3 days of pregnancy induced changes in embryo development and enhanced the growth of surviving fetuses.

Available results indicate the possibility of improving embryonic survival by exogenous progesterone administration as has been demonstrated in sheep (Parr et al., 1987). In the only recent experiment assessing the role of progesterone as a mediator of embryonic survival in gilts with high embryonic loss as a consequence of continuous ad libitum feeding, Ashworth (1991), used post-mating supplementary progesterone treatment from d4 of the pregnancy and reported an increase in embryonic survival on d30 from 66.4 to 82.8%. Though the estimates were based on relatively limited numbers of gilts per treatment, and plasma progesterone concentrations were not determined, the results indicate the potential for exogenous progesterone to improve embryonic survival in gilts.

It can be hypothesized that an earlier rise in post-ovulatory progesterone concentrations either as a result of changes in the feeding patterns during early pregnancy (Pharazyn, 1992; Chapter III) or by administration of exogenous progesterone one can modify the oviductal/uterine environment and embryonic transport. Generally, estradiol retards and progesterone enhances the transport of fertilized eggs from oviduct to uterus. So having higher, though within physiological limits, concentrations of progesterone during this time can reduce the time that embryos are resident in the oviduct. The faster rate of embryo transport can result into a slower development rate for the embryos, thus reducing embryonic diversity, and allowing relatively younger embryos to pass the oviductal phase along with the mature embryos. Therefore, most of the embryos will enter the uterus at the same time and will pass some extra time at the tubo-uterine junction. The relatively longer stay at the tubo-uterine junction and a slower developmental rate will give the younger embryos

sufficient time to develop to the extent that more mature embryos have reached, thus reducing embryonic diversity. Also, higher progesterone concentrations will advance uterine maturity, so when these embryos migrate further away from the junction, the uterus will be quite receptive for most of them. Since the embryonic diversity is less, the estrogens secreted by relatively mature embryos will be detrimental to fewer embryos, hence a lower embryonic mortality.

Based on the previous studies in our laboratory (Pharazyn, 1992), and also results of Experiments 1 and 2 (Jindal et al., 1996; Chapter III) the effective window during which changes in nutritional status can affect the periovulatory plasma progesterone concentrations and embryonic survival is critical and limited to the immediate post-mating period. Therefore, the present study was undertaken to test the hypothesis that nutritionally induced effects on embryonic survival in the gilt are mediated by differences in plasma progesterone concentrations in early pregnancy.

Material and methods

The study was conducted with 54 Pig Improvement (Canada) Ltd. Camborough gilts. During their first or second estrous cycle groups of gilts were individually fed twice daily a total of 2.5 kg of a standard commercial, barley-wheat-soybean grower feed, containing 12.5 MJ of DE/kg and 14% crude protein, with ad libitum access to water. Starting two days prior to expected date of next estrus, gilts were heat checked twice daily by fence line contact with a mature vasectomized boar. The gilts were artificially inseminated twice, 12 and 24h after the onset of estrus using pooled semen

from three boars specifically designated for the experiment to minimize any boar effect. All inseminations were done by the same trained person. The 24-h period from first observed standing heat was designated d0. All gilts were individually fed a 2 x maintenance ration daily, split between morning and afternoon meals. The maintenance requirements were calculated for individual gilts on the basis of their metabolic body weight ($B.Wt \cdot kg^{.75}$) to provide a maintenance energy allowance of 461 kJ DE/kg of metabolic body wt. After d15 of the pregnancy, all gilts were fed 1.8 kg feed per day as was done in Experiment 1 of these studies (Jindal et al., 1996). After the second insemination, the gilts were allocated randomly to two treatment groups, H (High plane of nutrition, 2 x maintenance from d1 of the pregnancy) and HP (High nutrition + Progesterone). Group HP gilts were given 3 ml i.m. injections of progesterone (25mg/ml solution in ethyl oleate) 24, 36, 48, 60, 72, and 84h from onset of estrus; group H gilts received vehicle alone. The dose of exogenous progesterone injections was determined in a preliminary experiment described below. The gilts that returned to estrus in the next cycle were removed from the experiment; therefore, data are from 23 and 21 gilts in groups H and HP, respectively.

Blood samples (2.5 ml) were collected from all gilts by acute venepuncture at 36, 48, 60, 84 and 108h from onset of estrus . The plasma was harvested immediately after collection of samples and stored at -30° C for later analysis for progesterone concentrations. Gilts were slaughtered on d28±3 of gestation at a local abattoir and reproductive tracts were recovered immediately after slaughter. Ovaries were examined for number of corpora lutea (ovulation rate). Embryos were recovered by dissecting uterine horns, and were classified as viable or non-viable as described

previously in Chapter II (Jindal et al., 1996) and the crown-rump length of each embryo within the amnion was recorded. Embryonic survival was calculated as percentage of corpora lutea represented by a viable embryo. The plasma samples were analysed for progesterone concentrations in a single assay (intra-assay coefficient of variation 4.1%) using 'Coat-a-Count' radioimmunoassay kits (DPC, Los Angeles), previously validated for pig plasma (Miller, 1996). The sensitivity of the assay, defined as 85% of total binding, was 0.02 ng/tube.

Preliminary dose study

Based on a dose response experiment by Stickney (1981), two doses of exogenous progesterone, expected to maintain high, but physiologically normal concentrations of plasma progesterone in the gilts, were selected for this experiment. A series of six i.m. injections of either 75 mg or 150 mg of progesterone per injection in ethyl oleate base was administered to two similarly maintained groups of 6 prepubertal (average body weight 72 kg) gilts, each at 12-h intervals and blood samples were collected 0, 12, 24, 36, 48, 60, and 72h after the first injection. The samples were collected just before each injection, and were assayed for plasma progesterone concentrations.

Statistical Analyses

The ovulation rate (total number of corpora lutea), total number of embryos, embryonic survival, crown-rump length of embryos and plasma progesterone concentrations at 36, 48, 60, 72, 84 and 108 h, were compared between two groups

using GLM procedures of SAS (1988). The average crown-rump length of all embryos from each gilt was adjusted to d28 using day of slaughter as a covariate. The variance (standard deviation) in embryonic survival in the two treatment groups were compared using the Student's t-test (SAS, 1988). Correlation analyses (SAS, 1988) were used to examine association between progesterone concentrations at different times and embryonic survival.

Results

Results of the preliminary study are shown in Table IV.1. As the dose of 75 mg/injection was sufficient to maintain higher, but near physiological concentrations of plasma progesterone, this was selected for the main experiment. The onset of the rise in plasma progesterone concentrations was observed within 12 h after the first injection, and the 12-h interval between subsequent injections was effective in maintaining higher plasma progesterone concentrations.

Mean plasma progesterone concentrations at 36, 48, 60, 84 and 108 h from onset of estrus, ovulation rates and embryonic survival in group H and HP are shown in Table IV.2. At all times, plasma progesterone concentrations in H gilts were lower ($P<0.001$) than for HP gilts. The ovulation rates were similar for the two groups. Mean embryonic survival (84.83 ± 2.58 %) was higher ($P= 0.004$) for HP gilts compared to H (70.03 ± 4.01 %) gilts. In H gilts, the plasma progesterone concentrations at 36, 48, 60, 84 and 108 h was positively correlated with embryonic survival ($r = 0.49$, 0.49, 0.79, 0.66 and 0.67, respectively; $P<0.01$). The relationship between plasma

progesterone concentrations 60h after onset of estrus and embryonic survival in H and HP gilts is shown in Figure IV.1. Further, there was a greater degree of variance in embryonic survival in H gilts than HP gilts ($P<0.05$). The mean crown-rump lengths were 22.52±0.42 and 22.63±0.36 mm in the groups H and HP, respectively.

Discussion

In Experiment 2 (Chapter III) plasma progesterone concentrations were determined to start rising, on an average, at 28h after the LH peak (nearly 37h after onset of estrus). Gilts on a normal plane of nutrition, compared to the gilts on high level of nutrition, had an earlier rise in plasma progesterone that was associated with greater embryonic survival. In the present study, administration of exogenous progesterone to gilts on the high plane of nutrition from 24h after the onset of estrus, maintained higher concentrations of progesterone from 36h and allowed us to test the hypothesis that nutritionally-induced effects on embryonic survival in the gilt are mediated by differences in plasma progesterone concentrations in early pregnancy. In a few historical studies (Reddy et al., 1958; Davis and Sorrensen, 1959; Morisette et al., 1963) effects of supplementation of progesterone to gilts with normal luteal phase concentrations on embryonic survival were equivocal. The only recent study addressing directly the effect of exogenous progesterone supplementation during early pregnancy (from d4) in improving the embryonic survival in gilts on ad libitum feeding was by Ashworth (1991).

In the present study, the gilts were individually fed a 2 x maintenance ration calculated on the basis of their metabolic body weight as described previously in chapter III (Jindal et al., 1996), so as to exactly repeat the experimental model used in previous studies (Pharazyn, 1992; Experiment 1 and 2). The dose of exogenous progesterone injections was determined in a preliminary experiment in which the selected dose was most appropriate to maintain high but physiological levels of plasma progesterone in the gilts on a high plane of nutrition.Results for plasma progesterone concentrations in the two treatment groups (Table IV.2) confirmed that this was achieved. Also, by giving the first of the series of six injections of progesterone, 24h after the onset of estrus (nearly 12h before the average time of onset of the endogenous rise), it was possible to ensure that gilts were exposed to elevated concentrations of plasma progesterone from 36h. This matched the early rise in endogenous progesterone detected in N group gilts in our previous study (Chapter III) which showed higher embryonic survival. The progesterone injections were given only up to 84h after onset of estrus because previous reports indicated that the window for nutritionally-induced effects on plasma progesterone and embryonic survival in gilts is in the very early phase of pregnancy (Dyck and Strain, 1983: Pharazyn, 1992; Experiment 1).

Enhanced fetal development/growth in response to progesterone supplementation during early pregnancy has been reported for cows (Garette et al., 1988) and ewes (Kleemann et al., 1994). In the present study, the average crown-rump-length of embryos was not different (22.5 vs 22.6) between the two groups. Similarly, in our previous study, no differences were found in the average crown-

rump-length between gilts on high or low planes of nutrition (Jindal et al., 1996) during early pregnancy. It appears that in gilts, the plasma progesterone concentration during early pregnancy may affect the growth/development of the embryos, and thus their viability, before or around attachment. However, for embryos that remain viable and complete a successful attachment, embryonic growth to d28 was not affected by progesterone treatment.

The results of the study substantiate the hypothesis that nutritionally-induced effects of high nutrient intake on embryonic survival in the gilt can be mediated by differences in plasma progesterone concentrations during early pregnancy. The critical window for exposing the gilt to higher plasma progesterone concentrations by administration of exogenous progesterone for improving embryonic survival is immediately after mating.

Table IV.1. Plasma progesterone concentrations (ng/mL) at 0, 12, 24, 36, 48, 60, 72 h from first injection of exogenous progesterone in two groups of gilts (Mean ± S.E.)

Group	0 h	12 h	24 h	36 h	48 h	60 h	72 h
A	.20 ± .04	12.58 ± 1.07	17.77 ± 2.98	19.51 ± 2.91	26.37 ± 4.44	34.64 ± 5.83	45.68 ± 5.27
B	.61 ± .36	6.28 ± .69	14.11 ± 1.61	13.67 ± 1.96	13.30 ± .32	17.44 ± 1.14	22.30 ± 2.33

A = gilts receiving 150 mg of exogenous progesterone per injection.

B = gilts receiving 75 mg of exogenous progesterone per injection.

Table IV.2. Plasma progesterone concentrations at 36, 48, 60, 84, and 108 h after onset of estrus, ovulation rates, and embryonic survival in two treatment groups of gilts (Mean ± S.E.)

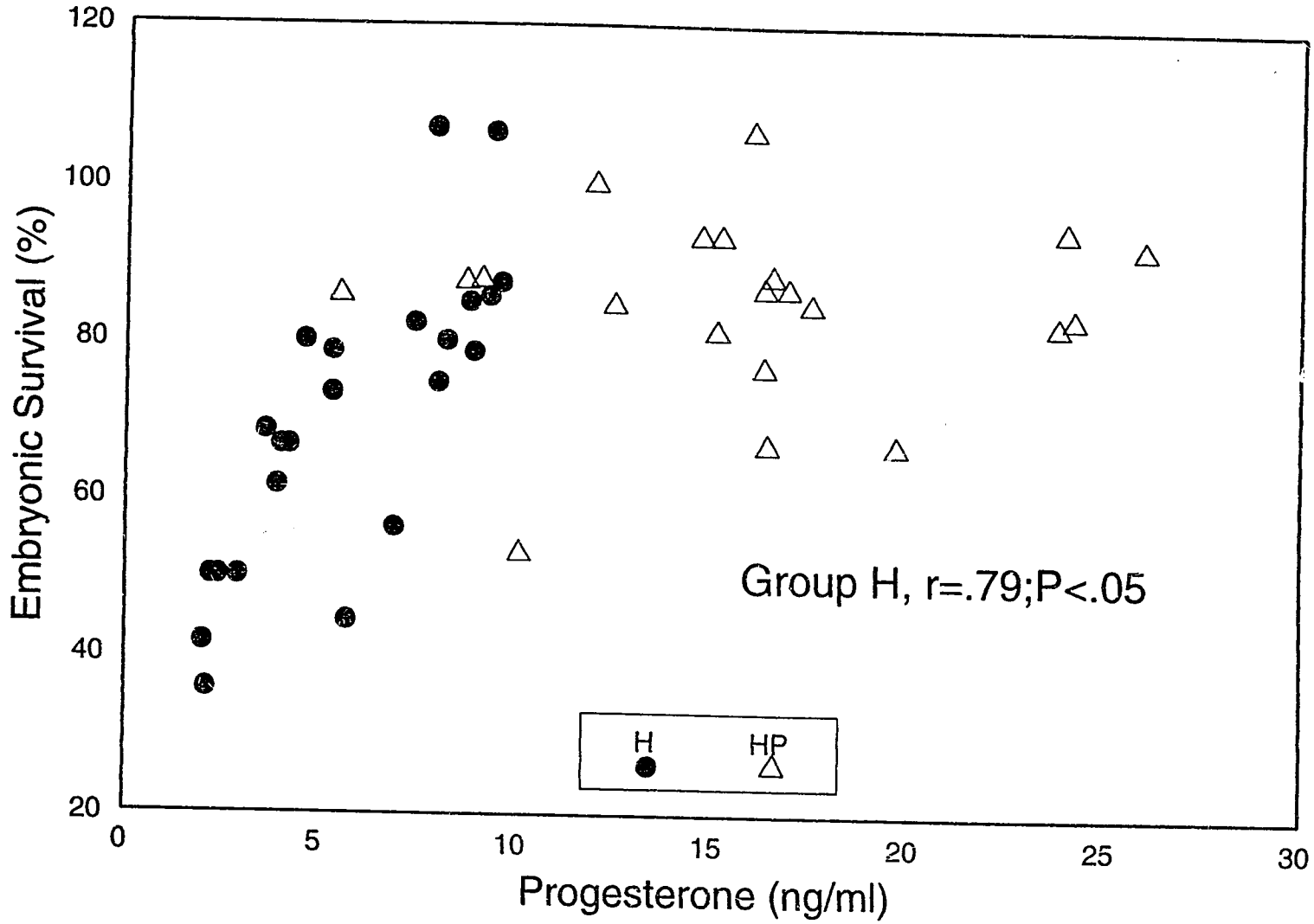
Group	36 h, ng/mL	48 h, ng/mL	60 h, ng/mL	84 h, ng/mL	108 h, ng/mL	Ovulation rate	Embryonic survival, %
H	1.88 ± .26[a]	2.96 ± .21[a]	5.79 ± .55[a]	8.97 ± .58[a]	13.53 ± 1.11[a]	14.86 ± .42	70.03 ± 4.01[a]
HP	14.26 ± 1.01[b]	14.67 ± .82[b]	16.04 ± .18[b]	20.27 ± 1.53[b]	20.92 ± 1.26[b]	15.12 ± .36	84.83 ± 2.58[b]

H = gilts on high plane of feeding (2 x maintenance) from d 1 of pregnancy.

HP = gilts on high plane of feeding + exogenous progesterone (75 mg/injection).

[a,b] Means differ significantly between two groups (P = .001).

Figure IV.1. Relationship between plasma progesterone concentration 60h after onset of estrus and embryonic survival in H (high feed-intake) and HP (high feed intake + progesterone supplementation) gilts.


120
100
80
60
40
20
Embryonic Survival (%)
0
5
10
15
20
25
30
Progesterone (ng/ml)
Group H, r=.79;P<.05
H
HP

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Chapter V

Plasma progesterone and embryonic survival in primiparous weaned sows

Introduction

The lactating sow has a high requirement for energy and protein. Depending upon the nutrient supply and feed intake, mobilization of body reserves may occur during late gestation and lactation. A reduction in feed intake during lactation leads to increased mobilization of body reserves for maintenance of milk production. Dietary effects on milk production increase with the progress of lactation (Tokach et al., 1992). There is also a positive relationship between feed intake and body weight gain during pregnancy and loss of appetite and body weight during lactation (see Einarsson and Rojkittikhun, 1993). The rate of mobilization of the fat depots of a lactating sow is reflected by loss of weight, and a decrease in back fat thickness. The rate of this mobilization is influenced not only by genotype (Rydhmer et al., 1992), but also by the back fat thickness at farrowing (Rojkittikhun et al., 1992), feed intake during lactation and size and weight gain of the litter (Sterning et al., 1990). Feeding level during lactation not only affects milk production but also the interval from weaning to estrus (Mullan and Williams, 1989), and a increase in weaning to estrus interval (WEI) may result in poor reproductive performance (Vesseur et al., 1994). Similarly, excessive weight loss in lactation can affect weaning to estrus interval, the proportion of sows returning to estrus, pregnancy rate and embryonic survival (Mullan and Williams, 1989; Kirkwood et al., 1987, 1990). It has, therefore, been suggested that the long term reproductive performance of sows is best served by minimizing body weight and fat loss during lactation (Einarsson and Rojkittikhun, 1993). An association between sow feed intake (and metabolic changes taking place in the body of a sow) and the

endocrine status during lactation and pregnancy is known (Kirkwood and Aherne, 1985; Baidoo et al., 1992; Foxcroft, 1992; Einarsson and Rojkittikhun, 1993). Baidoo et al., (1992) determined that plasma concentrations of FSH before weaning and FSH and LH concentrations after weaning were significantly higher in sows fed ad libitum during lactation than those receiving 50% of ad libitum intake; however, there was no effect of feed intake during weaning to estrus period. Low basal LH concentration during lactation in poorly fed sows have been reported (see Einarsson and Rojkittikhun, 1993). Also an association between LH concentrations during lactation and weaning to estrus interval has been reported (Tokach et al., 1992). In a recent study (Zak et al., 1996) plasma LH concentrations were lower in sows with restricted feed intake during the last week of a 28-day lactation. Whereas, sows with restricted feeding for first three weeks and ad libitum feeding during the last week had increased LH pulse frequency and mean LH concentrations. This indicated an effect of dynamic changes in nutritional status during lactation on LH secretion which could affect reproductive performance of the sow. Further, in sows on feed restriction, plasma insulin and insulin like growth factor-I (IGF-I) concentrations in plasma were also decreased compared to full-fed sows, indicating that the adverse effects on reproductive performance could be mediated through central as well as local effects at the ovarian level. Consistent with earlier data, feed restriction at any stage of lactation resulted in an increase in the weaning to estrus interval, whereas the pattern of lactational catabolism produced differential effects on ovulation rate and embryonic survival to d28. Although both patterns of feed restriction resulted in a significant reduction in ovulation rate compared to sows on ad libitum feed intake, feed restriction

during last week of the 28-day lactation reduced embryonic survival to 67% as compared to 88% for full fed sows. Refeeding of sows in the last week of lactation, even after three weeks of restriction, was associated with a high level of embryonic survival.

Lower plasma progesterone concentrations during early pregnancy have been associated with increased embryonic mortality in sheep (Ashworth et al., 1989), cattle (Maurer and Echternkamp, 1982) and pigs (Pharazyn, 1992). In Experiment 1, irrespective of feeding regimen, gilts with lower plasma progesterone concentrations had lower embryonic survival and greater variability in embryonic survival (Jindal et al, 1996). In a recent study in our laboratory (Zak et al., 1996) different patterns of lactational catabolism in primiparous sows produced differential effects on ovulation rate and embryonic survival. Sows fed ad libitum for 21 days and subjected to 50% feed restriction during the last week of a 28-d lactation (group AR) had a lower ovulation rates and embryonic survival compared to the full fed (group AA) sows and even more interestingly, similar ovulation rate but lower ES than sows feed-restricted for 21 d and fed ad libitum during last week of lactation (group RA). As the AR treatment selectively reduced ES, and increased variability in ES, we used this treatment (AR) to examine the relationship between plasma progesterone and ES in the weaned sow using regression analysis.

Therefore, to expand our studies in early pregnant gilts the present study tested the hypothesis that in primiparous sows effects of nutrition and body condition during lactation on reproductive performance are also mediated by plasma progesterone concentrations during early pregnancy.

Material and Methods

The study was conducted on 28 primiparous Camborough sows (Pig Improvement (Canada) Ltd.). From d1 to 21 of lactation, sows were fed ad libitum a standard wheat-barley, and soybean lactating sow ration providing 13.4 MJ ME/kg, 15.4 % crude protein and 0.74 % lysine. Feed consumption was determined by the weigh-back method. During the last week of a 28 day lactation, i.e., from d22 to 28, sows were fed only 50% of their mean daily feed consumption from d17 to 21, given as a single meal at 0800h. Sows and piglets had ad libitum access to water.

Using the same rationale as in our previous studies (Zak et al., 1996) litter size was standardized to six piglets within 24h of farrowing to optimize the potential for demonstrating nutritionally-induced changes in reproductive performance. The sows and the piglets were weighed at farrowing, and on d21 and 28 of lactation. Sow back fat was also measured at farrowing, d21 and 28 of lactation. After weaning on d28 to slaughter sows were allowed ad libitum access to a standard dry sow ration providing 13.4 MJ ME/kg, 13.7 % CP and 0.56 % lysine.

From the day of weaning, sows were heat checked twice daily at 0700 and 1900h by fence-line contact with a mature, vasectomized boar to determine the weaning to estrus interval (WEI). Sows were artificially inseminated 12 and 24 h after first observed standing heat (d0), using pooled semen from three boars specifically designated for this experiment and provided by the Alberta Swine Genetic Corporation. All inseminations were done by one of the two trained persons. Daily

blood samples were collected by acute jugular venipuncture on d1, 2, 3 and 4 of gestation. All sows were slaughtered on d28 ± 3 of gestation at a local abattoir, and the reproductive tracts were collected. The total number of corpora lutea were counted on both the ovaries as a measure of ovulation rate. Uterine horns were opened by blunt dissection, and the number of embryos present was counted. Embryos were categorized as viable or non-viable as described previously for gilts (Jindal et al., 1996). Plasma samples were analyzed for progesterone concentrations using 'Coat-a-Count' radioimmunoassay kits (DPC, Los Angeles) previously validated for use with porcine plasma (Miller, 1996). All samples were analyzed in a single assay with an intra assay variation of 4.6%. The sensitivity of the assay, defined as 85% of total binding, was 0.02 ng/tube.

Statistical Analyses

As the experiment was run in four replicates, possible effects of replicates on various parameters were determined by Analysis of Variance procedures of SAS (SAS, 1988). When replicate effects were significant ($P < 0.05$) homogeneity of regression coefficients on embryonic survival, with independent variables as covariates, were tested by GLM procedures (SAS, 1988). Data were subjected to Stepwise regression analysis on embryonic survival (ES) after forcing the effect of replicates into the model (Steel and Torrie, 1980) giving a multiple regression equation of:

$$ES = 202 - 6.41\ d3 + 13.24\ d4 - 5.33\ ov - 0.66\ wtf - 3.25\ loss21\text{-}28$$

(where ES = embryonic survival; d3 and d4 = plasma progesterone concentration on d3 and d4, respectively; wtf = body wt at farrowing; loss21-28 = body wt loss from d21 to 28 of lactation)

Finally the data were subjected to correlation analysis to assess the associations among various parameters (Steel and Torrie, 1980). All of the computations were done using SAS procedures (SAS, 1988).

Results

Mean sow body weight, back fat thickness and litter weights during the 28-d lactation are summarized in Table V.1, and data on feed intakes and body wt loss are in Table V.2. Data on feed intake, body weight and back fat changes from weaning to estrus, weaning to estrus interval, ovulation rate and embryonic survival and plasma progesterone concentrations on d1, 2, 3 and 4 of pregnancy are shown in Table V.3. All sows showed a loss in body wt and back fat from farrowing to d28. Out of a total body weight and back fat loss of 12.5 and 14 %, respectively during lactation, 6.5 and 9 % was during the last week of lactation. Body weight at d21 was directly related to feed intake during the first three weeks of lactation ($r = .41$; $P = .03$).

Weight loss during the last week of lactation was positively correlated with body weight on d21 ($r = .40$; $P = .03$). Total weight loss during the last week, the first 3 weeks and also from farrowing to d28 of lactation, were negatively correlated with back fat at d21 ($r = -.39$, $P = .04$; $r = -.40$, $P = .01$ and, $r = -.55$, $P = .005$, respectively).

A positive correlation was also detected between total wt loss during lactation and litter wt at d21 (r = .54; P = .003), and d28 (r = .54; P = .002).

Feed intake from weaning to estrus was negatively related to back fat at d21 (r = -.39; P = .03). Sows with a higher back fat on d28 also had higher back fat at estrus (r = .77; P = .001), and back fat at estrus had a negative correlation with overall body wt loss during lactation (r = -.45; P = .01). WEI tended to have a positive correlation with body weightwt at farrowing (r = .35; P = .06), but sows with low back fat at estrus had a longer WEI (r = -.52; P = .04). Interestingly ES was negatively correlated with back fat at farrowing (r = .39; P = .07).

No significant differences in ES were seen among various replicates. There was an effect of replicates on on plasma progesterone concentrations on d3 and 4 of gestation (P < 0.001). However, as shown by the test for homogeneity of coefficients of regression, the interactions between plasma progesterone on d3 and d4 by replicates on ES were not different (P > 0.05).

Analysis of variance on ES with plasma progesterone concentrations as the covariate detected highly significant effects of plasma progesterone concentration on d3 and 4 (P = .004 and .0004, respectively) on ES. Stepwise regression analysis indicated significant contributions of plasma progesterone concentrations on d3 and d4 (P=0.02 and 0.0007, respectively), body wt loss during last week of lactation (P=.009), ovulation rate (P=.01) and body weight at farrowing (0.03) to differences in ES. The relationship between plasma progesterone on d3 and embryonic survival in this study is shown in Fig. V.1.

Discussion

The loss of body weight (24.6 kg) and back fat (3.6 mm) during lactation was comparable to that from a previous study from this laboratory (Zak et al., 1996), and other workers (Baidoo, 1989; Prunier et al., 1993). The positive correlation between litter weight gain and loss of sow body weight is consistent with the suggestion of Aherne et al. (1991) that during lactation, the nutrients are partitioned to meet demands for milk production at the expense of maternal tissue catabolism. Thus, while an increase in feed intake results in an increase in milk production (den Hartog et al., 1984), when feed intake is reduced, substrate for milk synthesis from body reserves with little net effect on milk production and litter weight gain. Thus, in our earlier study, there was no difference in litter weights weaned among sows on three different feeding patterns associated with different patterns of lactational catabolism. A reduction in feed intake also results in an increase in the fat content of milk, and the fatty acid pattern of milk may change due to excessive mobilization of body lipid and protein (Mullan and Williams, 1989). In the present study, milk fat was not estimated, but litter weight at d21 of lactation had a positive correlation with body weight loss during lactation.

In this study, sows were allowed ad libitum access to feed after weaning. The sows with higher back fat at the time of weaning also had higher back fat at the time of first post-weaning estrus. However, sows with higher back fat during lactation (d21) had lower feed intake from weaning to estrus ($r = 0.39$; $P = 0.03$) compared to those

with lower back fat at d21. Therefore, it appears that the latter sows had a tendency to make up for their excessive catabolic loss before expressing estrus, and that this did not affect the benefit of having higher fat reserves in late lactation. A positive correlation between body wt loss during lactation and weaning to estrus interval also indicated a tendency for sows to restore their body condition before the next pregnancy. This effect can be extended by the use of 'skip a heat' breeding in which an increase in litter size is reported when the primiparous sows are bred at second instead of first estrus after weaning (Clowes et al., 1994). Although first and second parity sows start to deposit protein by the first post-weaning estrus they continue to break down fat to supply their energy needs for protein anabolism (see review of Foxcroft et al., 1995). In the present study the average weaning to estrus interval of 5.3 days was similar to the 5.1 days observed in AR group of our previous study (Zak et al., 1996), though an increase in weaning to estrus interval of 10d or more has been reported previously in catabolic sows (Koketsu, 1994). The shorter weaning to estrus interval in our studies could be due to lower suckling intensity because litter size was standardized to six piglets.

An average ovulation rate of 15.6 and embryonic survival of 58.5% is also comparable to those for AR gilts in our previous study (Zak et al., 1996). As suggested by Brooks (1982), sows that become catabolic during lactation may remain so after weaning and, as a consequence, have reduced ovulation rates. A 5-d period of ad libitum feed intake after weaning may not be sufficient to change the metabolic status of these sows to anabolic. Interestingly, the results of this study indicate a slightly negative trend between embryonic survival and back fat/wt. at farrowing ($r = -0.39$; $P=$

0.07). The reduction in body wt. during lactation as a percentage of body wt. at farrowing seems to be a good indicator of the way a sow's metabolism is regulated after weaning (Vesseur et al., 1994).

In the present study, there was a highly significant correlation between plasma progesterone on d3 and 4 and embryonic survival (Fig. V.1). A significant effect of plasma progesterone con ..rations during early pregnancy on embryonic survival has been reported for gilts (Ashworth, 1991; Pharazyn, 1992; Jindal et al., 1996). In sows, an effect of excessive loss of body wt. and back fat during lactation on hepatic blood flow and metabolic clearance rate of plasma progesterone has been suggested by Aherne and Kirkwood (1985). Results of the present study clearly support the hypothesis that progesterone could be an important mediator of the effects of metabolic status during lactation and after weaning on reproductive performance of the primiparous sow.

In the gilt model used in our laboratory (Pharazyn, 1992; Jindal et al., 1996; Chapter III and IV), the nutritional treatments were imposed very close to the time of ovulation. Therefore, although treatment could have little or no influence on the maturation of pre-ovulatory follicles it might affect the process of luteinization itself. The other possibility is that short-term changes in feed intake after mating could affect the rate of hepatic blood flow (Symonds and Prime, 1989) and metabolic clearance rate of progesterone (Parr, 1982), resulting in differences in plasma progesterone concentrations during early pregnancy which could affect embryonic survival. In contrast, dietary treatments in the sow model are imposed during lactation and cause in differences in metabolic status of sows. The induction of lactational catabolism may

influence progesterone secretion in two ways, neither of which would involve short-term effects of feed intake on progesterone metabolism. First, as already mentioned, some of the differences in metabolic status of a sow due to serious catabolism during lactation may persist into the periovulatory period. Of the various metabolites and metabolic regulators studied in our previous experiments (Zak et al., 1996), IGF-1 would appear to be a prime candidate to mediate such effects by affecting the gonadotropin and (or) gonadotropin releasing hormones, and thus influencing the maturation and luteinization of follicles, and hence post-ovulatory progesterone secretion. Secondly, as the follicles that are recruited into the ovulatory population at the first estrus after weaning undergo their initial stages of development during lactation they may be adversely affected by the catabolic state of the sow at this time. Therefore, as suggested by Foxcroft et al. (1995), nutritional/metabolic status during lactation may exert an 'imprinting' effect on the developing follicles. Such effects may limit the full maturation of the follicle, thus compromising the normal process of luteinization, and the pattern of the post-ovulatory progesterone secretion.

As documented in the Chapter III, a high plane of feed intake immediately after mating resulted in a 10-h delay in the post-ovulatory rise in progesterone which was associated with differences in embryonic survival in gilts. The onset of the post-ovulatory plasma progesterone rise has also been reported to be 20 h earlier in the Meishan pig, a breed known for its high prolificacy, as compared to Large White gilts (Hunter et al., 1996). Further, the ovarian hormones can control the development and secretory activity of the oviduct (Buhi et al., 1990; Murray, 1993) and endometrium (Roberts et al., 1993). Several major progesterone-responsive polypeptides have been

purified from porcine uterine secretions and their DNA cloned (Simmen and Simmen, 1990; Roberts et al., 1993). In Experiment 2 (Phase 2) of the present studies (Chapter III), gilts on different dietary regimens had differences in the uterine plasmin/trypsin inhibitor (UPTI) activity which has an important role in maintaining the integrity of uterine epithelium against the invasive potential of pig trophoblast, and hence the attachment of the early embryo. So, any process that can change plasma progesterone concentrations during early pregnancy can affect development and survival of an embryo.

Based on our gilt-studies, recent reports on Meishan pigs, and results of the present experiment, it can be concluded that effects of nutritional status and the patterns of change in body condition during lactation on subsequent reproductive performance of primiparous sows can be mediated by differences in plasma progesterone concentrations during early pregnancy.

Table V.1. Mean (± S.E.M.) sow body weights, back fat thickness and litter weights during a 28-d lactation.

Day of lactation	Sow Body weight	Back fat thickness	Litter weight
	kg	mm	kg
0 (Farrowing)	199.60 ± 2.75	18.43 ± 0.39	9.87 ± 0.23
21	186.96 ± 2.50	17.43 ± 0.35	45.52 ± 0.94
28	174.83 ± 2.30	15.85 ± 0.31	55.33 ± 1.10

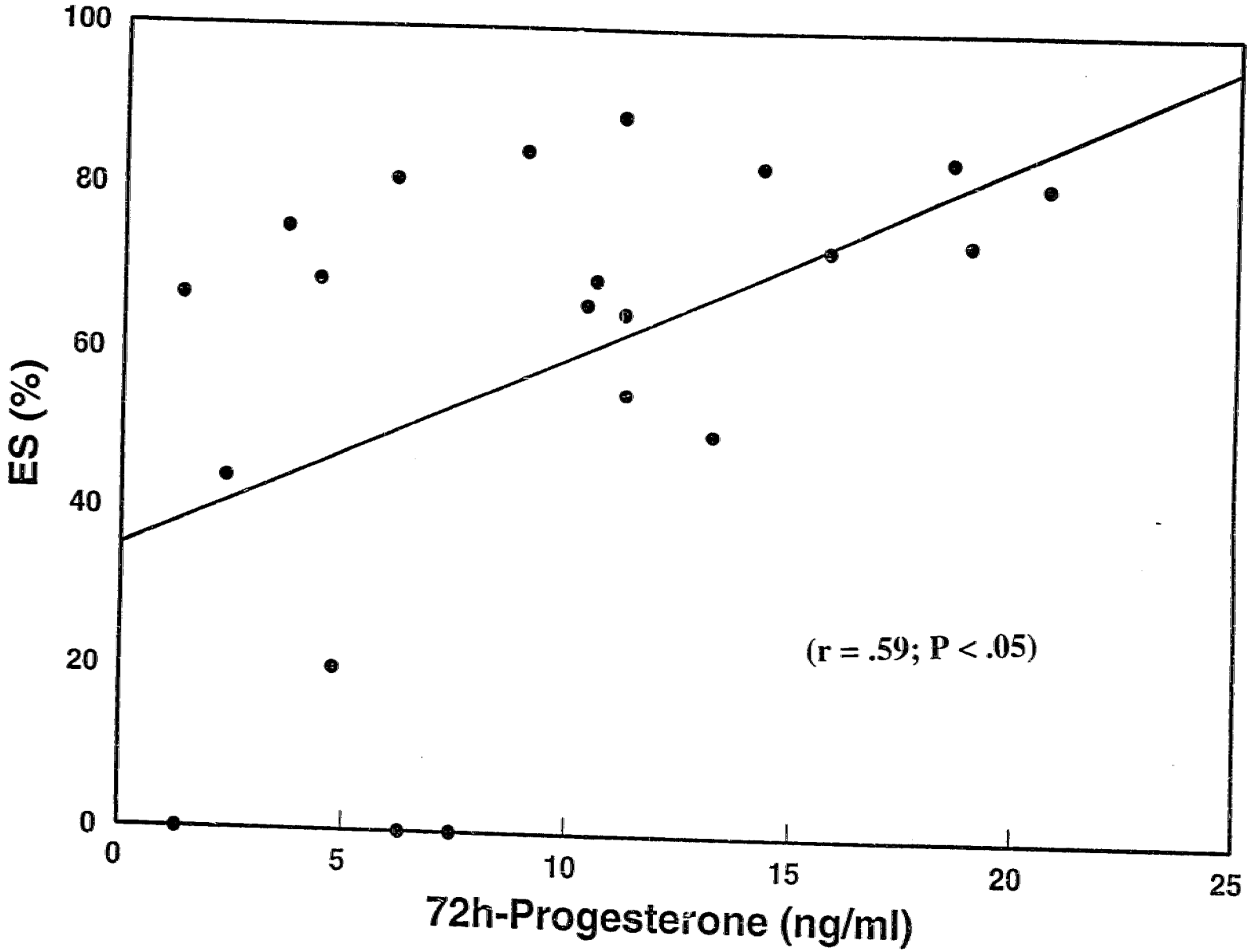
Table V.2. Mean (± S.E.M.) Daily feed intake and body weight loss during a 28-d lactation.

Period of lactation	Feed intake	Body weight loss
	kg/d	kg
d1 to d21	5.12 ± 0.11	12.65 ± 1.51
d22 to d28	2.66 ± 0.06	12.12 ± 0.77

Table V.3. Mean (± S.E.M.) Daily feed intake from weaning to estrus, body weight and back fat at estrus, change in body weight and back fat thickness from weaning to estrus, weaning to estrus interval, ovulation rate, embryo survival to d28 ± 3 and plasma progesterone concentrations on d1, 2, 3 and 4 of pregnancy.

Parameter	Mean (± S.E.M.)
Daily feed intake from weaning to estrus (kg/d)	4.57 ± 0.12
Daily feed intake during gestation (kg/d)	5.01 ± 0.11
Body weight at estrus (kg)	174.39 ± 2.38
Back fat at estrus (mm)	16.39 ± 0.29
Change in body weight from weaning to estrus (kg)	0.44 ± 0.35
Change in back fat from weaning to estrus (mm)	0.52 ± 0.19
Weaning to estrus interval (days)	5.3 ± 0.13
Ovulation rate	15.6 ± 0.61
Embryonic survival to d28 ± 3 (%)	58.52 ± 6.36
Plasma progesterone (ng/ml) on:	
d1	0.88 ± 0.61
d2	2.16 ± 0.34
d3	9.31 ± 1.05
d4	14.55 ± 1.23

Fig. V.1. Relationship between plasma progesterone on d3 of pregnancy and embryonic survival in primiparous weaned sows on restricted feed intake during last week of a 28-d lactation.


100
80
60
40
20
0
ES (%)
0
5
10
15
20
25
72h-Progesterone (ng/ml)
(r = .59; P < .05)

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Chapter VI

General Discussion

General Discussion

A significant loss of pig productivity due to embryonic mortality and the possibility of ameliorating this loss has been recognized for a long time. A series of studies have sought to establish whether changes in nutritional status might be partly responsible for increased embryonic loss but the lack of consensus in the literature (for example Dyck and Strain, 1983; Dyck, 1991; Ashworth, 1991; Pharazyn, 1992; Cassar et al., 1994) is frustrating. In the first experiment reported in Chapter III of this thesis, we wished to confirm the previous results obtained in our laboratory (Pharazyn, 1992) on the effects of different nutritional intakes during early pregnancy on embryonic survival, using the same model. Further, failure in one of the experiments done in our laboratory to demonstrate nutritionally-induced detrimental effects on embryonic survival (Pharazyn et al., 1991a), when dietary changes were made on d3 of pregnancy, led us to test the hypothesis that the timing after mating when the feed levels are changed could be critical. Significantly higher embryonic survival and plasma progesterone concentrations at 72h following onset of estrus after reducing feed-intake from d1 of pregnancy, compared to continuation of high feed intake after mating, and intermediate results after reducing the feeding level on d3, clearly supported our hypothesis, and may explain some of the inconsistencies in literature (Jindal et al., 1996). Precise control over feed-intake by individual gilts before and after mating could be another important factor in this type of experiment. Group feeding, compared to providing a fixed amount of feed to all animals, may lead to variable results. Based on this and previous studies, it is suggested that if 'flush

feeding' is used to increase ovulation rates in gilts, ad libitum feeding must be discontinued immediately after mating to avoid negative effects of increased feed intake on embryonic survival.

The gilts on lower feed intake in Experiment 1 had higher plasma progesterone concentrations, and, irrespective of the dietary regimens, gilts with higher plasma progesterone concentrations had higher embryonic survival. This confirms previous results of Pharazyn et al. (1991a,b) that suggested a potential role of progesterone as a mediator of nutritional effects on the viability of embryo. Therefore, a need for understanding the physiological basis for a relationship between nutritional status and changes in progesterone concentration led us to examine this relationship further in our second experiment (Chapter III). On average, there was a 10-h delay in the onset of the rise in post-ovulatory progesterone in gilts on high feed intake immediately after mating, compared to gilts on reduced feed intake. Substantial variation in the time interval from the preovulatory LH peak to the onset of the rise in progesterone concentrations, and subsequent concentrations during early pregnancy among gilts, could be related to variability in embryonic survival in gilts. At any given time, plasma progesterone concentrations reflect a balance between synthesis by the corpora lutea and metabolic clearance by the liver and kidney. Increased hepatic blood flow and metabolic clearance rate of progesterone in sheep (Parr et al., 1993a,b) and gilts (Symonds and Prime, 1989; Miller, 1996) on higher dietary intake has been documented. The difference in plasma progesterone profiles during early pregnancy, as observed in this study, may be related to the pattern of luteinization, and thus earlier and increased production of progesterone by more developed corpora lutea. However,

in the model used, the nutritional treatments were imposed very close to the time of ovulation and they may, therefore, have little or no influence on the maturation of preovulatory follicles. However, an effect on the process of luteinization itself, and thus production of progesterone is possible. In a recent study, Ashworth et al. (1995) reported preliminary data suggesting that dietary intake affects both the production and clearance of progesterone in gilts.

Within 24h of estrus onset progesterone concentrations in ovarian and oviductal veins are many times higher than in the jugular vein and in the veins draining the uterine horns (Pharazyn et al., 1991b), indicating the possibility of some local effects of rising progesterone on the oviduct and/or uterus. Oviductal (Murray, 1993) and endometrial (Roberts et al., 1993) secretions are both modified by ovarian hormones. Therefore, differences in the pattern of progesterone concentration between gilts on high or reduced feed intake during early pregnancy can affect embryonic survival by altering the oviductal and uterine environment. In Phase 2 of Experiment 2 (Chapter III), differences in plasma progesterone concentrations and UPTI activity in the two dietary treatment groups supports this hypothesis. Further, reduced variation in embryonic survival in gilts on normal feed intake in both Phase 1 and Phase 2 of Experiment 2 indicated that the impact of nutritional changes on embryonic survival could occur at a very early stage of pregnancy, even before an embryo has entered the uterus. Thus, nutritional management of gilts during early pregnancy may be very important.

Based on the information in the literature reviewed in Chapter II, differences in IGF-1 and UPTI activities in uterine flushings between the two groups of gilts in

Experiment 2 were expected. However, based on this preliminary study, the lack of an effect on uterine IGF-1 concentrations indicates that either uterine IGF-1 (unlike hepatic IGF-1) is not dependent on the nutritional status of the gilt, or that the timing of slaughter was not optimal to detect such differences. Another possibility is that the nutritional treatments and/or the plasma progesterone concentrations might have affected the time of the initial rise of IGF-1, and once peak concentrations were reached, no further differences could be detected. Further studies are clearly required to evaluate these alternatives.

In the pig, embryos are normally retained within the oviduct for about two days before entering the uterus. During this time oviductal secretions affect embryonic cleavage, transportation rate and potentially the development and viability of embryos (Fukui et al., 1988; Gandolfi and Moor, 1987). Additionally, time spent in the oviduct may be necessary to give the uterus sufficient time to prepare to nurture the embryo. As reviewed earlier, progesterone dominance of the uterus is required during transport of the embryo and its early existence in the uterus (Dziuk, 1985), and any change in progesterone concentrations during pregnancy can affect the length of time spent by the embryo in the oviduct, and thus indirectly its rate of development. A secondary objective in Experiment 2 was, therefore, to obtain information on the timing of the effect of nutritional treatments on embryonic development. Unfortunately, due to unavoidable variation in slaughter times, concerns about the reliability of available techniques for embryo collection, and problems in separating the entangled masses of the embryos at the filamentous stage, we decided not to include the analyses of data on embryonic recovery in this thesis, because such information could be quite misleading.

However, if the data presented in Chapter III have credibility they suggest that nutritional effects on embryonic survival may occur even at the oviductal stage.

In Chapter IV, we used a different and more direct approach to address the question of whether progesterone acts as a mediator of the nutritionally-induced effects on embryonic survival by testing the hypothesis that the detrimental effects of high nutritional levels immediately after mating could be reversed by progesterone supplementation. We used the same nutritional treatment as in Experiments 1 and 2, but gave a series of six injections of progesterone from 24h after onset of estrus, to ensure that gilts were exposed to elevated concentrations of plasma progesterone from 36h after onset of estrus, thus matching the time of rise in endogenous progesterone seen in gilts with reduced feed intake in Experiment 2 (Chapter III). In Experiment 3 embryonic survival in the H-group gilts was consistent with previous observations, and was significantly correlated with plasma progesterone concentrations. Further, exogenous progesterone was effective in counteracting the deleterious effects of high feed intake on embryonic survival in HP gilts. In other recent studies (Ashworth, 1991; Parr et al., 1993c; Parr et al., 1996) in which exogenous progesterone was given from d4 onwards, the results were equivocal, suggesting that the timing of progesterone treatment to improve embryonic survival is critical, and that delayed treatment may produce variable results.

Recently, in this laboratory, the pattern of catabolism by lactating sows with differet feed intakes has been associated with differences in reproductive performance and embryonic survival in primiparous sows. Having established a potential role for plasma progesterone in nutritionally-induced effects on embryonic survival in gilts, we

tested the hypothesis (Chapter V) that the effects of the pattern of catabolism during lactation on embryonic survival may be, in part, mediated by progesterone in primiparous sows. As discussed in Chapter V, the nutritional treatments imposed during lactation result in changes in lactational catabolism which do not apply to the gilt model. Any influence of nutrition on post-ovulatory progesterone concentrations may, therefore, involve effects on both the maturation and luteinization of developing follicles. A highly significant correlation between plasma progesterone concentration and embryonic survival supported the hypothesis that progesterone may influence embryonic survival in weaned sows.

Progesterone is considered to enhance oviductal transport of fertilized eggs. Therefore, faster embryonic transfer through the oviduct, and thus a relatively longer stay at the tubo-uterine junction as a result of changes in nutrient intake or exogenous progesterone administration, can result in slower rates of embryonic development and less variation among embryos. Also, an early rise in progesterone can advance uterine activities, thus making it more receptive to the embryo. Improved synchrony among embryos and between the uterus and embryos may improve embryonic survival.

Through the present studies we have made a major contribution to our understanding of factors regulating embryonic survival in swine, and developed a model for further studies. Regression analysis shows that nearly 50% of the variance in ES can be explained by differences in progesterone concentrations during early pregnancy. Further, the majority of embryonic deaths take place during the preimplantation period. Thus, once the role of periovulatory progesterone in ES is fully understood, appropriate exogenous progesterone administration may help in

ameliorating problems of embryonic mortality in pigs. At present, it is not known whether improvements in embryonic survival will result in an increase in number of piglets born. Also, the use of progesterone supplementation in swine breeding herds may not be economical. Treatment of gilts with high endogenous progesterone concentrations may have adverse effects on embryonic survival. Therefore, an important question is how can one identify pigs with lower progesterone concentrations during the periovulatory period. It is also not yet clear whether differences in embryonic survival associated with differences in progesterone secretion are repeatable in successive pregnancies by the same animal. If the problems proved to be mainly genetic, identification of animals with lower reproductive potential, and their treatment with exogenous progesterone or early exclusion from the breeding herd might be advantageous. These aspects, also merit further extensive research.

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