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THE UNIVERSITY OF ALBERTA

NUTRITIONAL EFFECTS ON EMBRYO SURVIVAL IN THE GILT

by

ANDREW PHARAZYN

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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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 **NUTRITIONAL EFFECTS ON EMBRYO SURVIVAL IN THE GILT** submitted by **ANDREW PHARAZYN** in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in **ANIMAL NUTRITION/REPRODUCTION.**

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Date: April 14, 1992

ABSTRACT

The effect of two levels of energy and two levels of protein intake (NRC, 1988 vs High) during early gestation (Day 3 to 15) on progesterone concentrations and embryo survival were evaluated. Neither changes in energy or protein intake affected plasma progesterone concentrations (Day 3, 9 and 15), or embryo numbers and percent survival at Day 28 of gestation in the gilt. Irrespective of treatment, mean percent embryo survival tended to be greater ($p<0.10$) and variance in survival less ($p<0.10$) with increased progesterone concentrations at Day 3. In a detailed endocrine study, the interval from the peal of the pre-ovulatory luteinizing (LH) surge to a significant rise in plasma progesterone varied from 31.5 to 52.9 hours. When feed intake was increased from 2.0 to 2.8 kg, there was a significant increase ($p<0.05$) in this interval (30.8 to 37.6 h). In a study to compare steroid concentrations in the vasculature of the reproductive tract, plasma progesterone concentrations in the oviductal veins were greater ($p<0.05$) than in the jugular or uterine veins while that in the ovarian veins were still greater ($p<0.01$). This same pattern was seen in all gilts sampled at day 1, 2, 4, 12 or 16 of gestation and on day 12 of the estrous cycle. In a fourth study, an increase in feed intake from 1.8 to 2.5 kg day^{-1} (from Day 1 to 15) decreased the number of viable embryos ($p=0.10$) and percent survival ($p=0.05$). The interval from the pre-ovulatory LH surge to progesterone rise was longer in

gilts on the high feed intake (42.3 vs 32.3 h). At 24 and 48 h post LH surge and at 72 h post-estrus, mean percent embryo survival was found to be greater ($p<0.05$), and variance in embryo survival less ($p<0.05$), with increased plasma progesterone concentrations. There appears to be a relationship between percent embryo survival and plasma progesterone concentrations in the first few days after estrus. Increased feed intake may therefore reduce embryo survival in gilts through effects on a progesterone-dependent mechanism.

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There are a number of others to thank for their invaluable help in completing these studies: to the personnel of the University Swine Unit for their help in the care and management of the gilts; to Dorothy Irwin for her help with the blood sampling and surgeries; to Dorothy Payne, Gordon Grant and Lynn Wright for their help in the lab work; to Ray Weingardt for his help in the statistical analysis of these studies and to Eduardo Beltranena and Sam Baidoo for their friendship and help and to the many others who made my stay in Edmonton an enjoyable and rewarding experience.

I would like to dedicate this manuscript to my wife Sharon, for her love and support and to my children, Joanna and Michael, who provided much love and laughter.

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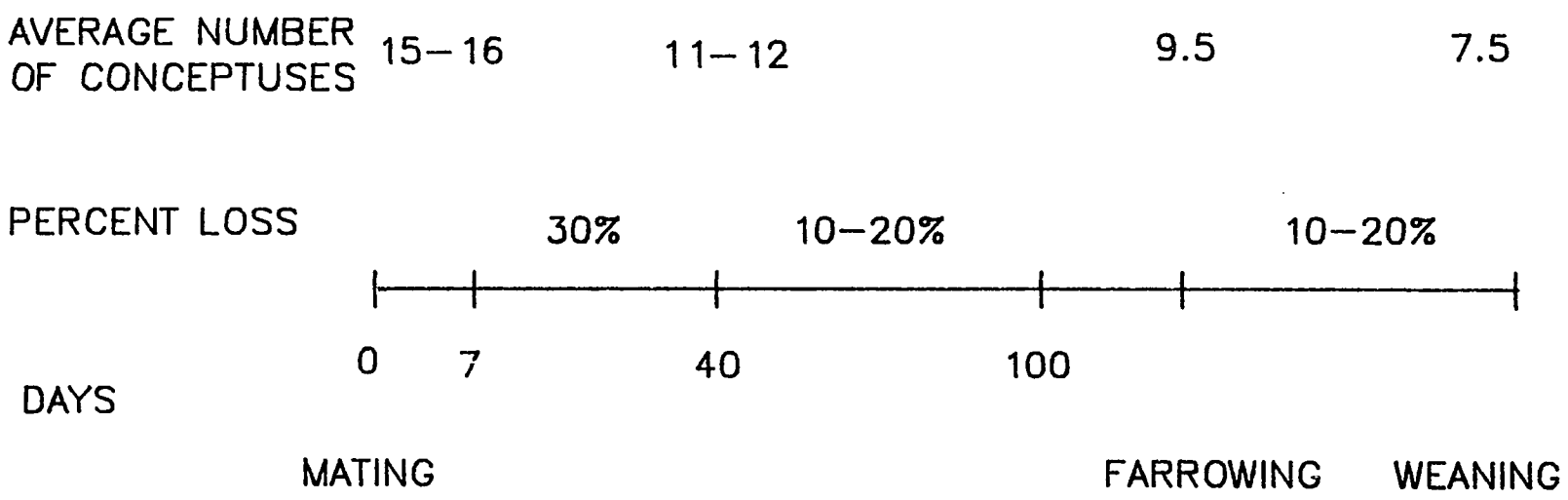
GENERAL INTRODUCTION

Increasing the number of piglets weaned per sow per year offers the greatest possibility of improving the profitability of a breeding swine enterprise. This number reflects the success in maximizing the number of piglets born per litter, the success of the sow in supporting the growth of those piglets during lactation and her ability to be rebred shortly after weaning.

Gilts represent 20 to 30% of farrowing females in many breeding herds. A major factor limiting productivity in a farrowing unit is the fact that gilts generally have smaller litter sizes than multiparous sows. Therefore, maximizing the reproductive performance of the gilt is critical in assuring good productivity in the herd as a whole. In multiparous sows, the metabolic demands of lactation may exert a major influence on the rebreeding interval and litter size at the subsequent farrowing. In the gilt, nutritional status during the rearing period (Beltranena et al., 1991a) and in the period before and after ovulation, may exert a major influence on puberty onset, ovulation rate and litter size (Christenson, 1986 ; den Hartog and van Kempen, 1980).

Through the period from mating to weaning, there is a substantial loss in the potential number of piglets weaned (Figure I.1). The stages at which these losses occur can be classified into three periods: embryonic, fetal and post-natal/pre-weaning. The loss of piglets in the latter period

I.1 Sources of potential piglet loss from ovulation to weaning (Pope and First, 1985)


AVERAGE NUMBER OF CONCEPTUSES
15–16
11–12
9.5
7.5
PERCENT LOSS
30%
10–20%
10–20%
0
7
40
100
DAYS
MATING
FARROWING
WEANING

reflects the influence of genetics, environment and nutrition, on the ability of the sow to raise the number of piglets that she has farrowed. Both embryonic and fetal loss determine the number of piglets farrowed. Litter size is dependent on ovulation rate or number of eggs shed, fertilization rate and on the loss of embryos and fetuses during the prenatal period. The number of eggs shed by a sow is the maximum potential litter size. As fertilization rate in the pig is generally assumed to be 98%, when females are inseminated at the optimum time, any observed reduction in litter size below the number of ovulations is largely due to prenatal losses.

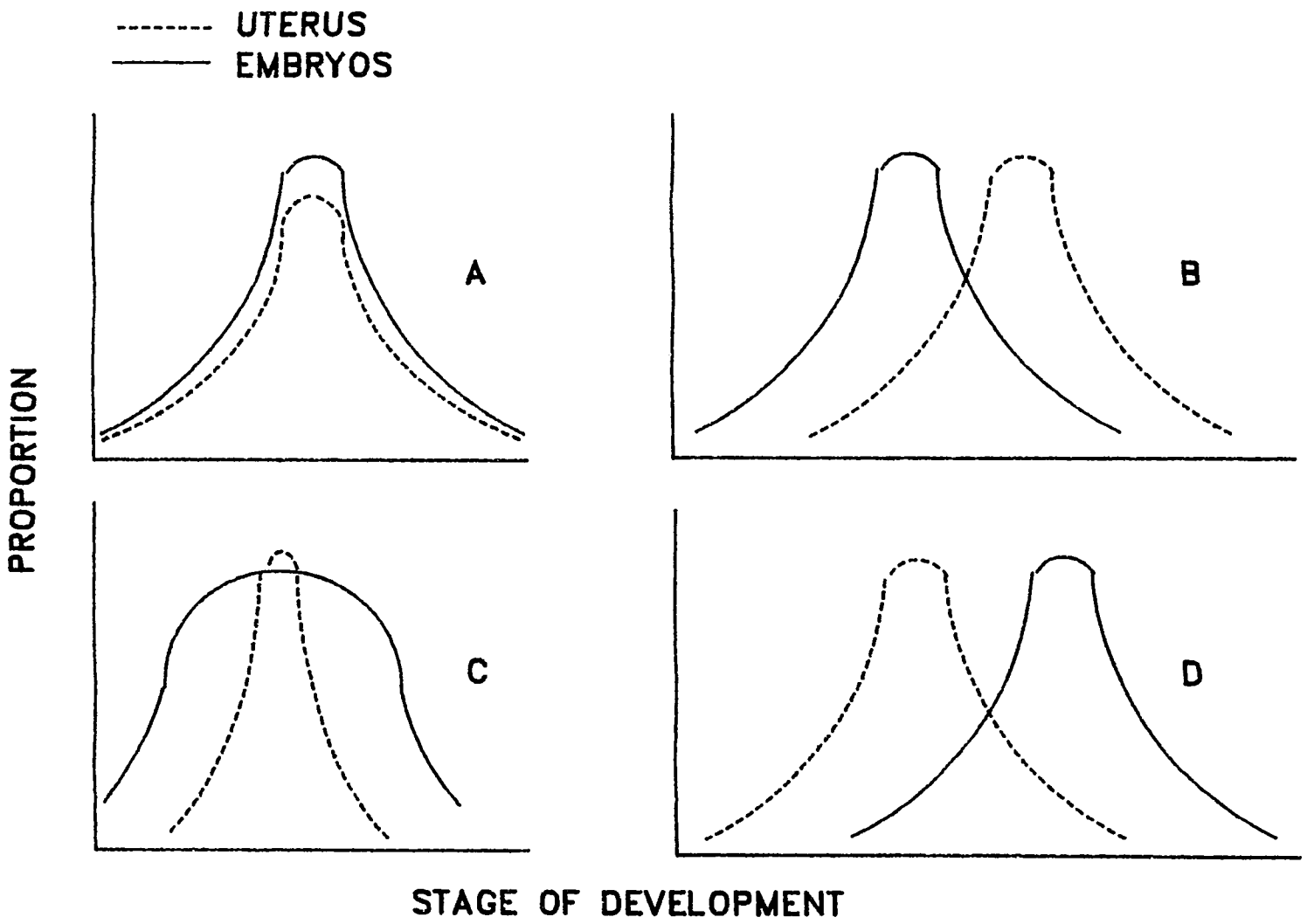
In practice, embryonic and fetal mortality represent a considerable loss in the potential number of piglets born. An estimated 30 to 40% of embryos die prior to Day 40 of gestation with an additional 10 to 20% of the remaining fetuses dying in the period from Day 40 to term (Pope and First, 1985 ; Wrathal, 1971). However, not all litters experience loss of embryos with approximately 20% of litters having 100% embryo survival (Dziuk, 1987). Thus, in any population of female pigs, embryonic loss can vary from 0 to 100%, with the average being approximately 30%.

The precise causes of embryonic loss remain an enigma that has defied explanation despite many years of research. In all likelihood, no one explanation may account for all embryonic loss. Among the many factors suggested to contribute to embryonic loss are genetic abnormalities, genetic variation in

rate of embryo development, abnormal maternal environment (immature), disease organisms within the uterus, improper nutrition and asynchrony between the embryo and the uterine environment. The latter has been suggested to be the underlying cause of most embryonic mortality in litters, with other factors modifying the degree of asynchrony (Pope and First, 1985 ; Wilmut et al., 1986 ; Dziuk, 1987 ; Pope et al., 1990).

Synchrony between the development of the uterine environment and that of the embryo is required for maximal embryonic development. The synchrony between the uterus and embryo need not be precise for optimal survival but as asynchrony increases, so does the possibility of embryo loss (Dziuk, 1987) as described in Figure I.2. In (A), embryos and uterus are in synchrony, with little loss of embryos. In (B), embryonic development is slower than that of the uterus, while the reverse scenario occurs in (D); in both situations, there would be increased loss of embryos, as a proportion of embryos are not synchronous with the uterus. In (C), a situation exists in which embryos vary widely in development and are both ahead and behind that of the uterus. Factors which retard or advance development of the embryos and/or uterus will influence the number of embryos which remain viable. Furthermore, the development of the uterine environment and embryos is not necessarily independent and one likely modifies the other. As discussed later, signals from the embryo can

I.2 Four possible scenarios for synchrony between the uterus and embryo (Dziuk, 1987). In (A), the embryo and uterine environment are in synchronous development. In (B), embryos develop at a rate slower than that of the uterine environment while the reverse occurs in (D). In (C), embryos vary widely in development, while some are more advanced and some lag behind that of the uterine environment.


UTERUS
EMBRYOS
A
B
C
D
PROPORTION
STAGE OF DEVELOPMENT

stimulate the release of uterine secretions (Geisert et al, 1982a).

To appreciate the importance of synchrony of the embryo and uterus in embryo survival, it is important to understand the changes that the embryo and uterine environment undergo in the period before and after implantation and the external factors which may influence each. The purpose of the literature review presented in this thesis is to provide such an understanding.

The first section covers the development of the porcine embryo from fertilization to the post-implantation stage, including physical changes and the biochemical development of the embryo, which culminates in the acquisition of the capacity for estradiol synthesis. This latter attribute is the major stimulus for the radical changes that occur during the process of implantation and may provide an explanation of the negative effects mature embryos have on less developed siblings. As well, the survival and success of embryos in the implantation process, is dependent on the development of the uterine environment and uterine receptivity to the signal received from the embryos. Initially, the uterine environment is largely dependent on the plasma steroid environment created by the corpora lutea and modified by several non-ovarian organs such as the liver and adipose tissue. The changes in this hormonal environment will be reviewed in the second section. The final section of the literature review will deal with the effects of feed intake during early pregnancy on

embryo survival and attempt to postulate a mechanism by which the two may be linked.

The objective of the series of studies described in the later section of the thesis was to further clarify the relationships between level of feed intake, the pattern of progesterone secretion and embryo survival and development in the gilt. The initial study posed a simple question. Is there a relationship between energy and protein intake in early pregnancy and hormonal profiles and embryo survival during that period? With the nutritional regimen adopted, the answer to that question was negative. However, that same pool of data indicated that there was a relationship between progesterone concentrations in plasma in the first three days after estrus and embryo survival, and that between gilt variation was fairly large. This led to the second study in which changes in plasma progesterone concentrations in the first ninety-six hours after estrus were monitored, again showing a wide range in times at which progesterone concentrations rose from basal levels. The third study arose out of literature suggesting that hormonal profiles in the local vasculature of the reproductive tract may be different than those seen in the systemic circulation, due to local counter-current transfer of steroids within the utero-ovarian vasculature. The design of the final study incorporated information arising from the observations of the previous studies in an experiment to detail the rise in peripheral concentrations of progesterone

in early pregnancy, the influence that quantity of feed intake has on this variable and their effects on embryo survival and growth.

These four studies are presented as four sequential chapters and are extended forms of papers submitted or accepted for publication. The experiments presented in Cahpters II and IV are published as Pharazyn et al., 1991a and 1991b, respectively.

I. LITERATURE REVIEW

The survival rate of embryos in early gestation is dependent on the rate of uterine development, as modified by the hormonal environment, on the development of the embryos and the suitability of the uterine environment to sustain the embryos present. In the following section, attention will be focused on the development of embryos prior to and at implantation and, with the onset of the capability to synthesize estrogens, how the embryo modifies the uterine environment to meet its needs. Changes in the hormonal milieu of gestation, factors which modify these changes and how these hormonal changes influence the uterine environment and its capacity to support embryo growth will be discussed. Finally, the relationship between nutrition and embryo survival and the perceived mechanisms of this interaction through changes in the hormonal environment and subsequent embryo development, will be discussed.

A. EMBRYO DEVELOPMENT IN EARLY PREGNANCY

FOLLICULAR HETEROGENEITY AND VARIATION IN EMBRYO DEVELOPMENT

The follicles from which the ova are derived are a heterogenous population. A diversity of follicular development exists which carries over into the pattern of ovulation and subsequent embryo development (Foxcroft and Hunter, 1985 ; Xie et al., 1988). Follicular development and oocyte maturation were skewed to a majority (76%) being more developed and a lesser developed minority (24%). This ratio was similar to the

pattern of ovulation seen in gilts observed by Pope et al. (1988) and the distribution of zygotes in the twenty-four to forty-eight hours after ovulation (Xie et al., 1990a). Removal of the late maturing follicles by electrocautery reduced the number of lesser developed embryos suggesting the later developing follicles gave rise to the immature embryos (Pope et al., 1988).

It has been suggested that the time at which an egg is shed will determine the probability of survival of the embryo arising from it. Pope et al. (1988) observed that 70% of ova were shed over a one hour period, while the remainder were shed over an extended period of time. This pattern of ovulation was highly correlated with the percentage of viable embryos found at Day 12 of gestation. This led to the hypothesis that the period of time over which ovulation occurred has a major part in determining the percentage of embryos remaining viable and their stage of development (Pope et al., 1990). The embryos which are ovulated later, or over a longer period of time, are at a competitive disadvantage to those ovulated earlier. The uterine flushings associated with the lesser developed embryos contained less estradiol, protein and acid phosphatase activity at Day 12 of gestation than did more developed siblings (Xie et al., 1990b).

This suggestion is supported by experiments comparing the Meishan and Yorkshire breeds of swine to assess the uterine and embryonic factors which influence embryonic viability.

Meishan and Yorkshire with similar ovulation rates had markedly different embryo survival rates, with the Meishans retaining a greater portion of their embryos (Bidanel and Legault, 1986). This has been attributed to the observation that ovulation in the Meishan occurs over a shorter period of time than in the Yorkshire, and to a greater uniformity of blastocyst development within the Meishan (Bazer et al., 1988). In the latter study, the variation in conceptus diameter was significantly less on Days 8, 10 and 11 in the Meishan than in the Large Whites. The authors speculated that the later maturing oocytes become the smaller embryos.

OVIDUCTAL INFLUENCES AND THE HORMONAL ENVIRONMENT

Though ova may be released over several hours, the rate of fertilization of the shed ova does not contribute to the variation seen in embryo development. The first cleavage division of the fertilized egg occurs between 60 and 108 hours after onset of estrus. As the number of cleavage divisions increases, so does the variation in developmental stages of the embryos.

As with other mammals, pig embryos go through distinct stages of development (Anderson, 1978). Embryos first enter the uterus approximately 48 hours after ovulation (approximately, 72 hours after estrus onset), with entry extending over a twenty-four hour period (Oxenreider and Day, 1965). Embryos are generally at the 4- to 8-cell stage when they enter the uterus but some will have progressed to the

morulae stage. The embryos generally remain near the uterotubal junction until Days 5 and 6 of gestation. On Day 7, the embryos hatch from the enveloping zona pellucida either by an expansion and contraction of the blastocyst, or by modification of the structure of the zona by the uterine environment (Oxenreider and Day, 1965).

The passage rate of the embryo through the oviduct is affected by the flow of secretions, beating of epithelial cilia, peristaltic contractions, local prostaglandins and gonadal steroids (Hunter, 1977b). Time spent in the oviduct is necessary to allow time for the uterus to be modified to nurture the embryo. As well, an obligatory period in the oviduct may be needed for the embryo to develop properly. The incidence of polyspermy in vitro is reduced when oocytes are co-cultured with oviductal epithelium than when they are not (Nagai and Moor, 1990). Embryos cultured from the 1- to 2-cell stage often fail to develop beyond the four cell stage with in vitro culture (Polge, 1982). Co-culture of one-cell embryos with oviduct epithelial cells allowed for greater transition from single cell to morulae, than if cultured with fibroblast cells in both pigs (White et al., 1989) and sheep (Gandolfi and Moor, 1987). Oviductal tissue has been demonstrated to synthesize specific glycoproteins at the time of fertilization and early cleavage divisions, the type and distribution of which changes from ampulla to isthmus (Buhi et al., 1990). In the embryo, a new pattern of protein synthesis occurs in the

lag phase at the four-cell stage which may be triggered by oviductal secretions. This suggested a role for secretions from oviductal cells in overcoming this restrictive phase in embryo growth. The role of estrogens and progesterone in the regulation of oviductal secretions has not been investigated in swine.

The zona pellucida of the porcine embryo consists of 71% protein and 19% carbohydrate in the form of complex glycoproteins (Hedrick and Wardrip, 1987). During oviductal transport, compositional changes in the zona pellucida were observed (Brown and Chang, 1986 ; Hedrick et al., 1987) which were thought to be due to the addition or deletion of several glycoproteins. Placement of oocytes into the oviduct for a thirty minute period increased the resistance of the zona pellucida to proteolytic digestion (Broermann et al., 1989), whereas placement into the uterus reduced resistance to proteolytic digestion. When the zona pellucida of morulae was exposed to the same oviductal and uterine environments, the same increase and decrease, respectively, was observed.

Gonadal steroids affect the excitability and conductivity of the oviduct and mucosal morphology (Hunter, 1977b). The smooth musculature of the oviduct is largely innervated with adrenergic receptors, with α-adrenergic activity restricting movement of the egg through the oviduct. α-Adrenergic activity is enhanced by estrogens, while progesterone enhances β-adrenergic activity, thereby increasing isthmic relaxation

and facilitating egg transport. Generally, estradiol retards transport of the fertilized egg whereas progesterone enhances its transport. Subcutaneous injections of progesterone before ovulation causes the eggs to appear in the uterus 8 hours after ovulation rather than the normal 48 hours (Day and Polge, 1968). Injection of estradiol results in "tube-locking" preventing descent of the fertilized ova. An alteration in plasma estradiol and/or progesterone concentrations during the time the fertilized eggs reside in the oviduct affects the length of time spent in the oviduct and when they enter the uterus. Therefore, factors affecting the ratios of these hormones can be expected to affect transit time through the oviduct and subsequent viability of the embryos.

Progesterone dominance of the uterus is required during the oviductal transport of embryos and their early existence in the uterus (Flint, 1982). Treatment with antibodies to progesterone altered oviductal transport, causing embryos to appear prematurely in the uteri of treated mice with subsequent loss of the embryos (Feinstein, 1983 ; Rider et al., 1987).

PRE-IMPLANTATION EMBRYO DEVELOPMENT IN THE UTERUS

After leaving the oviduct, the embryos remain stationary in the vicinity of the utero-tubal junction from Days 3 to 6 of gestation. As the embryos mature from Days 7 to 12 of gestation, they migrate from the oviductal junction, through the bifurcation of the uteri and become intermixed with

embryos from the other side (Dziuk et al., 1964 ; Dhindsa et al., 1967). Estradiol synthesized by the embryo mediates their intrauterine migration during this time (Pope et al., 1986c) by increasing the myometrial activity of the uterus (Pope et al., 1982b) and also influences their subsequent spacing in the uterus.

From Days 9 to 18, pig blastocysts undergo a series of profound morphological changes from spherical to ovoid, to tubular with embryonic disc and trophectoderm, and finally to formation of a filamentous embryo and the beginning of embryogenesis 18 days after mating (Anderson, 1978). On Day 9 post-mating, spherical and ovoid blastocysts are present 0.5 to 1.0 mm in diameter and 1.0 to 5.0 mm long. By Day 11 post-coitum, a few filamentous embryos are present (>5.0 cm long), while half of the conceptuses at Day 12 are filamentous blastocysts with lengths of 12 to 80 cm and a mean diameter of 0.2 to 0.4 mm. At Day 13, the majority of blastocysts are filamentous, though a few spherical, ovoid and tubular blastocysts still remain. At this stage, the filamentous embryos possess an embryonic disc which becomes more prominent the following day. By Day 15, embryogenesis is initiated in some of the embryos and a primitive vasculature is present by Day 18 of gestation (Anderson, 1978).

The embryos grow and differentiate at different rates and embryos at different morphological stages can cohabit the uterus at one time. For example, pig embryos at Day 12 of

gestation can vary from 1 to 800 mm in length and exist as ovoid, tubular or filamentous blastocysts. It has been estimated that embryos may be elongating at a rate of 30 to 45 mm h^{-1} in the transition to the filamentous state. The initial morphological change from spherical to the filamentous stage primarily involves cellular remodelling rather than an increase in cell proliferation (Giesert et al., 1982b). Implantation is initiated starting on Days 13 to 15 post-mating as a gradual attachment between the trophectoderm and endometrium of the uterus (Dantzer, 1985).

ROLE OF EMBRYONIC ESTROGENS IN EMBRYO DEVELOPMENT AND IMPLANTATION

Coincident with the growth and the development of the embryos is the acquisition of the capability to synthesize estrogen. Conversion of androgens to estrogen by porcine blastocysts was first demonstrated by Perry et al. (1973) and confirmed by Gadsby et al. (1980). Fischer (1982) demonstrated the conversion of progesterone to estrogen by 9 to 10 mm blastocysts but not by blastocysts that measured 5 to 7 mm in diameter. By Days 10 to 12, porcine embryos are capable of increasing synthesis of estradiol and estrone as the blastocysts progress from spherical to filamentous forms (Fischer et al. 1985 ; Stone et al., 1986), with the greatest estrogen content per cell occurring at Days 11 and 12 (Pusateri et al., 1990). As described earlier, uterine flushings associated with less developed embryos, contain less

estradiol than flushings associated with more developed siblings (Xie et al., 1990b). Estrone production was greater in Day 18 blastocysts compared to younger blastocysts, while the opposite was true for estradiol (Bate and King, 1988). Within the filamentous blastocysts, regional differences exist in the amount of estrogen present (King and Ackerley, 1985) and in the synthesis of estrogen, with the tissue adjacent to the embryonic disc having the greatest synthetic capability (Bate and King, 1988).

The increasing quantities of estradiol advance the secretion of uterine proteins and other factors (Geisert et al., 1982a, 1982b). Initially, the estradiol secreted by the blastocyst creates an advanced microenvironment in the blastocyst's immediate surroundings suitable for its development. Given the greater synthesis of estradiol in the area surrounding the embryonic disc, the modified microenvironment would exist in this region. The alteration in this area fits with the observation that placentation begins in the area of the embryonic disc and proceeds toward the chorionic tips (King et al., 1982). As the quantity of estradiol synthesized increases, the advance of the uterine environment becomes more generalized to the detriment of less developed embryos.

The timing of uterine exposure to estradiol is critical and has differential effects on embryo survival. Estradiol injected into a gilt at Day 9 is embryotoxic, whereas at Day 12, estradiol no longer is detrimental. This is coincident

with blastocyst estradiol synthesis (Pope et al., 1986a). As the dosage of estradiol given on Day 9 increased, embryonic mortality increased, with an increased incidence of pregnancy failure at the higher dosages. Exposure to the estrogenic mycotoxin, zeralenone, from Day 2 to Day 6 and from Day 10 to Day 15 had no effect on embryo survival, while exposure from Day 7 to Day 10 reduced embryo survival (Long and Diekman, 1986). Exogenous estradiol at Day 9 advances the uterine environment (Gries et al., 1989), changing the pattern of uterine secretions to make it less amenable to embryo viability. Filamentous embryos were present on Day 12 of gestation, but by Days 14 to 16, the embryos were fragmented and failed to implant (Morgan et al., 1987a ; Gries et al., 1989). Recent work has shown that premature exposure to estradiol alters the uterine endometrial surface, thereby preventing normal attachment of the embryos to the endometrium (Blair et al., 1991).

Polge et al. (1966) demonstrated that pigs bearing less than five embryos experience total pregnancy failure. Similarly, if a sufficient number of embryos fail to implant, the uterine endometrium releases $PGF_{2\alpha}$ into the venous circulation and via a counter-current mechanism, the $PGF_{2\alpha}$ is carried to the ovary, resulting in luteal regression.

The pig blastocyst exerts a luteotrophic or antiluteolytic effect before Day 11 to prevent luteal regression. Estradiol is an important blastocyst signal to induce maternal

recognition of pregnancy (Bazer and Thatcher, 1977). Thus, estradiol, either injected systemically (Gardner et al., 1963) or by infusion into the uterine lumen (Ford et al., 1982b), is luteotrophic in the pig. In ruminants, the signal sent from the uterus in response to the presence of the embryo is a protein. In pigs, estradiol, whether directly or indirectly, signals the presence of the embryo. Estradiol, injected on Day 11 of the estrous cycle, maintains the corpora lutea to Day 30. A state of pseudopregnancy can be extended beyond 60 days with additional estradiol injections on Days 14 to 16 (Geisert et al., 1987 ; Gardner et al., 1963). Venous concentrations of $PGF_{2\alpha}$ in the utero-ovarian vein declines and concentrations in the uterine lumen increases in response to estradiol adminstration (Frank et al., 1977). Estradiol produced by the blastocyst may act locally on the uterine endometrium, redirecting the $PGF_{2\alpha}$ towards the uterine lumen (Dubois and Bazer, 1991), or may be carried directly to the ovary via a counter-current mechanism and act directly on the ovary (Bazer and Thatcher, 1977 : Flint et al., 1982).

Estradiol, whether of blastocyst or exogenous origin, alters the nutrient and protein composition of the uterine fluid. An increase in the release of calcium, protein and acid phosphatase is seen with exposure of the uterine endometrium to estradiol (Geisert et al., 1982c). The profile of acidic proteins recovered in flushings were similar between control gilts and gilts injected with estradiol-valerate on Day 11.

The dominant protein recovered in all flushings was serum albumin. Two basic non-serum proteins were also recovered from control and treated gilts. Uteroferrin and two other acidic proteins were recovered from uterine flushings from both groups of gilts, though these proteins appeared earlier in the estradiol-treated gilts. In response to injected estradiol, calcium ion concentrations in the uterine flushings increased within six hours of injection and this increase preceded increases in luminal protein content (Young et al., 1987). It has been suggested that increased calcium release is the estrogenic mediator. Infusion of an estradiol antagonist or aromatase inhibitor failed to affect the concentrations of calcium, uterine protein or acid phosphatase during the period from Day 10 to Day 16 of pregnancy (O'Neill et al., 1991). While there was a reduction in estradiol concentrations, the authors suggested the level of antagonist or inhibitor may not have been sufficient to affect estradiol-mediated events.

A transient increase in uterine blood flow to the gravid uterus has been reported at the time of blastocyst implantation (Ford and Christenson, 1979). This is associated temporally with elevated concentrations of estrone and estradiol-17β in the uterine lumen (Ford et al., 1982a) and an elevated estrogen to progesterone ratio in peripheral plasma (Ford and Christenson, 1979).

The increase in calcium and protein in uterine fluid is time dependent; increases in luminal calcium were seen 12 hours

after estradiol injection in gilts at Days 11 and 14 of gestation, but not at Day 9 of gestation (Geisert et al., 1987). Uterine endometrial secretion of calcium and protein was not estrogen responsive prior to Day 10 gestation, suggesting that development of the endometrium's secretory capability was not fully developed. Administration of estrogen at Day 9 altered the release of calcium and protein in uterine flushings taken from gilts at Day 11 and 12 (Morgan et al., 1987a). Calcium levels were higher in estradiol-valerate injected gilts at Day 11 and declined on Day 12, while control gilts had lower calcium levels on Day 11 but experienced a four-fold increase on Day 12. Total protein in the uterine flushing was higher in the estradiol-valerate treated gilts and the increase from Day 11 to Day 12 was greater in the treated gilts. Gries et al. (1989) demonstrated a decrease or loss of several polypeptides (including a large acidic polypeptide) from the uterine milieu at Day 12 in response to estradiol administration at Days 9 and 10 of gestation. On Day 11, Morgan et al. (1987b) transferred embryos 24 hours younger than the recipient uterus and injected the recipient with estradiol-valerate or a vehicle control. None of the blastocysts in the gilts treated with estradiol developed while in the controls, the blastocysts developed normally. This suggested that the alteration in the uterine environment prevented the embryos from developing normally.

The acquisition of an estrogen synthesizing capability by an

embryo may determine if it is able to successfully compete with its sibling embryos. Estradiol synthesized by the embryo may be the stimulus used by the embryo to advance the uterine environment for its own further development. The closer the embryos are in their physiological development, the greater the possibility of their survival. Embryos less developed will find the uterine environment hostile to their requirements and may fail to implant.

UTERINE SECRETORY ACTIVITY

1. UTERINE PROTEINS

The uterine secretions or histotroph are composed of a variety of serum and non-serum proteins which provide an embryotrophic environment in the uterus (Roberts and Bazer, 1988). Uteroferrin, an iron containing protein with acid phosphatase activity, is the most thoroughly described of the uterine proteins. Uteroferrin is synthesized and stored by the glandular epithelium and its release, in response to estradiol, is calcium-mediated (Fazleabas et al., 1985). It is thought to be involved in iron transport to the developing embryo and fetus (Roberts and Bazer, 1980). Plasmin inhibitors are low molecular weight (M_r-14,000) basic polypeptides, synthesized and stored in the surface epithelium (Fazleabas et al., 1985), which function to control proteolytic activity in the uterus. The elongating pig blastocyst (Days 11 to 14) releases large quantities of the protease plasminogen activator (Fazleabas et al., 1983), which activates

plasminogen, originating as a serum transudate, to plasmin, which is a protease with broad proteolytic capacity. Plasmin is involved in the cellular remodelling and morphogenesis of the blastocyst (Bode and Dziadek, 1979). Plasmin inhibitors are synthesized under the influence of progesterone and injection of estradiol valerate on Days 12 to 14 increases the release of these inhibitors into the uterine lumen (Fazleabas et al., 1982 ; Young et al., 1987). Blastocysts transplanted to ectopic sites (ureter, outer uterine wall) are highly invasive and exhibit abnormal growth and development (Samuel, 1971). The increase in plasmin inhibitor release from the uterine endometrium in response to blastocyst estradiol prevents the proteolytic cascade initiated by the plasminogen activator. This in turn prevents damage to the uterine endometrium, while allowing the plasminogen activator within the blastocyst to promote cellular remodelling and outgrowth of embryonic tissue (Mullins et al., 1980).

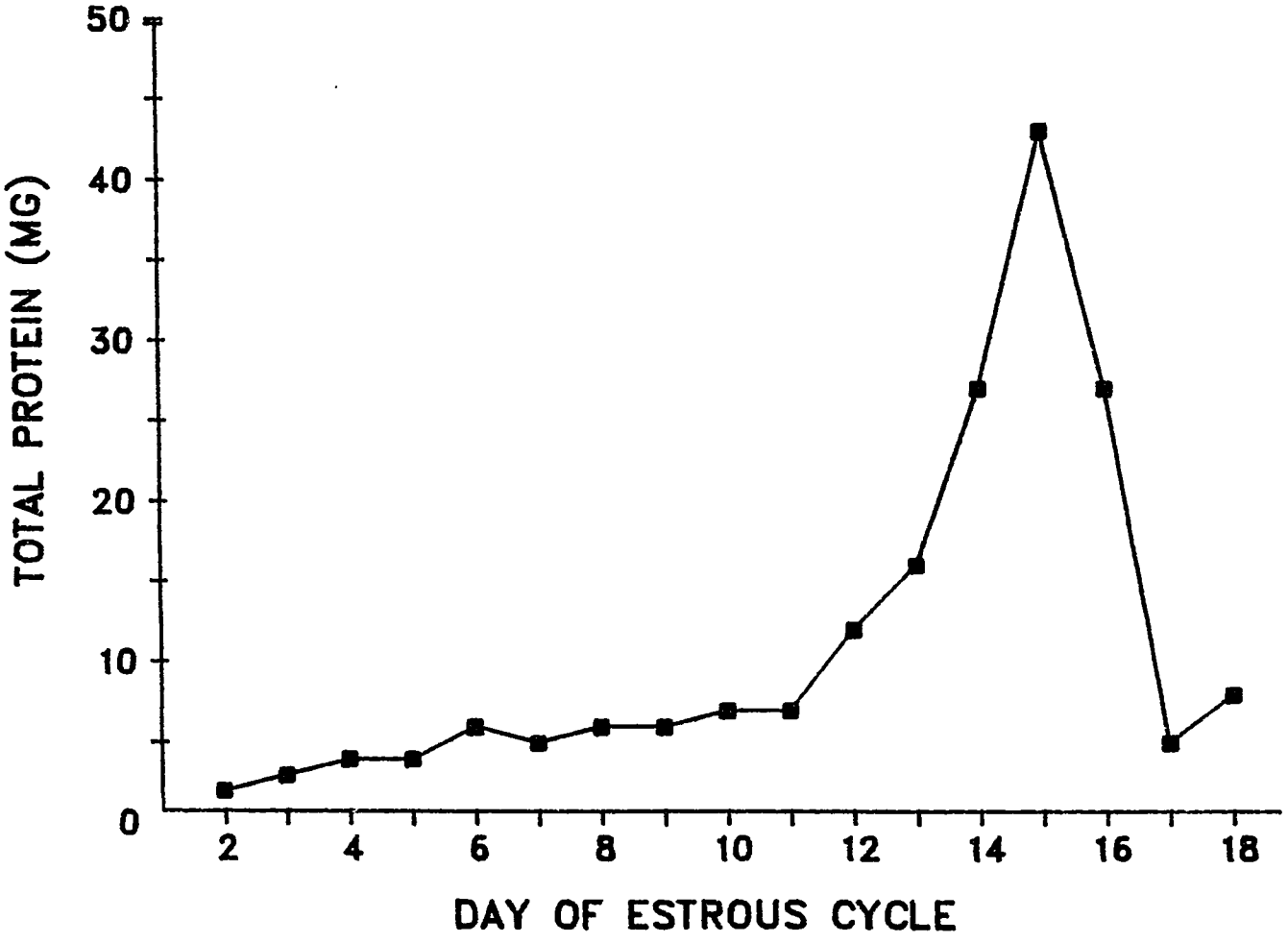
Several lysozymes have been identified in uterine secretions. Several low molecular weight acidic proteins are present and have been shown to be progesterone responsive. Two glycosidases with lysosomal-like functions, β-hexosaminidase and β-galactosaminidase have been isolated from porcine uterine flushings, the levels of which are stimulated by progesterone injection (Hansen et al., 1985). These enzymes may function in sperm capacitation, removal of the zona pellucida and in attachment of the conceptus and endometrium

(Roy et al., 1983).

The uterine endometrium is also a site for the synthesis of IGF-I and IGF-II which are thought to be involved in the cellular remodelling the developing blastocyst undergoes (Simmen and Simmen, 1990). IGF-I is released into the uterine lumen with increasing days of pregnancy and reaches maximal concentrations at Days 10 to 12 (Berthelot et al., 1991 ; Simmen et al., 1989); coincident with blastocyst elongation and estrogen synthesis. Incubation of embryonic tissue with IGF-I, in the presence of labelled leucine, showed that IGF-I stimulated protein synthesis (Estrada et al., 1991). Both treatment with progesterone and estradiol increased the levels of IGF-I in uterine flushings and tissue, indicating a role for these steroids in the release of IGF-I (Simmen et al., 1990).

The quantity of protein recovered in uterine flushings changes as the estrous cycle proceeds (Murray et al., 1972) with less than 8 mg protein present in each uterine horn until Day 9 of gestation (Figure I.3). These proteins were mainly of serum origin. From Days 12 to 15, there is rapid increase in the quantity of protein present (up to 50 mg protein), primarily due to an elevation in the levels of the basic iron-containing protein, uteroferrin. Until the blastocysts are capable of significant estrogen synthesis on Day 11, recoverable protein is similar between pregnant and non-pregnant gilts (Geisert et al., 1982a). Progesterone is

I.3 Total quantity of protein recovered in uterine flushings by day of the estrous cycle (Murray et al., 1972)


TOTAL PROTEIN (MG)
50
40
30
20
10
0
2
4
6
8
10
12
14
16
18
DAY OF ESTROUS CYCLE

primarily responsible for the qualitative and quantitative changes in the intraluminal protein milieu (Knight 1974a ; b), with a dose response relationship between the quantity of protein secreted by the uterus and the quantity of progesterone administered.

The uterine glandular epithelial cells of the pig accumulates secretory vesicles until the period of blastocyst elongation and estrogen synthesis on Day 11 or Day 12 post-coitum (Geisert et al., 1982b). The contents of the secretory vesicles are released by exocytosis into the uterine lumen in response to estradiol, resulting in marked changes in composition and amount of uterine fluid components (Roberts and Bazer, 1988). From Day 5 until the time of blastocyst elongation, the balance between acidic and basic proteins in the uterine lumen changes (van der Lende et al., 1988). A rapid decrease in levels of acidic proteins is observed during this period, with a concurrent increase in basic proteins. As a result, there is a change away from acidic protein dominance, to a mild basic protein dominance in the uterus, at the time of blastocyst elongation and implantation.

In a comparison between Meishan and Yorkshire females, Meishan gilts tended to have less recoverable uteroferrin in uterine flushings than Large Whites but greater acyl aminopeptidase activity (Bazer et al., 1991).

2. NON-PROTEIN ELEMENTS

In addition to secreted proteins, uterine fluid contains a

large number of other compounds, which vary in relation to estradiol concentrations. Steroid concentrations are greater in uterine washings than in the plasma of pregnant swine, suggesting an accumulation of steroids by the uterus (Stone and Seamark, 1985). Pregnenolone, progesterone, testosterone, estrone and estradiol concentrations were greater in the uterine washings of pregnant gilts than non-pregnant gilts. The difference reflects steroidogenesis by embryonic tissue and modification of enzyme activities in the uterine endometrium. Estradiol concentrations in the uterine flushings increase as the embryo acquires the ability to synthesize estradiol (Xie et al., 1990b). In addition, the prostaglandins F and E also accumulate in the uterine environment from Day 13 to Day 14 (Kennedy et al., 1986) and are stimulated by the embryo (Dubois and Bazer, 1991).

Several other components have been isolated and purified from uterine flushings. Accumulation of glucose and fructose is associated with the period of blastocyst elongation and estrogen synthesis on Days 14 to 18 (Bazer et al., 1984 ; Zavy et al., 1982). Ascorbic acid levels recovered in the uterine flushings increased five-fold from Days 6 to 18. Riboflavin (Murray et al., 1980 ; Moffat et al., 1980) and retinol (Adams et al., 1981) have been isolated from uterine flushings. A transient increase in total calcium and acid phosphatase is seen with the transformation of the spherical blastocyst to the 9 to 50 mm tubular form (Geisert et al., 1982a). In

pregnant gilts, there is a transient but well defined increase in calcium concentration which peaks on Day 12 of pregnancy (Young et al., 1987). Sodium and potassium concentrations increased several-fold from Days 10 to 14 of pregnancy. The relative amounts of several components of uterine flushings is correlated with the average blastocyst size in that horn (Geisert et al., 1982a). Total recoverable glucose, sodium, immunoglobulin A, PGF and PGE were higher in uterine flushings from Meishan gilts than for Yorkshire gilts (Bazer et al., 1991). The authors suggested that the Meishan conceptuses were more stimulatory to the uterine endometrium than the conceptuses of Yorkshires, or the endometrium of the Meishans are more sensitive to embryonic signals, as indicated by the greater accumulation of histotrophe in the Meishan gilts.

PROTEINS OF EMBRYONIC ORIGIN

The pre-implantation embryo, in addition to its steroidogenic capability, synthesizes and releases protein into the uterine lumen (Saunders et al., 1980 ; Rice et al., 1981 ; Godkin et al., 1982). The nature of the proteins synthesized changes with the age and morphological development of the blastocyst. Spherical, tubular and filamentous blastocysts synthesize several low molecular weight acidic proteins on Days 10 to 12 which have been identified as interferon-α-like proteins (Cross and Roberts, 1988), of which two species have been identified in the pig (Bonnadiere et al., 1991). These have an anti-viral activity which is highest

at Days 14 and 15 (Mirando et al., 1990): endometrial factors have a regulatory role on the secretion of these proteins (Beers et al., 1990). By the time of implantation on Days 12 to 16, larger basic polypeptides are the predominant proteins synthesized by the embryo (Godkin et al., 1982). Spherical blastocysts of 3 to 5 mm diameter, are first able to produce this high molecular weight glycoprotein (Mr 43,000), with maximal synthesis rates in the filamentous embryos (Baumbach et al., 1988). The protein was localized in the embryonic trophectoderm, secreted into the uterine lumen and thought to function in maternal-fetal interactions.

Porcine conceptuses have been demonstrated to secrete a retinol-binding protein prior to blastocyst elongation and continuing throughout the peri-implantation period (Harney et al., 1990). Retinol-binding protein is involved in the transport of retinol to the embryo (Adams et al., 1981) and antibodies raised to this particular protein result in increased embryonic mortality (Murty and Adiga, 1982).

Conceptus secretory proteins have been shown to stimulate the release of PGE and PGF from the uterine endometrium (Dubois and Bazer, 1991), though the identity of these proteins have not been determined. In the presence of estradiol, these proteins increase uterine length and weight, thereby increasing the total epithelial surface area available for normal elongation and placentation (Harney and Bazer, 1990).

B. PROGESTERONE SECRETION IN EARLY PREGNANCY

As the embryo undergoes rapid biochemical, morphological and physiological changes in the first two weeks of pregnancy, the secretory capacity of the uterine endometrium also develops to support these changes. The rate of uterine development is, to a large extent, determined by the development of the corpora lutea from the time of ovulation (Dziuk, 1987), primarily as it relates to the changes in plasma progesterone concentrations. The steroid environment generated by the ovary regulates the capacity for uterine endometrial secretions, which are necessary for the development and regulation of conceptus growth and differentiation.

PROGESTERONE PROFILES IN CYCLIC AND PREGNANT FEMALE PIGS

Prior to and during the period of corpora lutea formation, plasma progesterone concentrations in the peripheral circulation (Figure I.4) are low (<1 ng ml^{-1}) (Guthrie et al., 1972 ; Magness and Ford, 1983). They remain low until two to three days after onset of estrus, at which point systemic concentrations of progesterone begin to rise. Using an hourly bleeding schedule, van de Wiel et al. (1981) observed that plasma progesterone concentrations remained undetectable (<0.1 ng ml^{-1}) until twenty-seven to thirty hours after the occurrence of the LH peak (time at which LH concentration reached maximum). Progesterone concentrations in the plasma increased with time after estrus, reaching peak values at Days 12 to 15 of pregnancy (Guthrie et al., 1972 ; Robertson and

I.4 Progesterone concentrations in plasma and adipose tissue during the estrous cycle of the sow (Hillbrand and Elsaesser, 1983)

▲ PROGESTERONE (NG/ML PLASMA)
0
10
20
30
40
DAY OF ESTROUS CYCLE
-10
-8
-6
-4
-2
0
2
4
6
8
10
0
10
20
30
40
50
60
70
80
90
100
■ PROGESTERONE (NG/100 MG ADIPOSE)

King, 1974). When embryo implantation does not occur, the corpora lutea regress and plasma progesterone concentrations decline to minimal concentrations. With recognition of pregnancy, plasma progesterone concentrations decline from peak value but remain elevated. During the remainder of pregnancy, plasma progesterone remains relatively constant and only begins to decline ten to fifteen days prior to parturition.

Plasma progesterone concentrations are modified by the substantial uptake of progesterone into the adipose tissue (Hillbrand and Elsaesser, 1983). As progesterone concentrations rise in the plasma, a parallel but latent increase in the concentration of progesterone in the adipose tissue occurs (Figure I.4). Likewise, as plasma progesterone declines in the plasma, progesterone concentrations in the adipose tissue decline. The two pools are in equilibrium and the interchange between plasma and adipose tissue depends on the relative concentration of progesterone in the two pools. The total quantity of progesterone present in adipose tissue in the middle of the luteal phase is 36 mg, which is equivalent to the daily corpora luteal production of progesterone at Day 11 of the cycle. This large buffering capacity may delay the peripheral rise of plasma progesterone after corpora lutea formation, dampen fluctuations in plasma progesterone and maintain high systemic progesterone concentrations after luteal regression has occurred.

In contrast to progesterone concentrations, estradiol-17β concentrations in the plasma attain peak values (60 to 65 pg ml^{-1} plasma) at the onset of the estrous cycle and decline to 20 to 25 pg ml^{-1} by the third day of gestation (Magness and Ford, 1983 ; van de Wiel et al., 1981). The rise in plasma estradiol-17β over a two to three day period elicits the signs of behaviourial estrus in the female and triggers the "pre-ovulatory LH surge", which, in turn, elicits ovulation of the developing follicles. Estradiol-17β concentrations remain low during the remainder of the estrous cycle. During the estrous cycle, estrone concentrations in the plasma follow a similar pattern. However, a rise in estrone sulphate in the peripheral plasma is seen at Day 16 post-coitum and reaches peak concentrations at Day 30 of gestation. This corresponds to an increase in sulphated estrogens in uterine venous blood on Day 13 to 14 which is associated with increased uterine blood flow (Ford, 1982). Free estradiol-17β and unconjugated estrone are at minimal concentrations prior to Day 70 to 80 gestation at which point the concentrations of these steroids in the blood begin to rise and continue to do so until parturition.

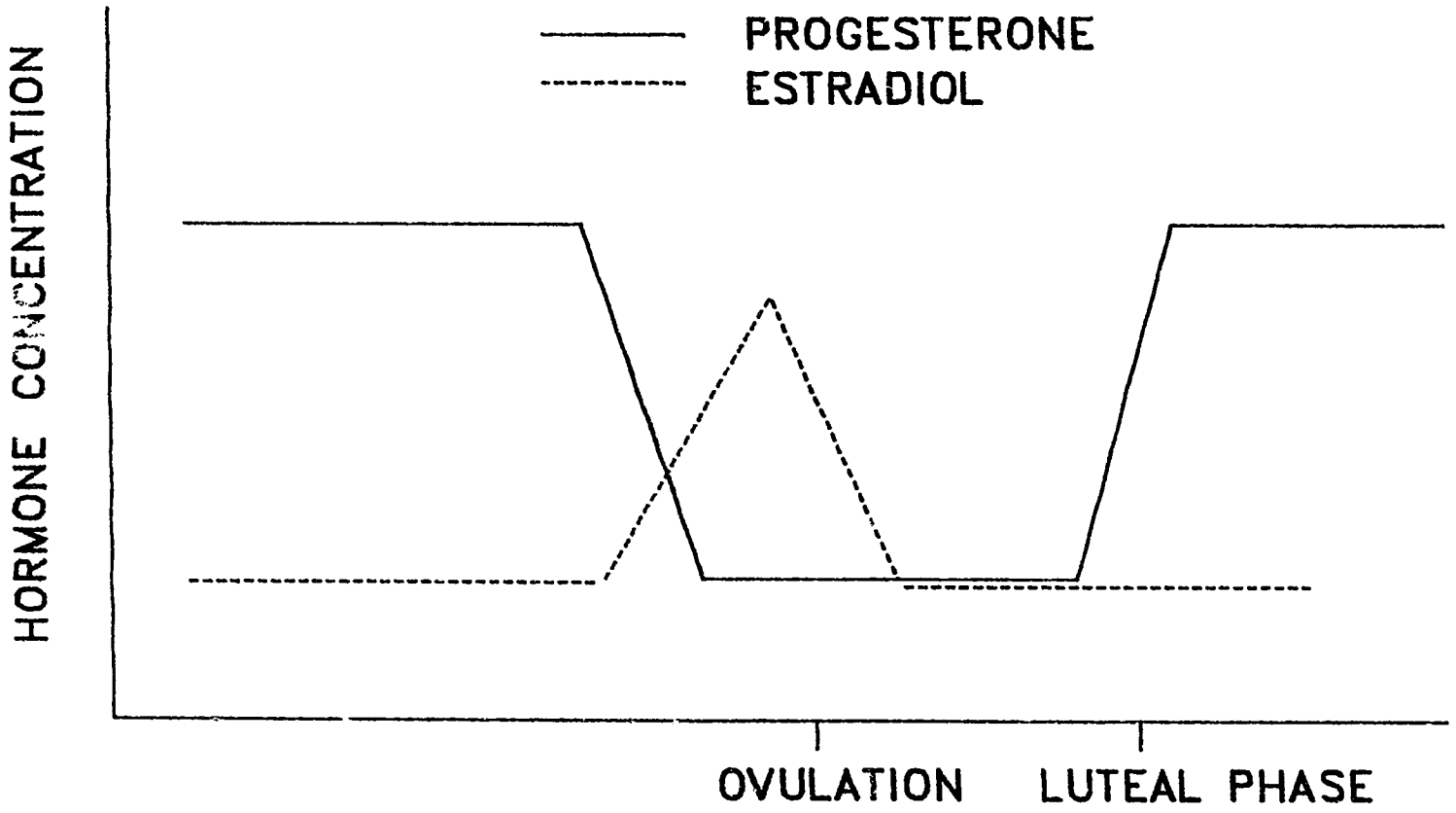
A minimum level of progesterone in the plasma is required for the maintenance of pregnancy. Removal of the corpora lutea in the pig results in the abrupt termination of pregnancy. In ovariectomized gilts, exogenous progesterone can maintain pregnancy provided that plasma progesterone concentrations remain above 4 to 5 ng ml^{-1} (Ellicott and Dziuk, 1973). In

studies utilizing a serial reduction in the number of corpora lutea present, a minimum of four to five corpora lutea were required to maintain pregnancy (Martin et al., 1977).

Several characteristic changes in plasma estradiol and progesterone concentrations, prior to, at and following ovulation are necessary for optimum uterine development and embryo survival (Figure I.5) as demonstrated using hormone replacement in ovariectomized sheep (Wilmut et al., 1986). This optimal steroid environment is characterized by a priming luteal phase concentration of progesterone prior to ovulation, followed by a high dose of estradiol sufficient to induce estrus. During the peri-ovulatory period, a low concentration of plasma progesterone followed several days later by luteal phase concentrations of progesterone in the plasma is required (Wilmut et al., 1986). The lack of luteal phase concentrations of progesterone prior to mating at pubertal estrus may partially explain the higher embryonic mortality associated with gilts bred at first, as opposed to third estrus (Archibong et al., 1987). Similarly, the need for low doses of exogenous progesterone in the peri-ovulatory period suggests the importance of a low progesterone environment during this time for optimal embryo survival in subsequent periods (Wilmut et al., 1985).

The importance of peri-ovulatory progesterone concentrations is supported by the association between plasma progesterone

I.5 Optimum hormonal pattern for embryo survival (adapted from Wilmut et al., 1986).


PROGESTERONE
ESTRADIOL
HORMONE CONCENTRATION
OVULATION
LUTEAL PHASE

after mating (peri-ovulatory period) and embryonic survival in prolific ewes (Ashworth et al., 1984 ; 1989) and beef cows (Maurer and Echternkamp, 1982). Progesterone concentrations in the peri-ovulatory period (Day 0 to 1) were significantly lower in ewes which experienced prenatal loss than in those which did not, which again suggests that there is a threshold progesterone concentration required during this period (Ashworth et al., 1984). The relationship with progesterone during the luteal period was not significant (Ashworth et al., 1984). In sheep, significant correlations were demonstrated to exist between embryo survival and the time after ovulation at which plasma progesterone concentrations begin to rise, and the subsequent rate of increase in plasma progesterone concentrations (Ashworth et al., 1989). Also, sows weaned at 10 days of lactation exhibited greater embryonic mortality in the subsequent pregnancy as compared to their counterparts weaned at five weeks (Varley and Cole, 1978): This was attributed to higher estradiol and progesterone concentrations in the immediate post-mating period in the sows weaned early (Varley et al., 1984), possibly resulting from an unfavourable uterine environment because of the altered estradiol/progesterone concentrations. Generally, the uterine environment necessary for the growth and differentiation of the developing conceptus is dependent on the steroid environment generated by the ovary and the development of functioning corpora lutea (Dziuk, 1987 ; Lamming et al.,

1989).

Luteal insufficiency has been postulated to contribute to embryonic mortality, but recent evidence refutes this. Induction of accessory follicles by injection of hCG (on Days 3 and 6) to increase luteal mass bore no benefits in terms of enhanced embryo survival in pigs (Stone et al., 1987). Plasma progesterone concentrations were correlated with the mass of corpora lutea present (Guthrie et al., 1974 ; Webel et al., 1975) but were not correlated with embryo numbers or percentage survival (Wetteman et al., 1980).

HORMONAL MANIPULATION OF UTERINE DEVELOPMENT

The relationship between luteal plasma progesterone concentrations or exogenous progesterone treatment during the luteal phase and increased embryo survival in gilts (De Sa et al., 1981 ; Wildt et al., 1976 ; Webel et al., 1975) is not clear. Injection of progesterone and estrone daily from Days 4 to 20 or from Days 20 to 30 had no effect on fetal numbers at Day 60, though the latter resulted in longer and heavier placentas and greater surface area than in non-injected controls (Dalton and Knight, 1983). Treatment of sows with 25 mg progesterone and 12.5 ug estrone daily from Day 14 to Day 23 of gestation, increased chorionic surface area at Days 30 to 35, but the differences were not significant at Day 60 of gestation (McGovern et al., 1981). Again, fetal numbers were not affected by exogenous hormones.

The physiological maturity of the uterine environment can be

manipulated through hormonal therapy by altering type, timing and quantity of steroid injected. Exogenous progesterone treatment of sows at Day 0 of gestation results in elevated embryo mortality and pregnancy failure (Spies et al., 1959), whereas progesterone injection from Day 4 onward has no effect on embryo survival. Exposure of the uterus in the immediate post-coital period to luteal phase concentrations of progesterone appears to advance the uterine environment prematurely; injecting 25 mg progesterone day^{-1} into estrous ewes for four days post-coitally advanced the uterine environment, to the point that Day 10 embryos are able to thrive in these sheep at Day 6 post-coitum (Lawson and Cahill, 1983). In a recent study, recipient dairy cows were injected with 100 mg progesterone daily from Days 1 to 5 post-estrus and received embryos from donors which had showed estrus seventy-two hours earlier (Geisert et al., 1991). The percentage embryo survival in the injected cows were the same as those recipients synchronous with the donor cows, demonstrating that uterine receptivity and development can be advanced by early administration of progesterone. Either increasing or decreasing the dosage of progesterone given to ovariectomized sheep enhanced survival of advanced and less advanced embryos, respectively (Miller and Moore, 1976). This indicates that asynchrony can develop between the embryo(s) released and the uterine environment. The degree of asynchrony is dependent on the time and rate at which progesterone

concentrations in the plasma increase and the rate at which the embryo(s) develop in utero. Alternately, estrogen administration post-coitally has been shown to delay progestational development of the uterine environment (Beier, 1974).

UTERINE ASYNCHRONY AND VARIATION IN EMBRYO DEVELOPMENT AND SURVIVAL

Embryos can tolerate a degree of asynchrony with the uterine environment. Studies in sheep have demonstrated that Day 4 embryos transferred to an advanced uterine environment (Day 7) are able to respond with an accelerated growth rate until Day 12 but then subsequently fail to implant (Lawson et al., 1983). In rats and mice, embryos physiologically younger than the uteri can be transferred, and will continue to grow until the time implantation is expected to occur, but will subsequently degenerate after failing to implant (Dickmann and Noyes, 1960 ; Doyle et al,. 1963). It has been suggested that though the blastocysts appear normal, they have failed to differentiate normally and are unable to either send an appropriate signal to the endometrium, or to respond correctly to the uterine environment. Blastocysts older than the physiological state of the uterus show reduced growth until the uterus becomes receptive to implantation.

The likelihood of an embryo surviving is related to its development relative to other embryos present. Embryo mortality is not a random event. Embryos at a more mature

stage of development have a higher survival rate than their less developed siblings (Pope et al., 1982a). Bazer (1968) suggested that embryos compete for a critical biochemical substance(s) that are necessary for growth and the more developed embryos will sequester a greater proportion of these. An alternative is the possibility that the more mature embryos may accelerate the physiological development of the uterine environment to the point where the immature embryos cannot survive. The uterine environment at Day 12 of gestation is qualitatively different when embryos at different stages of development are present (Xie et al., 1990b). Experiments with swine, involving the transfer of Day 5 and Day 7 embryos to a Day 6 recipient, showed that less developed embryos survived and developed normally in the absence of older embryos (Pope et al., 1986b). In the presence of Day 7 embryos, Day 5 embryos transferred to the recipient were less viable than the older embryos. The loss of younger embryos occurred after Day 11, presumably during implantation. Thus, an equal number of Day 5 and Day 7 embryos were present at Day 11 but not at Day 60.

Transfer of Day 6 embryos to Day 7 recipients resulted in a greater distribution of morphological stages than transferring Day 7 embryos to Day 6 recipients (Pope et al., 1986b). This correlated with the greater embryo mortality at Day 30 of gestation (64%) in the former group compared with the latter group (42%), though embryo numbers were again similar at Day

11.

PROGESTERONE EXCHANGE BETWEEN THE OVARIAN AND UTERINE VASCULATURE

Generally, studies exploring the relationship between embryo survival and plasma progesterone concentrations have focused on progesterone in the peripheral circulation. However, progesterone dynamics within the vasculature of the uterus and ovaries may differ from that of the systemic circulation, given the close apposition of the ovarian arteries and veins. Approximately 60 mm from the ovary, the ovarian artery forms two prominent branches, with one supplying the bursa, oviduct and the cranial portion of the uterine horn (Del Campo and Ginther, 1973). The remaining branch bifurcates into an ovarian branch and a branch which forms an anastomosis with the arterial network between the convolutions of the ovarian artery and the uterine artery. Beginning at this site, the ovarian branches are highly convoluted and closely associated with the veins arising from the ovary.

A local utero-ovarian pathway is involved in uterine induced luteolysis in a number of mammalian species (eg. swine, cattle, sheep, hamsters and rats) whereas several species rely on systemic pathways (e.g. horses). In the absence of a signal from an embryo(s), the uterine luteolysin $PGF_{2\alpha}$ is released from the uterus and carried to the ovary by a local veno-arterial pathway (Barrett et al., 1971). As measured using labelled $PGF_{2\alpha}$, exogenous $PGF_{2\alpha}$ injected into the uterine

vein passed directly into the ipsilateral ovarian artery.

There are three sites where counter-current transfer of steroids have been demonstrated in the reproductive tract: in the mesovarium or ovarian pedicle, in the mesosalpinx and vasculature of the oviduct and in the fine uterine arteries and veins in the mesometrium along the uterine horns (Krzymowski et al., 1990). A counter-current transfer of steroid hormones within the ovarian pedicle from the ovarian vein to artery (Krzymowski et al., 1982a) may occur by several mechanisms. A direct transfer of steroid from ovarian vein to ovarian artery by direct penetration of steroid through the adjoining vessel walls. The walls of the veins and arteries are significantly thinner in the area of apposition than those with no contact between vein and artery. The highly convoluted nature of the ovarian arteries increases the area over which this may occur. Steroid transfer may occur indirectly through interstitial tissue and the network of fine veins overlapping the branches of the ovarian artery. The tissues (other than the blood) in the ovarian pedicle retain a significant fraction of the steroids produced by the ovary (75 to 80 %). The surrounding tissues represent a significant steroid sink from which steroids may be subsequently released (Kryzmowski et al., 1982b) into the arterial supply of the ovary and the other tissues supplied by branches of the ovarian artery (i.e. oviduct and adjacent portions of the uterine horn). The transfer of steroids from the ovarian vein to the artery,

directly or indirectly, may play a role in local regulation of ovarian function.

The counter-current transfer of steroids within the mesosalpinx, in the arteries supplying the oviduct, may serve to further elevate the concentrations of steroids the oviduct is exposed to. Indirect evidence for this counter-current transfer was demonstrated by Hunter et al. (1983); estradiol and androstenedione concentrations were higher in the arterioles supplying the oviduct than in the jugular vein. The transfer of progesterone from the corpus luteum to the mesosalpinx vasculature has been demonstrated in the gilt (Adamowicz et al., 1989).

Progesterone transferred into the ovarian artery may have direct local effects on the luminal environment of the oviduct and uterine horn. Branches of the ovarian artery supply the oviduct and adjacent regions of the uterine horn. In sheep, progesterone concentrations, in the cranial uterine vein draining the uterine horn adjacent to the ovary bearing the corpus luteum, were higher than in the jugular vein or in the caudal uterine vein draining the same horn (Weems et al., 1989). In the horn opposite to the ovary bearing the corpora lutea, the concentration of plasma progesterone was similar between the jugular vein and the caudal and cranial uterine veins.

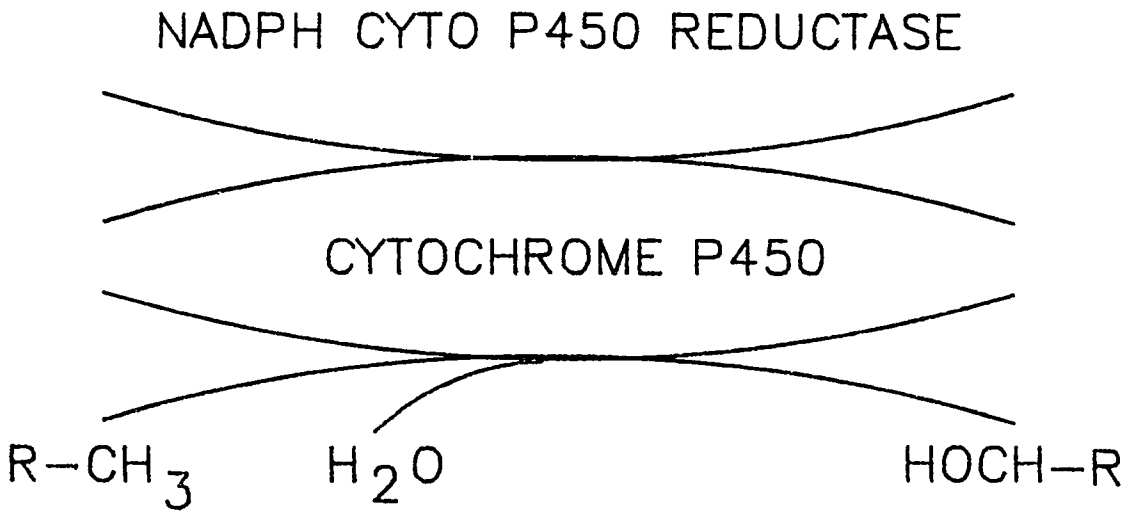
Uterine, and subsequently embryo development and survival, is dependent on the timing in local plasma progesterone

concentrations. This influence on embryo development and survival has been demonstrated with unilaterally ovariectomized rats. Embryonic development was retarded in the horn ipsilateral to the ovariectomy, suggesting a local pathway was involved in bringing progesterone to the uterus (Nuti et al., 1971). Transfer of bovine embryos to the uterine horn ipsilateral to the ovary bearing the corpus luteum resulted in more successful pregnancies than transfer to the horn opposite the corpora lutrea bearing ovary (Newcomb et al., 1980). Transferring embryos to both uterine horns demonstrated that embryos transferred to the horn ipsilateral to the corpora lutea bearing ovary had greater survival rates than those on the contralateral side (Del Campo et al., 1979).

THE ROLE OF THE MIXED FUNCTION OXIDASE SYSTEM (MFO)

Plasma progesterone concentrations reflect a balance between synthesis of progesterone by the corpora lutea and its metabolism and excretion by the liver and kidney. The mixed function oxidases (MFO) in the liver are primarily responsible for steroid metabolism and clearance from the blood. The MFO system is a series of enzymes (hydroxylases, reductases) with cytochrome P_{450} as the terminal oxidase (Figure I.6). These are involved in the synthesis of the steroid hormones from cholesterol and their subsequent interconversions (Waterman et al, 1986). They also have a primary role in the metabolism and excretion of drugs and other xenobiotics which find their way

I.6 Mixed function oxidase system in the liver (adapted from Gower and Honour, 1984)



CONJUGATION ENZYMES

1. SULPHOTRANSFERASE
2. UDP–GLUCURONYL TRANSFERASE
3. GLUTATHIONE–D–TRANSFERASE

into the body.

Hepatic metabolism of drugs and steroids occurs in two steps in what are referred to as Phase I and Phase II reactions. The Phase I reaction is carried out by the mixed function oxidase system. The particular mixed function oxidases are located in the endoplasmic reticulum of the liver. NADPH-cytochrome-P_{450} reductase and cytochrome P_{450} constitute the mixed function oxidase. The hydroxylations and reductions performed by these enzymes serve to make the steroids more hydrophilic and more susceptible to conjugation. Conjugation reactions can be completed by one or more enzyme systems. The enzymes generally involved are a sulfotransferase, UDP-glucuronyl transferase and glutathione-S-transferase. The resulting molecule is highly water-soluble and can be excreted in the bile or released into the plasma and excreted through the kidneys.

Several of the MFO enzymes are involved in the catabolism of progesterone. Progesterone catabolism usually involves reduction at c-20. There is also reduction of the A ring by $^4\Delta$-ene-5α- and 5β- reductases and reduction of the carbonyl at c-3 to a hydroxyl group (Gower and Honour, 1984). Up to six pregnanediols can be formed by this series of reactions with 5α-pregnane-3,20α-diol constituting the major metabolite excreted in the bile. The metabolites are complexed with a number of compounds (glucuronic acid, sulphates, etc.) to increase their solubility in water and are subsequently excreted into the bile (Adlercreutz and Martin, 1980). The

particular compound that progesterone is conjugated with determines its route of excretion and/or further metabolism. When the metabolites are excreted into the intestinal lumen with the bile, they are then subjected to metabolism (deconjugation) by microbes in the small intestine and reabsorption by the mucosa. Subsequently, there is reconjugation in the intestinal mucosa with uptake into the blood and excretion into the urine. In the sow, the predominant metabolites found in the urine are two pregnenolones, 5β-pregnan-3α-ol-20-one 5β-pregnan-3α-ol-20-one, as well as two pregnanediols, 5α-pregnan-3,20α-diol and 5α-pregnan-3,6α-diol-20-one (Edgerton et al., 1971).

Increased food or protein intake, or the associated increase in growth rate increases the metabolic activity of the liver (Argyris, 1971), increasing the uptake and metabolism of progesterone. Altering food intake alters the level of hepatic mixed function oxidase activity, though with different effects depending on the sex of the animal. In male rats, food restriction to 75 % ad libitum increased hepatic microsomal levels of cytochrome P_{450} when compared with rats given food ad lib. In contrast, hepatic cytochrome P_{450} was depressed by imposition of food restriction to female rats (Hashmi et al, 1986). The level and activity of cytochrome P_{450} differs between sexes and is greater in the male. Such differences are primarily due to the differential pattern of release of growth

hormone between males and females (Jansson et al, 1985 ; MacGeoch et al, 1985).

Increased dietary protein intake enhances the microsomal content of cytochrome P_{450} in the liver (Campbell and Hayes, 1974) and hence steroid and drug clearance. Plasma half-lives of theophylline and antipyrine (which are used as indicators of mixed function oxidase activity) in human subjects are shorter with high intakes of protein than with high carbohydrate intake (Kappas et al, 1976). The greater activity of the hepatic mixed function oxidase system in response to higher feed intake in females would increase the catabolism of progesterone from the plasma, resulting in lower plasma progesterone concentrations. These lower progesterone concentrations would suggest a mechanism by which alterations in food intake to the gilt in early pregnancy affects embryo survival. This will be discussed in more detail later in the review.

C. NUTRITION AND EMBRYO SURVIVAL IN EARLY PREGNANCY

PLANE OF FEEDING DURING EARLY PREGNANCY IN GILTS

Feed intake in early pregnancy has been suggested to impact on embryo survival in the gilt. Ad libitum feed intake, five to ten days prior to mating, increases ovulation rate above that of restrict-fed controls (Beltranena et al., 1991a; den Hartog and van Kempen, 1980). Continuation of the ad libitum feed intake into the post-mating period has been reported to increase embryonic mortality in comparison with restrict-fed

gilts (Robertson et al., 1951 ; Gossett and Sorenson, 1959 ; Dyck et al., 1980 ; Dyck and Strain, 1983 ; Christenson, 1986). Older studies maintained the same low or high feed intakes from the pre-mating period into the post-mating period. The increase in percentage embryo mortality associated with the higher feed intake may be due to an increase in ovulation rate with no concomitant change in number of embryos (Robertson et al., 1951 ; Haines et al., 1959 ; Sorensen et al., 1961 ; Frobish, 1970), reflecting the negative association between embryo survival and ovulation rate (Wrathall, 1971).

In a statistical evaluation of previous literature, Den Hartog and van Kempen (1980) reported that gilts fed ad libitum prior to mating and switched to a regimen of restricted feed intake at mating had a greater number of embryos present at Day 30 than gilts continued on ad libitum feed intake. The number of ova shed were not different between gilts given restricted or ad libitum feed intake. The critical window affecting embryo survival is the immediate post-coitum period. Feeding 2.5 vs 1.5 kg of feed in the first ten days after mating reduced embryonic survival at Day 30, while the same dietary treatment between Days 11 to 20 had no effect on embryo survival (Dyck and Strain, 1983). However, a later study utilizing crossbred gilts, providing the same level of feed intake from the day after estrus detection to Day 10, failed to produce any difference in embryo survival (Dyck,

1991).

PLANE OF FEEDING DURING LACTATION AND EARLY PREGNANCY IN SOWS

An effect of high feed intake during early pregnancy on embryo survival is not seen with mature sows (King and Young, 1957 ; Heap et al, 1967 ; Toplis et al., 1983 ; Dyck and Cole, 1986). Toplis (1983) observed no difference in embryo numbers or survival when multiparous sows were fed 2 or 4 kg feed per day starting at Day 3 post-breeding. Reduction in energy intake from 24.0 to 17.5 MJ DE day^{-1} from Days 1 to 10 of gestation did not affect pregnancy rate or the number of piglets born (Dyck and Cole, 1986). The authors suggested that the effects of nutrition during early pregnancy on the endocrine system of the sow would be overshadowed by the larger endocrine changes associated with lactation.

Whether the level of feed intake during lactation has an effect on embryo mortality during the subsequent pregnancy has been contested. Several studies (Reese et al, 1982 ; King and Dunkin, 1986) have shown no effect of reduced levels of energy and protein intake during lactation on litter size. Other authors (King et al, 1984 ; Hughes et al, 1984 ; Kirkwood et al., 1987 ; 1990) found increased embryonic mortality in sows given a low level of feed during lactation compared with those given high planes of nutrition. In the latter study, second parity sows were provided two levels of feed intake (3 vs 6 kg day^{-1}) which were factorialized with two levels of feed intake (1.8 vs 3.6 kg day^{-1}) during early pregnancy. Sows receiving

3 kg during lactation had significantly lower embryo survival than did sows fed 6 kg. Feed intake in early pregnancy had no effect, although sows fed the low feed intakes in both pregnancy and lactation had the lowest embryo survival of all four diet combinations. Several studies have failed to show a change in ovulation rate in sows fed low or high feed intake during lactation (den Hartog and van der Steer, 1981 ; King and Williams, 1984 a,b), though trends exist for lower embryo survival in low fed sows (Hughes et al, 1984). As in gilts, this may be related to low circulating concentrations of progesterone, as well as increased metabolic clearance of this steroid hormone, as suggested by Kirkwood and Aherne (1985). However, Prime et al. (1988) failed to demonstrate a difference in plasma progesterone concentrations to Day 25 in multiparous sows provided low or high feed intakes during lactation and/or early pregnancy. Alternatively, it has been speculated that a low pre-ovulatory surge of LH may affect luteinization of the corpora lutea and result in reduced progesterone concentrations during early pregnancy (Kirkwood and Aherne, 1985).

MECHANISM OF ACTION

The mechanism by which altered feed intake influences embryo survival is not known. Reduction in the metabolism of gonadal steroids allows the concentrations of these hormones to increase in the plasma. On a bodyweight basis, the metabolic clearance rate (MCR) of steroids decreases with age. The MCR

of estradiol on a metabolic body weight basis was greater in 80 day old prepubertal gilts than in gilts 160 days of age (Elsaesser et al., 1982). This was true for estradiol and testosterone in prepubertal boars (Christenson et al., 1984). The authors suggested that a maturational decrease in MCR may be partially responsible for increases in estradiol concentrations with age in the gilt. It has been suggested by Kirkwood and Aherne (1985) that alterations in the MCR of plasma steroids by the liver are induced by changes in feed intake in pregnancy and lactation. Female rats fed ad libitum possessed greater levels of steroid metabolizing enzymes than those restricted to 50 and 75% of ad libitum (Hashmi et al., 1986). The levels of hepatic steroid metabolising enzymes are enhanced by phenobarbital administration and increased feed intake. Both of these treatments increased ovulation rate and this was attributed to a greater metabolic clearance of estradiol by the liver (Thomas et al., 1987). However, neither plasma estradiol concentrations nor metabolic clearance rate were measured in this study.

Increased feed intake is associated with depressed plasma progesterone in sheep (Parr et al., 1982 ; 1987) and gilts (Dyck et al., 1980) and with increased metabolic clearance of progesterone from the plasma of sheep. Conversely, underfed sheep had higher circulating concentrations of plasma progesterone (Cumming et al., 1971). In the study reported by Dyck et al. (1980), gilts were fed one of three levels, 1.5,

2.25 or 3.50 kg d^{-1}, beginning at the day after observed estrus. Those gilts provided 3.5 kg per day had reduced progesterone concentrations in comparison to those given the lowest quantity of feed. The gilts provided 2.25 kg had plasma progesterone concentrations intermediate to the low and high intake groups. Increasing feed intake from 1 to 3 kg of feed per day in ovariectomized gilts increased the metabolic clearance rate of progesterone from 39.7 to 57.0 ml min^{-1} kg^{-1} (Symonds and Prime, 1988). Therefore, increased feed intake in gilts has been associated with increased metabolic clearance of progesterone from the plasma, resulting in lower plasma progesterone concentrations.

As discussed earlier, depression of plasma progesterone, particularly in the periovulatory period, has been suggested to decrease embryonic survival. Inverse relationships between plasma progesterone during this period and embryo mortality have been reported in sheep (Ashworth et al., 1989) and cattle (Maurer and Echternkamp, 1982). High feed intake continued after mating may be expected to depress plasma progesterone concentrations during the peri-ovulatory period, thereby increasing embryonic mortality.

Pope (1988) suggested that variability in progesterone synthesis (or catabolism) increased asynchrony between the embryo and uterus, thereby decreasing the viability of the embryos. The timing of the progesterone increase may be crucial in determining the synchrony between the uterus and

embryo and therefore the likelihood of an embryo remaining viable. As discussed earlier, the rate of uterine development is generally dependent on the development of the corpora lutea and their ability to produce progesterone (Dziuk, 1987 ; Lamming et al., 1989). If progesterone is metabolized to a greater extent under conditions of higher feed intake, the rise in plasma progesterone concentrations may be delayed or diminished, delaying the development of the uterine environment.

These concepts together provide a model by which increased feed intake in early gestation may reduce embryo survival in the gilt. Increased feed intake during early pregnancy may increase the metabolic clearance of progesterone from the plasma, delaying the rise in plasma progesterone concentrations by several hours. This may delay attainment of a threshold progesterone concentration delay initiation of the development of the secretory capacity of the oviduct and uterine endometrium.

The objective of the series of studies described in the next chapters was to provided evidence for such a link between changes in feed intake, plasma progesterone concentrations and embryo survival.

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II. THE INFLUENCE OF DIETARY ENERGY AND PROTEIN INTAKE DURING EARLY PREGNANCY ON PLASMA PROGESTERONE AND EMBRYO SURVIVAL

A. INTRODUCTION

Several experiments have shown that ad libitum feeding of gilts for at least four to five days prior to mating, will maximize ovulation rate (Beltranena et al., 1991a ; den Hartog and van Kempen, 1980). Continuation of the ad libitum feed intake into the post-mating period has been reported to increase embryonic mortality in comparison to gilts restrict-fed after mating (Robertson et al., 1951 ; Gossett and Sorenson, 1959 ; Dyck and Strain, 1983 ; Christenson, 1986). Where the same low or high feed intakes are continued from the pre-mating period into the post-mating period, the increase in percentage embryo mortality is associated primarily with an increase in ovulation rate with no concomitant change in number of embryos (Robertson et al., 1951 ; Haines et al., 1959 ; Sorensen et al., 1961 ; Frobish, 1970) reflecting the negative association between embryo survival and ovulation rate (Wrathal, 1971).

In a statistical evaluation of previous literature, den Hartog and van Kempen (1980) demonstrated that gilts fed ad libitum prior to mating and switched to a regimen of restricted feed intake at mating had a greater number of embryos present at Day 30 than gilts continued at ad libitum feed intake. Feeding 1.5 vs 2.5 kg of feed from Day 1 to Day

10 after mating reduced embryonic survival at Day 30, while the same dietary treatment provided from Days 11 to 20 had no effect on embryo survival (Dyck and Strain, 1983), indicating the critical window affecting embryo survival may be early in the post-mating period. A recently published work has reported no difference in embryo numbers or percent survival when feed intake was increased from 1.25 to 2.5 kg from Day 1 to 11 of gestation (Dyck, 1991).

The mechanism by which altered feed intake may influence embryo survival is not known. When gilts were given feed intakes of 1.5, 2.25 or 3.50 kg day^{-1}, gilts on the highest plane of nutrition exhibited reduced serum progesterone levels compared with those on the lowest level of feed intake (Dyck et al, 1980), though no difference in embryo survival was seen at Day 60 of gestation in this study. In cattle, increases in dietary protein intake during early pregnancy depressed plasma progesterone concentrations (Jordan and Swanson, 1979). An inverse relationship between plasma progesterone during the peri-ovulatory period and embryo mortality has been reported in sheep (Ashworth et al., 1989) and in beef cows (Maurer and Echternkamp, 1982).

The depression in plasma progesterone concentrations may be due to alterations in the metabolic clearance rate of plasma steroids by the liver, in response to changes in feed intake in pregnancy and lactation (Kirkwood and Aherne, 1985). Using ovariectomized ewes injected with progesterone during early

gestation, Parr (1982) demonstrated that sheep fed ad libitum had lower progesterone concentrations than their maintenance-fed counterparts, indicating increased catabolism of progesterone by the ad libitum fed ewes. Dziuk (1982) proposed that increased feed and/or protein intake increased the synthesis of hepatic mixed function oxidase, thereby increasing steroid metabolism in the liver.

The objective of this study was to examine the relative importance of energy and protein intake on plasma progesterone concentrations and on embryonic survival in the gilt during early pregnancy.

B. MATERIALS AND METHODS

ANIMAL MANAGEMENT

A total of fifty-five Yorkshire-Landrace gilts were used in this study. On reaching 90 to 95 kg bodyweight, gilts were induced naturally into puberty by mixing and exposure to a mature boar and allowed to complete one estrous cycle. Beginning three days prior to expected date of return, gilts were heat checked once daily. On attaining second estrus, they were bred three times at twelve hour intervals using a different boar at each insemination. The day on which second estrus occurred was designated as Day 0.

Prior to puberty, the gilts were raised on a standard 16% crude protein diet provided ad libitum. From puberty until the start of the study, gilts were provided a basal barley-wheat-soybean meal diet formulated to contain 12.2 MJ

DE kg^{-1} and 140 g CP kg^{-1} diet ad libitum.

On Day 3 of gestation, gilts were weighed before the first feeding of the day and were randomly assigned to one of the four dietary treatments. Littermates were not balanced over treatments. The gilts were also weighed prior to feeding on Day 9 and on the final day of the experiment on Day 15. The gilts were fed twice a day, receiving half their allotment at each meal. The animals were housed individually during the course of the study. On Day 16 post-coitum, the test diets were withdrawn and the gilts were fed the basal diet at the level recommended by the NRC (1988) until slaughtered at Day 28 of gestation.

At slaughter, the reproductive tracts were removed, the ovaries examined and the number of corpora lutea (CL) recorded. The uterus was opened and the number of viable embryos present was determined. Embryo survival was calculated as number of viable embryos/number of corpora lutea and expressed as a percent. The embryo and its associated placental tissue were removed from the uterine endometrium and weighed separately.

DIETARY TREATMENTS

A 2 X 2 factorial design was employed in this study with two levels of protein intake provided at each of two levels of energy intake. The low and high energy intakes were set at 1.6 times and 2.6 times the maintenance energy requirement, and represented NRC (1988) recommendations and a level of feed

intake approaching ad libitum energy intake, respectively, for the pregnant gilt. To establish the required feed intakes, the maintenance energy requirement was taken to be 452 KJ DE kg^{-1} $BW^{0.75}$ (Williams et al, 1985 ; NRC, 1988). The two levels of protein intake provided were equivalent to feeding a 14% crude protein diet at NRC (1988) recommended and a higher feed intake. Feed intake was adjusted with change in bodyweight. The dietary regimens were designed to provide the following intakes of energy and protein in the first two weeks of pregnancy:

1. LL - Low Energy (1.6 x ME_m), Low Protein (7.8 g BW $kg^{-0.75}$)
2. LH - Low Energy (1.6 X ME_m), High Protein (13.0 g BW $kg^{-0.75}$)
3. HL - High Energy (2.6 X ME_m), Low Protein (7.8 g BW $kg^{-0.75}$)
4. HH - High Energy (2.6 X ME_m),High Protein (13.0 g BW $kg^{-0.75}$)

The test diets were formulated to ensure that the intake of fibre, vitamins and minerals were relatively constant for all regimens and that only energy and protein intake varied. The test diets were formulated by providing the basal diet at 1.4 times the maintenance energy intake and supplying the additional energy and protein intake as corn starch/molasses and casein, respectively (Table II.1). The amount of feed to be provided was weighed on a scale, and fed over two meals at 0800 and 1500 h.

HORMONAL ANALYSIS

Blood samples (10 ml) were taken prior to feeding (time=0) and six hours after feeding from each gilt at six day

intervals beginning on Day 3 of gestation. The gilts were restrained with a rope snare and bled within five minutes of restraint. Blood samples were collected by jugular venipuncture into 10 ml heparinized vacutainers which were kept on ice until centrifuged at 2000 rpm for 10 minutes. Plasma was decanted and kept frozen at -20°C until analysed for progesterone concentration.

Plasma samples were analysed in duplicate for progesterone by radioimmunoassay (RIA) as described by Beltranena et al. (1991b) using an anti-serum raised against 4-pregnen-11α-ol-3,20-dione hemisuccinate:BSA. The supplier reported cross-reactions of this anti-serum to be: corticosterone 2.7%, pregnenolone 2%, 20α-progesterone 0.18%, estrone 0.12%, testosterone 0.06%, dihydrotestosterone 0.03%, cortisol 0.015%, cholesterol 0.003% and estradiol 0.002%) (Rawlings, personal communication).

For each tube, 50 ul of diluted or undiluted plasma sample was extracted with 4 ml of petroleum ether for 4 minutes. Dilutions were made using PBS gel buffer. Within an assay, extraction efficiency as determined by recovery of [1,2-^{3}H(N)]-progesterone was used to correct the quantity of progesterone determined in each tube. The mean recovery of tritiated progesterone was 90.4 +/- 4.9%. Standard curves ranged from 0.001 to 10 ng tube^{-1}. Sensitivity of the assay, defined as 85% of total binding, was 0.021.ng tube^{-1}. No significant deviation from parallelism was evident from

assaying 100, 50 and 25 ul of a standard plasma pool. The intra- and inter-assay coefficient of variation for the progesterone assay was 6.9 % and 17.6%, respectively. An equal number of gilts from each dietary treatment were included in each assay.

STATISTICAL ANALYSIS

Dietary effects on embryo numbers and percent survival were assessed using SAS (SAS Institute) general linear models procedure with the number of corpora lutea included as the covariate. The latter was included as a covariate since changes in energy/protein intake were initiated after ovulation had occurred. Embryo and placental weights and chorio-allantoic fluid volume were compared between dietary treatments by analysis of variance. Since all gilts could not be slaughtered at Day 28, day of pregnancy was included as a variable in analysing these variables. Contrasts were established to evaluate the effects of energy and protein independently if diet effects were significant. For ease of presentation, individual means for each diet combination are presented in the tables.

The progesterone data was analysed using an analysis of variance of a split plot design with diet as the main effect and day of gestation and time of sampling as the subplot. Interactions between the main and sub-plot effects were also evaluated.

Progesterone data from blood samples taken prior to

imposition of the dietary treatments were used retrospectively to designate animals as having either low, high or moderately low or high progesterone plasma concentrations. The mean and variance for embryo survival was then compared between these groups. Gilts were ranked from low to high according to initial progesterone concentrations and partitioned into the above four groups. Means for each group were determined and the homogeneity of variance in embryo survival between groups of animals assessed by the Bartlett-Box test (Snedecor and Cochrane, 1980).

C. RESULTS

The bodyweight of the gilts on Days 3 and 15, and the average daily gain (ADG) of the gilts in the four dietary groups are presented in Table II.2. The bodyweights of the gilts at Day 3 were similar between all energy and protein intakes. Day 15 weights and average daily gain were greater ($P<0.05$) for gilts on ad libitum energy intake (HL and HH) than on restricted energy intake (LL and LH). Increased protein intake did not have an effect on Day 15 weight or average daily gain. There was a significant interaction between energy and protein intake on average daily gain with the increased protein intake at restricted energy intake resulting in a small depression in ADG. This was mostly attributable to a depression in growth rate in the last six-day period in which the gilts on LH were given the test diet.

Plasma progesterone concentrations changed as gestation

progressed (Table II.3) increasing from Day 3 of gestation (9.4 ng ml^{-1}) to Day 15 of gestation (51.0 ng ml^{-1}). Progesterone concentrations were not significantly different between samples taken prior to feeding and six hours after feeding. The main effect of increased energy/protein intake was not significant on plasma progesterone concentration. The interaction between diet and day of gestation was also not significant.

The reproductive performance of the gilts is given in Table II.4. Average ovulation rate as measured by the number of corpora lutea was not significantly different between the four dietary groups. Using number of corpora lutea (ovulation rate) as a significant covariate, increasing energy and protein intake of the gilts separately or in combination, above that of the control group (LL), did not influence the number of viable embryos present in the uterus at Day 28 of gestation. Similarly, percent embryo survival was not influenced by a greater dietary intake of energy and/or protein. Embryo and placental weights and the volume of fluid collected from the allantoic sac were not affected by any of the dietary treatments. As would be expected, there was a significant effect ($p<0.05$) of day of pregnancy on the latter three variables.

Percentage embryo survival was plotted as a function of progesterone concentrations on the third day of gestation. In all dietary groups, there were gilts present that had 100%

embryo survival. It was observed that the lowest embryo survival was higher with increasing plasma progesterone concentrations; also that variance in embryo survival was greater at lower plasma progesterone concentrations (Figure II.1) than at higher concentrations. Consequently, partitioning gilts on the basis of their plasma progesterone concentrations on Day 3 of gestation revealed a tendency for mean percent survival to be greater ($p=0.09$) and variability in embryo survival to be smaller ($p=0.07$) at the higher concentrations of progesterone (Table II.5). Embryo survival increased from 84.9% in the low group to 93.0% in the group with higher progesterone concentrations at Day 3 of gestation, with the standard error for each mean decreasing from 4.7 to 2.3.

D. DISCUSSION

The lack of a significant response in embryo numbers or survival to either increased energy or protein intake is in sharp contrast to previous reports. Den Hartog and van Kempen (1981) showed that, following ad libitum feed intake prior to mating, lowered feed intake at mating reduced embryo mortality compared to gilts maintained at ad libitum feed levels. Adapting data from previous studies, Hughes (1989) showed a 5% difference in percent embryo survival in low and high fed gilts (77 vs 82%, respectively) which is comparable to the numbers found in the present experiment. Dyck and Strain (1983) reported increases in embryo mortality when food intake

was increased from 1.5 to 2.5 kg day^{-1} (17.6 and 29.4 MJ ME day^{-1}, respectively) which were lower than provided in the present study (26.3 and 42.7 MJ ME day^{-1}). The protein intake provided by Dyck and Strain (1983) were similar to the 260 and 440 grams of protein provided in the present study. Despite the higher energy intakes, embryo survival in the present study was greater than that of gilts on the high energy intake in the study of Dyck and Strain (1983), but similar to that of gilts on the lower energy intake. Gossett and Sorensen (1959) provided low and high energy diets at a constant energy intake prior to and after mating. No effect was shown on the number of eggs shed (11.4 vs 12.5) but there was a reduction in the number of live embryos (8.3 vs 7.0) present at Day 40. In contrast, providing energy intakes of 10.9 vs 25.1 MJ ME day^{-1}, but equivalent protein intakes before and after mating, did not alter ovulation rate (11.5 vs 12.3), number of embryos (10.2 vs 9.7) or percent embryo survival (93.8 vs 78.9) (Frobish, 1970). Dyck (1991) reported no difference in ovulation rate, number of embryo or embryo survival in gilts fed either 1.25 or 2.5 kg day^{-1}. This corresponded to energy and protein intakes of 15.7 and 31.4 MJ ME day^{-1} and 178 and 355 grams protein, respectively. Embryo survival at Day 30 of gestation in this study was 82.5 and 85.1%, respectively. This suggests that under circumstances of higher embryo survival, reduction in feed intake may have no benefit in terms of greater embryo survival. The average percent embryo survival

in the present study was higher than the reported average for percentage embryo loss in swine.

The lack of response may also be partially attributed to the delay in changing energy and protein intake to Day 3 of gestation rather than at the time of mating. The suggestion has been made that increasing feed intake at time of mating may increase ovulation rate (Brooks et al., 1972), thereby increasing percentage embryo mortality because of the negative association between ovulation rate and embryo survival (Wrathall, 1971). To avoid any of these complications, changes in feed intake were delayed. However, in doing so, the critical period in which feed intake may affect embryo survival may have been missed. Increasing feed intake from 1.5 to 2.5 kg day^{-1} beginning at Days 11 to 20 had no effect on percentage mortality though a similar increase from Days 0 to 10, reduced embryo survival (Dyck and Strain, 1983). In the study of Dyck (1991), no decrease in embryo survival was seen when gilts were fed low or high feed intakes from Days 1 to 10. However, this study was completed over a four year period and the 24 gilts assigned to each treatment were distributed over that time period.

In the present study, no significant difference was seen in plasma progesterone concentrations between the combinations of energy and protein intake. Peak plasma progesterone concentrations in the pig has been reported as high as 35 ng ml-1 (Guthrie et al., 1972 ; Robertson and King, 1974) on Day

12 of pregnancy, while in the present study, plasma progesterone concentrations were slightly greater (45 ng ml^{-1}) in Day 15 samples. In the only study in which plasma progesterone concentrations were examined in relation to changes in feed intake (Dyck et al., 1980), plasma progesterone concentrations during early gestation were reduced from 16.7 ng ml^{-1} to 11.8 ng ml^{-1} plasma with increased feed intake. Thus, concentrations were appreciably lower than the average concentration of 40 ng ml^{-1} in the present study. In circumstances where high plasma progesterone exist, a gilt's hormonal status may be less sensitive to changes in feed intake. Associations between increased feed intake, depressed plasma progesterone and increased metabolic clearance of progesterone from the plasma have been shown in other species (Parr, 1982).

The possibility that ad libitum feed intake reduces plasma progesterone concentration in certain situations and may therefore affect embryo survival must be tempered by the knowledge that little relationship has been demonstrated between luteal phase levels of progesterone and number or survival of embryos (De Sa et al., 1981 ; Wildt et al., 1976 ; Webel et al., 1975 ; Wettemann et al., 1980). In the study of Dyck et al. (1980), decreased plasma progesterone concentrations in early gestation due to increases in feed intake tended to be associated with a decrease in embryo survival, although trends were not significant. Stepwise

reduction in the number of corpora lutea had no effect on embryo survival until only four to five were left, when termination of pregnancy occurred (Martin et al., 1977). In ovariectomized gilts, maintaining a concentration of 4 to 5 ng ml^{-1} of progesterone was sufficient to maintain pregnancy. Increases above this concentration showed no benefit in terms of embryo survival (Ellicott and Dziuk, 1973). Injection of 0.55 mg progesterone kg^{-1} and 0.275 ug estrone kg^{-1} from Days 4 to 20 or from Days 20 to 30 had no effect on fetal numbers at Day 60 of gestation, but the latter did result in longer and heavier placentas and greater surface area than in non-injected controls (Dalton and Knight, 1983).

The positive relationship between percentage embryo survival and plasma progesterone concentrations at Day 3 of gestation supports the hypothesis that the timing of changes in plasma progesterone influences embryo survival. An estimated 30 to 40% of embryos die prior to Day 40 of gestation (Pope and First, 1985) with percent survival ranging from 0 to 100%. Not all litters experience embryo loss. It has been suggested that 20% of litters have 100% embryo survival (Dziuk, 1987). In the present study, when plasma progesterone was slow to rise (reflected by low progesterone concentrations at Day 3 of gestation), the variability in embryo survival was greater, ranging from 53.3 to 108.3%. With increasing progesterone concentration, embryo survival was greatest and less variable, suggesting an uterine environment more suitable for supporting

embryo viability. An association has been demonstrated between plasma progesterone concentrations after mating (peri-ovulatory period) and embryonic survival in both prolific ewes (Ashworth et al., 1984 ; 1989) and beef cows (Maurer and Echternkamp, 1982). In the study of Ashworth et al. (1989), progesterone levels in the peri-ovulatory period (Days 0 to 1) were significantly lower in ewes that experienced prenatal loss than in those that did not. The authors reported a significant correlation between embryo survival and the time after ovulation at which plasma progesterone concentrations began to increase and the rate at which plasma progesterone concentrations increased.

It has been suggested that variability in progesterone synthesis (or catabolism) is crucial in determining the synchrony between the uterine environment and the embryo and these may influence embryo viability (Pope, 1988). As an example, exposure of the uterus, in the immediate post-coital period, to luteal phase levels of progesterone advances the uterine environment prematurely. Injecting 25 mg progesterone.day^{-1} into estrous ewes for four days post-coitally advanced the uterine environment to the point that Day 10 embryos were able to thrive in these sheep at Day 6 post-coitum (Lawson and Cahill, 1983). Similarly, either increasing or decreasing the dosage of progesterone given to ovariectomized sheep enhanced survival of advanced and less advanced embryos, respectively (Miller and Moore, 1976). This

indicates that asynchrony can develop between the embryo(s) released and the uterine environment in response to changes in the timing of the rise in plasma progesterone concentration.

These results suggest that changes in dietary intake of energy and protein may only affect embryo survival in the period from mating to Day 3 of gestation. The effects of increased feed intake on plasma progesterone concentrations may be greater in gilts which are less capable of synthesizing progesterone than those where luteal synthesis of progesterone is quickly established. The observation that embryo survival is related to progesterone concentrations at Day 3 or in the peri-ovulatory period suggests that hormonal changes in the immediate post-ovulatory period may be of greater importance in determining embryo survival than hormonal changes later in gestation.

Table II.1. Composition of test diets[1]

Diet	LL	LH	HL	HH
		($g.kg^{-1}$ diet)		
Wheat	119.9	119.6	77.5	77.5
Barley	582.9	581.6	376.7	376.0
Soybean meal	34.7	34.6	22.4	22.4
Casein	14.9	105.1	9.6	67.9
Starch	136.5	50.3	446.8	390.8
Tallow	27.0	27.0	16.5	16.5
Molasses	50.0	50.0	30.0	30.0
Vitamin/ Minerals[2]	10.0	10.0	6.0	6.0
Iodized Salt	4.0	4.0	2.5	2.5
$CaCO_3$	7.2	7.2	4.3	4.3
Ca_2PO_4	12.9	10.6	7.7	6.4
	1000.0	1000.0	1000.0	1000.0
Analyses				
Crude Protein[3] ($g\ kg^{-1}$)	146.2	243.7	94.4	157.3
Digestible energy[4] ($MJ\ kg^{-1}$)	13.56	13.53	14.24	14.21

[1]Test diets are as follows:
LL - Low Energy, Low Protein LH - Low Energy, High Protein
HL - High Energy, Low Protein HH - High Energy, High Protein

[2]In the Low Energy diets, the vitamin/mineral premix provided per kg diet: 120 mg zinc, 12 mg manganese, 150 mg iron, 12 mg copper, 0.1 mg selenium, 5000 IU vitamin A, 500 IU vitamin D_3, 22 IU vitamin E, 12 mg riboflavin, 45 mg niacin, 24 mg calcium pantothenate, 840 mg choline chloride, 30 ug vitamin B_{12}, 200 ug biotin.

[3]Determined by chemical analyses

[4]Calculated digestible energy

Table II.2. Mean bodyweight gain of gilts in response to changes in dietary energy and protein intake during early pregnancy.

Feeding[2] Level	No. Gilts	Breeding Weight (kg)	Day 15 Weight (kg)	Avg. Daily Gain (kg)
LL	12	109.2	111.8[a]	0.311[a]
LH	15	108.3	110.7[a]	0.223[b]
HL	13	106.9	116.0[b]	0.666[c]
HH	15	108.3	117.3[b]	0.773[c]
S.e.m.[3]		±2.5	±0.51	±.040

[1]Means within columns with different superscripts are significantly different at $p<0.05$.
[2]Test diets are as follows:
LL - Low Energy, Low Protein LH - Low Energy, High Protein
HL - High Energy, Low Protein HH - High Energy, High Protein
[3]Standard error of the mean

Table II.3. Plasma progesterone concentrations ($ng.ml^{-1}$) from Day 3 to Day 15 post-mating in gilts provided differing energy and protein intakes in the post-mating period.

Feeding[1] Level	No. Gilts	Day of Gestation Day 3		Day 9		Day 15	
Time		0	6	0	6	0	6
LL	12	8.7	8.0	37.8	36.5	55.4	49.5
LH	15	10.0	11.3	45.7	42.6	51.6	47.3
HL	13	8.9	10.8	38.1	34.6	52.5	52.3
HH	15	9.2	7.9	42.8	39.2	48.0	50.9
S.e.m.[2]		±1.0	±1.0	±1.8	±1.8	±3.9	±3.8

[1]Test diets are as follows:
LL - Low Energy, Low Protein LH - Low Energy, High Protein
HL - High Energy, Low Protein HH - High Energy, High Protein
[2]Standard error of the mean
[3]Absence of superscripts indicate no significant differences

Table II.4. Mean number of eggs shed, number of embryos and percent embryo survival in response to changes in dietary energy and protein intake.

Feeding[1] Level	No. Gilts	Corpora Lutea (No.)	Embryo No.	Embryo Survival (%)	Embryo Weight[1] (grams)	Placenta[2] Weight (grams)	Fluid[3] Vol. (ml)
LL	12	13.7	12.2	85.7	1.27	17.5	146.5
LH	15	15.1	12.8	88.9	1.47	18.9	137.4
HL	13	14.2	12.4	85.6	1.49	18.8	147.4
HH	15	14.8	11.6	80.2	1.26	16.3	137.6
S.e.m.[4]		±0.60	±0.67	±3.54	±0.09	±2.2	±12.7

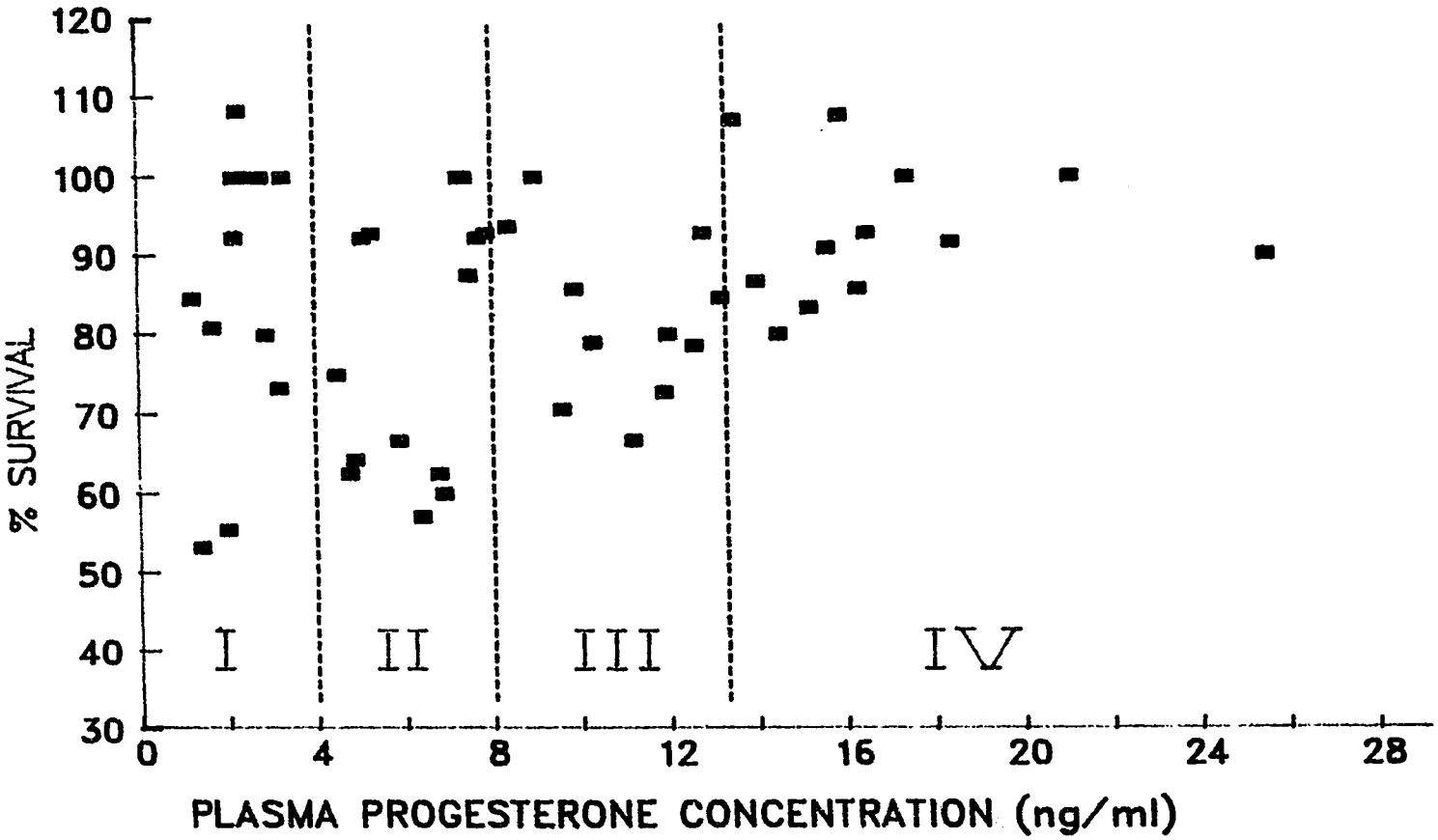
[1]Test diets are as follows:
LL - Low Energy, Low Protein LH - Low Energy, High Protein
HL - High Energy, Low Protein HH - High Energy, High Protein
[2]Expressed on a wet weight basis
[3]Volume of fluid of chorio-allantoic sac
[4]Standard error of the mean
[5]Absence of superscripts indicate no significant differences

Table II.5. Mean and standard error of percent embryo survival in gilts grouped on the basis of their initial plasma progesterone concentrations.

Grouping[1]	Gilts No.	Progesterone ($ng.ml^{-1}$ plasma)	% Embryo Survival Mean	Range	S.E.[2]
I	12	(1.3 - 3.3)	84.88	(53.3 - 108.3)	4.83
II	12	(4.5 - 7.5)	78.18	(57.1 - 100.0)	4.96
III	12	(7.7 - 12.8)	83.12	(86.7 - 100.0)	2.99
IV	12	(13.2 - 25.5)	93.00	(80.0 - 107.7)	2.59

[1]Plasma progesterone concentration
I - Low II - Moderately Low III - Moderately High IV - High
[2]Standard error

Figure II.1. Plot of percent embryo survival and plasma progesterone concentrations in samples taken at Day 3 of gestation in gilts. Gilts were grouped according to plasma progesterone concentration into the following categories. I - Low II - Moderately Low III - Moderately High IV - High


120
110
100
90
80
70
60
50
40
30
% SURVIVAL
I
II
III
IV
0
4
8
12
16
20
24
28
PLASMA PROGESTERONE CONCENTRATION (ng/ml)

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III. VARIABILITY IN PLASMA PROGESTERONE IN RELATION TO ESTRUS ONSET AND PEAK PLASMA LH DURING EARLY GESTATION IN THE GILT

A. INTRODUCTION

Plasma progesterone concentrations in the peripheral circulation during the peri-ovulatory period are low (<1 ng ml^{-1}) and then sharply increase two to three days after estrus onset (Guthrie et al., 1972). In sows bled on an hourly basis before and after estrus onset, progesterone concentrations remained below detectable levels (<0.1 ng ml^{-1}) until twenty-seven to thirty hours after the occurrence of peak LH (van de Wiel et al., 1981). These authors reported mean values for eight and four gilts each, with no indication of individual variation.

There are few reported studies examining plasma progesterone changes in the period following estrus onset. The previous study reported here (Chapter II), indicated substantial variation in plasma progesterone concentrations measured at Day 3 after onset of estrus, as progesterone concentrations in jugular vein plasma varied from 1.3 to 25.5 ng ml^{-1} at this time. Although this may partly reflect the uncertainty in the exact time of estrus onset or peak LH, considerable variation in progesterone secretion in the early luteal phase seems likely due to heterogeneity in corpora lutea development between gilts (Hunter and Wiesak, 1990).

In the same study, when progesterone data were used

retrospectively to designate animals as having either low, high or intermediate plasma progesterone concentrations, a trend for embryo survival to be greater and variability in survival to be smaller with higher initial progesterone was evident. Dyck et al. (1980) reported that as increased feed intake depressed plasma progesterone concentrations, embryo survival tended to be lower with decreased progesterone concentrations in the plasma. Significant correlations exist in sheep between embryo survival and the time after ovulation at which plasma progesterone concentrations begin to rise, and the subsequent rate of increase in plasma progesterone concentrations (Ashworth et al., 1989). Also, sows weaned on Day 10 of lactation exhibited greater embryonic mortality in the following pregnancy as compared to their counterparts weaned at five weeks (Varley and Cole, 1978). This was attributed to higher estradiol and progesterone concentrations in the immediate post-mating period in the sows weaned at 10 days of lactation (Varley et al., 1984). The rate of uterine development was suggested to be dependent on the development of the corpora lutea and their ability to produce progesterone (Dziuk, 1987). This variability in progesterone synthesis (or catabolism) may increase asynchrony between the embryo and uterus, thereby decreasing the viability of the embryos (Pope, 1988). The timing of the progesterone increase may therefore be crucial in determining the synchrony between the uterus and embryo and the likelihood of an embryo remaining viable.

The purpose of these studies was to assess individual variation in plasma progesterone concentrations in early gestation in relation to both time of estrus onset and maximum plasma concentration of LH (peak LH). The second study also examined the effect of feed intake during early pregnancy on the concentrations of plasma progesterone in the post-mating period.

B. MATERIALS AND METHODS

ANIMAL MANAGEMENT

Part 1: A group of eight gilts were fed a standard barley-wheat-soybean meal diet containing 16% crude protein and 12.5 MJ ME kg^{-1} ad libitum until puberty. At this point, the gilts were provided 2.2 kg of feed per day until the end of the study. Gilts were checked for estrus twice daily at twelve hour intervals with vasectomized boars. At detection of second heat (Day 0), the gilts were bred twice (at 12 hour intervals) by artificial insemination using fresh pooled semen obtained from the Alberta Swine Breeding Center, Leduc, Alberta.

Indwelling jugular cannulae were inserted surgically four days prior to expected date of second estrus. Anaesthesia was induced with an i.v. injection of a mixture of "Stresnil" (4 % solution of azaperon (4-fluro-4-2-pyridi-1-piperazinyl-butyrophenone) at a dose of 2.2 mg $(50 \text{ kg BW})^{-1}$ and "Hypnodil" (5% solution of metomidate; both Janssen Pharmaceutica) at a dosage of 3.3 ml $(50 \text{ kg BW})^{-1}$. A silastic cannula (i.d. 0.62 mm., o.d. 0.125 mm) was inserted into the external jugular

vein and externalized on the dorsal side of the neck. The patency of the cannula was maintained by daily flushing with heparinized (10 IU ml^{-1}) saline.

Blood sampling commenced three days prior to the expected onset of second estrus (Day -3). On predicted Days -3 and -2 prior to second estrus) samples were drawn at twelve hour intervals (0800 and 2000). On predicted Day 20 (Day -1), the frequency of blood sampling was increased to every six hours and continued up to 96 h from the time of estrus onset. Subsequent blood samples were taken at twenty-four hour intervals prior to feeding in the morning. At each sampling time, 5 ml of blood was collected into heparinized tubes, centrifuged and the plasma stored at -20°C until analyses.

Part 2: Samples taken from a group of eighteen gilts cannulated as part of a study to examine the influence of feed intake on hormonal profiles at estrus were used to examine the effects of feed intake on plasma progesterone concentrations after the LH surge. Blood samples were drawn at 4 hour intervals before and after estrus. The management, feeding and cannulation of these gilts was described previously (Beltranena et al., 1991b). Gilts were fed either 2.0 or 2.8 kg day^{-1} at the start of the study and continued at that level for the duration of the study. Progesterone concentrations were determined in samples taken from 36 h before until 96 h after the peak concentration of LH.

HORMONAL ANALYSIS

Plasma samples were analysed for progesterone by a double antibody radioimmunoassay (RIA) as described by Beltranena et al. (1991b), using an anti-serum raised in rabbit against 4-pregnen-11α-ol-3,20-dione hemisuccinate:BSA (Sigma Q3253) and obtained from The Endocrine Service Laboratory, University of Saskatchewan and goat anti-rabbit gamma-globulin as second antibody. Reported cross-reactions of this anti-serum were: corticosterone 2.7%, pregnenolone 2%, 20α-progesterone 0.18%, estrone 0.12%, testosterone 0.06%, dihydrotestosterone 0.03%, cortisol 0.015%, cholesterol 0.003% and estradiol-17β 0.002% (Rawlings, personal communication).

Duplicate 100 ul aliquots of diluted (with PBS gel buffer) or undiluted sample were extracted with 4 ml of petroleum ether for 5 min. Within an assay, extraction efficiency as determined by recovery of [1,2-^{3}H(N)]-progesterone was used to correct the progesterone concentration determined for each sample. The mean recovery of tritiated progesterone was 83.1±5.2 %. Standard curves ranged from 0.313 to 6.4 ng tube^{-1}. Sensitivity of the assay, defined as 85% of total binding, was 0.02 ng tube^{-1}. No significant deviation from parallelism was evident from assaying 100, 50, 25 and 10 ul of a standard plasma pool. Samples were diluted according to the day of gestation to keep samples on the linear portion of the standard curve. The intra- and inter-assay coefficients of variation for the assays were 5.2 and 17.7%, respectively.

Samples taken from 24 h prior to estrus onset to 48 h after were analysed for LH by a heterologous double antibody RIA as described by Cosgrove et al. (1991) to determine the time of peak LH concentration. Triplicate 100 ul aliquot samples were assayed. The sensitivity of the assay was 0.3 ng.tube^{-1}. The recovery of purified standard added to a control pooled plasma was 103.4 ($\pm$7.8%). A plasma dose response curve revealed no significant deviation from parallelism. The intra- and inter-assay coefficients of variation were 6.7 and 12.1 %, respectively. All samples from each gilt were analysed within an assay.

STATISTICAL ANALYSIS

In Part 1, plasma progesterone concentrations were analysed with respect to the time of estrus onset and the time at which the LH peak occurred. The time of the LH peak was defined as the time at which the greatest concentration was first seen. In Part 2, progesterone concentrations in plasma were related to the time of peak LH only. In individual animals, peri-ovulatory plasma progesterone concentrations, the time at which progesterone concentrations increased in relation to time of LH peak and estrus onset, and the rate of increase in plasma progesterone concentrations were determined. The time at which progesterone began to rise and the subsequent rate of increase was determined by using a two phase regression of plasma progesterone concentrations (BMDP, 1977) described by

the following equation.

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

where

$$b_a = b_1 - b_2$$

$$b_b = b_1 - b_2$$

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.

In Part 2, peri-ovulatory concentrations of progesterone and the time and rate of progesterone increase between treatment groups were compared by t-test. Data from all three groups were pooled and analysed by SAS (SAS Institute) using the general linear model procedure to determine the extent of correlations between peri-ovulatory concentrations and both the time of and rate of increase in progesterone concentrations.

C. RESULTS

The peri-ovulatory progesterone concentrations, time of progesterone increase relative to estrus onset and peak LH, and the rate of increase for gilts in Part 1 is given in Table III.1. A typical plasma progesterone profile for a gilt prior to and after detection of estrus is provided in Figure III.1.

The pre-ovulatory LH peak occurred on average 10.5 hours after first detection of estrus although this timing ranged from 6 hours prior to first detection to 18 hours after. On average, the rise in plasma progesterone began 50 hours after estrus onset which therefore corresponded to 40 hours after the occurrence of the LH peak. Times ranged from 29 to 58 hours after first detection of estrus. Although the shortest interval was observed in the gilt in which the LH peak had occurred before estrus onset, the time from LH peak to progesterone rise also varied from 31 to 52 hours.

Data for the gilts in Part 2 are given in Table III.2. The mean times from LH peak to beginning of the progesterone rise (30.8 and 37.6 hours for gilts provided 2.0 and 2.8 kg feed day^{-1}, respectively) were significantly ($p<0.05$) different between the two levels of feed intake. Neither peri-ovulatory concentrations (0.4009 vs 0.4017 ng ml^{-1}) nor the rate of increase (0.1868 vs 0.1817 ng.ml-1 h^{-1}) in plasma progesterone concentrations were significantly affected by the increase in feed intake. Overall, peri-ovulatory progesterone concentrations in these gilts were lower and the rate of

increase greater than those in the first study.

With correlation analysis of the pooled data, a significant positive ($r=0.6845$, $p<0.001$) correlation between the rate of increase in progesterone concentration and the timing of the increase was seen. There were no other significant correlations between the three variables considered.

D. DISCUSSION

In the present study, plasma concentrations of progesterone remained less than 1 ng ml^{-1} until 50 hours after the first detection of estrus. Although there may have been some error in determining when heat occurred as gilts were checked only at 12 hour intervals, the interval from estrus onset to increase in plasma progesterone is in agreement with that reported by Guthrie et al. (1972), who found that plasma progesterone concentrations remained low until 2 to 3 days after estrus onset. Similarly, the timing between the LH peak and plasma progesterone increase in the present study is in agreement with that reported in previous literature. Van de Wiel et al. (1981) reported that progesterone concentrations were below the limit of their assay sensitivity until 27 to 30 hours after the LH peak.

The variability in plasma progesterone profiles between gilts may be related to luteal heterogeneity (gross morphology, steroid content) within and between pigs. Heterogeneity in corpora lutea has been reported in the cow (Estergeen et al., 1968), sheep (Hunter and Southee, 1983) and

pig (Ottobre et al., 1984). The weight (Hunter and Wiesak, 1990) and progesterone content (personal communication, T. Wiesak) of individual pig corpora lutea on Day 12 of the estrous cycle and during early pregnancy can vary by at least two-fold within an animal.

Luteal heterogeneity may have its origins in the asynchrony existing in the pool of developing follicles which is perpetuated into the luteal phase (Hunter and Wiesak, 1990). With follicles collected from gilts at Day 20 of the estrous cycle, an increase in follicle size was associated with increased steroid, and in particular, progesterone concentrations (Grant et al., 1989); Progesterone concentrations in developing follicles varied from 74 ng ml^{-1} in smaller follicles (2.0 to 3.9 mm) to 501 ng ml^{-1} in larger follicles (>8.0 mm), demonstrating the considerable heterogeneity which exists between follicles within gilts. The morphological and biochemical changes associated with the LH surge do not necessarily occur simultaneously within and/or between follicles on the same ovary (Hunter et al., 1989). The effect of this asynchrony of follicular development on the distribution of embryo development is well documented (Pope et al., 1990). By extension, variation in luteal development and subsequent progesterone production by the follicles and developing corpora lutea may be reflected by differences in peri-ovulatory progesterone concentrations and the time needed for progesterone concentrations to increase in the peripheral

circulation.

In addition to considerable individual animal variation, changes in feed intake resulted in changes in the time at which progesterone concentrations began to rise in the plasma. Though peri-ovulatory concentrations were unaffected, there was a delay of seven hours to the time at which plasma progesterone began to rise when feed intake was greater. This agrees with observations that plasma progesterone was depressed from 16.7 to 11.8 ng ml^{-1} in gilts during early pregnancy when feed intake was increased from 1.5 to 3.0 kg day^{-1} (Dyck and Strain, 1983). Recent evidence has shown an increase in hepatic blood flow and progesterone turnover in response to increases in food intake in the gilt (Symonds and Prime, 1989). Although not determined in the present study, changes in progesterone concentrations may influence embryo survival in early gestation, as correlations have been determined between peri-ovulatory progesterone concentrations and embryo survival in cattle (Lee et al., 1985) and sheep (Ashworth et al., 1989).

Table III.1. Peri-ovulatory plasma progesterone concentrations and time and rate of increase in peripheral plasma progesterone in individual gilts in relation to onset of estrus and peak LH.

Gilt No.	LH peak post-estr. (h)	Time of progesterone rise post-estrus (h)	post LH peak (h)	Peri-ovul. conc'n (ng ml^{-1})	Post-rise rate[1]
101	18	57.85	39.85	0.5749	0.3279
104	6	58.86	52.86	0.3478	0.4955
250	6	51.30	45.30	0.7533	0.5396
253	12	51.41	39.41	0.7437	0.4806
281	-6	29.66	35.66	1.0242	0.3190
284	12	43.46	31.46	1.1036	0.4156
402	18	56.98	38.98	0.3630	0.1749
415	12	52.04	40.04	0.3453	0.2592
Mean		50.19	40.45	0.6570	0.3765
SEM		4.51	2.13	0.0681	0.0293

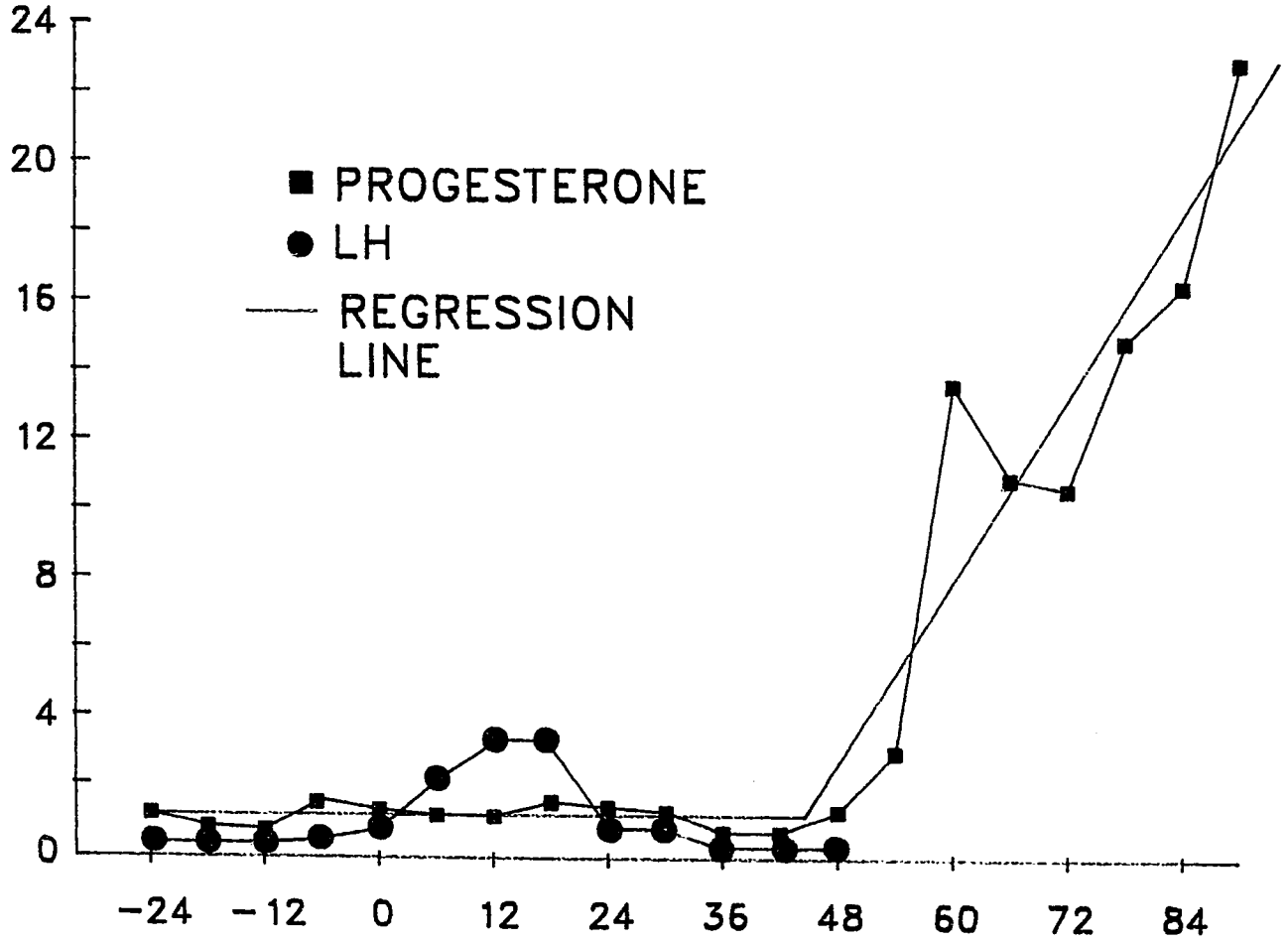
[1] Rate of increase in plasma progesterone concentration (ng ml^{-1} h^{-1})
[2] Standard error of the mean

Table III.2. Peri-ovulatory concentrations and time and rate of increase in peripheral plasma progesterone in individual gilts in response to one of two levels of feed intake (2.0 vs 2.8 kg per day)[1].

Gilt No.	Feed Intake	Time progesterone rise post LH peak (h)	Rate of increase[2]	Peri-ovulatory concentration (ng ml^{-1})
1	2.0	21.05	0.1912	0.5069
3	2.0	33.67	0.2702	0.4694
24	2.0	27.05	0.0859	0.4395
51	2.0	34.05	0.2344	0.1919
62	2.0	24.11	0.1503	0.2489
64	2.0	34.40	0.1719	0.4347
73	2.0	35.73	0.2091	0.3447
80	2.0	32.63	0.1783	0.4662
90	2.0	37.93	0.1948	0.4678
91	2.0	27.50	0.1818	0.4393
Mean		30.81[a]	0.1868	0.4009
SEM		1.85	0.0386	0.0354
4	2.8	46.89	----	0.6527
23	2.8	42.90	0.1789	0.2996
32	2.8	36.00	----	0.5068
43	2.8	44.00	----	0.3619
44	2.8	35.09	0.1674	0.2932
50	2.8	28.73	0.2195	0.4220
54	2.8	33.32	0.1585	0.3158
61	2.8	34.09	0.1842	0.4588
Mean		37.60[b]	0.1817	0.4017
SEM[3]		2.07	0.0431	0.0501

[1] Means within a column with different superscripts are significantly different at $p<0.05$).
[2] Rate of increase in plasma progesterone concentration (ng ml^{-1} h^{-1})
[3] Standard error of the mean

Figure III.1. Plasma progesterone profiles during early pregnancy from a gilt sampled at six hour intervals beginning 24 hours before until 90 hours after estrus detection, and fitted with two regression lines.


24
20
16
12
8
4
0
PROGESTERONE
LH
REGRESSION LINE
-24
-12
0
12
24
36
48
60
72
84

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IV. TEMPORAL RELATIONSHIP BETWEEN PLASMA PROGESTERONE CONCENTRATIONS IN THE UTERO-OVARIAN AND JUGULAR VEINS DURING EARLY PREGNANCY IN THE PIG

A. INTRODUCTION

The previous studies (Chapter II and III) suggested that between individual gilts, there are considerable differences in the time and rate at which plasma progesterone concentrations increase in early pregnancy. Progesterone concentrations in the peri-ovulatory period have been linked with embryo survival (Ashworth et al., 1989), while progesterone concentrations in the luteal phase have not been closely linked (De Sa et al., 1981). However, these studies reflect plasma progesterone concentrations in the peripheral circulation and do not necessarily reflect the concentrations the reproductive tract, and hence the embryos, are exposed to.

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., 1982a), either directly by penetration of steroids through the adjoining vessel walls or indirectly through interstitial tissue and the network of fine veins enmeshing the branches of the ovarian artery. The tissues in the ovarian pedicle retain a significant fraction of the steroids produced by the ovary (75 to 80 %). This represents a significant pool from which steroids may be subsequently released (Krzymowski et al., 1982b) into the arterial supply of the ovary and the other tissues supplied by

branches of the ovarian artery (i.e. oviduct and adjacent portions of the uterine horn) (Krzymowski et al., 1982c).

It is feasible that progesterone taken up from the ovarian vein may therefore exert local effects on the luminal environment of the oviduct and uterine horn. Indeed, in the limited study of Hunter et al. (1983), progesterone concentrations in arterioles supplying the oviduct were greater than in the systemic circulation during the pre-ovulatory period in gilts. In a recently published study, plasma progesterone concentrations in sheep were greater in the cranial uterine vein draining the uterine horn adjacent to the ovary bearing the corpus luteum (CL) than in the jugular vein and in the caudal uterine vein draining the same horn (Weems et al., 1989). In the horn opposite to the CL-bearing ovary, the level of plasma progesterone was similar in the jugular vein and the caudal and cranial uterine veins.

A possible influence of local progesterone concentrations on embryo development and survival has been demonstrated with unilaterally ovariectomized rats in which embryonic development was retarded in the horn ipsilateral to the ovariectomy (Nuti et al., 1971). Similarly, transfer of bovine embryos to the uterine horn ipsilateral to the ovary bearing the corpus luteum resulted in a greater number of pregnancies than transfer to the contralateral horn (Newcomb et al., 1980 ; Del Campo et al., 1979).

The purpose of this study was to provide in vivo evidence

for the presence of local veno-arterial progesterone transfer between the ovary, oviduct and the uterus in swine. Time related changes in progesterone concentrations in the utero-ovarian vasculature and systemic circulation in the estrous cycle and in early pregnancy were also examined.

B. MATERIALS AND METHODS

ANIMAL MANAGEMENT

Thirty Camborough*Canabrid gilts (PIC Canada, Ltd) weighing 110 kg and having completed one estrous cycle, were randomly assigned in groups of five to be sampled at either Day 1, 2, 4, 12 or 16 of gestation, or at Day 12 of the estrous cycle. These times were chosen to reflect periods when systemic plasma progesterone levels are low (Day 1, 2), beginning to rise (Day 4) and at peak luteal phase levels (Day 12 and 16). Gilts were 2.25 kg of feed daily. Gilts were checked for estrus twice daily with vasectomized boars. At detection of second estrus (Day 0), gilts to be sampled in pregnancy were bred twice by artificial insemination using fresh pooled semen obtained from the Alberta Swine Breeding Center, Leduc, Alberta.

Prior to surgery, gilts were fasted overnight. In preparation for sampling, anaesthesia was induced with a combined i.v. injection of "Stresnil" (4 % solution of azaperon (4-fluro-4-2-pyridi-1-piperazinyl-butyrophenone) at a dose of 2.2 mg (50 kg BW)$^{-1}$ and "Hypnodil" (5% solution of metomidate; both Janssen Pharmaceutica) at a dosage of 3.3 ml

(50 kg BW)$^{-1}$ and maintained with "Fluothane" (Halothane, Ayerst Laboratories). The ventral area posterior to the navel to the last pair of posterior teats was shaved and disinfected. The uterus was externalized by mid-ventral laparotomy and each uterine horn was identified as either the left or right.

Blood samples were taken from veins arising from sites in the uterine horns, oviducts and ovaries (Figure IV.1) of each gilt. Beginning at the caudal end (near the body of the uterus) of the left uterine horn, blood samples were drawn from the veins draining the caudal (near the uterine body) and mid portions of the uterine horn and from a venous branch draining the cranial portion adjacent to the oviduct. Samples were also taken from a vein arising in the region of the oviduct and an arterial/venous mixture of blood was obtained from the ovarian pedicle. Before taking samples from comparable locations in the right uterine horn, a peripheral blood sample was taken by jugular venipuncture. Approximately 2 to 5 ml of blood were collected from each sampling site with a 21 gauge butterfly catheter ("Surflo" Winged Infusion Set, Terumo Corporation). The blood was placed into heparinized tubes, centrifuged at 3000 rpm and the plasma stored at -20 °C until assayed.

At the conclusion of sampling, pregnancy was confirmed as follows; on Day 2, the oviducts were flushed with physiological saline to confirm the presence of fertilized eggs and/or zygotes. On Day 4, the cranial 10 cm portions of

each uterine horn and on Day 12 and 16, the entire length of the uterine horns, were flushed with physiological saline to confirm the presence of blastocysts. The occurrence of ovulation was confirmed in gilts on Day 1 by the presence of corpora hemorrhagica.

HORMONAL ANALYSIS

Plasma samples were assayed for progesterone by a double antibody radioimmunoassay (RIA) as described by Beltranena et al. (1991b) using an anti-serum raised in rabbit against 4-pregnen-11α-ol-3,20-dione hemisuccinate:Bovine Serum Albumin (Sigma Q3253) and obtained from The Endocrine Service Laboratory, University of Saskatchewan and goat anti-rabbit gamma-globulin as second antibody. Cross-reactions reported for this anti-serum were: corticosterone 2.7%, pregnenolone 2%, 20α-progesterone 0.18%, estrone 0.12%, testosterone 0.06%, dihydrotestosterone 0.03%, cortisol 0.015%, cholesterol 0.003% and estradiol-17β 0.002% (N.C. Rawlings, personal communication, 1987).

For each tube, 100 ul of diluted (with PBS gel buffer) or undiluted sample was extracted with 4 ml of petroleum ether for 5 min. Within an assay, extraction efficiency as determined by recovery of [1,2-^{3}H(N)]-progesterone was used to correct the progesterone concentration determined for each sample. The mean recovery of tritiated progesterone was 81.7 +/- 5.0 %. Standard curves ranged from 0.00313 to 6.4 ng tube^{-1}. Sensitivity of the assay, defined as 85% of total

binding, was 0.0195 ng tube^{-1}. No significant deviation from parallelism was evident from assaying 100, 50, 25 and 10 ul of a standard plasma pool obtained from cyclic gilts. Samples taken from the jugular and uterine horns were diluted from 10 to 50 times depending on the day of gestation. Plasma obtained from oviductal veins were diluted 10, 100 and 200 times. Samples from the ovarian vein were diluted 100, 500 and 1000 times. Potencies were determined from estimates on the linear portion of the curve. The intra- and inter-assay coefficients of variation for the assays were 7.2 and 18.4%, respectively. All samples from a gilt were analysed within an assay.

STATISTICAL ANALYSIS

As the variation in plasma progesterone concentration was not homogeneous between sampling sites as determined by the Bartlett-Box test for homogeneity of variance (Snedecor and Cochrane, 1980), log transformed data was subjected to analysis of variance. The values for comparable sites on the left and right horns were pooled for each gilt after confirming that left and right horns were not significantly different. For all pregnant gilts, an analysis of variance was performed on these means, testing for significant effects of day of gestation, sampling site and interaction between site and day of gestation. Means for plasma progesterone concentrations within each day of gestation were compared between sampling sites using the Student-Neuman-Keul's multiple range comparison test (Snedecor and Cochrane, 1980).

Using the log transformed data, the effect of pregnancy on progesterone concentrations was tested separately with the data from gilts sampled at either Day 12 of pregnancy or Day 12 of the estrous cycle. Analysis of variance was performed to separate the effects of pregnancy and sampling site.

Progesterone concentrations in the ovarian pedicle and oviductal vein on the left and right side were regressed to the number of corpora lutea on each respective ovary and to the day of gestation. A regression of progesterone concentrations in the plasma of the oviductal veins to that in the ovarian pedicle was also performed.

C. RESULTS

The progesterone concentrations measured were significantly affected by the day of gestation and the site of sampling (Fig. IV.2). However, there was no significant interaction between these two variables. As gestation progressed, plasma progesterone concentrations in the jugular vein increased from 2.5$\pm$.1 ng ml^{-1} at Day 1 to a peak (49.2$\pm$6.0 ng ml^{-1}) on Day 12 of gestation, with a small decline in concentration to 39.8$\pm$6.0 ng ml^{-1} at Day 16 of gestation. Within each day of gestation, plasma progesterone concentrations in the veins draining the cervical, mid- and cranial portions of the uterine horn did not differ significantly from those found in the jugular vein. Plasma progesterone concentrations in the uterine venous system rose with increasing day of gestation in parallel with concentrations in the jugular vein.

Plasma progesterone concentrations in the oviductal veins were several fold greater than in the jugular vein or in the uterine veins. At 1 day after estrus onset, the concentration of plasma progesterone in the oviductal veins (48.5±20.8 ng ml^{-1}) was significantly ($P<0.05$) higher than in the jugular vein or in the uterine venous system. At Day 2 and 4 of gestation, progesterone concentrations in the oviductal veins had tripled from 48.5 ng ml^{-1} on Day 1 to 132.8±48.0 and 138.9±60.2 ng ml^{-1}, respectively. There was a further increase in oviductal plasma concentrations of progesterone to Day 12 of gestation, with no subsequent increase to Day 16 of gestation.

Progesterone concentrations in the samples taken from the ovarian pedicle were significantly greater ($p<0.05$) than those in the jugular and uterine veins as well as the oviductal veins. As gestation progressed, the progesterone concentrations increased dramatically from 100±59.4 ng ml^{-1} on Day 1 to 1500±261.4 ng ml^{-1} at Day 12 and 16 reflecting the increasing production of progesterone by the maturing corpora lutea. The variation in plasma concentrations in samples taken was considerable. This may reflect the occasions when blood was drawn by puncturing the ovarian pedicle rather than a clearly identified ovarian vein.

Progesterone concentrations in Day 12 pregnant gilts were compared with concentrations in samples taken from a companion group of five gilts not bred at second estrus and sampled at

Day 12 of the estrous cycle. Analysis of variance revealed a significant effect of pregnancy ($p<0.05$) and sampling site ($p<0.001$) on plasma progesterone concentration but no significant interaction.

There were no ovulations or developing follicles on the left ovary of one gilt assigned for surgery on Day 2 of gestation. The absence of corpora lutea on the left ovary had a significant ($p<0.05$) effect on plasma progesterone concentrations in the veins draining the ipsilateral uterine horn and oviduct (Figure 3.3) compared to the contralateral side. The plasma progesterone concentration in the jugular vein was 8.8 ng ml^{-1} plasma. Progesterone concentrations in the veins draining the left and right uterine horns were similar to that seen in the jugular vein. With corpora lutea present on the right ovary, concentrations of progesterone in the right oviductal and ovarian veins were 102.4 and 369.6 ng ml^{-1} plasma, respectively, which were several-fold greater than jugular concentrations. On the left side, where no corpora lutea were present, progesterone concentrations in the oviduct and ovarian veins (8.3 and 6.1 ng ml^{-1}) were not significantly different from that seen in the peripheral circulation.

Twenty-four hours after estrus onset, one of the gilts had not ovulated but possessed well developed Graafian follicles while the remaining gilts had recently ovulated. The embryos collected from the oviduct of gilts at day 2 of gestation were either at the one or two-cell stage while embryos collected

from gilts at day four varied in stage of development from the four- to sixteen-cell stage. At Day 12 gestation, both spherical and filamentous blastocysts were collected from uterine flushings while all blastocysts obtained from gilts at Day 16 were in the elongated form.

Plasma progesterone concentrations in samples drawn from the ovarian pedicle were found to be significantly ($r=0.51$ $p<0.05$) correlated with the number of corpora lutea present on each ovary and day of gestation. No significant interaction was observed between number of corpora lutea and day of gestation. No significant curvilinear relationship existed between plasma progesterone concentrations and the number of corpora lutea present on the ovary indicating the relationship between ovarian vein concentrations and number of corpora lutea was strictly linear. Plasma progesterone concentrations in the oviductal vein were significantly correlated ($r= 0.15$, $p<0.05$) with the number of corpora lutea on the ipsilateral ovary, though oddly enough not with plasma progesterone concentrations in plasma taken from the ovarian pedicle.

D. DISCUSSION

Evidence obtained from the present study suggest the presence of a local transfer of progesterone from ovarian vein to branches of the ovarian artery. The resulting elevation in plasma progesterone concentrations was primarily restricted to the vasculature of the oviduct. However, our in vivo work suggests that these counter-current mechanisms do not result

in an increased plasma progesterone concentration in the uterine vasculature. Consistent with the report of Hunter et al. (1983) that progesterone, estradiol and androstenedione plasma concentrations were greater in arterioles supplying the distal portion of oviduct than in jugular vein samples during the pre-ovulatory period, we observed a significant elevation in plasma progesterone in the oviductal vein within 24 h of estrus. Our data extend earlier observations in showing that the high concentrations of progesterone in oviductal veins persists throughout early pregnancy.

The data obtained from the gilt with corpora lutea on the right ovary only, provided further confirmation of an ipsilateral counter-current mechanism operating between the ovary and oviduct. The similarity between progesterone concentrations in the left oviductal vein and peripheral circulation provided evidence that the elevation in the oviductal veins was not due to a sequestration of progesterone in the region from passing arterial blood or due to the presence of corpora lutea on the contralateral ovary. In contrast, several Day 1 gilts which had not ovulated but possessed pre-ovulatory follicles exhibited greater progesterone concentrations in the ovarian pedicle and oviductal veins than in peripheral plasma. After the LH surge, post-LH follicles contain high concentrations of progesterone (Grant et al., 1989) which can enter the ovarian blood. The absence of either follicles and/or corpora lutea as a source

of progesterone on the left ovary suggested that increases were due to the local counter-current transfer between the ovary and oviduct.

The positive correlation between oviductal plasma progesterone concentrations and the number of corpora lutea is consistent with the observation that plasma progesterone in the ovarian venous plasma was linearly related to weight of ovarian tissue and progesterone content of the corpora lutea (Masuda et al., 1967) and can also be taken as confirmation of the counter-current transfer of progesterone. Transfer of certain steroids such as testosterone is thought to be partly concentration dependent (Kotwica et al., 1981). However, the degree of progesterone transfer from ovarian vein to artery suggests that this phenomenon is not concentration dependent (Krzymowski et al., 1982b) and this would be consistent with the lack of a relationship between progesterone concentrations in the oviductal veins and the ovarian pedicle.

Our observation that progesterone concentrations in peripheral plasma were not closely correlated with the number of corpora lutea is also in agreement with previous studies. In pregnant gilts, there was little change in plasma progesterone concentrations following either unilateral ovariectomy (Thomford et al., 1984) or stepwise reduction in the number of corpora lutea (Martin et al., 1977). In non-invasive studies, there was no observed correlation between number of corpora lutea and plasma progesterone concentrations

when more than five corpora lutea were present (Guthrie et al., 1972 ; Webel et al., 1975). Confirmation of these different relationships, between luteal numbers and progesterone concentrations in different locations in the vascular system, in samples taken from the same animal, raises questions about the physiological significance of these differences. High ovulation rates may not necessarily affect mechanisms which are dependent on peripheral progesterone concentrations whereas they may influence physiological responses in adjacent areas of the reproductive tract.

The absence of local effects of the steroid counter-current transfer on progesterone levels in the venous effluent of the uterine horns is in contrast to that seen in sheep (Weems et al., 1989). In sheep, plasma progesterone concentrations in veins draining the area of the uterine horn adjacent to the oviduct were greater in the horn ipsilateral to the CL bearing ovary than in the jugular vein or veins draining the contralateral horn. In contrast, progesterone concentration in veins draining the area adjacent to the oviduct was not different from that in the jugular vein or that in the veins draining the caudal and middle regions of the uterine horns. This suggests that the local counter-current transfer of progesterone does not influence the development of the uterine horn in preparation for implantation in pigs, as is seen in sheep and cattle (Newcomb et al., 1980). Therefore, the counter-current transfer of progesterone to the oviductal

vasculature may primarily affect the embryo while it passes through the oviduct.

An alternative mechanism may be a peri-uterine counter-current transfer of steroid from uterine vein to uterine artery. In the mesometrium, branches of the uterine vein run parallel with branches of the uterine artery as well as with lymphatic vessels (Krzymowski et al., 1986a). The uterine artery is also covered by a venous mesh. Infusion of radiolabelled $PGF_{2\alpha}$ into the uterine myometrium and/or lumen led to the presence of the same label in uterine arterial blood (Koziorowski et al., 1988 ; Krzymowski et al., 1986b ; 1987) demonstrating the presence of a counter-current transfer of prostaglandin which may be involved in preventing luteal regression. Steroids, particularly progesterone, may be handled in a similar manner as prostaglandin, preventing high levels of progesterone entering the uterine venous system. Between species, the efficiency of steroid transfer from vein to artery may vary. This may explain why the increase in uterine venous progesterone concentration was seen in sheep but not in the present study with pigs. Areas of the uterine horn adjacent to the oviduct may be exposed to high arterial progesterone concentrations but the greater efficiency of transfer may prevent that from being reflected in venous blood.

In the immediate peri-ovulatory period, plasma concentrations of progesterone remain low (< 1 ng ml^{-1}) in the

peripheral circulation. The counter-current mechanism allows for local elevations of progesterone to moderate embryo transport through the oviduct while peripheral levels remain low. Progesterone concentrations were found to be higher in the fluid flushed from the oviducts of rabbits (Richardson and Oliphant, 1981) and monkeys (Wu et al., 1977) than in the plasma.

The elevation of local plasma concentrations of progesterone may change the excitability and contractile activity of the oviduct, altering sperm transport at the time at which ovulation occurs, as described by Hunter et al. (1983). The continuing elevation of progesterone into the peri-ovulatory period would similarly affect egg and zygote transport through the oviduct. Progesterone dominance is required during oviductal transport of embryos and during their early development (Flint et al., 1982). Gonadal steroids affect the excitability and conduction of the oviduct and mucosal morphology (Hunter, 1977a). The smooth musculature of the oviduct is highly innervated and α-adrenergic activity is enhanced by estrogens. Progesterone enhances α-adrenergic activity and increases isthmic relaxation and facilitates egg transport. Generally, estradiol retards transport of the fertilized egg whereas progesterone enhances its transport. Subcutaneous injections of progesterone before ovulation causes the eggs to appear in the uterus 8 hours after ovulation rather than the normal 48 hours (Day and Polge,

1968). Injection of estradiol results in "tube-locking" preventing descent of the fertilized ova. Treatment of mice with an anti-progesterone monoclonal antibody caused embryos to appear prematurely in the uteri of treated mice with subsequent loss of the embryos (Feinstein, 1983 ; Rider et al., 1987).

There may be an obligatory amount of time needed by the embryo to stay in the oviduct for optimal maturation. Embryos cultured from the 1- to 2-cell stage often fail to develop beyond the four cell stage with in vitro culture (Polge, 1982). Four-cell embryos recovered from the oviduct and grown in vitro develop at rates similar to that found in vivo (Davis, 1985). The requirement to co-culture zygotes with oviductal epithelial cells to obtain normal development suggests that secretions from the oviduct epithelium are necessary for transformation beyond the four-cell stage in pigs (White et al., 1989), in sheep (Gandolfi and Moor, 1987) and in cattle (Ellington et al., 1990). A new pattern of protein synthesis occurs in the lag phase at the four-cell stage which may be triggered by oviductal secretions. This suggested a role for secretions from oviductal cells in overcoming this restrictive phase in embryo growth. A role for progesterone in directing the synthesis of these oviductal secretions is very likely as the synthesis and storage of these glycoproteins by the oviductal epithelial cells in secretory granules is controlled by estrogen (Oliphant et al.,

1984) and released under the influence of progesterone (Leese, 1988). Changes in progesterone concentrations in the oviduct may therefore alter secretions from oviduct epithelial cells with the potential to alter embryo development and survival.

In summary, the present study points to the existence of a counter-current mechanism of steroid transfer as proposed by Krzymowski et al. (1982a) and a resulting elevation of plasma progesterone concentrations reaching the oviduct but not to adjacent areas of the uterine horn. The effects of the elevated plasma progesterone would be limited to the period in which the embryo resides in the oviduct and may have a two-fold effect. The movement of the embryo through the oviduct to the uterus may be influenced by the higher progesterone concentrations and secondly, the secretions of the oviductal epithelial cells which modulate embryo development may be modified.

Figure IV.1. Diagrammatic representation of the reproductive tract of the pig and the sites sampled. The sites sampled were: UCA- Uterine vein - caudal area, UM - Uterine vein - mid-area,
UCR- Uterine vein - cranial area, OD - Oviductal vein, OV - Ovarian pedicle.

OD
UCR
OV
UM
UCA

Figure IV.2. Plasma progesterone concentrations (ng ml^{-1}) in the venous system of the reproductive tract and in the jugular vein of gilts at Days 1, 2, 4, 12 and 16 of gestation and at day 12 of the estrous cycle. There were 5 gilts in each day group. The pooled standard error of the mean for the uterine veins, oviduct vein and ovarian pedicle were 5, 122 and 274 respectively.

* indicates plasma concentration significantly ($P<0.05$) different from jugular vein concentrations within days

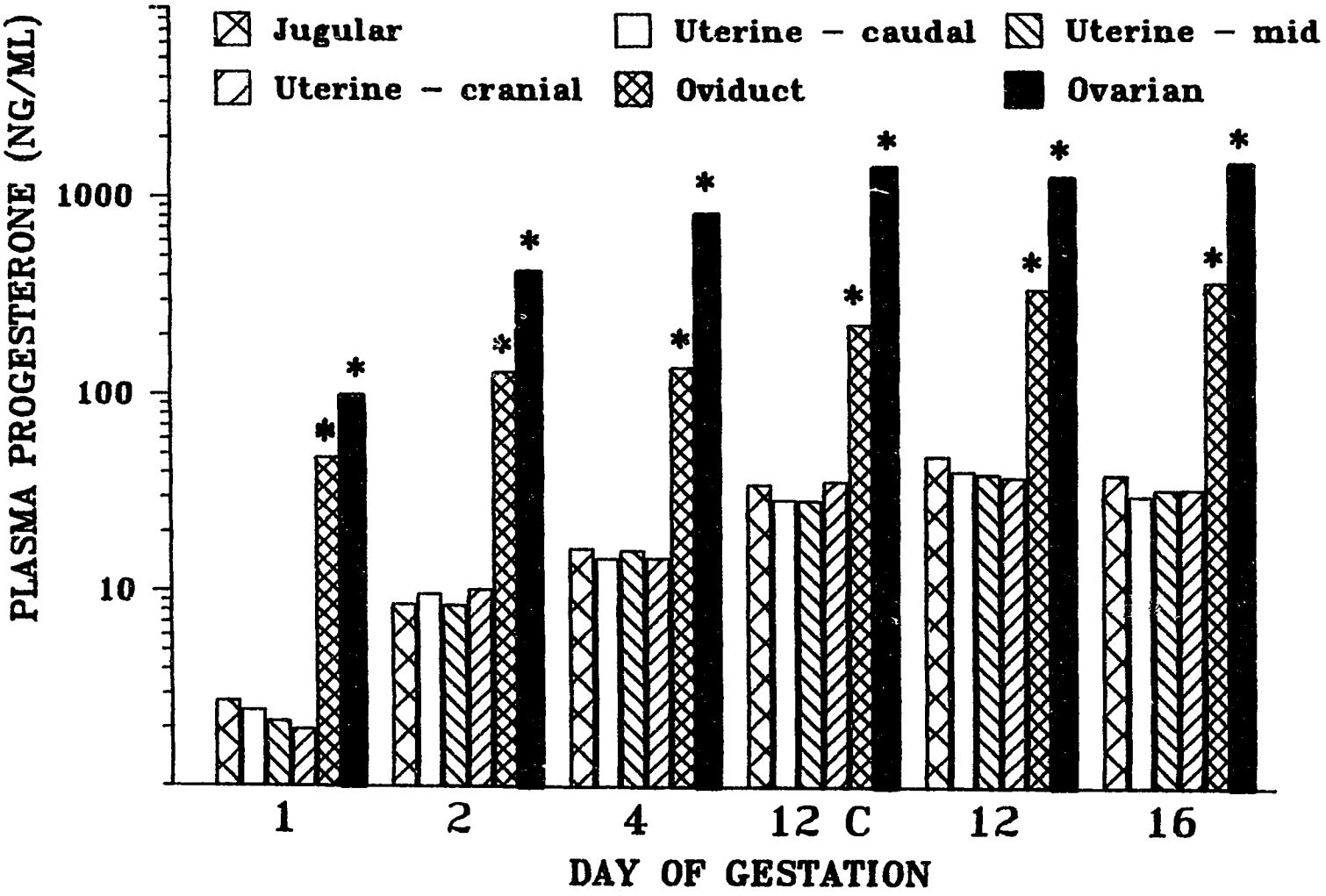
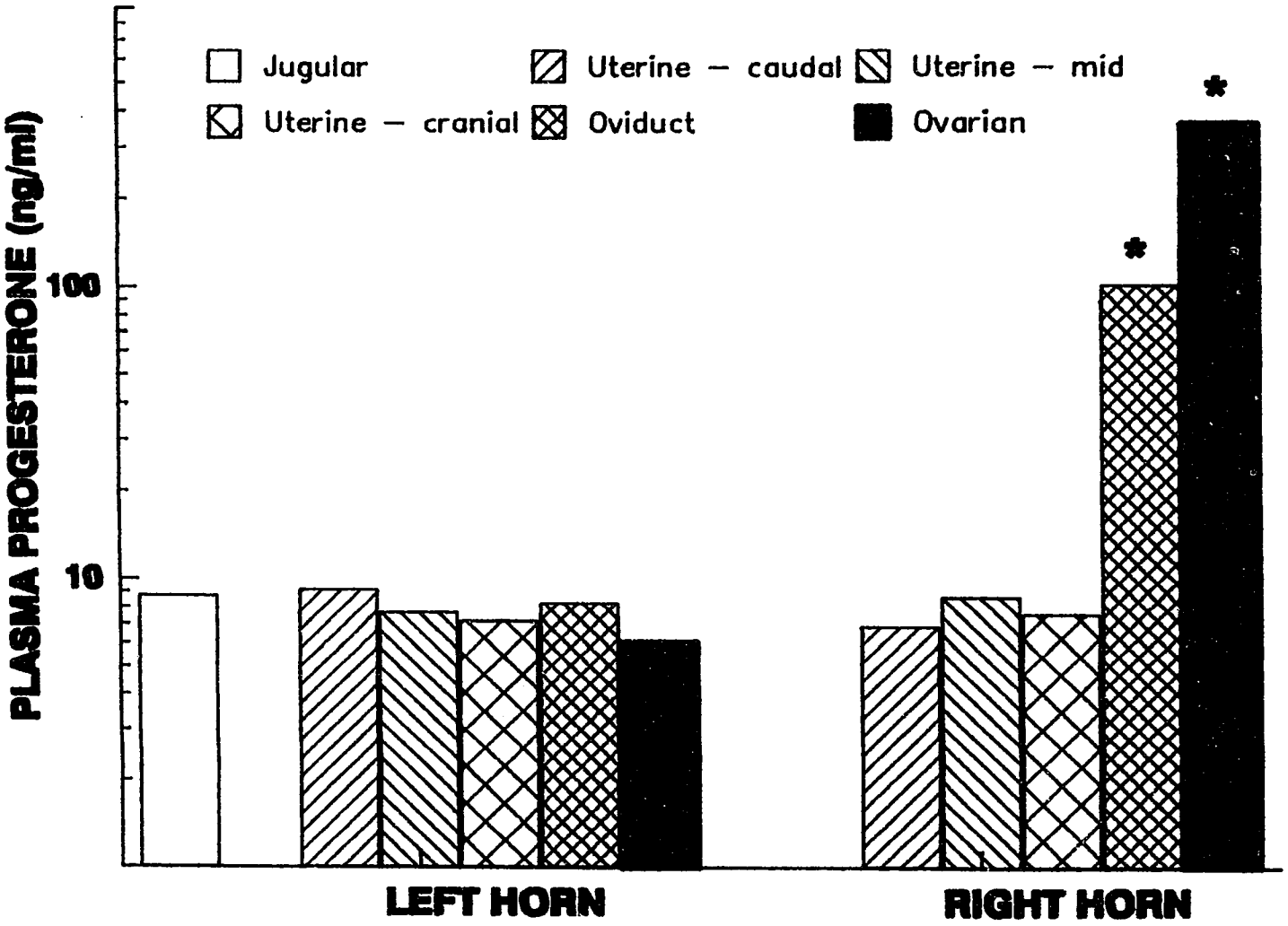

Jugular
Uterine – caudal
Uterine – mid
Uterine – cranial
Oviduct
Ovarian
PLASMA PROGESTERONE (NG/ML)
1000
100
10
1
2
4
12 C
12
16
DAY OF GESTATION

Figure IV.3. Plasma progesterone concentrations (ng ml^{-1}) in the veins of the reproductive tract of a gilt at day 2 of gestation with no corpora lutea on the left ovary. The right ovary had 11 corpora lutea.
* indicates significant ($P<0.05$) difference from jugular vein concentrations


Jugular
Uterine – caudal
Uterine – mid
Uterine – cranial
Oviduct
Ovarian
PLASMA PROGESTERONE (ng/ml)
100
10
*
*
LEFT HORN
RIGHT HORN

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V POSSIBLE RELATIONSHIPS BETWEEN FEED INTAKE, PROGESTERONE SECRETION AND EMBRYO NUMBERS AND DEVELOPMENT IN THE GILT

A. INTRODUCTION

Dyck et al (1980) reported that as plasma progesterone decreased, embryo survival tended to decrease as well. The study reported in Chapter II indicated that plasma progesterone concentrations in the first three days of gestation were positively associated with embryo survival at Day 28 of gestation. Gilts which had lower progesterone concentrations at 72 h after estrus had a lower mean embryo survival and greater variability while gilts with a higher progesterone concentration had a higher survival rate with less variation.

The changes in plasma progesterone concentrations in the peri-ovulatory period varies considerably among gilts and this variability may alter embryo survival. Recent work in sheep has suggested a high correlation between embryo survival, peri-ovulatory levels of progesterone and the rate and timing of the increase in plasma progesterone (Ashworth et al., 1989). Inverse relationships between plasma progesterone during the peri-ovulatory period and embryo mortality have been reported in sheep (Ashworth et al., 1989) and cattle (Maurer and Echternkamp, 1982).

An effect of increased feed intake on plasma progesterone concentrations has been well demonstrated in several species. Increased feed intake in gilts is associated with depressed

plasma progesterone (Dyck et al., 1980) and increased metabolic clearance of progesterone from the plasma in both sheep (Parr, 1982) and gilts (Symonds and Prime, 1988). The metabolic clearance rate of progesterone was increased from 39.7 to 57.0 ml min^{-1} kg^{-1} when the feed intake of ovariectomized gilts was increased from 1 to 3 kg of feed per day. A depression of plasma progesterone due to the increased feed intake, particularly in the peri-ovulatory period, could therefore result in a decrease in embryonic survival.

The critical period in which changes in feed intake affect embryo survival appears to be the immediate post-coitum period, which would be consistent with this hypothesis. Feeding 2.5 vs 1.5 kg of feed in the first ten days after mating reduced embryonic survival at Day 30, while the same dietary treatment from Days 11 to 20 had no effect on embryo survival (Dyck and Strain, 1983).

Evidence for functional relationships between plasma progesterone concentrations and both feed intake and embryo survival suggests a mechanism by which increased feed intake could result in an increased embryonic mortality. In the first study reported in this thesis, there was a relationship between embryo survival and plasma progesterone concentrations 72 h after estrus onset. However, plasma progesterone concentrations were highly variable. The subsequent study indicated that feed intake had an effect on the time at which plasma progesterone concentrations began to rise. Although, it

was hypothesized that the time at which progesterone rises will influence the rate of development of the oviductal and uterine environment and hence the percentage of embryos which remain viable through the first 30 days of pregnancy, embryo survival was not measured. The purpose of this final experiment was therefore to attempt to link changes in feed intake (NRC 1988 vs ad libitum) during early pregnancy with changes in plasma progesterone concentrations, embryonic development and survival.

B. MATERIALS AND METHODS

ANIMAL MANAGEMENT

A group of thirty Camborough*Canabrid gilts (PIC Canada, Ltd), with an average weight of 110 kg, were fed a standard barley-wheat-soybean meal diet containing 14% crude protein and 12.5 MJ ME kg^{-1} ad libitum until puberty. Gilts were then provided 2.0 kg of feed per day for a two week period after which they were provided 2.5 kg of feed per day until second estrus. Gilts were checked for estrus twice daily at 12 h intervals with vasectomized boars. At detection of second heat (Day 0), the gilts were bred twice by artificial insemination using fresh pooled semen provided by the Alberta Swine Breeding Centre, Leduc, Alberta. An equal number of gilts from each treatment were inseminated with a given batch of pooled semen.

The gilts were weighed on Day 1, prior to the first feeding and then assigned to one of two feed intakes. The gilts were

then weighed again at Day 15 of gestation, prior to the first feed of the day and prior to slaughter on Day 28. Backfat thickness was determined by ultrasonic probe (Scanoprobe, Scanco) at the 10 th rib at these same times.

After slaughter, the reproductive tracts were removed, the ovaries examined and the number of corpora lutea (CL) recorded. The uterus was opened and the number of viable embryos present was determined. Embryo survival was calculated as number of viable embryos/number of corpora lutea. The embryo and its associated placental tissue were removed from the uterine endometrium and weighed separately.

DIETARY TREATMENT

The study was set up with two levels of feed intake (NRC, 1988 and ad libitum feed intake) which corresponded to intakes of 1.8 and 2.5 kg of feed. The gilts were kept on their dietary regimens from the day after mating (Day 1) until Day 15 of pregnancy. Beginning at Day 16, gilts were provided 1.8 kg of feed until slaughter.

Gilts were randomly allocated within litters to their respective dietary intakes (either NRC, 1988 or high feed intake). When littermate gilts were available, an equal number of littermate gilts were allocated to each treatment to reduce genetic variation in reproductive characteristics.

HORMONAL ANALYSIS

Blood samples were taken when estrus was first detected and at twelve hour intervals thereafter, until 36 h after estrus

onset to estimate the time of the pre-ovulatory LH surge and by inference the time of ovulation (36 to 40 h later). Blood samples were also taken at 42, 48, 54, 60, 72, 84 and 96 h to determine the time of the critical rise in plasma progesterone concentrations; additional samples were taken on Days 6, 9 and 15 of gestation to estimate peak progesterone concentrations in early pregnancy. Previous studies had demonstrated that progesterone concentrations begin to rise approximately 50 to 56 hours after estrus onset.

Five ml of blood were collected by jugular venipuncture into heparinized tubes and centrifuged; gilts were subject to short-term (less than 2 minutes) nose-rope restraint at the time of sampling. Plasma from samples taken up to 36 h was divided into two lots; 1 ml reserved for progesterone analysis and the remaining plasma for LH analysis. Subsequent samples were kept in single lots. All plasma samples were stored at -20°C until analysed.

Plasma samples were analysed for progesterone concentration by a double antibody radioimmunoassay (RIA) as described by Beltranena et al. (1991b). Within an assay, extraction efficiency as determined by recovery of [1,2-^{3}H(N)]-progesterone was used to correct the progesterone concentration determined for each sample. The mean recovery of tritiated progesterone was 80.7±2.1 %. Sensitivity of the assay, defined as 85% of total binding, was 0.01 ng.tube^{-1}. Samples were diluted according to the expected concentration

of steroid to ensure extrapolation of potencies on the linear portion of the standard curve. The intra- and inter-assay coefficients of variation for the assays were 5.8 and 13.0%, respectively.

Samples taken up to 36 h after estrus onset were analysed for LH concentration by a heterologous double antibody RIA as described by Cosgrove et al. (1991) to determine the time of peak LH concentration. Triplicate 100 ul plasma aliquot samples were assayed. The sensitivity of the assay was 0.03 $ng.tube^{-1}$. All plasma LH concentrations were determined in one assay and the intra-assay coefficient of variation was 8.6 %.

STATISTICAL ANALYSIS

Dietary effects on number of corpora lutea, embryo numbers and percent embryo survival were assessed using SAS (SAS Institute) general linear models procedures (Snedecor and Cochran, 1980). Embryo weight and crown-rump length and weight of associated placental tissue were compared between dietary treatments by analysis of variance. Since all gilts could be not slaughtered at Day 28, day of pregnancy was included as a variable in analysing these data.

Plasma progesterone concentrations were analysed with respect to both the time of estrus onset and the time at which the LH peak occurred. In individual animals, peri-ovulatory plasma progesterone concentrations, the time at which progesterone concentrations increased in relation to time of the LH peak and estrus onset, and the rate of increase in

plasma progesterone concentrations were determined. The time at which progesterone began to rise and the subsequent rate of increase was determined by using a two phase regression of plasma progesterone concentrations (BMDP, 1977) described by the following equation.

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

where

$$b_a = b_1 - b_2$$

$$b_b = b_1 - b_2$$

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.

The values obtained for peri-ovulatory progesterone concentrations, time and rate of increase in progesterone concentrations were then compared between treatments by using the t-test procedure in SAS.

Data from all gilts were pooled within treatments, and overall and then analysed by SAS using the general linear

model procedure to determine the extent of correlations between peri-ovulatory concentrations and both the time and rate of increase in progesterone concentrations.

The number of corpora lutea, numer of embryos, percent embryo survival and the crown-rump length and weight of viable embryos were compared between treatments by analysis of variance. A Pearson correlation analysis between percent embryo survival and the hormone parameters determined above (peri-ovulatory concentrations, time of progesterone rise) was performed to examine the relationships between embryos and plasma progesterone concentrations. The same procedure was performed between percent embryo survival and plasma progesterone concentrations at 48 h post LH and at 72 h post-estrus.

Progesterone data from the gilts were pooled and the gilts were sorted into two groups based on plasma progesterone concentrations in samples taken at 24 and 48 h after the LH surge. The mean and variance in embryo survival was compared between groups of gilts with the lowest and highest progesterone concentrations. For example, data from 24 gilts was used in samples drawn at 48 h. The gilts with the 12 lowest progesterone concentrations were assigned to the Low group and those with the 12 highest concentrations were assigned to the High group. Variance in embryo survival was compared between the groups using the Bartlett-Box test for homogeneity of variance (Snedecor and Cochrane, 1980).

The same procedure was used for plasma samples taken 72 h post-estrus, as in Chap II. Embryo survival was plotted against progesterone concentration for these samples. Again, mean and variance in embryo survival were compared in gilts divided into Low or High progesterone concentrations.

Finally, the data from samples taken at 72 h from the present study were pooled with those obtained from samples taken at 72 h in Chap II after comparing the mean and variance in plasma progesterone and embryo survival of the two populations to confirm that the two groups represented a homogeneous population. Embryo survival was plotted as a function of plasma progesterone concentrations. The gilts were ranked from low to high based on progesterone concentrations at 72 h after estrus and split into Low and High on that basis. Mean embryo survival was compared using a simple t-test and the homogeneity of variance using the Bartlett-Box test (Snedecor and Cochran, 1980).

C. RESULTS

The bodyweight of the gilts on Days 1 and 15 of gestation, average daily gain (ADG) and change in backfat thickness of the gilts in the two dietary groups are presented in Table V.1. The bodyweight of the gilts at Day 1 were similar. Day 15 weights and ADG were greater ($p<0.05$) for gilts provided 2.5 kg feed compared with those gilts provided 1.8 kg of feed per day. Backfat was not significantly different between the two treatment groups at either breeding or at Day 15 of gestation.

However, gilts provided 1.8 kg feed per day experienced a slight decrease in backfat thickness, while the high fed group gained 1 mm of backfat during the two week period the gilts were on trial.

The reproductive performance of the gilts is shown in Table V.2. Ovulation rate, as determined from the number of corpora lutea, was not significantly different between the two levels groups. The average number of embryos present was also not significantly affected by the quantity of feed provided (12.2±0.9 vs 10.0±0.9, p=0.12). However, the percentage embryo survival was different between gilts given 1.8 and 2.5 kg feed per day; the gilts continued on the high plane of nutrition had a lower (p=0.07) embryo survival (70.0±6.7) than those gilts provided only 1.8 kg of feed (87.7±5.5). The average embryo weight and crown-rump length, and the weight of the associated placental tissue (all corrected for day of gestation) were not significantly affected by the post-mating feeding level.

The progesterone data for the gilts are presented in Table V.3. The mean times from LH peak to the beginning of the progesterone rise (32.3±5.2 and 42.3±4.4 h for gilts provided 1.8 and 2.5 kg day^{-1}, respectively) were not significantly different between the two levels of feed intake. Similarly, neither the peri-ovulatory concentrations (0.393±.043 vs 0.398±.031 ng ml^{-1}) nor the rate of increase (0.142±.009 vs 0.146±.020 ng ml^{-1} h^{-1}) in plasma progesterone concentrations

were significantly affected by the increase in feed intake.

The Pearson correlation analysis revealed no correlation between embryo survival and any of the characteristics of progesterone secretion (peri-ovulatory concentrations, time and rate of rise of plasma concentrations and plasma progesterone concentrations at 48 h post LH or at 72 h post-estrus), either using data within treatments or pooled over the entire study.

However, when percentage embryo survival was plotted as a function of progesterone concentrations at 24 and 48 h after the LH peak (Figure V.1, and V.2, respectively), variation in embryo survival appeared to decrease as progesterone concentration increased. A statistical evaluation of the homogeneity of variance of the data for each time period, confirmed a significant difference in variance at 24 ($p<0.05$) and 48 ($p<0.01$) (Table V.4). Mean embryo survival was lower and standard error greater in the Low group than in the High progesterone group at 24 ($p<0.05$) and 48 ($p<0.05$) h. Not all gilts were represented in these plots as some had no samples taken at that particular time. As samples were taken relative to estrus onset, some gilts did not have samples which corresponded to 24 and 48 h post LH peak. Data from several gilts were removed due to abnormally high progesterone concentrations. If progesterone concentrations deviated more than two standard deviations from the mean, they were excluded from the analysis.

The data obtained from samples taken at 72 h after first detection of estrus (rather than post LH) revealed the same pattern, when embryo survival was plotted against plasma progesterone concentrations (Figure V.3). As progesterone concentrations increased, the variation in embryo survival decreased. The mean embryo survival was significantly greater ($p<0.05$) and variation in embryo survival smaller ($p<0.01$) in the higher than the lower plasma progesterone group (Table V.5).

Having determined that the data on progesterone concentrations and embryo survival from Chapter II and the present study represented a homogeneous population (Bartlett-Box), the data was pooled for further analysis. The same pattern was observed when embryo survival was plotted as a function of embryo survival (Figure V.4). Mean embryo survival was greater in gilts with the highest plasma progesterone concentrations (79.4 vs 87.4, respectively; $p<0.05$) (Table V.6). Variation in embryo survival was smaller in gilts with the higher plasma progesterone concentrations ($p<0.05$).

C. DISCUSSION

The gilts in the present study received lower energy intakes than in Chapter II, as evidenced by the lower growth rates observed in this study. Energy and protein intake for the gilts provided 1.8 and 2.5 kg day^{-1} were 21.9 and 30.5 MJ ME day^{-1} and 252 and 350 g protein, respectively. These are lower

than the energy intakes provided in the initial study (26.3 and 42.7 MJ ME day^{-1}) though protein intakes were similar. In the first experiment, the additional energy and protein was provided as either starch or casein. In the present study, a single diet was fed at either 1.8 or 2.5 kg day^{-1}.

There was no significant effect of feed intake on embryo numbers but there was an effect on the percentage of eggs shed represented by viable embryos that approached statistical significance. This is in contrast to the initial study where no differences in embryo survival were seen in gilts on different energy and protein intakes. Several differences between the two studies may explain the discrepancy in results. First, the genotype of the gilts (Yorkshire*Landrace gilts versus Camborough*Canabrid) used were different between the two studies. Genetic differences in embryo survival have been demonstrated between prolific Yorkshire and Meishan gilts (Bazer et al., 1988). Secondly, the initiation of the changes in dietary treatments also varied between studies. In Chapter II, dietary changes were made on Day 3, whereas, in the present study, feed intake was changed the day after estrus was detected. If this was a critical factor, the data would be consistent with the observation that increased feed intake from Day 1 to Day 10 of gestation decreased embryo survival, while changes in feed intake from Day 11 to Day 20, did not (Dyck and Strain, 1983). Finally, there were differences in energy intake between Chap II and the present study. In

Chap II, energy intake in the low and high energy groups were 26.3 and 42.7 MJ ME day^{-1}, respectively. The energy intakes in the present study (21.9 and 30.5 MJ ME day^{-1}) were similar to those of 17.6 and 29.4 MJ ME day^{-1} reported in the work by Dyck and Strain (1983) where evidence of a feed related effect on embryo survival was seen. Recently however, Dyck (1991) provided 15.7 and 31.4 MJ ME day^{-1}, which was a wider range of energy intakes than is provided in the present study, and reported no difference in number of corpora lutea, number of embryos or embryonic survival rate between these two levels of feed intake. The average embryo survival reported by Dyck (1991) was 85%.

Additional differences between the experiment in Chapter II and the present may have affected the determination of a nutritional effect on embryonic survival. In the present study, littermates were equalized over treatments. As well, pooled semen from the same three boars were used to inseminate all gilts whereas this was not done in Chapter II.

In the present experiment, the difference in the period between the LH peak and the rise in progesterone concentration, although not significant, was similar to that found in the second experiment. This may be partially due to greater variability in the timing of the progesterone increase between gilts in the present study than in Chap III. A major difference between the two studies was the length of time gilts had been fed on the particular dietary regimen. In Chap

III, the same level of feed intake that had been provided before mating was continued for a five day period. In the present study, gilts fed the low level of feed intake had been previously fed 2.5 kg day^{-1} and feed intake was only reduced on the day after estrus. This may have been insufficient time for the hepatic levels of mixed function oxidase to adjust, though hepatic blood flow and hence the quantity of steroid exposed to hepatic metabolism would be fairly responsive.

When embryo survival was plotted against progesterone concentrations at 24 and 48 h after the LH peak, the same distribution of percent survival was seen as in the initial study. The variance in embryo survival decreased as progesterone concentrations in plasma increased. When plasma progesterone concentrations were slow to rise (reflected by low progesterone concentrations at 24 and 48 h from LH peak), the variability in embryo survival was greater, ranging from 13 to 100.0%. With increasing progesterone concentration, embryo survival was greater and less variable, possibly suggesting a uterine environment more suitable for supporting embryo viability.

These observed relationships are consistent with the hypothesis that variability in progesterone synthesis may alter the degree of synchrony between the embryo and uterine environment and the probability the embryo remains viable (Pope, 1988). An association has been demonstrated between plasma progesterone concentrations in the peri-ovulatory

period and embryonic survival in both prolific ewes (Ashworth et al., 1984 ; 1989) and beef cows (Maurer and Echternkamp, 1982). Progesterone concentrations in the peri-ovulatory period (Day 0 to 1) were significantly lower in ewes that experienced prenatal loss than those that did not (Ashworth et al., 1989). The work of Ashworth et al. (1989) demonstrated that significant correlations existed between embryo survival and the time after ovulation at which plasma progesterone concentrations begin to rise and the subsequent rate of increase.

Our data suggest a consistent relationship between plasma progesterone concentrations and embryo survival. At low progesterone concentrations, embryo survival can be as high as 100% but is also highly variable. As plasma progesterone concentrations in the post-ovulatory period increase, embryo survival improves and becomes less variable. It could be suggested that increasing plasma progesterone concentrations tends to make the oviductal and uterine environment more supportive of embryo growth and survival. At lower progesterone concentrations, the uterine environment may be less supportive and other factors (e.g. range of embryo development) may have a more critical role in determining the proportion of embryos which survive. Alternatively, the rate of luteinization and hence the time of the initial rise in progesterone, may reflect the maturational state of the follicles from which the corpora lutea have developed. This

may in turn, directly or indirectly, effect the developmental potential of the oocyte and early embryo.

Table V.1. Mean bodyweight gain and change in backfat thickness in gilts in response to changes in feed intake during early pregnancy.

Feeding Level	No. Gilts	Bodyweight, (kg) Day 1	Day 15	Daily Gain	Backfat thickness, mm Day 1	Day 15	Change
LOW	13	112.7	114.8	0.16	15.5	15.3	-0.3
HIGH	17	116.0	122.1	0.43	14.6	15.7	1.1
S.E.M.[1]		2.9	3.0	0.07	0.5	0.6	0.3
P		N.S.	0.05	0.01	N.S.	N.S.	0.01

[1]Standard error of the mean

Table V.2. Mean number of eggs shed, number of embryos and percent embryo survival in gilts in response to changes in feed intake in early pregnancy[1].

Feeding Level	No. Gilts	Corpora Lutea (No.)	Embryo No.	Survival (%)	Weight[2] (grams)	Crown-Rump Length (mm)	Placenta[2] Weight (grams)
LOW	13	14.0	12.2	87.7[a]	1.00	23.6	16.7
HIGH	17	14.5	10.0	70.0[b]	1.23	24.4	19.6
S.E.M.[3]		0.58	0.90	6.5	0.11	0.6	1.8
P		N.S.	0.11	0.06	N.S.	N.S.	N.S.

[1]Means within a column are significant at $p<0.05$
[2]Expressed on a wet weight basis
[3]Standard error of the mean

Table V.3. Peri-ovulatory concentrations and time and rate of increase in peripheral plasma progesterone concentrations in gilts fed either 1.8 or 2.5 kg feed per day in early gestation.

Feeding Level	No. Gilts	Pre-rise conc'n ($ng.ml^{-1}$)	Time of post-estrus rise (hrs)	Post-rise rate[1]
LOW	13	0.393	32.3	0.142
HIGH	14	0.398	42.3	0.146
S.E.M.[2]		0.036	4.6	0.016
P		N.S.	N.S.	N.S.

[1]rate of increase in plasma progesterone concentration ($ng\ ml^{-1}\ hr^{-1}$)
[2]Standard error of the mean

Table V.4. Mean and standard error of percent embryo survival in gilts grouped on the basis of their plasma progesterone concentrations at 24, 36 and 48 h after LH peak[1].

Grouping	Gilts No.	Progesterone ($ng\ ml^{-1}$ plasma)	% Embryo Survival Mean	Range	S.E.[2]
Time = 24					
Low	11	(0.1 - 0.4)	77.2^a	(13.0 - 100.0)	8.5^a
High	11	(0.4 - 3.1)	91.0^b	(61.0 - 100.0)	3.3^b
Time = 48					
Low	12	(0.3 - 3.1)	64.0^a	(12.5 - 100.0)	9.4^a
High	12	(3.2 - 9.5)	88.9^b	(20.0 - 100.0)	3.2^b

[1]Different superscripts within a column and within time indicates a significant difference at $p<0.05$.
[2]Standard error

Table V.5. Mean and standard error of percent embryo survival in gilts grouped on the basis of their plasma progesterone concentrations at 72 h post-estrus[1].

Grouping	Gilts No.	Progesterone (ng ml^{-1} plasma)	% Embryo Survival Mean	Range	S.E.[2]
LOW	13	(1.5 - 7.0)	84.0[a]	(13.0 - 100.0)	4.3[a]
HIGH	13	(7.4 - 15.1)	93.7[b]	(84.6 - 100.0)	1.5[b]

[1]Different superscripts within a column and within time indicates a significant difference at $p<0.05$.
[2]Standard error

Table V.6. Mean and standard error of percent embryo survival in gilts pooled from Chapter II and V and grouped on the basis of their plasma progesterone concentrations at 72 h post-estrus[1].

Grouping	Gilts No.	Progesterone (ng ml^{-1} plasma)	% Embryo Survival Mean	Range	S.E.[2]
Low	38	(1.3 - 7.6)	79.4[a]	(13.0 - 108.3)	3.6[a]
High	38	(7.7 - 25.5)	87.4[b]	(26.7 - 107.7)	2.5[b]

[1]Different superscripts within a column and within time indicates a significant difference at $p<0.05$.
[2]Standard error

Figure V.1. Percent embryo survival plotted against plasma progesterone concentrations in samples drawn at 24 h after LH peak. (n= 22, 2 observations hidden). Three gilts were excluded as their plasma progesterone concentration was greater than 3 standard deviations. Their values were 5.5, 8.3 and 10.2 ng ml^{-1} with percent embryo survival of 100.0, 86.0 and 108.0., respectively.

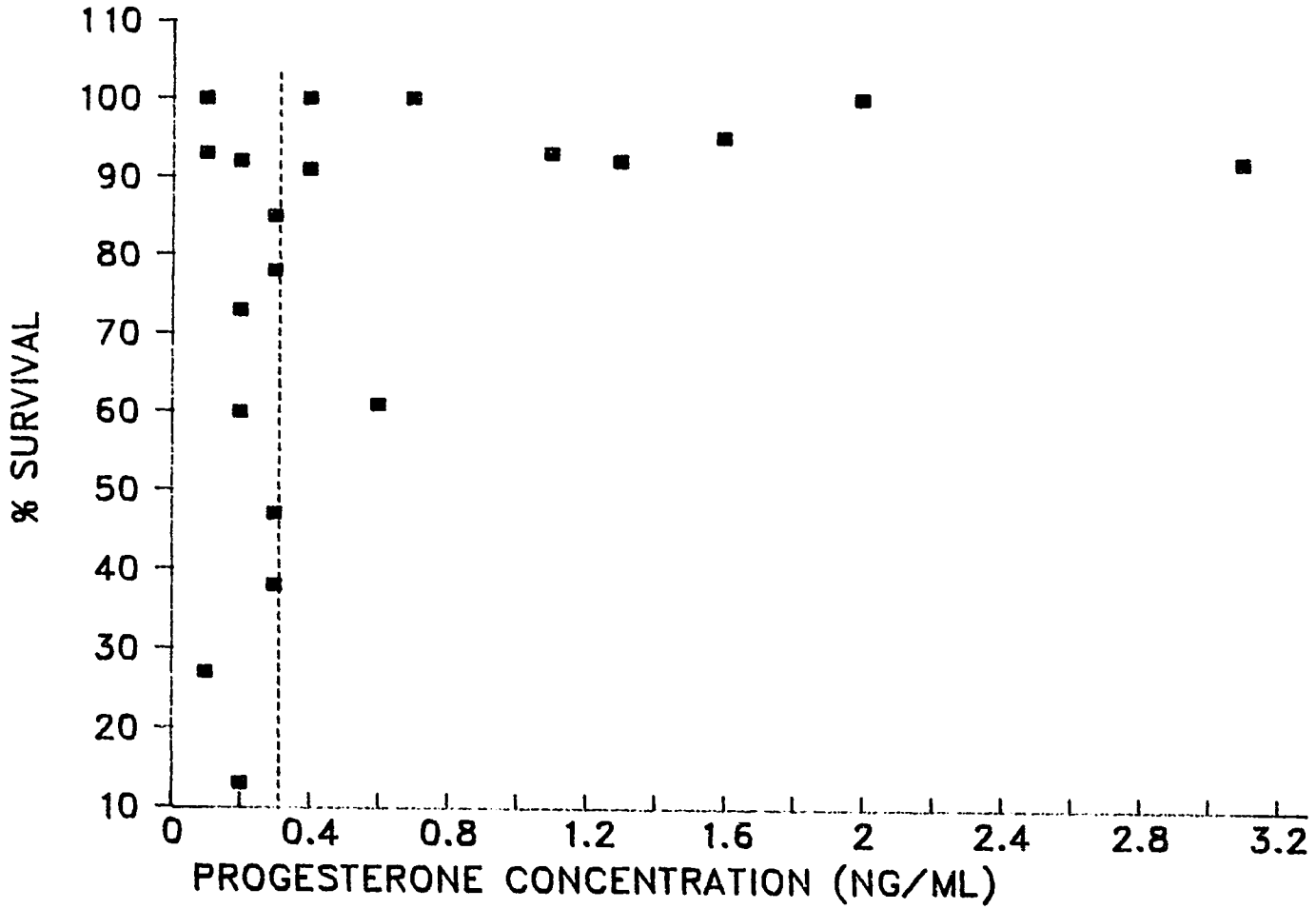

110
100
90
80
70
60
50
40
30
20
10
% SURVIVAL
0
0.4
0.8
1.2
1.6
2
2.4
2.8
3.2
PROGESTERONE CONCENTRATION (NG/ML)

Figure V.2. Percent embryo survival plotted against plasma progesterone concentrations in samples drawn at 48 h after LH peak. (n=24) One gilt was excluded as its plasma progesterone concentration was greater than 3 standard deviations. Its value was 15.6 ng ml^{-1} with percent embryo survival of 108.0.

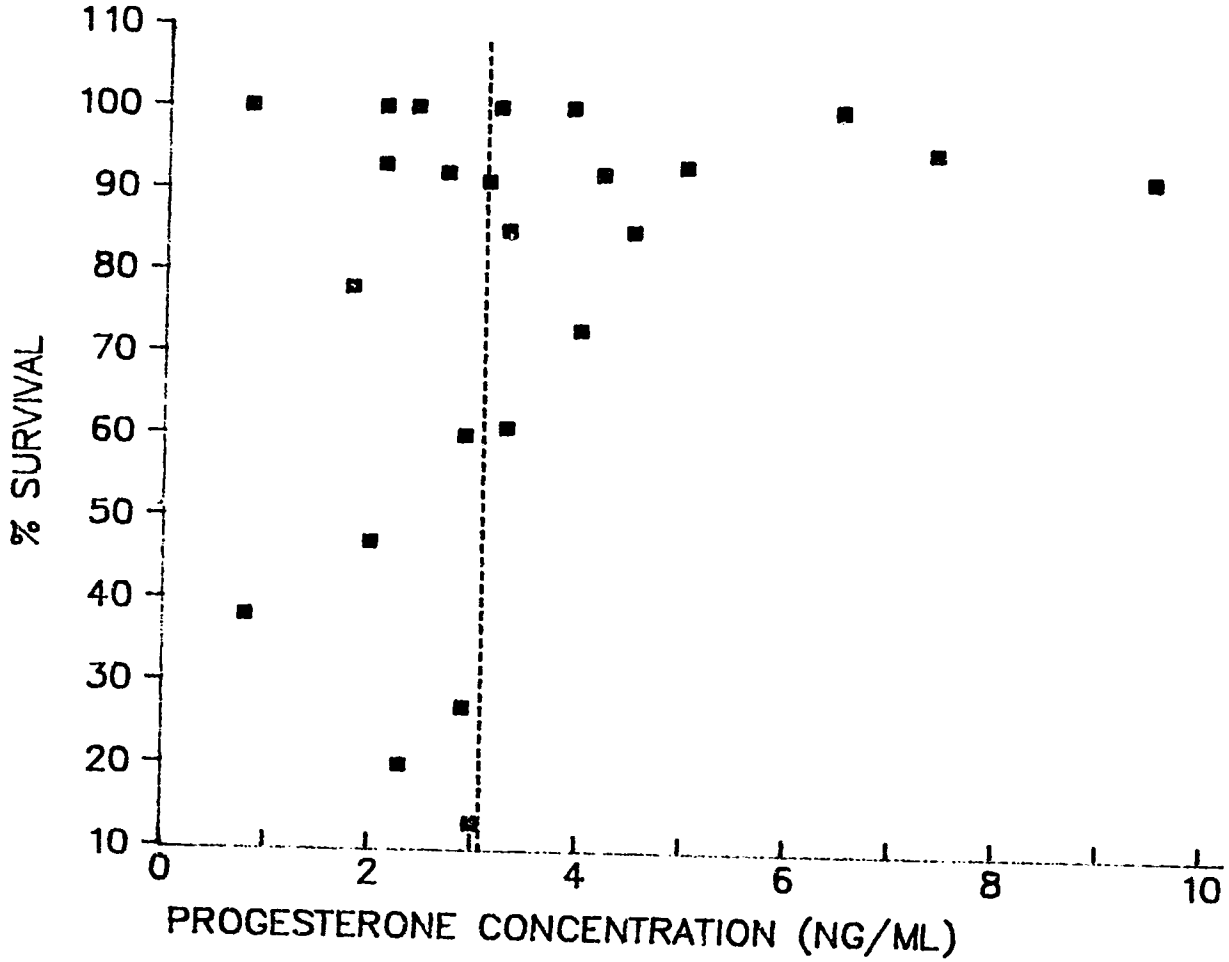

110
100
90
80
70
60
50
40
30
20
10
% SURVIVAL
0
2
4
6
8
10
PROGESTERONE CONCENTRATION (NG/ML)

Figure V.3. Percent embryo survival plotted against plasma progesterone concentrations in samples drawn at 72 h after first detection of estrus. (n=26, 1 observation hidden)

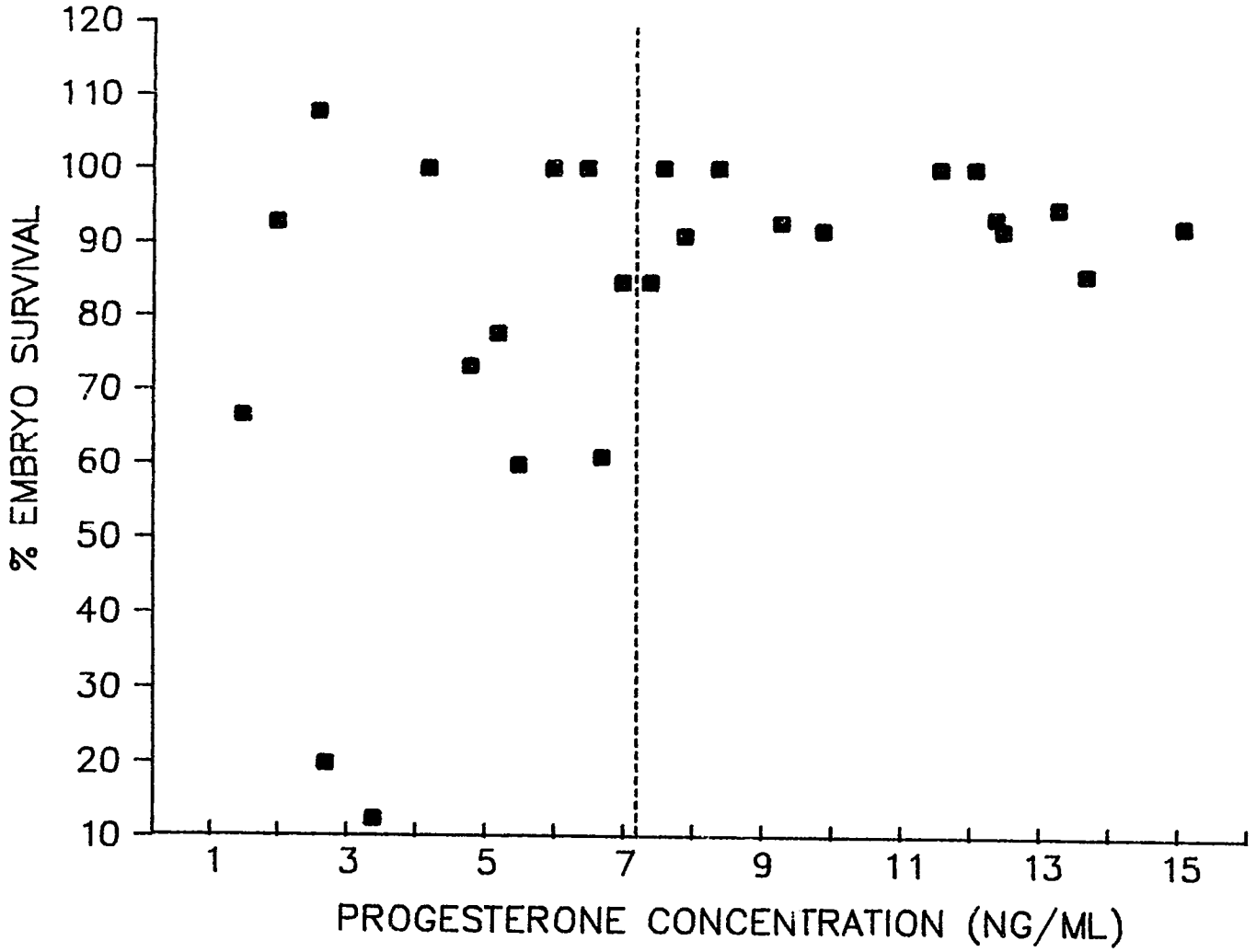
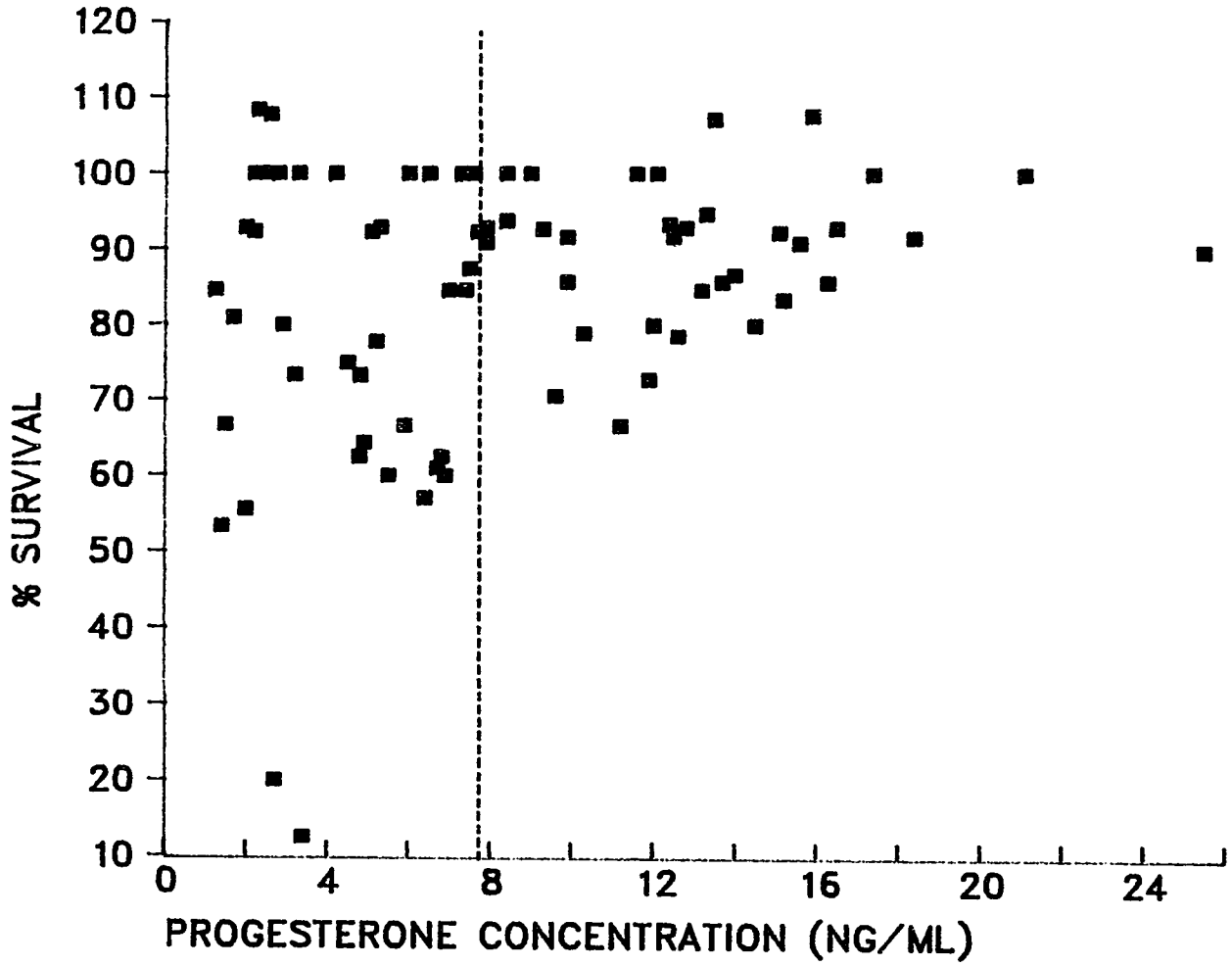

120
110
100
90
80
70
60
50
40
30
20
10
% EMBRYO SURVIVAL
1
3
5
7
9
11
13
15
PROGESTERONE CONCENTRATION (NG/ML)

Figure V.4. Percent embryo survival plotted against plasma progesterone concentrations in gilts pooled from Chapters II and V in samples drawn at 72 hours after first detection of estrus.
(n=76)


120
110
100
90
80
70
60
50
40
30
20
10
% SURVIVAL
0
4
8
12
16
20
24
PROGESTERONE CONCENTRATION (NG/ML)

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VI GENERAL DISCUSSION

There is a lack of consensus as to whether increased feed intake in early pregnancy affects embryo survival in the gilt. Several studies report a reduction in survival (Dyck and Strain, 1983) while others report no difference in embryo survival due to increased feed intake (Dyck, 1991). It has been suggested that increased protein intake induced hepatic mixed function oxidase activity and that this increased metabolic clearance of progesterone from the plasma (Dziuk, 1982). Increased dietary protein intake in dairy cattle reduced plasma progesterone concentrations (Jordan and Swanson, 1979). In the first study, we found no evidence that changes in energy and/or protein intake affected plasma progesterone concentrations or embryo survival. However, in our final study, in which lower energy levels were fed, increased feed intake lowered embryo survival in gilts.

The effects of increased feed intake on embryo survival may be dependent on when changes in feed intake are made. Chapters II and V would indicate that reducing feed intake on the day after mating improves the rate of embryo survival. If gilts are continued on the high plane of feeding until Day 3 of gestation, a subsequent decrease in feed intake may not affect embryo survival, as increased feed intake may exert its negative influence in the intervening two days. Thus, the days immediately after mating may be of critical importance in determining embryo loss, such that subsequent diet changes

have little effect on embryo survival. This suggestion would be consistent with the observations of Dyck and Strain (1983) that higher feed intake from Days 1 to 10 increases embryo mortality, while feed intake after this period has no effect. The experiments reported here would suggest that the critical period in which increased feed intake would affect embryo survival is from mating to Day 3 of gestation. A study incorporating two levels of feed intake at either Day 1 or 3 of gestation would determine whether the intervening period is the critical period in which embryo survival may be affected.

The period between Day 1 and Day 3 is coincident with the period in which plasma progesterone concentrations are beginning to rise in the plasma (van de Wiel et al., 1981). Increased feed intake at this time may affect the changes in plasma progesterone concentrations as Symonds and Prime (1988) demonstrated an increased metabolic clearance rate of progesterone from the plasma in response to increased feed intake. The study of plasma progesterone profiles in Chapter III showed that there was substantial variation between gilts in the time after peak LH at which plasma progesterone concentrations began to rise. Chapters III and V indicated that the rise in plasma progesterone concentration is delayed when high levels of feed intake are provided. In Chapter III, the average time of rise was delayed from 30 h to 37 h, when feed intake was increased from 2.0 to 2.8 kg day^{-1}. In the last experiment, when feed intake was increased from 1.8 to 2.5 kg

day^{-1}, the time of the progesterone rise was delayed from 32 to 42 hours after the LH peak. Measuring both luteal synthesis of progesterone and hepatic clearance of progesterone in response to changes in feed intake would determine if synthesis or catabolism is altered by feed intake.

Changes in plasma progesterone concentrations reflect a balance between synthesis and release, and the metabolic clearance of progesterone by the liver and kidney. There is considerable heterogeneity in luteal development, as indicated by weight and progesterone content of corpora lutea, both within and between gilts (Hunter and Wiesak, 1990). Progesterone concentrations in corpora lutea can vary two-fold within a gilt. Due to this heterogeneity, the release of progesterone into the plasma and changes in plasma progesterone concentrations would vary between gilts as has been demonstrated in this series of studies. With greater metabolic clearance of progesterone due to increased feed intake, the corpora lutea would need to release a greater quantity of progesterone before a rise in plasma progesterone concentrations was seen. Given the heterogeneity in corpora lutea between gilts, the increased MCR would have a greater effect in those gilts where the corpora lutea have developed at a slower rate and are less capable of synthesizing and releasing progesterone, than those which have well developed corpora lutea.

As a result of this balance between release and clearance,

the concentration of progesterone needed to trigger the synthesis of uterine proteins may be reached earlier in some gilts than others. The difference in the time at which progesterone increases in the plasma suggests that the time at which the uterine endometrium receives the initial stimulus to begin synthesis of secretory products would also differ due to changes in feed intake. This situation would be analogous to the exogenous administration of progesterone at different intervals from mating. Early administration advances the uterine environment and makes it more receptive to the more advanced embryos. Therefore, the uterine environment of gilts continued on a high plane of feeding would be less advanced than those where feed intake was reduced after mating, due to the delay in the time at which progesterone increases in the plasma. The consequences of decreasing the interval from peak LH to progesterone rise on embryo survival could be determined by the placing of PRIDs (Progesterone Releasing Intravaginal Devices) after estrus onset at sequentially earlier intervals relative to when progesterone normally occurs in the gilt

There appears to be a relationship between embryo survival and progesterone concentrations in blood samples drawn up to 72 hours after estrus. The relationship is not linear as demonstrated by the lack of statistical correlation between embryo survival and progesterone concentrations in this period. The plot of embryo survival against plasma progesterone concentration suggests that the response of

embryos to the uterine environment changes as the concentration of progesterone increases in the period from estrus to 72 h post-estrus, suggesting that a threshold value is reached to stimulate uterine development. Gilts which have lower progesterone concentrations tend to have lower embryo survival and greater variation in embryo survival. While it is possible for gilts in this group to have 100% embryo survival, they are more likely to have very low embryo survival. As progesterone concentrations increase, gilts tend to have an improved rate of embryo survival with smaller variability. The majority of gilts have very high rates of embryo survival. Low plasma progesterone concentrations may be a pre-disposing factor in embryo loss but not the only cause; other factors may take on a more critical role in determining embryo viability in this situation.

The portion of embryos which die during early gestation ranges from 25 to 35 percent, which is coincidental with the observations that on Days 6 to 9 there is a distinct minority (20%) of smaller embryos with lower protein content. It has been suggested that it is this minority that fails to survive (Pope et al., 1990). The more mature embryos alter the uterine environment through the release of estradiol and the less developed embryos fail to survive in this altered environment. However, not all females experience embryo loss and other factors may overcome this developmental variation. The variability in embryo development, especially as it relates to

estradiol synthesis, may only come into effect under conditions where uterine development is limited or delayed, due to delays in exposure of the uterus to threshold concentrations of progesterone.

In a situation of high progesterone concentrations or an earlier rise in progesterone, the uterine environment may be more supportive of a wider variation in embryo development. Where lower progesterone concentrations are present or the rise in progesterone is later, the embryos may need to be in a narrow range of development, or at comparable stages of development, to survive in this particular environment. Bazer et al. (1968) suggested that embryos compete for critical biochemical substances to develop and that those which fail to survive do not sequester sufficient quantities of these. A number of these substances have been identified, such as IGF-I which stimulates protein synthesis in the blastocysts (Estrada et al., 1991) and is synthesized in response to increasing progesterone and estradiol concentrations (Simmen et al., 1990). Experiments using ferritin or horse radish peroxidase have demonstrated that blastocysts are capable of absorbing macromolecules intact by pinocytosis (Stroband et al., 1984) and that this varies between embryos (Stroband, personal communication).

With lower progesterone concentrations, there would be a more critical shortage of required growth factors or nutrients in the uterine environment. Siblings at comparable stages of

growth may compete equally for marginal factors and thus all would continue to develop albeit at a slower rate but with a large portion surviving. Where there is a mix of immature and mature embryos present, the more mature embryos may sequester a greater share as the smaller embryos are less competitive. In this scenario, smaller embryos may get none or very little of the required growth factors and would not be able to develop. Moreover, those larger embryos able to sequester more material may develop at a greater rate, increasing their release of estradiol and changing the nutrient and protein composition of the uterine environment. Smaller embryos would thereby be further disadvantaged, as their needs would not have been satisfied before the uterine environment was changed. These embryos may then find the uterine environment less hospitable and may not survive (Pope et al., 1990). Many of the uterine proteins are synthesized in direct proportion to progesterone concentrations in the plasma, as demonstrated by the dose-dependent recovery of uterine protein after exogenous progesterone administration (Dalton and Knight, 1974a,b).

With higher progesterone or an earlier rise in progesterone concentrations, growth factors and nutrients may be present in sufficient quantities, that even in situations where there is a large variation in embryo development and the larger embryos may sequester a disproportionate share of these factors, there may still be sufficient secretory material for the smaller

embryos to satisfy their needs and develop normally. In this scenario, the smaller embryos could develop sufficiently to respond to changes in the uterine environment elicited by the larger embryos and survive. As a result, embryo survival could be as high as 100%.

An uterine endometrium exposed to lower progesterone concentrations may be less sensitive to embryonic signals and release less histotrophe to nurture the embryo. It has been suggested that Meishan and Yorkshire females differ in the sensitivity of their uteri to release histotrophe in response to signals from the embryos or in the ability of their embryos to stimulate histotrophe release (Bazer et al., 1991).

The relationship between embryo survival and progesterone concentrations seen in the pig is similar to that seen in the sheep. In sheep, embryo survival was directly correlated with plasma progesterone concentrations in the peri-ovulatory period and the time of the increase in progesterone concentrations (Ashworth et al., 1989). Embryo survival in sheep is an all or none situation; either there is sufficient progesterone to support uterine development and, as a result meet the nutrient demands of the embryo, or there is not, in which case the embryo does not remain viable. With greater progesterone concentrations, there would be greater synthesis of uterine proteins to nurture the developing embryo. In the case of pigs, a polytoccus species, the competition between embryos for available nutrients and the competitive advantage

of more developed embryos, complicates the relationship between survival and progesterone concentrations. The sooner the stimulus for the uterine endometrium to begin synthesis of uterine protein, the greater the probability of a greater proportion of embryos surviving.

Plasma progesterone concentrations can vary substantially between pigs and between organs, particularly in the reproductive tract. The ovarian pedicle has extremely high concentrations of steroid. Arterioles supplying the oviduct have been demonstrated to have substantially greater steroid concentrations than the arteries outside the area of the reproductive tract (Hunter et al., 1983). These local elevations in steroid concentrations occur through counter-current mechanisms that have been shown to exist in several locations in the reproductive tract (Krzymowski et al., 1990). The third experiment confirmed the presence of local elevations in progesterone concentrations in the oviductal veins but not in the veins originating from the uterine horn. In sheep, veins draining the area of the uterine horn adjacent to the oviduct and ipsilateral to the CL bearing ovary, were also found to have elevated progesterone concentrations (Weems et al., 1989). The progesterone concentrations measured in the oviductal veins in our study were fifty-fold greater than in samples taken from the jugular vein. This difference was evident twenty-four hours after detection of estrus, at which time jugular concentrations were minimal (<1.0 ng ml^{-1}) but in

the oviductal veins concentrations already exceeded 40 ng ml^{-1}. These proportions persisted at all times sampled. This indicated that the oviductal epithelium (an possibly portions of the uterine endometrium adjacent to the oviduct) are exposed to an effective progesterone stimulus prior to any increase in peripheral plasma progesterone concentrations. The embryos would be exposed to this hormonal environment for the 48 hours from ovulation to the time they enter the uterus, and possibly for the 72 hours in which they remain in the vicinity of the utero-tubal junction.

The role of progesterone in facilitating the transport of the egg and embryo through the oviduct is clear (Hunter, 1977b), as progesterone dominance is required for tubal transport of the fertilized ova and embryo. However, the role of elevated progesterone concentrations in the oviduct in regulating oviductal protein synthesis or its resulting influence on embryo survival is not clear. Co-culture of one-cell porcine embryos with oviduct epithelial tissue resulted in a greater number of embryos advancing to the morula stage than if cultured with fibroblast tissue (White et al., 1989). The incidence of polyspermy was also reduced when ova were co-cultured with oviduct epithelial cells (Nagai and Moore, 1990). The local elevations in progesterone concentrations would aid embryo development by stimulating the synthesis of the proteins needed to stimulate the transformation of the embryos to the morula stage.

Local elevations in plasma steroids have been demonstrated to influence embryo viability in the rat. In unilaterally ovariectomized rats, embryo development was retarded in the horn ipsilateral to the ovariectomy (Nuti et al., 1971). In the third experiment, plasma progesterone concentrations in the oviductal veins were significantly correlated with the number of corpora lutea on the ipsilateral ovary. The greater number of corpora lutea would release greater quantities of progesterone, providing a stronger stimulus to synthesis of oviductal proteins, ensuring the optimum development of the embryos originating from that ovary. Variability in the process of luteinization could also change the concentrations of steroid the oviductal epithelium is exposed to. Restriction of embryos to the horn of origin and relating this to progesterone concentrations in the oviductal veins would be a method by which to test the influence of elevated steroid concentrations in the ipsilateral oviduct on embryo survival. If the observed differences in the initial rise in progesterone in the oviductal circulation does have a critical effect on the oviductal environment, this could also influence final sperm maturation and fertilization. Although such effects may not result in any immediate failure to penetrate the oocyte, the potential for latent effects on early embryonic development could be another contributory factor to differences in embryonic survival.

Embryo survival in the gilt reflects an interplay of many

factors e.g. genetics, hormonal profiles, local elevations in steroid concentrations, variation in embryo development and in the uterine environment. Where one element is altered, other factors may assume a more critical role in determining the viability of an embryo. Where progesterone concentrations are slow to rise and uterine development is reduced, a predisposition to loss may arise and greater variability in embryo development may increase embryo mortality rates. Because an alteration in hormonal concentrations through increased feed intakes or exogenous administration may not be the sole regulator of embryo mortality, the lack of treatment effects in a number of experiments may simply reflect the complexity of the situation. Similarly, if the early rise in luteal progesterone secretion exerts its critical effects through the sub-ovarian counter-current mechanism, the exogenous treatment with progesterone may be relatively ineffective in mimicking the changes occurring in vivo.

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