Mechanisms mediating nutritional effects on embryonic survival in pigs

G. R. Foxcroft

Alberta Pork Research Centre, Department of Agricultural, Food and Nutritional Science, 310-C Agriculture-Forestry Centre, University of Alberta, Edmonton, Alberta, T6G 2P5, Canada

The inconsistency of data from experiments designed to show nutritional effects on embryonic survival is perplexing. However, a number of experimental models have provided some insight into the mechanisms that potentially mediate interactions between nutrition, metabolic state and embryonic survival. The developing ovarian follicle provides the maturational environment for the oocyte, and differences in follicular maturation are associated with differences in the ability of these follicles to support oocyte maturation. In turn, the rate of oocyte maturation and the maturational state of the oocyte immediately before ovulation may contribute to differences in embryonic survival. Therefore, evidence that nutritionally induced differences in metabolic state affect follicular development and the maturational state of the oocyte in the late follicular phase may constitute a mechanism by which nutrition affects the very early stages of conceptus development. Once ovulation occurs, the process of fertilization and early cleavage occurs in the environment of the oviduct. Nutritional state might affect the secretory and motile activity of the oviduct both directly, by influencing the physiology of the oviductal cells, or indirectly by affecting the secretion of key regulatory hormones. Thus evidence for nutritionally dependent effects on plasma progesterone concentrations in early pregnancy and associations with differences in embryonic survival may be partly mediated at the oviductal stage of development. Nutritional effects on circulating progesterone concentrations may also affect the uterine environment. However, the metabolic state of the gilt or sow, or specific nutrients in the diet, may directly affect the integrity of the endometrium and thus affect embryonic survival at this stage of development.

Introduction

An adequate understanding of the physiology of gamete production and of early pregnancy in pigs is essential as background information for a review of the mechanisms affecting embryo survival. The presentation of such background information is beyond the scope of this review but an excellent summary of most of the relevant information was presented by Pope (1994). In the same publication Ashworth (1994) described much of the data linking nutritional state to differences in embryo survival and provided some insight into the mechanisms involved. Such reviews of the literature should leave little doubt that various nutritional factors can affect embryo survival in pigs. The purpose of this review is to identify some of the key stages in the reproductive cycle at which nutrition may affect embryo survival and extend the discussion of the possible mechanisms that might mediate these effects. Initially, however, it seems appropriate to discuss the reasons for the inconsistency in much of the literature regarding the effects of nutrition on embryo survival. This inconsistency has been a major problem in developing good experimental models with which to explore the mechanistic aspects of nutritional effects. By combining a critical approach to the better design of experiments with information already available on the putative mechanisms involved, it should be possible to achieve greater progress in describing the physiological mechanisms by which nutrition affects embryonic survival.
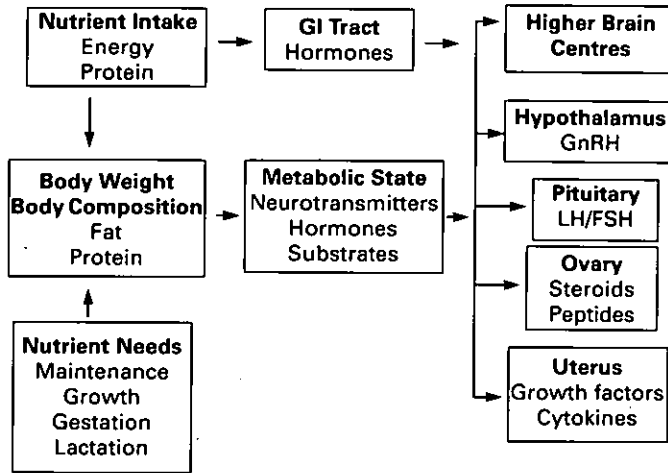
Fig. 1. Schematic representation of the interactions between metabolic state of the gilt or sow and the reproductive axis. Nutrient intake can affect reproduction via gut-mediated mechanisms throughout the gastro-intestinal (GI) tract or, depending on the nutrient demands of the animal, may affect metabolic state and therefore mechanisms at all levels of the hypothalamic–pituitary–ovarian–uterine system.

Problems in Consistently Demonstrating Effects of Nutrition on Embryonic Survival

How to define nutritional effects

Much of the confusion that has arisen in defining the effects of nutrition on reproductive function comes from the reluctance to move from an empirical to a mechanistic approach to this problem. The advantages of adopting a mechanistic approach have been discussed in detail by I'Anson *et al.* (1991) but some of the key issues are worth considering. Firstly, if the metabolic theory of nutritional effects on reproduction is accepted, then even long-term effects of nutritional state on reproduction must be explained on the basis of metabolically mediated mechanisms. Experiments that simply explore the relationships between changes in body composition and reproductive function, without information being provided on the metabolic and endocrine status of the animal, are unlikely to make a significant contribution to our understanding of the problem. In many instances the mechanisms that mediate the effects of nutrition on reproduction will operate in an additive or even synergistic manner. Thus for instance, the point at which nutritional manipulations result in measurable changes in protein or adipose tissue will be relatively late in a cascade of events that will already have involved very rapid responses to changes in energy balance. It is equally likely that the same 'nutritional' change will exert its effects at different levels of the hypothalamo–hypophysial–ovarian axis (Fig. 1).

If nutritional treatment involves changes in total feed intake, it is likely that different components of the diet will be exerting many effects on the reproductive system. Thus, although studies in gilts suggest that changes in energy intake may be a key regulator of follicular development and hence ovulation rate (see Cox, 1997), in sheep, responses to the protein component of the diet may be equally, if not more, effective when used during 'flush feeding' to increase ovulation rate (see Ashworth, 1994). Furthermore, while there may be effects of nutrition on embryo survival that reflect something as general as energy balance mediated through the actions of, for example, insulin and insulin-like growth factors, other nutritional effects may relate to an effect of specific nutrients, such as minerals or vitamins, that regulate essential mechanisms for successful embryo development. Given all these alternatives, it is clear that only a critical and reductionist approach to dissecting the mechanisms mediating effects of nutrition on embryonic survival is likely

to be successful. A further advantage of embracing the metabolic approach to considering nutritional effects on reproduction is the realization that nutrient intake *per se* is only one component of the complex interactions (Fig. 1) that determine the final outcome in terms of fertility. By relating the pool of available nutrients to the needs of the gilt or sow for maintenance and productive functions like growth, gestation and lactation, it should be possible to develop greater comparability between different experimental paradigms.

A further problem in defining nutritional effects is that the response to nutrition at one stage of the reproductive cycle may confound responses at a later stage. This problem was addressed in the review of Aherne and Kirkwood (1985) in relation to the fertility of gilts maintained on a high plane of nutrition. Although high feed or energy intakes (flushing) may be beneficial in maximizing ovulation rate, the negative impact of continued high planes of nutrition after mating, discussed later, may negate this effect. Therefore, the continuous application of dietary treatments throughout the preovulatory, periovulatory and postovulatory periods does not allow us to define the stage of the reproductive cycle that is being critically affected.

The general problem of achieving comparability among nutritional experiments was highlighted by Schultz *et al.* (1966) and in more recent studies that have sought to establish effects of nutrient intake during the postmating period on embryo survival in the gilt; the inconsistency of the results may be related to the precise time in relation to ovulation and fertilization at which nutritional changes were implemented (Jindal *et al.*, 1996). Therefore, as suggested by Pope (1994), a consideration of the potential effects of nutrition on embryo mortality should try to differentiate clearly between effects at each stage of oocyte and embryonic development.

How to optimize the chance of defining effects at a mechanistic level

One very obvious feature of any set of data on embryonic survival in gilts or sows is the variability involved. Populations of gilts that show greater overall embryonic survival also tend to show less variability in survival (Jindal *et al.*, 1996). Interestingly, in populations of gilts and sows subjected to adverse nutritional regimens, some animals invariably show 100% survival to the postimplantation stage. Given this variability, it is frustrating that many studies are still conducted with inadequate numbers of animals. Assuming the variance in embryonic survival that we have encountered in populations of sows (Zak *et al.*, 1997a; L.J. Zak, R. Jindal and G.R. Foxcroft, unpublished; J. Mao, L.J. Zak, J.R. Cosgrove and G.R. Foxcroft, unpublished) in which embryo survival was compromised (mean±SD embryo survival of 70.7±14.0), we calculate that to observe a difference in embryo survival in response to treatment of 5% or 10% would require 65 and 16 observations, respectively. Comparable data from our recent experiments in gilts (Pharazyn *et al.*, 1991a; Pharazyn, 1992; Jindal *et al.*, 1996; Jindal *et al.*, 1997) in which mean embryo survival was 84% indicate a need for 44 and 17 animals, respectively, to demonstrate a 5% and 10% improvement in embryo survival in response to experimental treatments. In many reports the level of possible response may be below 5%, yet limited numbers of sows have been used to estimate treatment effects. Experimental paradigms that seem to provide a level of response that may be conducive to effective mechanistic studies include comparisons between prolific and less prolific genotypes and inbred lines (see Pope, 1994), variants of postmating nutritional manipulation in gilts (see Ashworth, 1994; Jindal *et al.*, 1996) and nutritional manipulation in lactating and weaned sows (Zak *et al.*, 1997a).

Inconsistent responses to nutritional manipulation may also be related to differences in embryo survival in the 'control' population used for comparison. This may be particularly true where research is conducted in large commercial units and careful selection of the population of sows under study may be beneficial, even though large numbers of animals may be available. A comparison between the data of Tremblay *et al.* (1989a) and Zak *et al.* (1997a; shown in Table 1) illustrates this point, in that the highest embryonic survival in any treatment group in each of these experiments was 67.4% and 87.5%, respectively. Thus the baseline against which treatment effects were being assessed was very different.

G. R. Foxcroft

Table 1. Effects of pattern of feed intake in lactation on postweaning fertility in primiparous sows*

Parameter	Treatment 1	Treatment 2	Treatment 3
Weight loss in lactation (kg)	11.00[a]	21.12[b]	24.75[b]
Backfat loss in lactation (mm)	2.19[c]	4.61[b]	5.38[b]
Ovulation rate	19.86[c]	15.44[b]	15.43[b]
Embryo survival (%)	87.53[a]	64.43[b]	86.50[a]
Weaning to oestrus interval (h)	88.71[a]	122.33[b]	134.71[b]

* This experiment involved three patterns of feeding during lactation: treatment 1 sows were fed *ad libitum* (100%) throughout lactation; treatment 2 sows were fed *ad libitum* from day 0 to day 21 and then restrict fed to 50% of *ad libitum* feed intake from day 22 to day 28 of lactation: Treatment 3 sows were restrict fed from day 0 to day 21 and then *ad libitum* from day 22 to day 28 of lactation. All sows suckled litters of six piglets to reduce the direct inhibitory effects of suckling *per se* and thereby allow the effects of nutrition to be more clearly demonstrated (from Zak *et al.*, 1997a)
[a] and [b] denote treatment differences at $P < 0.002$, and [c] denotes differences at $P < 0.05$.

Given the reported association between nutritionally induced increases in ovulation rate and reduced embryo survival in pigs (see review of Anderson and Melampy, 1972), nutritionally induced changes in ovulation rate may confound the study of embryo survival. In a number of studies in cyclic gilts, this problem was avoided by imposing nutritional treatments only after the expected time of ovulation (Pharazyn *et al.*, 1991a; Liao and Veum, 1994). However, the later studies of Pharazyn (1992) and Jindal *et al.* (1996, 1997) suggest that this may have been unnecessary and indeed, as discussed later, the critical period for an effect on embryonic survival may have been missed. If the confounding effect of nutrition on ovulation rate cannot be avoided, then as in the study of Liao and Veum (1994), it may need to be removed by covariance analysis. A better solution would be to develop experimental paradigms in which nutrition differentially affects ovulation rate and embryo survival. The recent studies of Zak *et al.* (1997a) and J. Mao, L.J. Zak and G.R. Foxcroft (unpublished) suggest that nutritional manipulation during lactation provides this opportunity, in that different patterns of feed restriction resulted in a similar reduction in ovulation rate compared with sows fed to appetite, but substantial differences in embryo survival (Table 1).

The confounding effect of inducing variable ovulation rate is also clearly demonstrated in the experiment of Tremblay *et al.* (1989a) in which PMSG was used as a deliberate strategy to increase ovulation rate and thereby the chance of showing a beneficial effect of folic acid treatment on embryo survival (discussed later). In their control (no folic acid treatment) sows given PMSG, an ovulation rate of 28.6 was reported, compared with 19.2 and 21.5 ovulations, respectively, in the two control groups not treated with PMSG. These ovulation rates were associated with estimated embryonic survival rates of 41.8% in the PMSG-treated control sows compared with 60.8 and 63.0%, respectively, in the other groups. Thus, as in other studies, the increase in ovulation rate was completely offset by the increase in embryo loss and the estimated number of live fetuses at day 31–36 after mating was not different (12.2, 11.4 and 13.4, respectively). Personal discussions with breeding companies in recent months also raise another potentially important confounding effect that needs consideration when interpreting experiments on embryo survival. The advent of prolific dam lines for use with crosses to terminal line boars means that very different animals are used compared with those used in earlier experiments. Litter size in these prolific dam lines shows some increase with parity (for example 11 increasing to 13 born live) but this is apparently associated with an even greater parity effect on ovulation rate. In cyclic gilts that have not been mated, ovulation rates of 14 to 16 are typical, increasing to 18 to 20 ovulations in parity 2. However, in higher parity sows it appears that ovulation rates of 25 or greater are not uncommon. This discrepancy between the magnitude of the increase in litter size and ovulation rate suggests that prenatal survival over different parities is very different, probably decreasing from about 70% at parity 1 to 50% or less at

higher parities. Whether this relates to differences in embryo survival in the preimplantation period, or to fetal losses due to problems with uterine capacity in later gestation needs to be clarified. The data of Harper *et al.* (1996) suggest that the former is the case, as in their control groups, an increase in ovulation rate from 14.4 in parity 1 to 19.5 in parity 3 was associated with a decrease in embryo survival from 77.8% to 59.3% at day 40–48. However, on the basis of an expected litter size born alive of 10.9 in first parity sows in our herd fed to appetite during a 28 day lactation and mated at first oestrus (Clowes *et al.*, 1994), the data in Table 1 suggest that relatively low embryonic mortality (13%) in sows fed to appetite (Treatment 1) would be associated with losses in later gestation of the order of 35%. Parity and genotype may therefore result in very different patterns of prenatal loss and these differences should be appreciated when selecting populations of animals in which to study potential nutritional effects on embryo survival, and when the results of different experiments are compared.

Finally the contribution of the semen used and the pattern of breeding management to observed differences in embryo survival merits consideration. The standardization of any 'boar' effect among different experimental treatments is often difficult using natural matings, and yet an unbalanced use of boars will probably contribute unwanted non-treatment effects to the results of studies on embryo mortality. The logical and usually very practical solution is to use artificial insemination. If this is combined with the use of pooled semen from a group of boars designated to a particular experiment, then it is usually possible to inseminate gilts from all treatments with semen from the same collections and even to equalize the use of day 1 and day 2 semen among treatments.

Potential Mechanisms Mediating Nutritional Effects at Different Reproductive Stages

The preovulatory period

A range of follicle maturational stages has been reported in both gilts (Grant *et al.*, 1989; Hunter *et al.*, 1989) and sows (Foxcroft *et al.*, 1987) at the time of recruitment into the ovulatory population. This prompted speculation about the consequences of this follicular 'heterogeneity' for oocyte maturation, luteal function and subsequent embryo survival (Foxcroft and Hunter, 1985; Hunter and Wiesak, 1990). The essential stages of maturation through which the follicle and oocyte progress during the oestrous cycle were summarized by Foxcroft and Hunter (1985) and Pope (1994). These reviews included reference to a number of studies that indicate that nutritional effects on the size of the preovulatory pool of follicles is one factor determining ovulation rate in swine (Clark *et al.*, 1972; Dailey *et al.*, 1972; Clark *et al.*, 1973; Dailey *et al.*, 1975a,b). As the estimated time for an antral follicle to mature to the preovulatory stage is 19 days (Morbeck *et al.*, 1992), differences in nutritional and metabolic state could exert a protracted effect on the quality of the follicle and oocyte.

The essential role of the follicle in supporting the maturation of mammalian oocytes is well established and the hormonal microenvironment of the follicle has associations with oocyte maturation (Xie *et al.*, 1990a). Direct evidence for a functional relationship between follicular status and oocyte maturation came from studies of Ding and Foxcroft (1992, 1994) in which the origin of the follicle, or follicle conditioned medium, used to support the *in vitro* maturation of standardized pools of immature oocytes affected the outcome of the maturation process. More recently, as part of a series of studies comparing the endocrine and follicular characteristics of prolific Meishan to European commercial White-type gilts, Faillace and Hunter (1994) reported that oocyte maturation to metaphase II of the second meiotic division occurred earlier in the Meishan females. This raises the possibility that differences in the preovulatory development of the follicle and its enclosed oocyte could already be a contributory factor to differences in embryo survival. It seems likely that nutritional state could exert similar effects.

The results of an earlier study in pigs (Bazer *et al.*, 1968) and more recent studies in sheep (McKelvey *et al.*, 1988) led Ashworth (1994) to conclude that "...these data suggest that nutritional modifications affect embryo survival by altering maternal function rather than by influencing embryo viability per se". However, the series of experiments reported by McEvoy *et al.* (1995)

provided evidence for effects of nutritionally mediated differences in circulating progesterone in the preovulatory period in ewes on the developmental potential of the early embryo *in vitro*.

The extensive studies of Pope and his colleagues have provided compelling evidence that in pigs the preovulatory development of the follicle and oocyte has consequences for subsequent embryo survival (see Pope *et al.*, 1990). Variability in follicular development within the recruited pool of follicles affects the sequence of ovulation and use of electrocautery to change the population of ovulatory follicles also affected the variability in embryo development at day 11 of pregnancy (Pope *et al.*, 1988). Subsequently it was shown that the maturational state of oocytes in the periovulatory period was associated with their development as early zygotes (Xie *et al.* 1990b) and that later ovulating oocytes developed into the smallest embryos *in utero* and were less effective in stimulating uterine secretory activity (Xie *et al.*, 1990c). We therefore used the lactating and weaned sow paradigm of Zak *et al.* (1997a) described earlier to demonstrate that nutritionally induced differences in metabolic state in late lactation affect the state of nuclear maturation in oocytes recovered in the late follicular phase preceding the first post-weaning oestrus (Zak *et al.*, 1997b). Furthermore, follicular fluid recovered from the larger follicles of sows that were previously the most catabolic were less able to support nuclear maturation of oocytes in a standard *in vitro* maturation system. Although further evidence is required to relate such differences in preovulatory oocyte maturation to development after fertilization either *in vivo* or *in vitro* in pigs, the potential impact of nutritionally mediated effects on follicular development and hence on oocyte maturation cannot be ignored. The laborious nature of such studies in lactating and weaned sows has prompted the development of a comparable experimental paradigm involving nutritional manipulation within the 21 day oestrous cycle.

The periovulatory period

Clearly, any effects on the preovulatory development of the oocyte will carry over into the periovulatory period and the same nutritional effects could continue to influence pre- and post-fertilization events within the oviduct. Although it has been suggested that nutritional effects on the fertilization process probably contribute little to reproductive failure in pigs (Ashworth, 1994; Hunter, 1994), definitive information as to whether nutrition might influence oviductal function is lacking. Only a brief discussion of potential mechanisms that might be involved will therefore be presented.

As nutrition can affect both the number of follicles in the preovulatory pool and their steroidogenic activity, as measured by the concentration of follicular fluid oestradiol (Zak *et al.*, 1997b), it seems increasingly likely that there will be carry-over effects on the pattern of luteinization of these follicles. Therefore, assuming that oviductal secretions are functionally important during the periovulatory period (see Buhi, 1997) and that this secretory activity will be controlled by the prevailing steroidogenic stimulation at the level of the oviductal circulation, the timing of the switch from oestrogen to progesterone dominance could be critical. Hunter *et al.* (1988, 1996) described local mechanisms that appear to allow steroids to control periovulatory events, and evidence for the existence of effective local countercurrent exchange mechanisms in the sub-ovarian plexus was extensively reviewed by Krzymoski *et al.* (1990). The effectiveness of these mechanisms in concentrating progesterone in the oviductal circulation was demonstrated by Pharazyn *et al.* (1991b). Therefore, if a high plane of nutrition in the immediate postmating period results in a delayed rise in peripheral plasma progesterone concentration (Pharazyn, 1992; Jindal *et al.*, 1997), this will be reflected in an even earlier difference at the oviduct and there is a potential mechanism by which nutrition can exert a critical effect on oviductal function in the periovulatory period. If oviductal secretions affect the development of the cleaving embryo, nutrition could exert a direct effect via changes in the steroid environment of the Fallopian tube. Alternatively, nutritionally dependent changes in the steroid environment could affect the rate of tubal transport and hence the precise synchrony between the development of the uterus and the preimplantation embryo.

In the context of the earlier discussion of the need to define clearly the stage of development at which embryo mortality might be affected by nutrition, it is important to realise that when changes
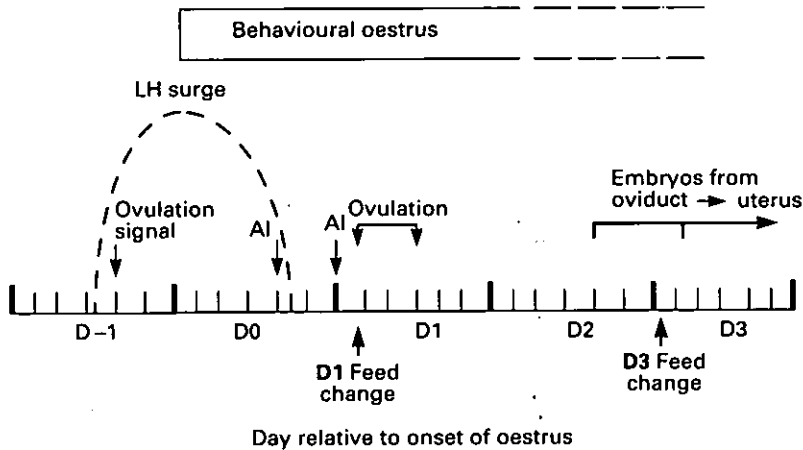
Fig. 2. Representation of the relationship between key events in the periovulatory period in the gilt and sow and key elements in the experimental design of the study of Jindal *et al.* (1996). By implementing a reduction of feed intake at day 1, effects on progesterone concentrations could exert effects at both the oviductal and uterine stages of development. By delaying the feed change to day 3, only uterine stages of development could be affected.

in nutrition are made in the immediate post-mating period there is the possibility that both the oviductal and uterine stages of development are affected (Fig. 2). However, if changes in nutritional state are delayed by only 1 or 2 days, only uterine effects would be involved. If future experiments do provide convincing evidence for nutritionally mediated effects at the oviductal stage of development, from a mechanistic viewpoint this may also help to explain why differences in the timing of nutritional treatments have produced such inconsistent effects on embryo survival.

The uterine phase of development

The literature on the physiology of the embryo and uterus in the preimplantation stages of pregnancy is already vast and has been reviewed by Bazer *et al.* (1982). A key feature of this period of development is the complex interaction between the developing blastocyst and the uterus (see Geisert *et al.*, 1982; Roberts *et al.*, 1993; Geisert and Yelich, 1997). For successful implantation to occur it is essential that the development of the embryo and uterus is synchronous (Pope, 1988). In some of the earliest embryo transfer experiments in pigs that addressed this question, Bazer *et al.* (1969) and Webel *et al.* (1970) established that a lack of synchrony of only 12 h affected blastocyst survival in the recipient uterus. Compared with monotocous species, in litter bearing species like the pig, asynchrony among embryos within a litter is an important factor in embryo survival. The relationships between follicular maturation, the time of ovulation and subsequent diversity of early embryo development has already been discussed and leads to functionally important differences in embryonic development at the crucial transitional stage of development (see Pope, 1994; Fig. 3). Therefore, if nutrition affects the developmental competence of the early embryo, or the variance in embryo development within a litter, this will compromise the ability of embryos to mature appropriately at the transitional stage and therefore survive the implantation process.

On its part, the uterus is required to progress through a sequence of developmental changes that prepare the endometrium for implantation and provide essential support to the developing blastocyst. Nutritional factors may affect this process indirectly by effects on progesterone secretion, or directly at the uterus.
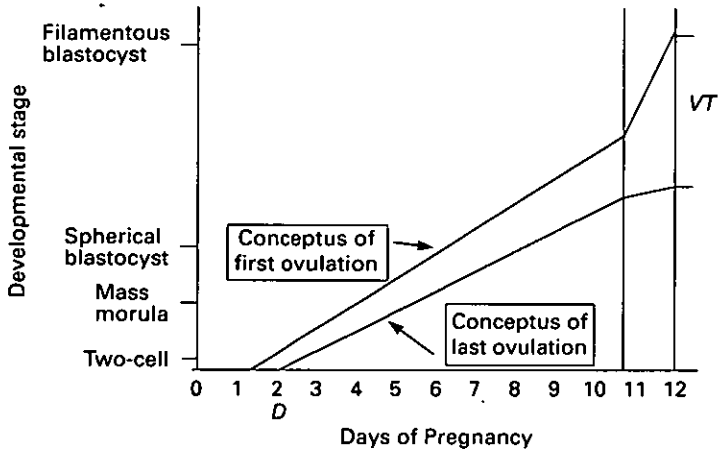
Fig. 3. Diagrammatic representation of the relationship between the
duration of ovulation (D), representing the time of ovulation of the first to
the last ovulating ovarian follicle, and the variance (VT) in embryo
diversity during the transitional period of development when blastocysts
undergo rapid elongation. Although the immediate impact of the spread
in ovulation time may appear limited, the complex interactions between
embryos of different maturity with the uterine environment greatly
amplifies developmental differences in this transitional stage of
development on day 11 to 12, and places the smaller embryos at risk.
(Modified from Pope, 1994.)

Progesterone-mediated effects. Effects of progesterone-induced changes in uterine secretory activity
on embryo development were first reported by Knight *et al.* (1974a) and the dose dependency of this
secretory effect of progesterone by Knight *et al.* (1974b). A more comprehensive review of the
regulation and functions of uterine secretions was presented by Roberts and Bazer (1988). There is
both indirect (Ashworth, 1991; Pharazyn, 1992; Jindal *et al,* 1996) and direct (Ashworth, 1991; Jindal
et al., 1997) evidence that reduced plasma progesterone concentrations in early pregnancy in gilts
may mediate the detrimental effects of high planes of nutrition on embryo survival. A positive
association between plasma progesterone concentrations 72 h after the onset of oestrus and embryo
survival has also been observed in primiparous weaned sows in which embryo survival was
depressed by nutritionally induced catabolism in late lactation (L.J. Zak, R. Jindal and G.R. Foxcroft,
unpublished); furthermore, Clowes *et al.* (1994) reported that the initial increase in plasma
progesterone concentrations was earlier in primiparous sows mated at the second compared with
the first oestrus after weaning and was associated with a significant increase in litter size born.
Finally, in a study of the endocrinology of the prolific Meishan breed, an earlier increase in plasma
progesterone concentrations was observed compared with European commercial·type gilts (Hunter
et al., 1996). On the strength of these observations, it seems reasonable to suggest that effects on
circulating progesterone concentrations may be an important mechanism by which nutrition and
metabolic state can influence embryo survival, and similar effects have been clearly demonstrated in
sheep (Parr *et al.,* 1982, 1987).

   Several mechanisms may contribute to nutritionally induced changes in progesterone secretion.
Prime and Symonds (1993) reported effects of the plane of nutrition on hepatic portal blood flow and
the metabolic clearance rate of progesterone in ovariectomized gilts, again supporting comparable
observations in sheep (Parr *et al.,* 1993a,b). However, it is also possible that the marked changes in
splanchnic circulation generally as a consequence of changes in feed intake may divert blood away
from the ovarian circulation. Even if progesterone secretion rates remain constant, changes in the
perfusion rate of the utero–ovarian vasculature may markedly affect the efficiency of the
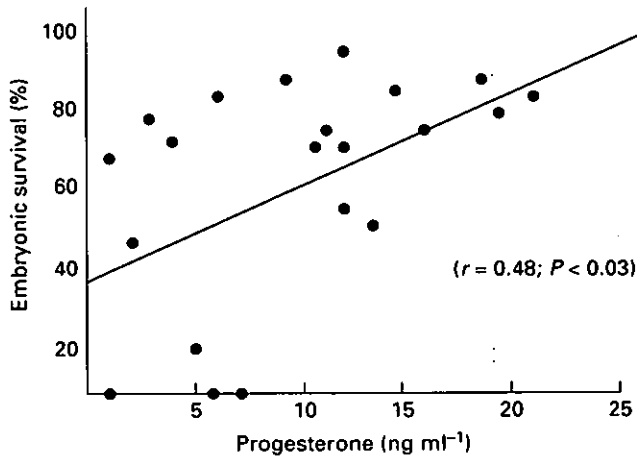
Fig. 4. Relationship between plasma progesterone at 72 h after onset of oestrus and embryonic survival (ES) at day 28 of pregnancy in primiparous sows subjected to severe feed restriction during the last week of a 28 day lactation and mated at the first postweaning oestrus (L.J. Zak, R. Jindal and G.R. Foxcroft, unpublished data).

countercurrent exchange mechanism and thus the distribution of steroids between the local and peripheral circulation. Finally, there is also the possibility, alluded to previously, that the prior effects of nutrition on the steroidogenic competence of the periovulatory follicle may translate into important differences in the pattern of luteinization of these follicles in the periovulatory period. Again, the time at which critical thresholds for progesterone actions were reached, either locally or peripherally, may be functionally much more important than the eventual peak concentrations of progesterone in the circulation.

Returning to the need for effective synchrony between embryonic and uterine development, the above studies may indicate the essential timing involved in demonstrating these effects of nutrition on embryo survival. By delaying the time at which plasma progesterone reaches the threshold for stimulating the normal cascade of endometrial changes, high planes of nutrition may effectively deprive less developed embryos of essential support and thus compromise their ability to survive the transitional stage of development. Furthermore, the ability to demonstrate that exogenous progesterone can reverse this effect may rely to a great extent on the fact that the nutritionally induced increase in embryo survival is indeed progesterone mediated. In other instances in which progesterone therapy has failed to improve embryo survival this situation may not apply. Because the pig is polyovulatory, the likelihood of plasma progesterone concentrations being below some essential threshold in established pregnancy is probably low; in contrast in monovulatory species like the ewe and cow, and in women, luteal insufficiency is more likely to be a cause of infertility in early pregnancy. Thus in pigs, the critical window of time during which progesterone-mediated effects of nutrition (or any other factor) will be observed is probably limited to the first 3–4 days after ovulation. If progesterone mediated effects of nutrition on early embryo survival can be consistently demonstrated, these experimental paradigms need to be exploited to determine the changes in uterine function involved.

Direct nutritional effects on the conceptus or uterus. Although a multitude of nutritional effects on embryo survival have been reported that might exert their effects at the uterus, the effects of folic acid, β-carotene and vitamin A provide some insight into the mechanisms that might be involved. The discussion of the effect of specific nutrient effects at the uterus will therefore be limited to these effects.

Although an effect of folic acid treatment on litter size and embryonic survival has not been consistently established using accepted statistical criteria, in summarizing published data from previous experiments, Lindemann (1993) concluded that "The likelihood of 11 positive responses in 11 trials, if in fact the response is not real, is 1/2,048..." and therefore that folic acid treatment effects on embryo survival were real. After some initial experiments which identified the potential for obtaining beneficial effects with folic acid treatment, the physiological basis for effects of folic acid was explored in a series of studies by Matte, Tremblay and their colleagues. A decline in serum folate concentrations after weaning and through the breeding and early gestation period in sows (Matte *et al.*, 1982) suggested that the demand for folic acid was not being met from dietary sources in this critical period of the reproductive cycle. This deficiency could be corrected with folic acid injections (Matte *et al.* 1984a), and treatment at weaning, mating and periodically up to week 12 of gestation resulted in a significant improvement in litter size born (Matte *et al.*, 1984b). Later studies demonstrated the effectiveness of dietary supplementation with folic acid in maintaining serum folate concentrations (Tremblay *et al.*, 1986) but variable responses in both litter size and embryonic mortality to such dietary supplementation have been reported (Lindemann and Kornegay, 1989; Thaler *et al.*, 1989; Tremblay *et al.*, 1989a; Harper *et al.*, 1994, 1996). In general, the greatest responses to folic acid supplementation were observed in situations in which potential litter size was increased by an initial increase in ovulation rate, due to 'flush' feeding in the preovulatory period (Matte *et al.*, 1984b), treatment with PMSG (Tremblay *et al.*, 1989a) or increased parity of the sows studied (Harper *et al.*, 1996). The potential for folic acid supplementation to be more effective when the number of implanting embryos increases is consistent with the suggestion that folic acid may act by supporting DNA synthesis at critical stages of embryonic development (see Lindemann, 1993). Even if embryonic survival is not improved by folic acid treatment, an increase in fetal protein content (Tremblay *et al.*, 1989a; Harper *et al.*, 1996) and a trend for increased placental growth (Harper *et al.*, 1996) suggest that postimplantation survival may also contribute to overall effects on litter size at term. Beyond the suggestion that folic acid supplementation may prevent deficiencies in DNA synthesis in the early conceptus, little else is known about its mechanism of action, although Tremblay *et al.* (1989b) reported interactions with serum zinc concentrations during early gestation.

The role of vitamin A and its provitamin, β-carotene, in pig reproduction were reviewed by Chew (1993). Because of reported effects of retinoic acid *in vitro* on progesterone secretion from porcine luteal cells (Talavera and Chew, 1988), effects of vitamin A might be mediated indirectly through differences in circulating progesterone, with implications for effects on any progesterone-dependent changes in endometrial function. However, in the light of the existence of retinol-binding proteins in both the endometrium (Adams *et al.*, 1981) and conceptus (Harney *et al.*, 1990) and cellular mechanisms by which retinoic acid can affect cellular metabolism and gene expression by binding to nuclear receptors (see Chew, 1993), the retinoids can potentially have very direct effects on uterine function, and conceptus growth and differentiation.

Consistent with some of these possible mechanisms of action, Chew *et al.* (1982) reported an increase in uterine-specific proteins on day 15 of gestation after injecting gilts daily with β-carotene from the day of mating. This work was followed by the study of Brief and Chew (1985) in which various combinations of dietary and injected vitamin A and β-carotene were administered to gilts for one oestrous cycle before mating until weaning at day 21. Although there is extensive reference in the results to significant effects of both vitamin A and β-carotene injections on 'embryonic survival', only an increase in final litter size born was actually recorded and clear evidence that effects on embryonic development was an important component of the response was not provided. Actual improvements in embryo survival *per se* were later reported by Britt *et al.* (1992) in gilts in response to injections of retinol palmitate on day 15 of the oestrous cycle before breeding, and this treatment was also able to counteract the detrimental effects of high plane feeding after mating. There was also a suggestion in this report that the effectiveness of the treatment might have been related to reduced variance in embryo development at day 11–12 of gestation. A subsequent study (Coffey and Britt, 1993) involved large numbers of primiparous and multiparous sows already provided with diets supplemented with vitamin A. In Expt 1, sows received single injections of increasing doses of β-carotene (n = > 166 sows per dose) on the day of weaning. Although an overall effect of β-carotene
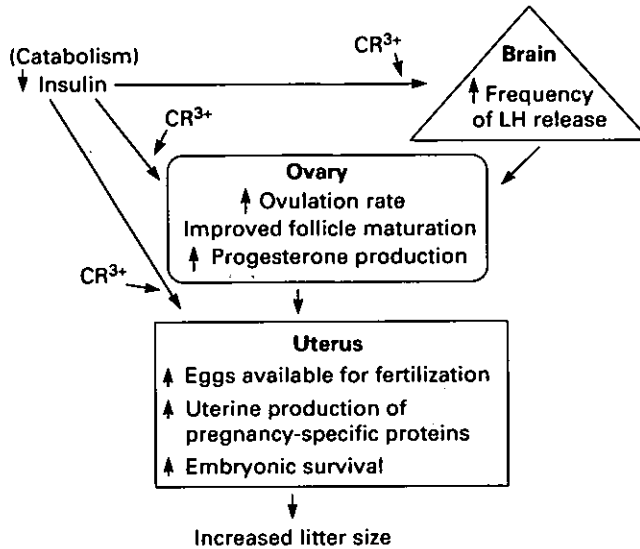
Fig. 5. Modification of the conceptual diagram of Trout (1995) indicating the mechanisms by which chromium (Cr^{3+}) may potentiate the effects of insulin and therefore improve reproductive function in insulin-deficient sows. The critical point is that chromium alone has no direct beneficial effects on reproduction; rather it exerts its effects by altering the overall metabolic state of the sow.

on litter size born failed to reach significance, there was evidence for a treatment by parity interaction, and in multiparous sows a linear increase in litter size born alive was recorded over the dose range of 0–200 mg. These effects were associated with an increase in plasma β-carotene on day 6 and 13 after injection but not at day 18, and no increase in plasma vitamin A compared with controls. No effect of treatment on plasma progesterone was recorded, although as samples were taken at approximately day 14 of pregnancy, this may not be very meaningful. On the assumption that these effects of β-carotene were direct (i.e. not mediated by changes in plasma vitamin A concentrations), and that increased plasma concentrations of β-carotene may need to be maintained beyond day 13 of treatment, in Expt 2 sows received repeated injections of either vitamin A, β-carotene or control vehicle (n = 140 per treatment) on the day of weaning, the day of mating and on day 7 after mating. A marginal effect of both vitamin A and β-carotene treatments on litter size born alive (10.6 in both treated groups compared with 10.0 in controls; $P < 0.10$) was reported. In the light of these generally positive responses to treatment with either vitamin A or β-carotene, it was suggested that effects on endometrial glandular secretion might be involved.

As with the studies on folic acid, there therefore appears to be evidence that there may be a beneficial response to vitamin A and β-carotene on embryo survival, but that in this case an inherent deficiency in either vitamin A or β-carotene may not be needed to show a response to supplementation. In the different studies reviewed, it is not always certain whether vitamin A and β-carotene are exerting independent effects and it will be important to clarify this in future studies. It is also uncertain whether treatment in the preovulatory period is essential for producing consistent responses. Therefore the mechanism of action may include effects on follicle and oocyte maturation, as well as effects on the uterus.

Indirect nutritional effects on the conceptus or uterus. A good example of how this might occur is seen in recent literature related to potential effects of chromium on reproductive function in sows.

The mechanisms mediating effects of chromium supplementation on growth performance in swine was recently extensively reviewed by White *et al.* (1993) and these authors stressed a number of important points. Firstly, chromium is a nutrient and not a therapeutic agent, and therefore its mechanism of action must be explained in terms of its metabolic effects. Secondly, effects of chromium were seen only in situations of deficiency but there was evidence that traditional diets used in swine production could be chromium deficient. Finally, at a mechanistic level chromium acted as an essential cofactor for insulin and therefore many of the clinical responses to chromium deficiency were analogous to insulin-dependent diabetes.

Given this scenario, and the discussion of insulin-mediated effects on reproductive function reviewed above, it is not unexpected that evidence for effects of chromium deficiency on sow fertility have been identified (Lindemann *et al.*, 1995). In addition, given the proven and potential effects of insulin at all levels of the reproductive system, it is difficult to identify the precise mechanism by which chromium supplementation would affect embryo survival *per se*. The various possibilities were summarized by Trout (1995) and a modification of his conceptual outline of these mechanisms is shown in Fig. 5. The importance of this particular example is that it illustrates that by understanding that the mechanism of action of chromium is to potentiate insulin-mediated effects on all tissues, we can also appreciate why it may have very diverse effects on reproductive function. In some of the examples of specific nutrient effects discussed earlier, it appears that the mechanism of action may be much more specific.

Conclusion

Building on previous reviews, this paper specifically addressed the question of nutritional effects on embryo survival at a mechanistic level. There seems to be convincing evidence that there are effects of nutrition and metabolic state on embryo survival in gilts and sows. Not surprisingly, the clearest responses to changing nutritional state occur when nutrition is limiting and in this situation many effects at all levels of the hypothalamo–hypophysial–ovarian axis can be anticipated. Furthermore, in situations in which embryo survival is limited by inadequate nutrition, a number of specific nutrients, as well as an overall increase in total dietary intake may produce positive responses.

From a general perspective, there seems to be increasing evidence that a number of the effects of nutrition and metabolic state on embryonic survival are mediated by progesterone. However, the precise mechanisms involved need to be elucidated and a concerted effort is needed to determine the timing and the location of progesterone-mediated effects.

A final comment relates to the conceptual outlines shown in Figs 1 and 3. The hypothalamic–hypophysial release of gonadotrophins, and particularly LH, is the essential driver of ovarian development. In many situations in which the nutrition of the gilt or sow is compromised, episodic release of LH is suppressed (see review of Foxcroft *et al.*, 1995). In these situations the lack of tonic LH secretion may have consequences for follicular development that will carry over to the time of ovulation and affect oocyte quality and luteal function. However, although there has been speculation that differences in LH secretion may affect subsequent fertility (for instance in the catabolic, first parity sow bred at first oestrus after weaning), there is no convincing evidence that these reproductive characteristics are functionally linked. It is possible that the pattern of LH secretion merely reflects the energy or protein status of the sow and that effects of metabolic state at the ovary or uterus mediate the decrease in subsequent fertility. A clearer definition of these possibilities is therefore needed. We are presently using GnRH-induced increases in LH secretion during periods of feed restriction in late lactation in sows to address this question.

References

Adams KL, Bazer FW and Roberts RM (1981) Progesterone-induced secretion of a retinol binding protein in the pig uterus *Journal of Reproduction and Fertility* 62 39–47

Aherne FX and Kirkwood RN (1985) Nutrition and sow prolificacy *Journal of Reproduction and Fertility Supplement* 33 169–183

Anderson LL and Melampy RM (1972) Factors affecting ovulation rate in the pig. In *Pig Production* Ed. DJA Cole. Butterworths, London

Ashworth CJ (1991) Effect of pre-mating nutritional status and post-mating progesterone supplementation on embryo survival and conceptus growth in gilts *Animal Reproduction Science* 26 311–321

Ashworth CJ (1994) Nutritional factors related to embryonic mortality in the domestic species. In *Embryonic Mortality in Domestic Species* pp 179–194 Eds MT Zavy and RD Geisert. Boca Raton, Florida

Bazer FW, Clawson AJ, Robinson OW, Vincent CK and Ulberg LC (1968) Explanation for embryo death in gilts fed a high energy intake *Journal of Animal Science* 27 1021–1026

Bazer FW, Clawson AJ, Robinson OW and Ulberg LC (1969) Uterine capacity in gilts *Journal of Reproduction and Fertility* 18 121–124

Bazer FW, Geisert RD, Thatcher WW and Roberts RM (1982) The establishment and maintenance of pregnancy. In *Control of Pig Reproduction* pp 227–252 Eds DJA Cole and GR Foxcroft. Butterworths, London

Brief S and Chew BP (1985) Effects of vitamin A and β-carotene on reproductive performance in gilts *Journal of Animal Science* 60 998–1004

Britt JH, Whaley SL and Hedgpeth VS (1992) Improvement of embryo survival by injection of vitamin A in gilts fed normal or high-energy diets before and after mating *Journal of Animal Science* 70 (Supplement 1) 271

Buhi WC (1997) Oviductal regulation of fertilization and early embryonic development *Journal of Reproduction and Fertility Supplement* 52 285–300

Chew BP (1993) Effects of supplemental β-carotene and vitamin A on reproduction in swine *Journal of Animal Science* 71 247–252

Chew BP, Rasmussen H, Pubols MH and Preston RL (1982) Effects of vitamin A and β-carotene on plasma progesterone and uterine protein secretions in gilts *Theriogenology* 18 643–654

Clark JR, Daily RA, First NL, Chapman AB and Casida LE (1972) Effect of feed level and parity on ovulation rate in three genetic groups of swine *Journal of Animal Science* 35 1216–1222

Clark JR, Edey TN, First NL, Chapman AB and Casida LE (1973) The effects of four genetic groups and two levels of feeding on ovulation rate and follicular development in pubertal gilts *Journal of Animal Science* 36 1164–1169

Clowes EJ, Aherne FX and Foxcroft GR (1994) Effect of delayed breeding on the endocrinology and fecundity of sows *Journal of Animal Science* 72 283–291

Coffey MT and Britt JH (1993) Enhancement of sow reproductive performance by β-carotene or vitamin A *Journal of Animal Science* 71 1198–1202

Cox NM (1997) Regulation of follicular development and ovulation rate *Journal of Reproduction and Fertility Supplement* 52 31–46

Dailey RA, Clark JR, First NL, Chapman AB and Casida LE (1972) Effects of high and low feeding at two stages of the oestrous cycle on follicular development in gilts from four genetic groups *Journal of Animal Science* 35 1210–1222

Dailey RA, Clark JR, First NL, Chapman AB and Casida LE (1975a) Loss of follicles during the follicular phase of the estrous cycle of swine as effected by genetic group and level of feed intake *Journal of Animal Science* 41 835–841

Dailey RA, Clark JR, First NL, Chapman AB and Casida LE (1975b) Effect of short-term 'flushing' on follicular development at estrus and ovulation rate of gilts of different genetic groups *Journal of Animal Science* 41 842–848

Ding J and Foxcroft GR (1992) Follicular heterogeneity and oocyte maturation *in vitro* in pigs *Biology of Reproduction* 47 648–655

Ding J and Foxcroft GR (1994) Conditioned media produced by follicular shells of different maturity affect maturation of pig oocytes *Biology of Reproduction* 50 1377–1384

Faillace LS and Hunter MG (1994) Follicle development and oocyte maturation during the immediate preovulatory period in Meishan and white hybrid gilts *Journal of Reproduction and Fertility* 101 571–576

Foxcroft GR and Hunter MG (1985) Basic physiology of follicular maturation in the pig *Journal of Reproduction and Fertility Supplement* 33 1–19

Foxcroft GR, Shaw HJ, Hunter MG, Booth PJ and Lancaster RT (1987) Relationship between luteinizing hormone, follicle-stimulating hormone, prolactin secretion and ovarian follicular development in weaned sows *Biology of Reproduction* 36 175–191

Foxcroft GR, Aherne FX, Clowes EJ, Miller HM and Zak LJ (1995) Sow fertility: the role of suckling inhibition and metabolic status. In *Animal Science Research and Development: Moving Towards a New Century* pp 377–391 Ed. M Ivan. Center for Food and Animal Research, Ottawa

Geisert RD and Yelich J (1997) Regulation of conceptus development and attachment *Journal of Reproduction and Fertility Supplement* 52 133–149

Geisert RD, Renegar RH, Thatcher WW, Roberts RM and Bazer FW (1982) Establishment of pregnancy in the pig. I. Interrelationship between preimplantation development of the pig blastocyst and uterine endometrial secretions of the pig blastocyst and uterine endometrial secretions *Biology of Reproduction* 27 925–939

Grant SA, Hunter MG and Foxcroft GR (1989) Morphological and biochemical characteristics during ovarian follicular development in the pig *Journal of Reproduction and Fertility* 86 171–183

Harney JP, Mirando MA, Smith LC and Bazer FW (1990) Retinol binding protein: a major secretory product of the pig conceptus *Biology of Reproduction* 42 523–532

Harper AF, Lindemann MD, Chiba LI, Combs GE, Handlin DL, Kornegay ET and Southern LL (1994) An assessment of dietary folic acid levels during gestation and lactation on reproductive and lactational performance of sows: a cooperative study *Journal of Animal Science* 72 2338–2344

Harper AF, Lindemann MD and Kornegay ET (1996) Fetal survival and conceptus development after 42 days of gestation in gilts and sows in response to folic acid supplementation *Canadian Journal of Animal Science* 76 157–160

Hunter MG and Wiesak T (1990) Evidence for and implications of follicular heterogeneity in pigs *Journal of Reproduction and Fertility Supplement* 40 163–171

Hunter MG, Southee JA and Lamming GE (1988) Function of abnormal corpora lutea *in vitro* after GnRH-induced

ovulation in the anoestrous ewe *Journal of Reproduction and Fertility* 84 139–148

Hunter MG, Grant SA and Foxcroft GR (1989) Histological evidence for heterogeneity in the development of preovulatory pig follicles *Journal of Reproduction and Fertility* 86 165–170

Hunter MG, Picton HM, Biggs C, Mann GE, McNeilly AS and Foxcroft GR (1996) Periovulatory endocrinology in high ovulating Meishan sows *Journal of Endocrinology* 150 141–147

Hunter RHF (1994) Causes for failure of fertilization in domestic species. In *Embryonic Mortality in Domestic Species* pp 1–22 Eds MT Zavy and RD Geisert. Boca Raton, Florida

I'Anson H, Foster DL, Foxcroft GR and Booth PJ (1991) Nutrition and reproduction *Oxford Reviews of Reproductive Biology* 13 239–311

Jindal R, Cosgrove JR, Aherne FX and Foxcroft GR (1996) Effect of nutrition on embryonal mortality in gilts: association with progesterone *Journal of Animal Science* 74 620–624

Jindal R, Cosgrove JR and Foxcroft GR (1997) Progesterone mediates nutritionally induced effects on embryonic survival in gilts *Journal of Animal Science* 75 1063–1070

Knight JW, Bazer FW and Wallace HD (1974a) Effect of progesterone induced increase in uterine secretory activity on development of porcine conceptus *Journal of Animal Science* 39 743–746

Knight JW, Bazer FW, Wallace HD and Wilcox CJ (1974b) Dose–response relationship between exogenous progesterone and estradiol and porcine uterine protein secretions *Journal of Animal Science* 39 747–751

Krzymowski T, Kotwica J and Stefanczyk-Krzymowska S (1990) Uterine and ovarian countercurrent pathways in the control of ovarian function in the pig *Journal of Reproduction and Fertility Supplement* 40 179–191

Liao CW and Veum TL (1994) Effects of dietary energy intake by gilts and heat stress from days 3 to 24 or 30 after mating on embryo survival and nitrogen and energy balance *Journal of Animal Science* 72 2369–2377

Lindemann MD (1993) Supplemental folic acid: a requirement for optimizing swine reproduction *Journal of Animal Science* 71 239–246

Lindemann MD and Kornegay ET (1989) Folic acid supplementation to diets of gestating lactating swine over multiple parities *Journal of Animal Science* 67 459–464

Lindemann MD, Wood CM, Harper AF, Kornegay ET and Anderson RA (1995) Dietary chromium picolinate additions improve gain: feed and carcass characteristics in growing–finishing pigs and increase litter size in reproducing sows *Journal of Animal Science* 73 457–465

McEvoy TG, Robinson JJ, Aitken RP, Findlay PA, Palmer RM and Robertson IS (1995) Dietary-induced suppression of pre-ovulatory progesterone concentrations in superovulated ewes impairs the subsequent *in vivo* and *in vitro* development of their ova *Animal Reproduction Science* 39 89–107

McKelvey WAC, Robinson JJ and Aitken RP (1988) The use of reciprocal embryo transfer to separate the effects of pre- and post-mating nutrition on embryo survival and growth of the ovine conceptus *Proceedings of the 11th International Congress for Animal Reproduction and Artificial Insemination, Dublin* (Abstract 176)

Matte JJ, Girard CL and Brisson GJ (1982) Serum folates levels during the reproductive cycle of sows *Journal of Animal Science* 55 (Supplement 1) 284

Matte JJ, Girard CL and Brisson GJ (1984a) Folic acid and reproductive performance of sows *Journal of Animal Science* 59 1020–1025

Matte JJ, Girard CL and Brisson GJ (1984b) Serum folates during the reproductive cycle of sows *Journal of Animal Science* 59 158–163

Morbeck DE, Esbenshade KL, Flowers WL and Britt JH (1992) Kinetics of follicle growth in the prepubertal gilt *Biology of Reproduction* 47 485–491

Parr RA, Cumming IA and Clark IJ (1982) Effects of maternal nutrition and plasma progesterone concentrations on survival and growth of the sheep embryo in early gestation *Journal of Agricultural Science* 98 39–46

Parr RA, Davis IF, Fairclough RJ and Moss MA (1987) Overfeeding during early pregnancy reduces peripheral progesterone concentration and pregnancy rate in sheep *Journal of Reproduction and Fertility* 80 317–320

Parr RA, Davis IF, Miles MA and Squires TJ (1993a) Feed intake affects metabolic clearance rate of progesterone in sheep *Research in Veterinary Science* 55 306–310

Parr RA, Davis IF, Miles MA and Squires TJ (1993b) Liver blood flow and metabolic clearance rate of progesterone in sheep *Research in Veterinary Science* 55 311–316

Pharazyn A (1992), *Edmonton, Canada Nutritional Effects on Embryo Survival in the Gilt* PhD Thesis, University of Alberta, Edmonton

Pharazyn A, Den Hartog LA, Foxcroft GR and Aherne FX (1991a) The influence of dietary energy and protein intake during early pregnancy on plasma progesterone and embryo survival *Canadian Journal of Animal Science* 71 949–952

Pharazyn A, Foxcroft GR and Aherne FX (1991b) Temporal relationship between plasma progesterone concentrations in the utero–ovarian and jugular veins during early pregnancy in the pig *Animal Reproduction Science* 26 323–332

Pope WF (1988) Uterine asynchrony: a cause of embryonic loss *Biology of Reproduction* 39 999–1003

Pope WF (1994) Embryonic mortality in swine. In *Embryonic Mortality in Domestic Species* pp 53–77 Eds MT Zavy and RD Geisert. Boca Raton, Florida

Pope WF, Wilde MH and Xie S (1988) Effect of electro-cautery of nonovulated day 1 follicles on subsequent morphological variation among day 11 porcine embryos *Biology of Reproduction* 39 882–887

Pope WF, Xie S, Broermann DM and Nephew KP (1990) Causes and consequences of early embryonic diversity *Journal of Reproduction and Fertility Supplement* 40 250–260

Prime GR and Symonds HW (1993) The influence of plane of nutrition on portal blood flow and the metabolic clearance rate of progesterone on ovariectomized gilts *Journal of Agricultural Science* 121 389–397

Roberts RM and Bazer FW (1988) The function of uterine secretions *Journal of Reproduction and Fertility* 82 875–892

Roberts RM, Xie S and Trout WE (1993) Embryo–uterine interactions in pigs during week 2 of pregnancy *Journal of Reproduction and Fertility Supplement* 48 171–186

Schultz JR, Speer VC, Hays VW and Melampy RM (1966) Influence of feed intake and progesterone on reproductive performances in swine *Journal of Animal Science* 25 157–160

Talavera F and Chew BP (1988) Comparative role of retinol, retinoic acid and β-carotene on progesterone secretion by pig corpus luteum *in vitro*. *Journal of Reproduction and Fertility* 82 611–615

Thaler RC, Nelssen JL, Goodband RD and Allee GL (1989) Effect

of dietary folic acid supplementation on sow performance through two parities *Journal of Animal Science* 67 3360–3369

Tremblay GF, Matte JJ, Lemieux L and Brisson GJ (1986) Serum folates in gestating swine after folic acid addition *Journal of Animal Science* 63 1173–1178

Tremblay GF, Matte JJ, Dufour JJ and Brisson GJ (1989a) Survival rate and development of fetuses during the first 30 days of gestation after folic acid addition to a swine diet *Journal of Animal Science* 67 724–732

Tremblay GF, Matte JJ, Girard CL and Brisson GJ (1989b) Serum zinc, iron and copper status during early gestation in sows fed a folic acid supplement diet *Journal of Animal Science* 67 733–737

Trout WE (1995) Hypothesis provides possible explanation as to chromium's effect on reproductive efficiency in swine *Feedstuffs* 67 12–23

Webel SK, Peters JB and Anderson LL (1970) Synchronous and asynchronous transfer of embryos in the pig *Journal of Animal Science* 30 565–568

White M, Pettigrew J, Zollitsch-Stelzl J and Crooker B (1993) Chromium in swine diets. In *Proceedings of the 54th Minnesota Nutrition Conference and National Renderers Technical Symposium* pp 251–261

Xie S, Broermann DM, Nephew KP, Ottobre JS, Day ML and Pope WF (1990a) Changes in follicular endocrinology during final maturation of porcine oocytes *Domestic Animal Endocrinology* 7 75–82

Xie S, Broermann DM, Nephew KP, Bishop MD and Pope WF (1990b) Relationship between oocyte maturation and fertilization of zygotic diversity in swine *Journal of Animal Science* 68 2027–2033

Xie S, Broermann DM, Nephew KP, Geisert RD and Pope WF (1990c) Ovulation and early embryogenesis in swine *Biology of Reproduction* 43 236–240

Zak LJ, Cosgrove JR, Aherne FX and Foxcroft GR (1997a) Pattern of feed intake and associated metabolic and endocrine changes differentially affect postweaning fertility in primiparous lactating sows *Journal of Animal Science* 75 208–216

Zak LJ, Xu X, Hardin RT and Foxcroft GR (1997b) Impact of different patterns of feed intake during lactation in the primiparous sow on follicular development and oocyte maturation *Journal of Reproduction and Fertility* 110 99–106