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Control of follicular development and ovulation rate in pigs

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There is considerable evidence that nutritional and metabolic control of follicular growth is mediated by metabolic hormones and growth factors, particularly with processes mediated by insulin-like growth factor I (IGF-I) and its binding proteins (IGFBPs). From knowledge that hormones and growth factors which can be affected by diet also positively affect ovarian function, the concept has emerged that metabolic modifiers of gonadotrophin action, rather than gonadotrophins themselves, could affect follicle development. While ovulation rate can be enhanced under certain conditions in cyclic gilts, assessing influences of metabolic modifiers on the post-lactational sow is confounded by variability in the return to oestrus after weaning. In a series of studies involving insulin administration between weaning and oestrus, successive experiments produced different results, but several measures of reproductive performance were enhanced. Administration of somatotrophin (ST) has also been shown to increase follicular development in both gilts and sows. Both insulin and ST increase IGF-I production by pig ovarian follicles, and insulin is more effective than IGF-I in reducing atresia and increasing progesterone in cultured pig follicles. Whether increases in litter size are achieved after an increase in ovulation rate involves many factors, including the quality of ova and whether the increase in ovulation rate exceeds the uterine capacity to maintain pregnancy. Given the variation in genetics and management practices, development of treatments to enhance follicle quality leading to maximal litter size is challenging.

Introduction

Although it has long been known that exogenous gonadotrophins increase follicular development and ovulation rate, the number of ovulations is variable and may have negative effects on embryo survival. Nutritional and hormonal manipulation may enhance follicular development, both by enhancing gonadotrophin secretion directly and by enhancing the ovarian response in the face of unvarying gonadotrophin concentrations. Given the knowledge that hormones and growth factors that can be affected by diet also positively affect ovarian function, the concept that metabolic modifiers, rather than gonadotrophins themselves, could be applied to affect follicle development has emerged. This review will concentrate mainly on metabolic interfaces between nutrition and reproduction rather than nutritional manipulations themselves. Emphasis will be on follicle development rather than embryo survival; effects of metabolic manipulations on embryo survival are discussed by Foxcroft (this volume).

When consideration is given to increasing ovulation rate, there are practical limitations. First, while ovulation rate and litter size are positively correlated up to approximately 18 ovulations, ovulations beyond that point result in little or no increases in litter size (Wu *et al.*, 1987). In mature sows and gilts on full feed, ovulation rates are usually maximal, and manipulation of follicular

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development to increase ovulation rate will not be useful. However, in animals with lower ovulation rates, such as gilts on the first or second oestrous cycle and primiparous sows, augmentation of ovulation rate may be useful.

Current Concepts of Follicle Growth, Atresia and Ovulation Rate

It is well established that the ovulatory population of follicles increases growth between about day 14 and day 16 of the oestrous cycle. Granulosa and theca cell proliferation decreases between day 15 and oestrus, which indicates differentiation (Fricke *et al.*, 1996). Between day 16 and oestrus, there is a loss of 40–50% of the medium-sized follicles due partly to growth of follicles to the next size category but mostly to atresia (Guthrie *et al.*, 1995a). Exposure to FSH during the last stages of granulosa cell mitosis appears to be required so that some follicles can escape atresia (Hsueh *et al.*, 1994). In pigs, FSH patterns were temporally correlated with atresia, decreasing at the time atresia increased (Guthrie *et al.*, 1993). Apoptotic cell death in the ovary has been characterized by several biochemical markers including decreased DNA turnover, decreased oestrogen production, increased progesterone production, a decrease in the number of gonadotrophin receptors and increased production of IGF-binding protein (Hsueh *et al.*, 1994). Thus follicular atresia is considered to be a result of a balance between survival and atretogenic factors. Follicle survival factors include, but are not limited to, epidermal growth factor, nerve growth factor, insulin-like growth factor I (IGF-I), gonadotrophins, activin and oestrogens, while atretogenic factors include testosterone, GnRH and interleukins (Hsueh *et al.*, 1994).

In the pig ovary as in the ovaries of other species, apoptotic cell death is the mechanism by which follicular atresia occurs (Guthrie *et al.*, 1995a). Guthrie *et al.* (1995a) demonstrated that apoptosis could be assessed in gilts by flow cytometric analysis and that these measures were correlated highly with oestradiol production and with morphological assessment of atresia. Atresia was highest in medium-sized (3–6 mm) follicles (87%) on day 5 after withdrawal of Altrenogest (Guthrie *et al.*, 1994), which corresponds to the late stages of preovulatory maturation before the LH surge. In contrast, the percentage of atretic granulosa cells was 17% in medium-sized follicles on days 1 and 3 after Altrenogest withdrawal, which corresponds to earlier stages of preovulatory follicular development (Guthrie *et al.*, 1994). These researchers also demonstrated that during the early luteal phase, there is a wave of follicular growth followed by atresia (Guthrie *et al.*, 1995a). In contrast, during follicular growth in the latter part of the luteal phase between day 7 and day 15, there was no change in the percentage of atretic follicles, indicating that follicular growth in pigs may not be characterized by repeated waves of follicular development (Guthrie and Cooper, 1996). The most severely affected enzyme was p450 aromatase, which was undetectable in atretic follicles (Garrett and Guthrie, 1996). In contrast, 3β-hydroxysteroid dehydrogenase and $P450_{c17}$ lyase were not affected by atresia, which is in agreement with other work which demonstrates that progesterone production continues in atretic follicles (Guthrie *et al.*, 1994). Figure 1 illustrates identification of Ki-67, a nuclear antigen associated with cell proliferation, aromatase and detection of apoptosis in atretic and nonatretic follicles. Since oestrogen is a follicle survival factor that reduces atresia (Hsueh *et al.*, 1994), aromatase expression may be critical for survival of follicles.

Techniques for enhancing follicular development include methods that may either influence negative feedback relationships with gonadotrophins or intrafollicular control mechanisms. Immunization against inhibin (King *et al.*, 1993) and androstenedione (McKinnie *et al.*, 1988) increase ovulation rate but their effects on litter size have not been evaluated. However, immunization against the α-subunit of inhibin prevented the compensatory increase in corpora lutea following unilateral ovariectomy, suggesting a local effect of inhibin (King *et al.*, 1995). There is increasing evidence for intraovarian paracrine regulation by inhibin and related peptides, and further refinement of these relationships may result in more predictable control of ovulatory processes (Findlay *et al.*, 1996). Manipulation of the steroid environment may also influence ovulation rate. When testosterone was administered between day 13 and oestrus, ovulation rate and the number of recovered blastocysts were increased (Cardenas and Pope, 1997). Part of the mechanism may be provision of a substrate for oestradiol production, but the mechanism is not known.

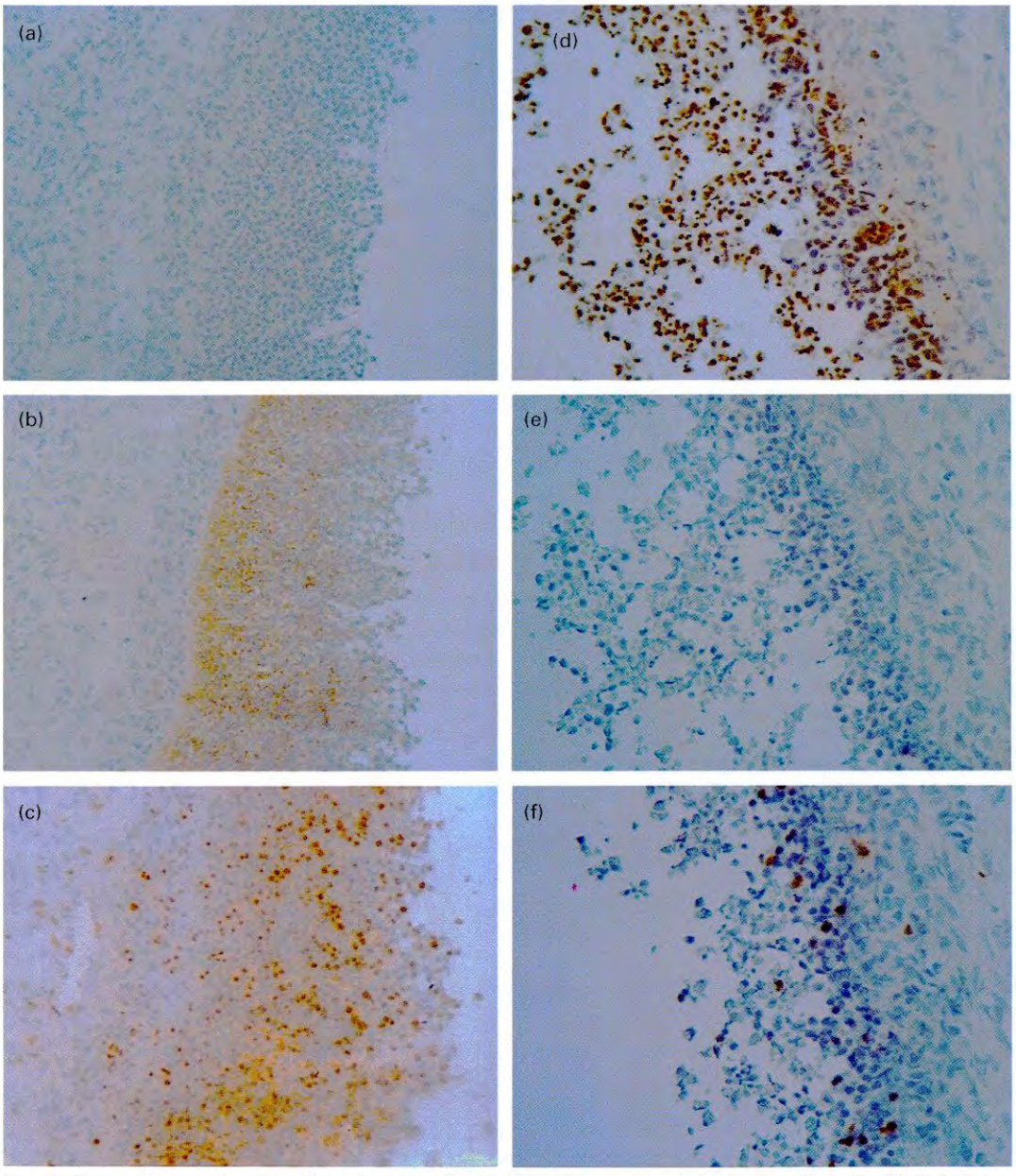

Fig. 1. Photomicrographs of sections from ovarian follicles from non-atretic (a–c) and atretic (d–f) follicles. Sections (a) and (d) are 3′ end-labelled to detect apoptosis; sections (b) and (e) are immunostained for aromatase, and sections (c) and (f) are immunostained for cell proliferation-associated antigen, Ki-67 (W. Garrett and D. Guthrie, unpublished).

Metabolic Influences on Components of the Hypothalamo–Hypophyseal–Ovarian Axis

Although positive associations between level of feed intake and ovulation rate or litter size are well established (reviewed by Einarsson and Rojkittikhun, 1993; Cosgrove and Foxcroft, 1996), the mechanisms by which increased nutritional status affects ovarian function have not been

established. In gilts, the principal effect of feed restriction is to compromise the GnRH pulse generator, and realimentation or glucose infusion restores LH pulses (Booth, 1990), while insulin restores follicular growth (Britt *et al.*, 1988). Charlton *et al.* (1993) observed lowered ovarian IGF-I mRNA with restricted feeding, but refeeding did not increase IGF-I mRNA. In feed-restricted lactating sows, glucose infusion did not affect pulsatile LH secretion (Tokach *et al.*, 1992b). These results suggest that metabolic state can affect the LH response to a stimulus such as glucose.

When extra feed was given to cyclic gilts, increases in ovulation rate were accompanied by increases in LH pulse frequency and FSH, as well as insulin (Cox *et al.*, 1987; Flowers *et al.*, 1989). However, in the studies of Cox *et al.* (1987), increases in ovulation rate stimulated by dietary energy or exogenous insulin were not necessarily accompanied by increased LH. In ovariectomized gilts with insulin depleted by diabetes mellitus and without insulin therapy for 4 days, pulsatile LH secretion was not altered (Angell *et al.*, 1996). When ovary-intact diabetic gilts were depleted of insulin on day 12 of the oestrous cycle, LH pulse frequency increased between day 12 and day 18, and LH increased again at the time at which normal gilts had preovulatory LH surges (Cox *et al.*, 1994). In spite of the absence of adverse effects on gonadotrophins, follicle growth decreased and atresia increased (Meurer *et al.*, 1991; Cox *et al.*, 1994). Taken together, these results suggest that the effect of metabolic modifiers of reproduction on ovarian function does not require changes in gonadotrophins.

In considering the applicability of using metabolic modifiers to enhance ovulation rate in gilts, it must be considered that maximal feed intake will produce maximal ovulation rates (Beltranena *et al.*, 1991). In contrast, sows, in which weaning triggers preovulatory development, are more vulnerable to the metabolic influences of the previous lactation. Despite the increased LH pulsing that is stimulated by weaning, there is considerable variability in follicular development and steroid secretion (Foxcroft *et al.*, 1987). The primiparous sow is particularly susceptible to delayed oestrus after weaning or smaller litter sizes, suggesting that when metabolic factors interface with enhanced gonadotrophin secretion at weaning, follicle development can be affected. In support of this concept, Clowes *et al.* (1994) observed greater litter sizes (12.8 versus 10.4) and increased insulin concentrations when remating was delayed until the second post-weaning oestrus. Understanding of metabolic influences in sows is further complicated by a non-linear relationship between interval to oestrus and litter size and perhaps ovulation rate (Vesseur *et al.*, 1994). Thus metabolic manipulations that affect the interval from weaning to oestrus could alter litter size.

Research assessing influences of nutritional manipulations on ovarian function in sows has demonstrated that ovarian function after weaning can be affected by metabolic state during lactation. Tokach *et al.* (1992a) and Koketsu *et al.* (1996) demonstrated that concentrations of insulin and glucose and the number of LH pulses during lactation were greater in sows with normal intervals to oestrus (Fig. 2). Zak *et al.* (1997) observed that restricting feed during weeks 1–3 or week 4 of lactation decreased ovulation rate and increased the weaning to oestrus interval in primiparous sows. Circulating insulin and IGF-I were reduced during feed restriction, and embryo survival was decreased only in sows receiving less feed during the last week of lactation. Feeding starch as an energy source during lactation may also facilitate LH production and ovarian function after weaning (Kemp *et al.*, 1995). Thus dietary manipulation clearly can affect ovarian function after weaning, perhaps by affecting follicles during the lactation phase or before, as well as embryo survival.

Role of Metabolic Hormones on Ovarian Function

The insulin-like growth factor I system

A preponderance of evidence confirms positive associations between IGF-I and follicle function in swine, although most of these studies were performed *in vitro* (reviewed by Hammond *et al.*, 1993; Spicer and Echternkamp, 1995). Although, as indicated above, nutrition modulates systemic concentrations of IGF-I, there is local control of IGF-I as well as other intraovarian compounds, and this explains the marked heterogeneity of follicles in the face of a similar endocrine milieu (Tonetta

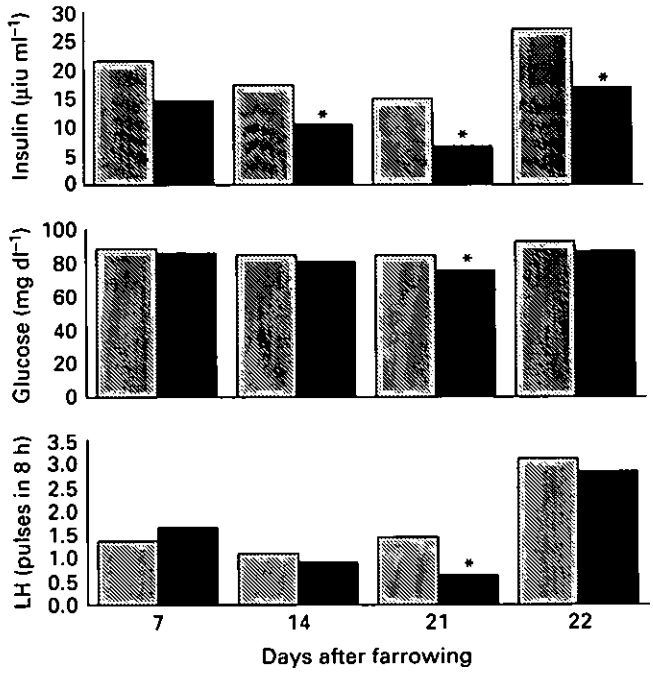

Fig. 2. Patterns of insulin, glucose and LH in sows categorized on the basis of days to oestrus (▨ in oestrus < 7 days after weaning $n = 23$; and ■, in oestrus > 7 days after weaning, $n = 11$). Day 22 represents the first day after weaning. In sows with delayed oestrus, serum insulin was lower on days 14, 21 and 22, and glucose and number of LH pulses were lower on day 21 ($P < 0.05$). SEMs ranged from 1.43 to 2.67, 1.35 to 2.88, and 0.16 to 0.35 for insulin, glucose and LH pulses, respectively. Redrawn from Koketsu *et al.* (1996).

and diZerega, 1990). Synthesis of IGF-I by granulosa cells from pigs has been demonstrated, and production of IGF-I is increased by gonadotrophins and growth hormone *in vitro* (Hammond *et al.*, 1993). Messenger RNA for both IGF-I and -II increased with increasing size of follicles (Yuan *et al.*, 1996). In contrast, Zhou *et al.* (1996) observed that IGF-I and IGF-I receptor mRNA were concentrated in healthy follicles, whereas mRNA encoding IGF-II was found in all follicles regardless of the degree of atresia. This finding suggests a functional paracrine role for IGF-I but does not allow conclusions for IGF-II, although IGF-II affects steroidogenesis (Spicer and Echternkamp, 1995). The IGF-binding proteins modulate effects of IGF-I systemically as well as in the ovary and are in general considered to inhibit follicular development (Hammond *et al.*, 1993).

Recent evidence indicates that IGFBPs, as well as IGF-I, are related to follicular function in swine. Expression of mRNA encoding IGF-I increased as follicles increased in size during the oestrous cycle, whereas expression of the IGFBP-2 gene was greater in smaller follicles, and IGFBP-3 was not detected in follicles using Northern blot analysis (Hammond *et al.*, 1993). Yuan *et al.* (1996) demonstrated that most IGFBP-2 gene expression was in the theca interna cells and was lower in large antral than in small antral follicles in that cell type, but decreased less markedly in granulosa cells. Concentrations of IGFBP-2 in follicular fluid were also inversely related to follicular diameter or oestradiol production (Howard and Ford, 1992) and were positively related to atresia in the early luteal phase as well as during preovulatory maturation (Guthrie *et al.*, 1995b). Despite the association with atresia, mRNA encoding IGFBP-2 was detected in healthy, preovulatory follicles (Zhou *et al.*,

1996). In contrast, IGFBP-3 was not associated with atresia (Guthrie *et al.*, 1995b; Edwards *et al.*, 1996), although addition of IGFBP-3 to cultured rat follicles prevented IGF-I from acting as a survival factor (Chun *et al.*, 1994). Messenger RNA for IGFBP-3 was not detected in the ovary at the follicular phase (Zhou *et al.*, 1996), but recent evidence from our laboratory using a sensitive ribonuclease protection assay in individual follicles suggests that mRNA for IGFBP-3 is present in the walls of preovulatory follicles (Cox and Qiu, 1995). Associations between atresia and IGFBP-4 and -5 have been reported, but these IGFBPs are present at low concentrations in the follicular fluid of pigs (Guthrie *et al.*, 1995b). However, Zhou *et al.* (1996) observed that mRNA for IGFBP-4 was associated with the presence of LH receptor gene expression in granulosa cells, indicating a role that is more complex than mediation of atresia. In addition to alterations in gene expression, IGFBPs in follicular fluid may be altered by post-translational processing, including proteolysis. Recent evidence in swine indicates that proteolytic activity for IGFBP -2, -4 and -5 increased during follicular growth and was decreased during atresia (Besnard *et al.*, 1997). Thus factors that influence activity of proteases for IGFBPs represent another level of control of follicle function.

Insulin-like growth factor I is a strong survival factor for rat follicles, attenuating apoptosis in a similar way to gonadotrophins (Chun *et al.*, 1994). Insulin also acted as a survival factor in that study, but was less potent than IGF-I. Growth hormone, which increased IGF-I, nevertheless did not reduce atresia in the rat early antral or preovulatory follicle (Chun *et al.*, 1994, 1996). In contrast to the rat model, we have obtained evidence that insulin is more potent in reducing apoptosis and increasing progesterone than IGF-I in pig follicles (Purvis *et al.*, 1997). The model we developed was aimed at studying the population of medium follicles (4 mm diameter) representative of days 16–18 of the oestrous cycle. It was considered that this population, although non-atretic when obtained, has a high potential for atresia. We have previously determined that follicles cultured in the presence of FSH (100 ng ml^{-1}) produce oestradiol linearly over time in culture (Edwards *et al.*, 1996). Addition of insulin to FSH reduced apoptosis, whereas IGF-I had no effect (Fig. 3). Insulin (50 ng ml^{-1}) also increased progesterone concentrations, but 5000 ng IGF-I ml^{-1} did not affect progesterone. The mechanism of insulin action does not appear to involve mediation by IGF-I, because intrafollicular IGF-I concentrations were higher in insulin-treated follicles but were similar to those produced by addition of IGF-I. Interestingly, insulin increased IGFBP-2 in medium after culture for 23 h, but not in follicular fluid, whereas IGF-I did not alter IGFBP-2 compared with controls. In cultures of pig preantral follicles that already contained 1% insulin in the medium, IGF-I enhanced growth although the increase was less than with FSH or epidermal growth factor (Flowers and Turner, 1996). There have been few attempts to administer IGF-I *in vivo* to affect follicular function in pigs.

Both insulin and ST increase IGF-I production systemically and in the ovarian follicle, and both affect IGFBP production, although each hormone responds to circulating glucose in opposite ways (Spicer and Echternkamp, 1995). In our laboratory the combined influences of pST and insulin on follicular development were examined by inducing diabetes mellitus in gilts that had been immunized against growth hormone-releasing hormone (provided by Jeffrey D. Armstrong of North Carolina State University) (Howell *et al.*, 1993). The two treatments were compared with control animals in a 2×2 factorial arrangement. Immunoneutralization of GHRH lowered follicular IGF-I concentrations from 245 to 113 ng ml^{-1} but did not affect oestradiol concentrations on day 18 of the oestrous cycle, after half the diabetic gilts were without insulin therapy from day 12 (Fig. 4). In contrast, diabetes mellitus significantly lowered both IGF-I (104 versus 254 ng ml^{-1}) and oestradiol (71 versus 301 ng ml^{-1}), as well as the number of follicles greater than 2 mm (31.5 versus 58.3). Another unique effect of diabetes mellitus, but not GHRH immunoneutralization, was that IGFBP-2 was increased in follicles to 186% of control values. However, follicular IGFBP-3 was reduced to 50% of control values as a result of GHRH immunoneutralization. Therefore, although the functional absence of either ST or insulin decreased IGF-I in follicular fluid, reduction in IGFBP-3 activity may have ameliorated negative effects of lowered IGF-I. In contrast, the combined influence of increased IGFBP-2 and decreased IGF-I in follicles of diabetic gilts may account for decreased follicular function. Interactions among systemic insulin, ST, IGFBPs and IGF-I as well as intrafollicular interactions beween the last two factors may function to control normal follicular responses to alterations in metabolism.

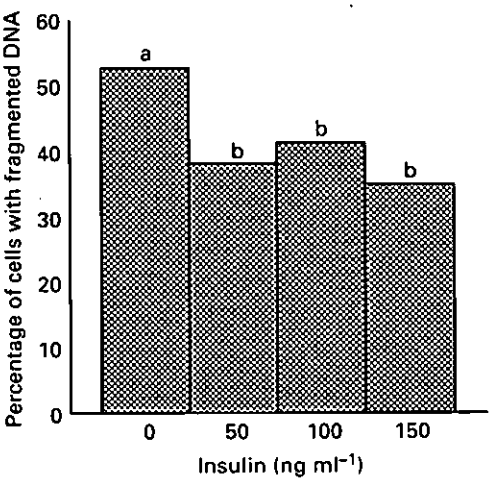

Fig. 3. Percentage of granulosa cells with fragmented DNA identified using cell flow cytometry in 4 mm follicles (six per treatment) from luteal phase ovaries cultured for 23 h with 100 ng ml^{-1} porcine FSH supplemented with insulin. All doses of insulin decreased apoptosis ($P < 0.05$). The SEM was 5.3%. Redrawn from Purvis *et al.* (1997).

Insulin

Follicle development in gilts. We have previously observed that administration of insulin to cyclic gilts during the first pubertal oestrous cycle increased ovulation rate (Cox *et al.*, 1987) and reduced follicle atresia (Matamoros *et al.*, 1990) in gilts at the second postpubertal oestrus. In prepuberal, eCG-treated gilts, we observed an increase of IGF-I in medium-sized follicles of gilts given insulin (Matamoros *et al.*, 1991). In contrast, when insulin was administered to gilts during days 13–17 of the fifth postpubertal oestrous cycle, IGF-I and oestradiol concentrations were decreased and atresia was not affected (Hughey *et al.*, 1993). This result suggests that effects of insulin may be related to age, maturity and (or) related metabolic changes.

We demonstrated that normal ovarian function could be maintained in streptozotocin-induced diabetic pigs given daily insulin injections from 35 days of age until initiation of oestrous cycles, but that withdrawal of insulin therapy reduced follicle diameter and oestradiol production and increased atresia (Meurer *et al.*, 1991; Cox *et al.*, 1994; Edwards *et al.*, 1996). Upon withdrawal of insulin replacement therapy, the incidence of follicular atresia increased within 2 days. Gene expression for IGF-I in follicle walls, as well as IGF-I in follicular fluid, decreased rapidly upon withdrawal of insulin, and IGFBP-2 in follicular fluid increased (Cox and Qiu, 1995; Fig. 5). Neither mRNA encoding IGFBP-3 nor IGFBP-3 in follicular fluid (not shown) was affected by diabetes. Both lowered IGF-I and increased IGFBP-2 probably contributed to the increased atresia and lowered oestradiol.

There is evidence that 4–6 mm follicles are particularly affected by insulin. First, it was the medium-sized follicle population that was maintained from day 17 (39.8% of total follicles) to 19 (35.1%) of the cycle by insulin injections beginning on day 15, compared with a reduction from 41.7 to 16.6% in saline-treated animals. A corresponding increase in atretic follicles from 15.5 to 38.2% occurred in control animals, while a reduction in atretic follicles on both days 17 (6.3%) and 19 (10.7%) was observed in insulin-treated animals (Matamoros *et al.*, 1991). This evidence is supported by the *in vitro* data presented in Fig. 3. Further evidence that the 5 mm follicle population is

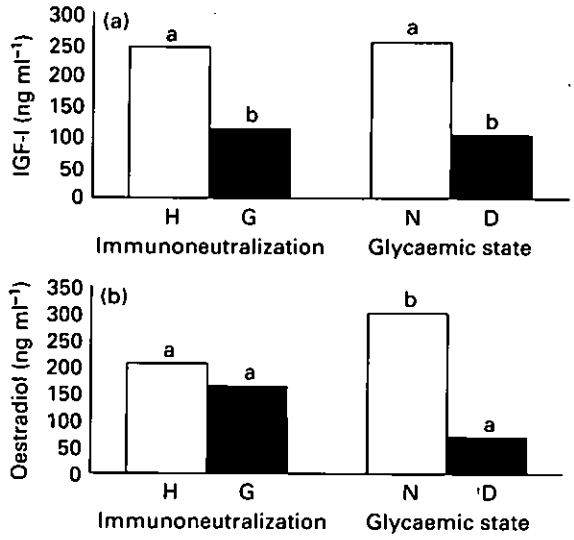

Fig. 4. Influence of immunoneutralization of GHRH on concentrations of (a) IGF-I and (b) oestradiol in the five largest follicles present on day 18 of the oestrous cycle in gilts ($n = 22$). The letters H and G designate gilts immunized against human serum albumin (HSA) and an HSA–GHRH conjugate, respectively, and the letters N and D represent normal and streptozotocin-diabetic gilts, respectively. Insulin replacement therapy was removed from diabetic gilts on day 12 of the oestrous cycle. There were no interactions, and both main effects significantly reduced IGF-I, but only diabetes mellitus reduced oestradiol ($P < 0.05$). SEMs ranged from 15.3 to 25.0 and from 13.3 to 19.1 for IGF-I and oestradiol, respectively. Redrawn from Howell *et al.* (1993).

vulnerable to altered metabolic support was obtained by Edwards *et al.* (1996). In diabetic gilts, the largest follicles present on the ovary were 5 mm in diameter, and oestradiol production *in vitro* was similar to that of follicles from control and diabetic insulin-treated gilts. However, IGF-I was severely reduced in those follicles, indicating that either insulin, IGF-I or a combination thereof is necessary for follicle growth beyond the 5 mm stage. While research has demonstrated that insulin is required for normal follicle function, manipulating follicle development in gilts with insulin is not of practical use, because, as noted above, maximal ovulation rates can be obtained with feeding. Table 1 illustrates the lack of an effect of insulin on ovulation rates and litter size in gilts fed *ad libitum*, for which ovulation rates were maximal, and is in agreement with the observations of Beltranena *et al.* (1991) cited above. Insulin was not given to limit-fed gilts because of the danger of hypoglycaemia.

Effects on reproductive performance in primiparous sows. In sows after weaning, there is potential for manipulating reproduction with metabolic modifiers such as insulin, because ovulation occurs in the face of the recent negative energy balance of lactation. On the basis of initial studies with gilts, in several studies in commercial settings we have administered insulin during the period between weaning and oestrus and have observed inconsistent, but positive effects on reproductive function (Table 2). These effects include increasing farrowing rate in a herd with typically low farrowing rates and increasing litter size in another herd with greater than 90% farrowing rate (Ramirez *et al.*, 1997). These results contrast with those of Kirkwood and Thacker (1991), who did not find a significant effect of exogenous insulin on litter size, using a higher daily dose of insulin (0.75 versus 0.40 iu kg^{-1} day^{-1})

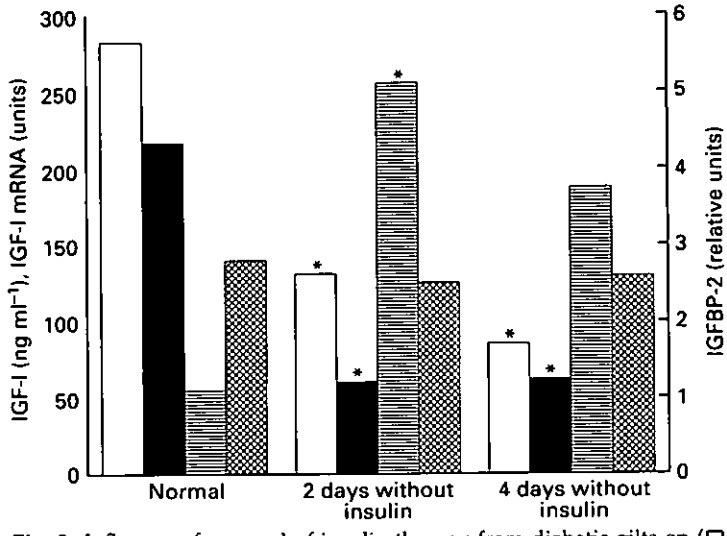

Fig. 5. Influence of removal of insulin therapy from diabetic gilts on (□) IGF-I and (▤) IGFBP-2 in follicular fluid and mRNA for (■) IGF-I and (▩) IGFBP-2 in follicle walls. Insulin removal for 2 or 4 days decreased IGF-I and its mRNA and increased follicular fluid IGFBP-2 ($P < 0.05$) but did not affect its mRNA. SEMs ranged from 9.8 to 15.6 and 4.9 to 10.4 for IGF-I concentration and mRNA and from 0.3 to 0.5 and 0.9 to 1.5 for IGFBP-2 in follicular fluid and mRNA, respectively. Redrawn from Cox *et al.* (1995).

and mixed parity sows. Their results indicate that insulin did not influence ovulation rate, that uterine capacity was insufficient, or that mature sows already ovulated maximally. We also failed to increase litter size in multiparous sows treated with insulin (Cox *et al.*, 1995). When primiparous and multiparous sows were compared, total litter size was not affected by insulin but was higher, as expected, for multiparous sows compared with primiparous sows (11.8 and 9.8 pigs born in total, SEM = 0.3; $P < 0.001$). Farrowing rates (89.7 ± 3% overall) were not influenced by parity or insulin treatment.

In a study with primiparous sows lactating for 30 days, doses of insulin were higher and given for longer than in other studies (Table 2), and insulin treatment lowered ovulation rate (Rojkittikhun, 1992). This finding may be related to production of a negative metabolic signal by the increased duration of injection, the small sample sizes or to the fact that some of the treated sows gave birth to a smaller number of piglets in their previous litter, indicating a predetermined low ovulation rate.

Since increased feed intake alone between weaning and oestrus might be sufficient to increase insulin, we examined the influence of insulin injections daily for 4 days between weaning and postweaning oestrus in primiparous sows given extra feed between weaning and postweaning oestrus in a 2 × 2 arrangement (Cox *et al.*, 1995). Total litter size in primiparous sows (in oestrus within 10 days after weaning) was affected differently by insulin depending on the level of feed intake between weaning and oestrus (treatment by feed level interaction, $P < 0.02$) and was also positively related to feed intake during lactation ($P < 0.05$). When litter sizes, adjusted for lactation feed intake, were compared with the mean of insulin-treated sows given 3.63 kg feed (11.6 piglets in total), means for the insulin/2.72 kg and saline/3.63 kg treatments (9.6 piglets each) were lower ($P < 0.05$) and the mean for the saline/2.72 kg (10.5 piglets) treatment was similar ($P = 0.10$; overall SEM = 0.7). It should also be noted that despite the larger litter size in the insulin-treated sows fed 3.63 kg, farrowing rate tended ($P < 0.07$) to be lower in this treatment group (74 ± 8%), compared with 86 ± 5, 89 ± 5, and 93 ± 8% for saline/2.72 kg, insulin/2.72 kg and saline/3.63 kg treatment combinations, respectively. Although the greatest litter size was achieved with insulin plus the high level of feed, the tendency for lower farrowing rates suggests that there are negative effects of this combination of insulin and extra feed. The results show that primiparous sows may have a critical need to be in a

Table 1. Effects of exogenous insulin and feed intake on ovulation rate and litter size in gilts

Treatment	Number of gilts	Corpora lutea	Number of fetuses at 60 days
Limit-fed [a]	14	13.2[b]	Not mated
Ad libitum feed	36	17.5	12.0
Ad libitum + insulin once a day [c]	37	17.2	11.4
Ad libitum + insulin twice a day	31	17.9	12.3
SEM		0.9	0.5

[a]A diet based on corn and soybean meal (14% protein) was limit-fed to provide 5400 kcal metabolizable energy (1.8 kg day^{-1}) or fed *ad libitum*. Data from Ramirez, 1994.
[b]Significantly different from the other treatments ($P < 0.05$).
[c]Each insulin treatment provided a total of 0.80 IU kg^{-1} day^{-1} Lente insulin (Eli Lilly and Company, Indianapolis, IN).

certain metabolic condition for insulin to be effective. More appropriate feeding regimens may permit an augmented and repeatable reproductive response to insulin. More recently interval to oestrus was decreased but ovulation rate (18.3 average) and litter size (10.3) were unaffected by insulin (N. Whitley, unpublished observation, Table 2). Taken together, these results are consistent with the notion that insulin increased the anabolic state of sows, but the positive effects were manifested in different ways.

Manipulation of post-weaning follicle development in sows. In lactating sows, follicle growth advances as lactation advances, but follicles reach only about 5 mm in diameter by 3–4 weeks of lactation (Britt *et al.*, 1985). Follicular growth patterns during lactation and after weaning measured ultrasonically illustrate that within 2 or 3 days after weaning, the 4–5 mm follicles are replaced by larger follicles (M. Lucy, personal communication). We have conducted one study in which follicle populations were monitored after insulin administration to primiparous sows for 3 or 5 days after weaning (Whitley *et al.*, 1997a). Insulin increased atresia and the number of 6 mm follicles at 3 days after weaning and the overall number of follicles present at day 5, but at both 3 and 5 days after weaning follicular fluid IGF-I, IGFBP-2 and oestradiol were reduced. These results suggest that insulin may have increased numbers of follicles without increasing steroidogenesis by attenuating IGF-I. Perhaps the lowered IGFBP-2 allowed follicles to escape atresia. However, in another study, insulin increased oestradiol in medium- and large-sized follicles but did not affect IGF-I or IGFBP-2 (Whitley *et al.*, 1997b). It is assumed that in the latter study insulin may not have lowered peripheral glucose concentrations as much (not measured), because animals received 0.45 kg more feed per day between weaning and oestrus, and the duration of lactation was 21 days, compared with 30 days in the former study. In addition to increasing oestradiol, insulin administration in the second study also resulted in increased follicular testosterone (not shown) and progesterone in a pattern similar to that for oestradiol.

The effects of insulin reported to date, while generally positive, are not consistent. However, the results are consistent with an overall anabolic effect that could variously affect follicles, oocytes or embryos, reproductive tract environment or gonadotrophin actions. Administration of insulin may provide a physiological signal of increased anabolism before the time such a state would normally occur after weaning, but coincident with preovulatory follicle growth.

Pig somatotrophin

Results of studies investigating the control of follicular development and ovulation rate after administration of pig somatotrophin (pST) are contradictory. Positive effects of pST have been

Table 2. Summary of effects of exogenous insulin on reproductive function of sows during lactation or after weaning

Number in experiment	Insulin dose and duration	Significant effects of insulin	Authors
120 Mixed parity	0.40 iu kg^{-1} day^{-1} 2 days before to 4 days after weaning	No effects	Kirkwood and Thacker, 1991
8 Primiparous	0.75 iu kg^{-1} day^{-1} 1day pre-weaning through oestrus	Lowered ovulation rate	Rojkittikhun, 1992
26 Primiparous	0.75 iu kg^{-1} day^{-1} from weaning to oestrus (up to 30 days)	Higher incidence of anoestrus (10/13 versus 5/13)	Johnston *et al.*, 1994
28 Primiparous	Infusion 130 iu day^{-1}, day 20 to 26 post-farrowing	No effects	Tilton *et al.*, 1996
138 Primiparous	0.40 iu kg^{-1} day^{-1} 4 days after weaning	Farrowing rate increased (92.3 versus 76.7%)	Ramirez *et al.*, 1997
491 Primiparous	0.40 iu kg^{-1} day^{-1} 2 or 4 days after weaning	Insulin for 4 days increased total litter size (10.3 versus 9.3) and live born pigs (10.0 versus 9.0)	
231 Multiparous	0.40 iu kg^{-1} day^{-1} 4 days after weaning	None	Cox *et al.*, 1995
171 Primiparous	0.40 iu kg^{-1} day^{-1} 4 days after weaning	Increased return to oestrus in 10 days (86 versus 78%)	
143 Primiparous	0.40 iu kg^{-1} day^{-1} 4 days, increased feed	Interaction to increase litter size in insulin-treated sows on higher feed intake	
43 Primiparous	0.40 iu kg^{-1} day^{-1} 4 days after weaning	Decreased interval to oestrus (5.0 versus 6.9 days)	N. Whitley and N. Cox, unpublished

observed for IGF-I production *in vitro* by pig granulosa cells (Spicer and Echternkamp, 1995) as well as other species, and synergism of ST with gonadotrophins has been demonstrated in humans undergoing superovulation (Homburg *et al.*, 1988). In early antral rat follicles in culture, rbST increased IGF-I messenger RNA but did not reduce apoptosis (Chun *et al.*, 1996).

Many attempts *in vivo* to increase follicular development involved administration of pST during follicular development (Table 3). Administration during the prepubertal period has had few permanent effects on reproductive characteristics, with the exception of the study of Echternkamp *et al.* (1994), who used low doses of pST in sustained release implants. Treatment with 4 mg pST day^{-1} interacted with body type to increase the number of medium-sized follicles, with a greater initial number in control compared with lean and obese body types (12, 2 and 4, respectively) and 23, 9 and 11 follicles in control, lean and obese gilts treated with 4 mg pST day^{-1}. Importantly, pST decreased IGFBP-2 concentration in follicular fluid but not in serum.

In cyclic animals, pST can have potent effects on the occurrence of oestrus. Kirkwood *et al.* (1988) administered pituitary-derived pST from day 14 of the oestrous cycle to 24 h after oestrus. This dose increased insulin and glucose concentrations on day 20 of the oestrous cycle, and ovulation rate was increased by pST (12.4 for control and 14.3 for pST-treated). However, nine of 20 pST-treated gilts were anoestrous, whereas none of the control animals were anoestrous. The anoestrous gilts tended to have lower insulin and glucose values, which may have been beneath a threshold necessary for continued cycling. Despite *ad libitum* feeding, pigs did not gain weight during the experimental period, adding additional credence to the idea that there is a critical threshold of metabolism below which metabolic modifiers can have harmful peripheral effects. A critical period for inhibition of oestrus with pST was determined by Kirkwood *et al.* (1989). They observed that administration of pST beginning on day 14, but not day 17, suppressed the subsequent oestrus, illustrating that pST may affect follicles negatively during follicle selection. In a follow-up experiment, treatment was shortened to days 14–17 of the oestrous cycle, and that treatment resulted in an increase in percentage in oestrus (91%) compared with gilts treated from day 14 to day 22 (73%), but no increase in ovulation rate. It should be noted that the ovulation rate of the controls in this experiment was 14.0, compared to 12.3 in the study of Kirkwood *et al.* (1988). Thus ovulation rate must be below a physiological maximum in order to be increased by metabolic modifiers, which may explain the inconsistent results for the two studies.

Preovulatory development in sows may also be manipulated by pST, although farrowing rate and litter size in multiparous sows were not affected by treatment with 6 mg pST day^{-1} from 2 days before weaning to 4 days after weaning (Kirkwood *et al.*, 1993). Recently we treated primiparous sows with 40 μg pST kg^{-1} day^{-1} (dose of approximately 6 mg day^{-1}) beginning one day after weaning and observed increased IGF-I on day 5 (Whitley *et al.*, 1997b). The only IGFBPs increased by pST treatment in this model were the 30 and 22 kDa species, but the relevance of this increase is questionable since these IGFBPs are present in small quantities and proteolysis of these IGFBPs is related to follicle development (Besnard *et al.*, 1997). Follicular fluid oestradiol and testosterone were also increased by pST and insulin, indicating that ST may have positive effects on follicular development in sows if administered during the 5 days after weaning. In summarizing the potential applicability of manipulation with ST, increased follicular development is possible with appropriate dosages and administration, but the influence on pregnancy establishment has not been evaluated.

Conclusion

Parallel with information on the intrafollicular control of ovarian function, progress has been made in the practical application of metabolic modification of reproductive function. Metabolic manipulation of follicles is most successful in follicles of intermediate development, which have the potential for atresia or ovulation, depending on metabolic and gonadotrophin support. Progress in control of follicular development and ovulation rate will depend on a better understanding of these factors as well as the influence of metabolic state when metabolic hormones are administered. It can be concluded that metabolic modifiers may be harmful if body condition is below average and may

Table 3. Summary of representative experiments to affect reproductive function of gilts and sows with porcine somatotrophin (pST)

Dose/duration	Reproductive period or age	Effect of pST[a]		Reference
	Prepubertal administration			
70 μg kg^{-1} for 30 or 65 days	72 kg weight at start	+	Follicle IGF-I, progesterone, number of follicles	Bryan *et al.*, 1989
70 μg kg^{-1} day^{-1}, 21 or 42 days	Age 159 day to puberty	0	Corpora lutea	Bryan *et al.*, 1990
35 or 70 μg kg^{-1} day^{-1} for 50 days	From age 140 day	0	Age at puberty	Andres *et al.*, 1993
		0	Corpora lutea	
implant; 2 or 4 mg day^{-1} for 6 weeks	From age 160 day	+	Medium follicles (4 mg day^{-1})	Echternkamp *et al.*, 1994
		+	follicle IGF-I	
	Oestrous cycle administration			
90 μg kg^{-1} day^{-1}; day 14 of cycle to 24 h after oestrus	First cycle	+	Corpora lutea (12.4 versus 14.3)	Kirkwood *et al.*, 1988
		–	% in oestrus (55 versus 100%)	
90 μg kg^{-1} day^{-1}; day 14–17 or 14–22	First cycle	0	Corpora lutea (15.1 versus 14.3 and 14.0 for day 14–17, day 14–22 and control)	Gilbertson *et al.*, 1991
	Postweaning follicular period			
6 mg day^{-1}; daily or alternate	–2 to +4 days from weaning	0	Farrowing rate	Kirkwood *et al.*, 1993
		0	Litter size	
40 μg kg^{-1} day^{-1}	Days 1 to 5 after weaning	+	Follicular IGF-I	Whitley *et al.*, 1997b
		+	Follicular progesterone, oestradiol, testosterone	

[a] +, – or 0 denote positive, negative or absence of effects of pST, respectively.

be unnecessary if body condition is above average. Enhancing ovulation rate by manipulating the preovulatory follicle is possible in gilts and appears to be possible in sows, although less reliable. Gilts can be managed such that ovulation rate is not limiting to litter size. However, follicle development in sows may benefit from anabolic signals applied for a short enough time to enhance reproduction without causing deleterious peripheral metabolic effects. Thus it is more practical to consider application of metabolic modifiers to produce anabolic signals during preovulatory follicle growth in sows. Future progress in this area will involve assessing effects of metabolic manipulation on embryo quality, as well as integration of research on intrafollicular factors which control fertility.

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