Insulin-like Growth Factor-I (IGF-I) and IGF Binding Proteins: Potential Mediators of the Influence of Nutrition on Ovarian Function in the Heifer and Gilt

AM Benoit, K Swanchara, P Schoppee and JD Armstrong

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Nutrition influences age at puberty, duration of postpartum anestrus, and ovulation rate. Mechanisms mediating these effects, however, have not as yet been fully elucidated. Shifts in feed intake are accompanied by changes in circulating levels of insulin-like growth factor-I (IGF-I), and somatotropin (ST). Feed restriction increases ST, decreases IGF-I, and shifts the proportions of IGF binding proteins (IGFBP). Active immunization against growth hormone releasing factor (GRFi) has been used to examine the effects of alterations in the somatotropic axis, independent of feed intake, on reproduction in prepubertal gilts and heifers. GRFi decreased the number of large follicles present on the ovaries prior to puberty, delayed onset of puberty in heifers, and decreased ovulation rate in gilts. GRFi altered follicular turnover in prepubertal heifers. Replacement therapy with somatotropin restored follicle numbers. GRFi decreased serum and follicular fluid IGF-I and IGFBP-3 and increased IGFBP2. Feed restriction also reduced IGF-I in follicular fluid. We speculate that the population of follicles affected by GRFi or feed restriction is related to their sensitivity to IGF-I. Possibly, alterations of ovarian or peripheral IGF-I at critical times during prepubertal follicular growth result in decreased follicle numbers, culminating in delayed onset of puberty in heifers and decreased ovulation rate in gilts.

Introduction

Energy balance influences age at puberty (den Hartog and Noordervier, 1984) and interval from weaning to estrus (Britt et al., 1988) in swine. Restriction of dietary energy, delays puberty in heifers (Kinder et al., 1995; Moran and Roche, 1989), reduces follicular development in prepubertal heifers (Bergfeld et al., 1994), lengthens the interval from calving to conception (Jolly et al., 1995; Randel, 1990), and impacts resumption of estrous cycles in cattle (Bishop et al., 1994; Villa - Godoy et al., 1988). Potential signals mediating effects of nutrition on reproduction have been the subject of several reviews (Cameron, 1991; Jolly et al., 1995; Keisler and Lucy, 1996; Petersen, 1996). However, direct manipulation of the hormonal environment in vivo to test its specific role in the interaction between nutrition and reproductive function has proven difficult, and precise mechanisms through which alterations in metabolism affect reproduction have only been partially elucidated. Recent reviews have described possible roles of IGF-I during follicular development (Adashi, 1994; Erickson and Danforth, 1995; Giudice, 1992; Spicer and Echternkamp, 1995).

The majority of IGF-I circulates bound to specific binding proteins (Clemmons, 1991; Cohick and Clemmons, 1993; Jones and Clemmons, 1995), the largest of which, IGFBP-3, forms a ternary complex with IGF-I and an acid labile subunit. In fasted and diabetic conditions the amount of acid labile subunit is altered and may influence the ability of IGF-I to bind to IGFBP-3 (Barrera et al., 1995). A second complexity of the IGF/IGFBP family is that these proteins are produced by the liver, as well as by the ovary (Adashi, 1994; Monget and Monniaux, 1995; Spicer and Echternkamp, 1995). Therefore, the quantity of these proteins within the antrum of the follicle, is likely derived from multiple sites of synthesis and from serum transudation. Degradation of IGFBP by specific proteases may also regulate bioavailability of IGF-I (Binoux et al., 1991; Chandrasekher et al., 1995).

Effect of Feed Restriction on IGF-I and IGFBP

An increase in ST typically results in elevated serum IGF-I and IGFBP-3 (Clemmons and Underwood, 1991; Cohick and Clemmons, 1993; McGuire et al., 1992). Feed restriction alters the ST axis such that ST and IGF-I are 'uncoupled'; ST increases and IGF-I decreases after feed restriction in ruminants (Elsasser et al., 1989) and swine (Armstrong et al., 1993c). In feed restricted heifers, serum IGF-I, liver IGF-I mRNA and liver ST receptor mRNA were decreased (Vandehaar et al., 1995), but although CL size was decreased, neither IGF-I nor ST receptor mRNA in luteal tissue were altered.

Feed restriction may also alter the bioavailability of IGF-I by shifting the proportions of serum IGFBPs (Clemmons and Underwood, 1991; McGuire et al., 1992) and exacerbating the already low levels of IGF-I. IGFBP-2 increased during feed restriction or negative energy balance in cattle (Sharma et al., 1994; Vandehaar et al., 1995). Serum IGFBP-3 was higher and IGFBP-2 was lower in cyclic than in anestrous postpartum cows (Roberts et al., 1994) but feed restriction had no effect on serum IGFBP-2 or -3 (Armstrong et al., 1993b) in beef cattle.

If suppression of IGF-I is causally related to

aberrant ovarian function during negative energy balance, elevation of IGF-I should also alter ovarian function. ST increased number of medium sized follicles in cows (Lucy et al., 1993; Gong et al., 1991; 1993a)and increased the number of small follicles in gilts (Echternkamp et al., 1994b).

An In Vivo Model to Lower IGF-I and Alter IGFBP
GRFi effectively lowers serum IGF-I and ST (Armstrong et al., 1994b; Trout and Schanbacher, 1990) in a variety of physiological states, independent of nutritional influences per se. GRFi suppresses IGF-I to a level lower than that achieved by feed restriction. GRFi increases circulating IGFBP-2 and decreases IGFBP-3, but IGFBP are not consistently affected by feed restriction (Armstrong et al., 1993b; Kirby et al., 1993; Stanko et al., 1994). Recent reports, however, (Sharma et al., 1994; Vandehaar et al., 1995) showed that energy balance was inversely related to IGFBP-2.

GRFi at 3 mo of age delayed puberty in over 70% of heifers (Cohick et al., 1996), while GRFi at 6 mo of age delayed puberty in 40% of heifers, (Armstrong et al., 1994b), suggesting that the timing of GRFi may be important. The number of large follicles present at 6 mo of age was decreased by GRFi at 3 mo of age. In heifers GRFi at 4.5 mo of age, the number of follicles (6-9 mm) present at 7.5 mo of age was lower than in controls (Benoit and Armstrong, unpublished). Ultrasonography at 6 mo of age indicated that follicular turnover was reduced by GRFi. In acyclic GRFi heifers although the diameter of the largest follicle was increased after 3 days of bST replacement therapy , a chronic effect on ovarian function was not detected (Stanko et al., 1994). GRFi heifers given bST at an earlier age, however, had similar follicle numbers, and reached puberty at the same age as controls (Benoit and Armstrong, unpublished).

In gilts GRFi at 90 d of age, onset of puberty was not affected (Britt et al., 1993).GRFi initiated at 35 d of age decreased ovulation rate after third estrus, but did not delay puberty (Armstrong et al., 1993a; 1994b). Swanchara (unpublished) found that at 90-100 d of age, the number of large ($\geq$ 6 mm) follicles was lower in GRFi than in control gilts. Moreover, decreased ovulation rate after third estrus was associated with a comparable decrease in the number of preovulatory follicles. Thus, although GRFi failed to alter age at puberty in gilts, ovarian function was significantly altered. This species difference may reflect a different manifestation of a similar mechanism; alteration of follicular development early in life by suppression of IGF-I delays puberty in heifers and decreases ovulation rate in gilts.

Putative Mechanisms by which GRFi affects Follicular Growth
In GRFi heifers and gilts, follicle populations were altered 2-4 mo before observed effects on timing of puberty or ovulation rate. Such changes in follicular growth could result from altered ovarian responses to gonadotropins. In GRFi heifers, pulsatile

administration of GnRH resulted in similar gonadotropin profiles, but lower peak estradiol (Schoppee et al., 1996) or a delayed increase in estradiol as compared to controls, suggesting a reduced ovarian response to LH and FSH. Treatment with ST increased the superovulatory response of heifers (Gong et al., 1993b), and increased the number of medium follicles in cattle (Gong et al., 1991, 1993a; Lucy et al., 1993). In prepubertal heifers, GRFi slowed apparent follicle turnover (Benoit and Armstrong, unpublished), however Gong et al. (1993a) found no effect of ST on pattern of follicle growth in heifers.

Stanko et al. (1994) evaluated follicular fluid concentrations of IGF-I and IGFBP in cycling cattle and found that administration of bST elevated, and GRFi lowered, follicular IGF-I. Follicular fluid IGF-I was decreased in follicles of 6 mo old heifers which had been immunized at 3 mo of age (Cohick et al., 1996). Administration of GnRH to GRFi heifers increased follicular fluid concentrations of IGF-I in large follicles (Schoppee et al., 1996). Cohick et al. (1996) reported no effect of GRFi on whole ovarian expression of IGF-I; however, effects on specific follicle populations were not examined. In gilts, intrafollicular IGF-I mRNA increased concomitant with increased follicle size (Samaras et al., 1993).

GRFi may also change bioavailable IGF-I by altering intraovarian concentrations of IGFBP. In gilts, IGFBP-2 mRNA decreased as follicle size increased (Samaras et al., 1993). In heifers, GRFi decreased IGFBP-3 in dominant and subordinate follicles and increased IGFBP-2 and -4 in subordinate follicles (Stanko et al., 1994). Follicular IGFBP-3 was decreased and IGFBP-2 increased in heifers GRFi at 3 mo of age (Cohick et al., 1996). IGFBP-2, -4 and -5 were greater in dominant than subordinate follicles (Thatcher et al., 1996), and IGFBP-4 and -5 were greatest in atretic follicles (Erickson and Danforth, 1995). Collectively, these results indicate that GRFi is associated with increased inhibitory forms of IGFBP in follicular fluid. Potentially GRFi alters follicular growth by decreasing the facilitory input of IGF-I and increasing the inhibitory input of IGFBP. Echternkamp et al. (1994a) and Erickson and Danforth (1995) demonstrated that follicular diameter and follicular IGFBP-2 are inversely related; however, Grimes et al. (1994) found that after adjusting for atresia, follicular diameter was not correlated to IGFBP-2. Moreover, follicular fluid IGFBP-2 was greater in atretic than in nonatretic follicles in the gilt (Guthrie, et al., 1995). Grimes et al (1994) demonstrated that follicular IGFBP-3 levels were greater in large growing follicles than in inactive follicles. However, Guthrie et al. (1995) found no difference in IGFBP-3 in atretic and nonatretic follicles in the gilt. In heifers, whole ovarian IGFBP-2, -3, -4 and -5 mRNA were not affected by GRFi (Cohick et al., 1996); however, Samaras et al. (1993) found that whole ovarian mRNA for IGFBP-2 decreased concomitant with resumption of preovulatory follicular growth. Interpretation of these results is difficult as whole ovarian levels may not

adequately reflect differences in distribution of IGFBP among different follicle populations.

A Working Model

Aberrations in ovarian function observed at 6 mo of age in heifers immunized at 3 mo of age, and observed at 100 d of age in gilts immunized at 35 d of age, represent an intricate interaction of events which occur approximately half-way between birth and expected onset of puberty. In the heifer, the percentage of animals that reach puberty is lower when they are first immunized at 3 vs 6 mo of age. Moreover, active immunization of gestating cows actually hastens the resumption of estrous cycles (Moore et al., 1992). In the gilt, GRFi at 35, but not at 90 d of age decreased ovulation rate (Armstrong et al., 1994a; Britt et al., 1993). Ovulation rate was not affected in gilts immunized after puberty (Armstrong et al., 1994a). Thus, in both the heifer and the gilt, the physiological state of the female influences the ability of GRFi to alter ovarian function.

We speculate that the population of follicles affected by GRFi is related to their sensitivity to altered serum IGF-I and IGFBP. In the gilt, the period from birth to 90 d of age corresponds to a period of rapid activation of primordial follicles (Oxender and Colenbrander, 1979). Follicles activated one week after birth would reach 1 mm in diameter at 90 d of age (Morbeck et al., 1992). Thus, we speculate that GRFi-induced alterations in IGF-I occur during a time of enhanced follicular growth. The heifer is born with a full complement of oogonia and primordial follicles (Erickson, 1966), and number of vesicular follicles peaks at 6 mo of age. Lussier et al. (1987) estimated that 60 d are required for a follicle that has just formed an antrum to reach the preovulatory stage. Extrapolating from data for the gilt (Morbeck et al., 1992), one could speculate that the time required from activation at the primordial stage to preovulatory size is approximately 100 days. Although these effects are manifested within a few weeks of initiation of immunization as a decrease in follicle numbers, the detrimental effects on puberty (heifer) and ovulation rate (gilt) are only observed several months later.

We believe a similar sensitivity of follicles to low serum IGF-I exists during feed restriction. Feed restriction clearly decreases serum IGF-I; however, several reports have failed to show a decrease in concentrations of IGF-I in large follicles (Kirby et al., 1993; Schoppee et al., 1996; Spicer et al., 1992). In contrast, follicular fluid IGF-I in follicles less than 7 mm in diameter were decreased by feed restriction to a degree similar to that observed in serum (Schoppee et al., 1996). It is possible that follicular growth during this period of activation of follicles is particularly sensitive to alterations in serum IGF-I.

Based on work demonstrating that negative energy balance during the first two weeks postpartum exerted a latent effect on progesterone secretion (Villa-Godoy et al., 1988), Britt (1992) speculated that ovulatory follicles that develop during postpartum negative energy balance occurring after parturition would be most affected. This was corroborated by data showing that progesterone concentrations were similar during the first two cycles or during the nadir in energy balance but were lower during the third, fourth, and fifth estrous cycles in cows that had lost more body condition. This line of reasoning is consistent with our observed effects of lowered IGF-I on follicular growth, and the latent effect of GRFi on onset of puberty in heifers and ovulation rate in gilts.

In conclusion, elucidation of mechanisms through which lowered serum IGF-I or altered IGFBP affect follicular development will lead to a better understanding of the mechanism through which nutrition alters reproductive function. These data provide evidence that particular stages of prepubertal follicular development and thus ovarian function are especially sensitive to the negative effects of poor nutrition via lowered serum IGF-I, and that these effects are manifested in delayed onset of puberty in heifers and reduced ovulation rate in gilts.

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