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Control of follicular growth: Local interactions and nutritional influences1,2

R. Webb*3, P. C. Garnsworthy*, J.-G. Gong†, and D. G. Armstrong†

*Division of Agricultural and Environmental Sciences, School of Biosciences, University of Nottingham, Loughborough LE12 5RD, United Kingdom and †Division of Integrative Biology, Roslin Institute, Roslin, Midlothian EH25 9PS, United Kingdom

ABSTRACT: Regulation of ovarian activity is an integrated process encompassing both extraovarian signals and intrafollicular factors. Initiation of primordial follicle growth and the early stages of folliculogenesis can occur without gonadotropins, but FSH may affect the rate of preantral follicle growth. Antral follicle development from 1 to 4 mm in sheep and cattle is completely gonadotropin dependent. These recruited follicles express a range of mRNA encoding steroidogenic enzymes, gonadotropin receptors, and local regulatory factors and their receptors. As follicles continue to mature, there is a transfer of dependency from FSH to LH, which may be part of the mechanism involved in selection of follicles for continued growth. Locally produced growth factors, such as the IGF and members of the transforming growth factor-β superfamily, work in concert with gonadotropins throughout the follicular growth continuum and can have significant effects on follicle selection. Environmental influences, such as changes in nutrition, also have an effect on ovarian activity. This can occur without significant variation in circulating gonadotropin concentrations and can be correlated with changes in circulating concentrations of metabolic hormones, including insulin, IGF-I, GH, and leptin. Nutrition can also affect the expression of mRNA encoding components of the ovarian IGF system to regulate the sensitivity/response of follicles toward gonadotropins. Hence, the roles of growth factors in follicular development and survival depend on gonadotropin status and differentiation state of the follicle. In conclusion, it is the integration of these extraovarian signals and intrafollicular factors that determine whether a follicle will continue to develop or be diverted into atretic pathways.

Key Words: Bovine, Follicle, Gonadotropins, Growth Factors, Nutrition

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Introduction

In monovulatory ruminants, such as cattle, as in other species, primordial follicle growth, once initiated, continues until the follicle either becomes atretic or proceeds to ovulation. The precise mechanisms controlling the initiation and the number of primordial follicles that start to grow are still not known. Ovarian autograft studies have confirmed that the early stages of folliculogenesis can occur without gonadotropins, but FSH may affect the rate of preantral follicle growth. Antral follicle development from 1 to 4 mm in sheep and cattle is completely gonadotropin dependent. These recruited follicles express a range of mRNA encoding steroidogenic enzymes, gonadotropin receptors, and local regulatory factors and their receptors. As follicles continue to mature, there is a transfer of dependency from FSH to LH, which may be part of the mechanism involved in selection of follicles for continued growth. Locally produced growth factors, such as the IGF and members of the transforming growth factor-β superfamily, work in concert with gonadotropins throughout the follicular growth continuum and can have significant effects on follicle selection. Environmental influences, such as changes in nutrition, also have an effect on ovarian activity. This can occur without significant variation in circulating gonadotropin concentrations and can be correlated with changes in circulating concentrations of metabolic hormones, including insulin, IGF-I, GH, and leptin. Nutrition can also affect the expression of mRNA encoding components of the ovarian IGF system to regulate the sensitivity/response of follicles toward gonadotropins. Hence, the roles of growth factors in follicular development and survival depend on gonadotropin status and differentiation state of the follicle. In conclusion, it is the integration of these extraovarian signals and intrafollicular factors that determine whether a follicle will continue to develop or be diverted into atretic pathways.

Moreover, in dairy cows, increased milk yields and associated metabolic demand have been associated with longer postpartum anestrous intervals and abnormal estrous cycles (Royal et al., 2000; Lucy, 2003). However, the detailed physiological mechanisms through which nutrition exerts many of these effects remain to be fully characterized.

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3Correspondence: Sutton Bonington Campus, Loughborough, Leicestershire (phone: 0115 951 6061; fax: 0115 951 6069; e-mail: bob.webb@nottingham.ac.uk).

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Figure 1. The follicular growth continuum. Schematic representation of the requirement for growth factors, such as the TGFβ and IGF families, and gonadotropins at different stages of ovarian follicle development in cattle. Growth factors seem to be important in both the initiation of and in early follicle growth, whereas gonadotropins are essential for the final stages of follicle growth. In this regard, the dominant follicle switches its requirement from FSH to LH. There is also increasing evidence that gonadotropins can influence follicle development before antrum formation and growth factors can influence follicle development throughout the follicular growth continuum.

This review will focus on the interaction between gonadotropins and intrafollicular factors in bovine follicular development. It will also discuss the mechanisms through which changes in nutritional status directly influence folliculogenesis, oocyte quality, and embryo survival.

Follicle Growth and Maturation

Preantral Follicle Growth

Mechanisms regulating the activation and subsequent growth of primordial follicles still remain poorly understood. However, their growth probably depends on the presence of oocyte/granulosa cell interactions (e.g., C-KIT/KIT ligand) and the secretion of a range of local factors (e.g., growth differentiating factor [GDF]-9, bone morphogenetic proteins [BMP], activins, inhibins, basic fibroblast growth factor [bFGF], and epidermal growth factor [EGF]; Figure 1; McNatty et al., 1999; Knight and Glistser, 2001; Smitz and Cortvindt, 2002; Webb et al., 2003). For example, sheep with a BMP15 gene mutation are infertile, with follicle growth arrested at the primary stage of growth (Galloway et al., 2000). Both GDF-9 and BMP15 have been localized in the oocyte and immunization against these peptides arrests ovine follicular development at either the preantral Type 1a stage or the Type 2 primary stage (Juengel et al., 2002).

Messenger RNA for the steroidogenic enzymes, cytochrome P450 side-chain cleavage (P450scce), cytochrome P450 17α-hydroxylase (P450c17), and 3β-hydroxysteroid dehydrogenase (3β-HSD) are first expressed soon after formation of the theca interna. Cytochrome P450 aromatase (P450arom) is localized solely to granulosa cells (Bao and Garverick, 1998) and preantral follicles seem capable of producing estradiol early in development (Thomas et al., 2001; K. J. Dugan, M. Lopez-Bejar, D. G. Armstrong, and R. Webb, our unpublished observations).
Gonadotropins are probably not involved in the initiation of follicle growth (Wandji et al., 1992; McNatty et al., 1999; Campbell et al., 2000; Fortune et al., 2000), although FSH receptor (FSHR) mRNA can be detected in follicles with only one or two layers of granulosa cells (Bao and Garverick, 1998). Importantly, in vivo (Campbell et al., 2000) and in vitro (Hulshof et al., 1995; Gutierrez et al., 2000) studies have demonstrated that FSH can accelerate the rate of preantral follicle development. A role for LH in these early stages of development has not been described, although expression of LH receptor (LHR) mRNA is first detected when the theca interna forms around the granulosa cells (Bao and Garverick, 1998).

Locally produced factors also have important roles in these early stages of development (Figure 1). In a recent study, Armstrong et al. (2002a) demonstrated that bovine granulosa cells of preantral follicles express mRNA encoding both IGF-binding protein (IGFBP)-2 and Type 1 IGF receptor. Theca externa of antral follicles and the stromal tissue surrounding preantral follicles were also shown to be a site of IGFBP-3 mRNA expression. In contrast, the expression of IGF-I in granulosa cells remains controversial, with reports (Spicer and Echternkamp 1995; Yuan et al., 1998; Perkins et al., 1999; Webb et al., 1999a; Schams et al., 2002) demonstrating either the presence or absence of IGF-I in granulosa cells of both preantral and antral follicles. Insulin-like growth factor-II has been detected in the theca cell layer of bovine antral follicles (Yuan et al., 1998; Webb et al., 2003). This suggests that IGF regulates preantral follicle growth primarily via endocrine mechanisms, with IGFBP-2 and -3 regulating the bioavailability of extravarian IGF-I and IGF-II derived from adjacent antral follicles.

Insulin-like growth factor-I, as well as EGF, has been shown to stimulate preantral follicle growth in vitro (Gutierrez et al., 2000; Saha et al., 2000). In contrast, higher concentrations of IGF-I may have a negative effect on oocyte growth (McCaffery et al., 2000). Hence, the role of locally produced IGFBP is probably to maintain IGF in an optimum range for preantral follicle and oocyte growth but not for the initiation of growth of primordial follicles. As discussed, other growth factors are undoubtedly involved in both the initiation and subsequent growth of primordial follicles (McNatty et al., 1999; Juengel et al., 2002). For example, it has recently been demonstrated that BMP can stimulate estradiol production by preantral follicles in culture (K. J. Dugan, M. Lopez-Bejar, D. G. Armstrong, and R. Webb, our unpublished observations).

**Antral Follicle Growth**

The later stages of antral follicle development in cattle are characterized by two or three waves of follicular growth during each estrous cycle. Follicular waves appear to be constitutive and have been observed prior to puberty and during other periods of anestrus (Adams, 1999; Ireland et al., 2000). Each wave of growth in cattle is characterized by recruitment of a group of follicles, which continue to grow to approximately 6 to 8 mm in diameter. In monovulatory species, such as cattle, one follicle is selected for continued growth and becomes dominant.

Antral follicle growth, at least from 2 mm in diameter, is under gonadotropic control (Campbell et al., 1995) as demonstrated by treatment of hypogonadotropic cattle with bovine follicular fluid and estradiol (Figure 1; Campbell et al., 2003). Each wave of follicular growth is preceded by a transient increase in FSH secretion (Adams, 1999). It has recently been suggested that peripheral inhibin-A and FSH concentrations influence the number of follicular waves (Parker et al., 2003) and that concentrations of FSH control the interval to the emergence of the subsequent follicular wave (Ginther et al., 2002a). As reviewed (Bao and Garverick, 1998; Webb et al., 1999a), changes in the expression patterns of mRNA for the gonadotropin receptors (FSHR and LHR) and the key steroidogenic cytochrome P450 enzymes, including P450scc, P450c17 and P450arom, and 3β-HSD, occur at this later stage of development from approximately 2 mm in diameter. Specifically, growth of follicles to approximately 5 mm in diameter (recruitment) and above is characterized by induction of mRNA expression for P450scc and P450arom in granulosa cells and by an increase in mRNA of the gonadotropin receptors and steroidogenic enzymes with increasing follicular size. In two recent studies, FSH infusion in cattle, in which pituitary gonadotropin secretion had been significantly reduced by GnRH agonist (GnRHa) or GnRH immunization, stimulated follicle growth up to 8.5 mm in diameter (Crowe et al., 2001; Garverick et al., 2002). This follicular growth was accompanied by increased expression of mRNA for P450scc and P450arom in granulosa cells and by an increase in mRNA of the gonadotropin receptors and steroidogenic enzymes with increasing follicular size. In two recent studies, FSH infusion in cattle, in which pituitary gonadotropin secretion had been significantly reduced by GnRH agonist (GnRHa) or GnRH immunization, stimulated follicle growth up to 8.5 mm in diameter (Crowe et al., 2001; Garverick et al., 2002). This follicular growth was accompanied by increased expression of mRNA for P450scc and P450arom in granulosa cells and by an increase in mRNA of the gonadotropin receptors and steroidogenic enzymes with increasing follicular size. In two recent studies, FSH infusion in cattle, in which pituitary gonadotropin secretion had been significantly reduced by GnRH agonist (GnRHa) or GnRH immunization, stimulated follicle growth up to 8.5 mm in diameter (Crowe et al., 2001; Garverick et al., 2002). This follicular growth was accompanied by increased expression of mRNA for P450scc and P450arom in granulosa cells and by an increase in mRNA of the gonadotropin receptors and steroidogenic enzymes with increasing follicular size. In two recent studies, FSH infusion in cattle, in which pituitary gonadotropin secretion had been significantly reduced by GnRH agonist (GnRHa) or GnRH immunization, stimulated follicle growth up to 8.5 mm in diameter (Crowe et al., 2001; Garverick et al., 2002). This follicular growth was accompanied by increased expression of mRNA for P450scc and P450arom in granulosa cells and by an increase in mRNA of the gonadotropin receptors and steroidogenic enzymes with increasing follicular size. In two recent studies, FSH infusion in cattle, in which pituitary gonadotropin secretion had been significantly reduced by GnRH agonist (GnRHa) or GnRH immunization, stimulated follicle growth up to 8.5 mm in diameter (Crowe et al., 2001; Garverick et al., 2002). This follicular growth was accompanied by increased expression of mRNA for P450scc and P450arom in granulosa cells and by an increase in mRNA of the gonadotropin receptors and steroidogenic enzymes with increasing follicular size. In two recent studies, FSH infusion in cattle, in which pituitary gonadotropin secretion had been significantly reduced by GnRH agonist (GnRHa) or GnRH immunization, stimulated follicle growth up to 8.5 mm in diameter (Crowe et al., 2001; Garverick et al., 2002). This follicular growth was accompanied by increased expression of mRNA for P450scc and P450arom in granulosa cells and by an increase in mRNA of the gonadotropin receptors and steroidogenic enzymes with increasing follicular size. In two recent studies, FSH infusion in cattle, in which pituitary gonadotropin secretion had been significantly reduced by GnRH agonist (GnRHa) or GnRH immunization, stimulated follicle growth up to 8.5 mm in diameter (Crowe et al., 2001; Garverick et al., 2002). This follicular growth was accompanied by increased expression of mRNA for P450scc and P450arom in granulosa cells and by an increase in mRNA of the gonadotropin receptors and steroidogenic enzymes with increasing follicular size.
BMPR-1A, and BMPR-1B), inhibins, and activins. The precise roles of these factors are not known, but, similarly to the IGF system, it is likely that they are involved in follicular differentiation by enhancing the action of gonadotropins (Campbell and Baird, 2001; Knight and Glister, 2001; Montgomery et al., 2001; Souza et al., 2002).

It is also around the time of antrum formation that IGF-II mRNA is first detected in thecal tissue. Type 1 IGF receptor and a range of IGFBP (IGFBP-2, -3, and -4) have also been detected at this stage of development (Armstrong et al., 1998, 2000). However, in situ hybridization has failed to detect the presence of IGF-I mRNA in granulosa tissue at any stage of development (Armstrong et al., 1998, 2000; Perks et al., 1995, 1999). In contrast, Leeuwenberg et al. (1995) in sheep and Yuan et al. (1998) in cattle have demonstrated the presence of mRNA encoding IGF-I in granulosa cells from both subordinate and dominant follicles. Previous work using bovine granulosa cell cultures (Spicer et al., 1993; Spicer and Echternkamp, 1995; Spicer and Chamberlain, 2000) detected both IGF-I mRNA and IGF-I. Nevertheless, there is general agreement that IGF-II, produced by theca cells, is the major intrafollicular IGF ligand regulating the growth of bovine antral follicles (Yuan et al., 1998; Armstrong et al., 1998, 2000; Webb et al., 1999a) acting through the Type 1 IGF receptor (Adashi et al., 1990; Lucy, 2000).

**Follicle Selection and Dominance**

Transrectal ultrasound technology has greatly increased our understanding of dominant follicle selection and the temporal associations between changes in follicle dynamics and peripheral hormone concentrations (Adams, 1999; Ireland et al., 2000; Ginther et al., 2001). However, the precise mechanism of selection and dominance remains to be fully elucidated. It has been suggested that the decrease in FSH secretion after the emergence of a follicular wave may be a key mechanism in follicle selection (Figure 1). All recruited follicles appear to contribute to the initial decline in peripheral FSH (Gibbons et al., 1997), the largest follicle has the major role in decreasing further the circulating FSH concentrations to levels below that required to support the continued growth of the cohort of smaller follicles (Campbell et al., 1995; Webb et al., 1999a; Ginther et al., 2001). The major factors produced by the growing and selected follicles that act to suppress the secretion of FSH are estradiol and inhibin (Webb et al., 1999a,b; Bleach et al., 2001). This suppression results in a rapid deviation in the size of the future dominant follicle and the largest subordinate follicle, which can be detected within an 8-h window when the future dominant follicle is approximately 8.0 to 8.5 mm in diameter (Kulick et al., 1999). It has been shown that infusion of FSH can override the process of follicle selection (Mihm et al., 1997). This deviation in diameter between the two largest follicles has been associated with a transient increase in peripheral LH concentrations (Kulick et al., 1999) and reduced intrafollicular estradiol, induced by treatment with estradiol antiserum (Beg et al., 2003).

Around the time of selection of the dominant follicle (approximately 8 to 9 mm in diameter) LHR and 3β-HSD mRNA expression can be detected in granulosa cells (Bao and Garverick, 1998; Webb et al., 1999a), supporting the concept that the dominant follicle can utilize LH to support its continued growth even when circulating FSH concentrations are declining (Figure 1). Certainly, the lifespan of the dominant follicle can be extended with optimum pulsatile LH support (Fortune, 1994). Infusion studies have demonstrated that FSH alone, or in combination with LH, at physiological concentrations stimulated follicles of <4 mm in diameter to develop to the preovulatory stages and these preovulatory follicles were capable of ovulating in response to hCG (Webb et al., 2003). Furthermore, adequate pulsatile LH support appears to be required to maintain the ovulatory competence of large follicles (>9 mm in diameter) when FSH concentrations are decreased. Indeed, the duration of exposure to FSH is critical for normal follicle selection and dominance, as FSH infusion for longer than 48 h, when the recruited follicles had reached a diameter of 6 to 7 mm, always induced a superovulatory response, whereas FSH exposure for approximately 48 h resulted in selection of one to two dominant follicles (Webb et al., 2003). These data are comparable with those generated using a similar model in sheep (Campbell et al., 2000) and agree with our current understanding of the role of declining FSH and subsequent LH support in selection of the dominant follicle (Campbell et al., 2003).

These effects of gonadotropins are almost certainly mediated in conjunction with locally synthesized growth factors. Utilizing a culture system where the cellular phenotype of granulosa cells is maintained (Campbell et al., 1995; Gutierrez et al., 1997a; Nicholas et al., 2000), it has been demonstrated that FSH can induce estradiol production by bovine granulosa cells and that this induction is related to an increase in P450 arom mRNA expression (Silva and Price, 2001). Utilizing similar culture systems, a wide range of local factors, including members of the TGFβ superfamily, FGF, EGF/TGFα, and cytokines, have been shown to be involved in the regulation of follicular growth (Armstrong and Webb, 1997; Webb et al., 1999a,b). For example, it has been demonstrated that IGF-I, as well as insulin, interacts with FSH to stimulate granulosa cell estradiol production (Gutierrez et al., 1997b; Spicer et al., 2002).

In the cow, IGF-II gene expression is restricted to the theca of antral follicles (Armstrong et al., 2000), providing support that IGF-II is the major intraovarian IGF. Insulin-like growth factor-binding proteins also have a regulatory role in follicle development. In healthy bovine antral follicles up to 9 mm in diameter, IGFBP-2 and -4 mRNA expression are restricted to granulosa and theca tissue, respectively (Armstrong et
members of TGF-β superfamily of ligands, operating through Smad signaling pathways. Certainly, BMP are involved in follicular maturation as indicated by the marked increase in ovulation rate in sheep with a BMP receptor mutation (Montgomery et al., 2001; Monget et al., 2002). Bone morphogenetic proteins have been localized in the bovine follicle (Glisher et al., 2004). Similarly, in bovine granulosa cells, BMP-4, -6, and -7 increased estradiol, inhibin-A, activin-A, and follistatin production (Glisher et al., 2004). Furthermore, activin and TGF-β have been shown to affect bovine granulosa cell steroidogenesis and proliferation (Knight and Glisher, 2001). Overall, these results provide evidence for a functional role of BMP, acting in concert with other locally produced factors. However, the exact mechanisms through which these factors operate and degree of redundancy need to be elucidated.

Nutritional Influences on Follicular Development

There is increasing evidence of the effect of nutrition on follicle development (Garnsworth and Webb, 1999). For example, short-term changes in the plane of nutrition have been shown to influence small antral follicle (1 to 4 mm) recruitment, without affecting circulating concentrations of FSH (Guthrie et al., 1997c; Armstrong et al., 2001, 2002b; Gong et al., 2002a), resulting in a larger number of ovulations after a superovulatory gonadotropin challenge (Gong et al., 2002a). Diet has also been positively correlated with the growth rate and size of the ovulatory follicle (Rhodes et al., 1995; Bergfeld et al., 1994; Mackey et al., 1999; Bossis et al., 2000; Armstrong et al., 2001). During lactation, the extent of the negative energy balance deficit is a major factor controlling follicle growth (Beam and Butler, 1999; Butler, 2000). Recent studies have also highlighted the link between dietary intake and oocyte developmental competence (O’Callaghan and Boland, 1999; Boland et al., 2001). Extraovarian factors, such as metabolic hormones (Figure 2), and locally produced growth factors are involved in mediating these nutritionally induced changes in follicle dynamics and oocyte quality and some of this evidence is summarized.

Growth Hormone

Treatment with GH has been shown to have a significant effect on ovarian follicle development in both nonlactating (Figure 2; Gong et al., 1991, 1993) and lactating (Lucy et al., 1999; Lucy, 2003) cattle. The suggestion that GH is involved in mediating the actions of nutrition by acting directly on follicles has been questioned. Messenger RNA encoding GH receptor was not detected in bovine follicles (Lucy et al., 1999), and some in vitro experiments (Gong et al., 1994; Jimenez-Krassel et al., 2002) have shown that GH does not affect the proliferation and steroidogenesis of bovine granulosa cells in serum-free culture. In contrast, large luteal cells of bovine corpora lutea have been shown to express the GH receptor and respond to GH treatment (Lucy et al., 1999).

An in vivo bovine somatotropin dose-response study demonstrated that GH is acting through increased peripheral concentration of insulin and/or IGF-I to alter follicle development in heifers (Gong et al., 1997). Furthermore, the association between acute changes in di-
The influence of metabolic factors on ovarian function. Interaction between metabolic factors and ovarian function. There are a large number of metabolic factors that have been implicated in the regulation of ovarian function, a number of which are shown. These include hormones and growth factors, such as insulin, glucagon, leptin, GH, thyroid hormones, and hepatic IGF and their binding proteins, as well as metabolic fuels, such as glucose, fatty acids, and low- and high-density lipoproteins. LDL = low-density lipoproteins; HDL = high-density lipoprotein; T3 = triiodothyronine; T4 = thyroxine.

Insulin

There is increasing evidence to associate the decrease in dairy cow fertility with negative energy balance postpartum and decreased IGF-I and insulin concentrations (Beam and Butler, 1999; Butler, 2000). A number of studies have demonstrated the importance of insulin as a signal mediating the effects of acute changes in nutrient intake on follicle dynamics in cattle. Circulating insulin concentrations exhibit diurnal variation, but also change during the estrous cycle with significantly increased concentrations during the preovulatory period (McCann and Hansel, 1986; Armstrong et al., 2001). Estrogen is a prime candidate for mediating these changes as these increases in serum insulin concentrations parallel the increase in estrogen associated with the development of the dominant follicle. Estrogen has been shown to stimulate both the expression of mRNA encoding insulin and its secretion from the pancreas in a number of species (Figure 2; Morimoto et al., 2001).

The initiation of the first ovulation is delayed in dairy cows selected for high genetic merit for milk yield and this has also been shown to be associated with a lower circulating insulin concentration (Butler, 2000). In contrast, feeding diets specifically designed to increase circulating insulin concentrations during early lactation can advance the first ovulation postpartum (Table 1; Gong et al., 2002b). Infusion of insulin into beef heifers increased both the diameter of the dominant follicle (Simpson et al., 1994) and ovulation rate in energy-deprived beef heifers (Harrison and Randel, 1986).

A large number of granulosa and theca cell in vitro studies have demonstrated the direct action of metabolic factors (Webb et al., 1999a,c; Lucy, 2000; Spicer et al., 2002; Armstrong et al., 2003). Indeed, cell culture studies have shown bovine granulosa cells to be critically dependent on the presence of physiological concentrations of insulin (Gutierrez et al., 1997b; Gliester et al., 2001). Moreover, we have recently correlated diet-induced increases in circulating concentrations of insulin with increased estradiol production in cultured granulosa cells from small antral (1 to 4 mm) follicles (Armstrong et al., 2002b), demonstrating a direct action of metabolic hormones on follicle function.
The high-insulin diet significantly ($P < 0.05$) increased the proportion of cows ovulating within 50 d of calving. Fewer high-genetic-merit cows ovulated within this time frame, but the effect was not significant. Furthermore, the high-insulin diet significantly ($P < 0.01$) decreased the interval from calving to first ovulation. Genetic merit also had a significant ($P < 0.05$) effect on the interval from calving to first ovulation. Conception rate to the first insemination, and the effects of genetic merit, but not diet, were significant ($P < 0.05$). Adapted from Gong et al. (2002b).

### IGF System

As with insulin, there is increasing evidence linking nutritionally induced changes in systemic IGF-I concentrations with ovarian activity (Webb et al., 1999c). The liver is the main source of systemic IGF-I and GH is the primary regulator of hepatic IGF-I gene expression and secretion (Etherton and Bauman, 1998). Utilizing a hyperinsulinemic-euglycemic clamp, which maintained glucose within 10% of baseline concentrations, insulin has been shown to increase plasma IGF-I concentrations in lactating dairy cows (McGuire et al., 1995) and to interact with GH to control hepatic IGF-I production (Molento et al., 2002). In dairy cattle, decreased circulating concentrations of IGF-I are associated with both the periparturient period and acute feed restriction (Kobayashi et al., 1999, 2002). These changes have been associated with decreased GH receptor expression in the liver during the periparturient period, but not during acute feed restriction (Kobayashi et al., 2002).

Diet-induced changes in circulating levels of the components of the IGF-I system have been described (Clemmons and Underwood, 1991; McGuire et al., 1992; Thissen et al., 1994; Monget and Martin, 1997), with circulating IGF-I concentrations being positively correlated with level of feeding (Vandehaar et al., 1995; Bossis et al., 2000; Armstrong et al., 2001; Rausch et al., 2002). Acute changes in feed intake alter circulating IGF-I concentrations following an artificially induced ovulation in estrus-synchronized heifers, with nonlactating heifers fed to twice maintenance showing higher circulating concentrations of IGF-I than those fed to maintenance levels. These results, when combined with earlier studies (Gutierrez et al., 1997c; Armstrong et al., 2001, 2002b) show considerable between experiment variation in the magnitude of the changes in IGF-I concentrations associated with changes in nutritional status, suggesting that a number of other endocrine systems are interacting with GH to regulate hepatic IGF-I secretion. Estrogen, as well as being linked to changes in insulin, has been shown to increase concentrations of GH (Grigsby and Trenkle, 1986), stimulate IGF-I secretion (Richards et al., 1991), and increase circulating IGF-I concentrations in ovariectomized cattle (Simpson et al., 1997).

The bioavailability of circulating IGF-I and its clearance from serum is controlled by IGFBP (Thissen et al., 1994). Peripheral concentrations of binding proteins are regulated by feed intake, with IGFBP-3 being positively correlated with dietary intake (Rausch et al., 2002) and increased growth rate in cattle associated with elevated levels of IGFBP-3 (Vestergaard et al., 1995). These effects may be modulated by other factors since in dairy cows insulin has been shown to decrease peripheral IGFBP-2 concentrations, but not affect IGFBP-3 concentrations (McGuire et al., 1995).

Metabolic hormones may therefore directly affect the follicular IGF system, which in turn increases the response of bovine granulosa cells from small antral follicles to FSH (Armstrong et al., 2001). Specifically, it is hypothesized that increased dietary energy decreases the steady-state concentration of mRNA encoding IGFBP-2 and -4 in small antral follicles, which in turn increase the bioavailability of locally produced IGF-II and systemically derived IGF-I in these follicles (Webb et al., 2003; Armstrong et al., 2003).

#### Leptin

There is increasing evidence that leptin, which is produced primarily by adipocytes, may act as a signal linking nutritional status with reproductive performance (Keisler et al., 1999; Spicer, 2001). Peripheral leptin concentrations have been linked to body condition in lactating cows (Ehrhardt et al., 2000), and to level of feeding, in nonlactating cows (Delavaud et al., 2002). Short-term fasting, which decreases serum concentrations of insulin and IGF-I, was also shown to decrease expression of mRNA encoding leptin in adipocytes (Amstalden et al., 2000). Acute changes in feed intake also change circulating leptin concentrations with maximum concentrations of leptin being observed 2 d after the onset of feeding at twice maintenance levels (Armstrong et al., 2003). Indeed, leptin inhibits the synergistic interaction between gonadotropins and insulin (Spicer, 2001).

In a serum-free culture system, we have recently demonstrated that physiological concentrations of leptin inhibit estradiol and androstenedione production by granulosa and theca cells, respectively (Armstrong et al., 2003). These results are similar to those described previously that showed leptin to inhibit the action of insulin on steroidogenesis (Spicer and Francisco, 1997; Spicer et al., 2001).
In summary, nutritionally induced alteration in a range of metabolic hormones can be correlated with changes in ovarian function. Ovarian steroids can also modulate the action and production of these metabolic hormones resulting in interactive positive and negative feedback loops. Furthermore, nutritionally induced changes in the concentrations of these metabolic hormones have the potential to interact directly with gonadotropins to regulate follicle growth and steroidogenesis, since, as discussed, gonadotropins provide the primary drive for antral follicle development.

**Oocyte Quality and Embryo Survival**

A range of diets has been shown to affect not only follicular growth but also oocyte quality (Armstrong et al., 2001; Boland et al., 2001). For example, acute changes in dietary energy intake influence both the morphology and developmental competence of the oocyte (McEvoy et al., 1995; O’Callaghan et al., 2000). Increased intake of highly degradable protein resulted in increased concentrations of ammonia in follicular fluid, but decreased peripheral concentrations of insulin (Sinclair et al., 2000). These changes were associated with altered follicular growth patterns and reduction in both the number of ova that cleaved and the proportion that developed to the blastocyst stage. However, embryo survival rates have been shown not to be affected in vivo, although the outcome may be different in high yielding dairy cows (Kenny et al., 2002). In addition, enhancement of early embryo development has recently been demonstrated in heifers fed a diet, that increased the insulin:glucagon ratio (Mann et al., 2003).

The ovarian IGF system, which as discussed can be influenced by diet, has the potential to interact directly with the oocyte through the Type 1 IGF receptor (Armstrong et al., 2001, 2002a,b). Small follicles from heifers offered high-energy diets had significantly reduced levels of mRNA encoding IGFBP-2 and -4 (Armstrong et al., 2001), potentially regulating the bioavailability of IGF. This is probably a critical factor controlling oocyte developmental capacity (Armstrong et al., 2002a). Indeed, overstimulation by IGF, and possibly insulin, may be detrimental to oocyte development (Armstrong et al., 2001). Recent studies have shown that concentrations of IGF-I that are optimal for the growth of follicles in vitro may be detrimental to oocyte maturation (McCaffery et al., 2000). It appears that nutritionally induced changes in both circulating concentrations of insulin and IGF-I and the ovarian IGF system are important for follicle recruitment. However, these changes may be detrimental to the quality of the oocyte within the growing follicle.

**Implications**

Despite recent progress, the precise mechanisms underlying the follicular growth continuum have yet to be fully elucidated. Gonadotropins are certainly the main driving force for antral follicle development, but also interact with a range of local growth factor systems. Extraovarian factors, such as nutritionally mediated changes in metabolic hormones, also directly affect follicle development and oocyte quality. Diets that are optimal for follicle growth may not necessarily be optimal for oocyte quality. Hence, diets are required that both optimize oocyte quality while maintaining follicle development. This is of key importance because oocyte quality will impinge on subsequent embryo survival. All these factors must be considered when developing approaches to first halt and then reverse the downward trend in dairy cow fertility.

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