

Control of follicular growth: Local interactions and nutritional influences^{1,2}

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

©2004 American Society of Animal Science. All rights reserved.

J. Anim. Sci. 2004. 82(E. Suppl.):E63–E74

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 it takes months for a primordial follicle to reach the preovulatory stage (Campbell et al., 2000; Figure 1). Although this follicular growth continuum is controlled primarily by gonadotropins and locally produced growth factors, a number of

environmental factors, such as nutrition, can influence follicular development and oocyte quality, and hence fertility (Garnsworthy and Webb, 1999; Webb et al., 1999a,b, 2003).

For example, nutritional status is a key factor influencing reproduction, and a number of reviews have been published during the last 5 yr discussing various aspects of nutrition on fertility in ruminants (Beam and Butler, 1999; Garnsworthy and Webb, 1999; Webb et al., 1999a; Butler, 2000; Lucy, 2000, 2003; Armstrong et al., 2003). Dietary intake acts at various levels within the hypothalamus-pituitary-ovarian axis to control ovarian activity. Nutritional status has also been correlated with embryo survival and is a major factor controlling efficiency in assisted reproduction technologies. Moreover, in dairy cows, increased milk yields and associated metabolic demand have been associated with longer postpartum anestrus 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.

¹This article was presented at the 2003 ADSA-ASAS-AMPA meeting as part of the Triennial Reproduction Symposium.

²Original research from our laboratories, presented in this review, was supported by BBSRC, Defra, and SEERAD.

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

Received July 28, 2003.

Accepted December 3, 2003.

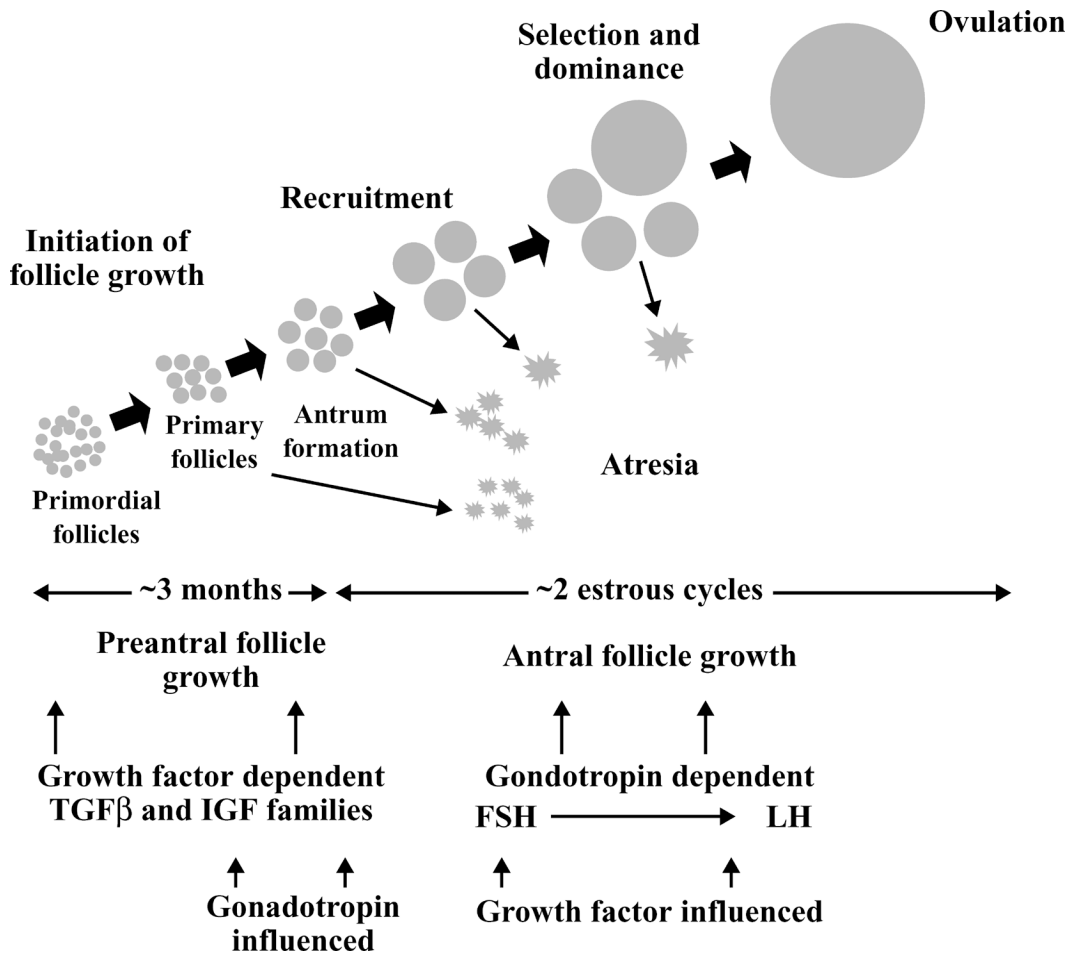


Figure 1. *The follicular growth continuum.* Schematic representation of the requirement for growth factors, such as the TGF β and IGF families, and gonadotropins at different stages of ovarian follicle development in cattle. Growth factors seem to be important in both the initiation of and in early 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 Glister, 2001; Smits 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 (P450_{scc}), cytochrome P450 17 α -hydroxylase (P450_{c17}), and 3 β -hydroxysteroid dehydrogenase (3 β -HSD) are first expressed soon after formation of the theca interna. Cytochrome P450 aromatase (P450_{arom}) 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; Perks 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 extraovarian 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 3 β -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 P450c17 in theca compared with recruited follicles of similar size in cows with normal estrous cycles. Furthermore, infusion of FSH for 48 h (Garverick et al., 2002) also induced an increase in mRNA expression for P450scc and P450arom in granulosa cells in small (1 to 4 mm) follicles compared with follicles in control animals. This is comparable to sheep carrying the major *FecB* gene, which increases ovulation rate and causes precocious follicular differentiation. (Souza et al., 2003; McNatty et al., 2003; Mulsant et al., 2003). The *FecB* gene is a mutation in the BMP-1B receptor and increases the expression of mRNA for P450arom and inhibin- β A subunit in granulosa cells (Webb et al., 1999a), clearly demonstrating the importance of local control mechanisms.

A number of locally produced growth factors are known to be important for follicle development (Figure 1). It has recently been demonstrated that BMP can alter bovine granulosa cell steroidogenesis and proliferation in vitro (Glister et al., 2004). Other members of the TGF β superfamily that also seem to be involved in local control mechanisms include additional BMP and their receptors (BMP-15, BMP receptor [BMPR] -2,

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 P450arom mRNA expression (Silva and Price, 2001). Utilizing similar culture systems, a wide range of local factors, including members of the TGF β superfamily, FGF, EGF/TGF α , 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

al., 1998). Indeed, the conversion of a subordinate follicle to a future dominant follicle has been associated with a transient increase in follicular fluid activin A and estradiol, but a decrease in IGFBP-2 (Ginther et al., 2002a; Kojima et al., 2003). These results are also in agreement with those of Echternkamp et al. (1994) and Austin et al. (2001), who observed that IGFBP-2, and possibly IGFBP-4 and -5 concentrations, are higher in the follicular fluid of small- and medium-sized follicles and follicular fluid of large atretic follicles, but are significantly reduced or undetectable in follicular fluid of large and/or dominant follicles (Nicholas et al., 2002), possibly due to FSH acting indirectly to inhibit IGFBP-2 expression (K. J. Woad, B. K. Campbell, H. A. Garverick, C. G. Gutierrez, J.-G. Gong, G. Baxter, C. O. Hogg, R. Webb, and D. G. Armstrong, our unpublished observations). Furthermore, a reduction in follicular fluid IGFBP-4 and -2 concentrations, coupled with an increase in estradiol concentrations, has been demonstrated in the future dominant follicle in cattle (Mihm et al., 2000; Austin et al., 2001; Ginther et al., 2002b). Indeed, low amounts of IGFBP-2 and increased LH receptors in granulosa cells appear to be associated with the establishment of the dominant follicle.

One clear possibility lies in an altered susceptibility of IGFBP to cleavage by IGFBP proteases. It has been suggested that lower levels of IGFBP-2 in estrogen dominant follicles of cattle are not due to increased proteolysis, unlike decreases in IGFBP-4 and -5. The presence of IGF-dependent regulation of IGFBP-4 proteolytic degradation has been demonstrated in bovine follicular fluid (Mazerbourg et al., 2000) and along with IGFBP-5 proteolytic activity was found to be highest in the dominant follicle (Rivera et al., 2001; Rivera and Fortune, 2003a). The protease that degrades IGFBP-4 and -5 has been shown to be the pregnancy-associated plasma protein-A (PAPP-A; Monget et al., 2002; Rivera and Fortune, 2003b). Furthermore, it has recently been shown that PAPP-A is responsible for IGF-dependent degradation of IGFBP-2 leading to increased IGF bioavailability (Monget et al., 2003). Expression of PAPP-A mRNA in bovine and ovine granulosa cells was maximal in preovulatory follicles and positively correlated with expression of both aromatase and LH receptors. Post-translational modifications of the IGFBP are also known to occur and recently it has been demonstrated that at least 51 isoforms of IGFBP are present in bovine follicular fluid (Nicholas et al., 2002), but whether they have a physiological role or increased susceptibility to proteolytic enzyme action is not known.

Additional locally produced growth factors include 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 (Glister et al., 2004). Similarly, in bovine granulosa cells, BMP-4, -6, and -7 in-

creased estradiol, inhibin-A, activin-A, and follistatin production (Glister et al., 2004). Furthermore, activin and TGF β have been shown to affect bovine granulosa cell steroidogenesis and proliferation (Knight and Glister, 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 (Garnsworthy 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 (Gutierrez 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-

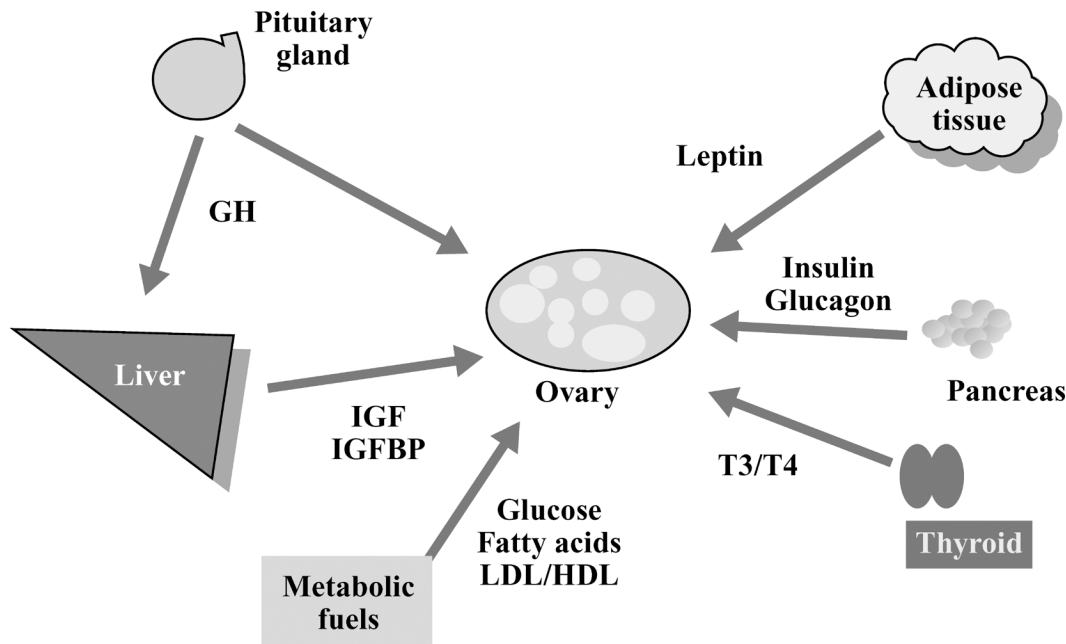


Figure 2. The influence of metabolic factors on ovarian function. Interaction between metabolic factors and ovarian function. There are a large number of metabolic factors that have been implicated in the regulation of ovarian function, a number of which are shown. These include hormones and growth factors, 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.

etary intake and small antral follicle (1 to 4 mm) recruitment (Gutierrez et al., 1997c; Armstrong et al., 2001) occurred despite decreased circulating GH concentrations and no differences in peripheral FSH concentrations between treatments (Gutierrez et al., 1997c). In contrast, peripheral GH concentrations are higher in lactating dairy cows with higher genetic merit for milk yield, coupled with a delayed first ovulation postpartum, compared with a lower genetic merit line (Webb et al., 1999b; Gong et al., 2002b). Taken together, these results suggest that GH acts via other metabolic hormones, such as insulin and IGF-I, to influence follicular development.

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; Glister 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.

Table 1. Effect of diets designed to alter peripheral insulin concentrations on reproductive performance in high- and low-genetic-merit postpartum dairy cows selected for milk yield (n = 10/group)^a

Genetic merit	Diet			
	Low insulin		High insulin	
	Low	High	Low	High
Ovulated in first 50 d postpartum, %	60	50	100	80
Days to first ovulation	43	54	28	41
Conception rate to first insemination, %	63	38	67	44

^aThe 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 (Vandelaar 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 concentra-

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

Literature Cited

- Adashi, E. Y., C. E. Resnick, and R. G. Rosenfeld. 1990. Insulin-like growth factor-I (IGF-I) and IGF-II hormonal action in cultured granulosa cells: mediation via I but not II IGF receptors. *Endocrinology* 126:754–760.
- Adams, G. P. 1999. Comparative patterns of follicle development and selection in ruminants. *J. Reprod. Fertil. Suppl.* 54:17–32.
- Amstalden, M., M. R. Garcia, S. W. Williams, R. L. Stanko, S. E. Nizielski, and C. D. Morrison. 2000. Leptin gene expression, circulating leptin, and luteinizing hormone pulsatility are acutely responsive to short-term fasting in prepubertal heifers: Relationships to circulating insulin and insulin-like growth factor-I. *Biol. Reprod.* 63:127–133.
- Armstrong D. G., G. Baxter, C. G. Gutierrez, C. O. Hogg, A. L. Glazyrin, B. K. Campbell, T. A. Bramley, and R. Webb. 1998. Insulin-like growth factor binding protein-2 and -4 mRNA expression in bovine ovarian follicles: Effect of gonadotropins and developmental status. *Endocrinology* 139:2146–2154.
- Armstrong, D. G., G. Baxter, C. O. Hogg, and K. J. Woad. 2002a. Insulin-like growth factor (IGF) system in the oocyte and somatic cells of bovine preantral follicles. *Reproduction* 123:789–797.
- Armstrong, D. G., J. G. Gong, J. O. Gardner, G. Baxter, C. O. Hogg, and R. Webb. 2002b. Steroidogenesis in bovine granulosa cells: the effect of short-term changes in dietary intake. *Reproduction* 123:371–378.
- Armstrong, D. G., J. G. Gong, and R. Webb. 2003. Interactions between nutrition and ovarian activity in cattle: Physiological, cellular and molecular mechanisms. *Reproduction Suppl.* 61:403–414.
- Armstrong D. G., C. G. Gutierrez, G. Baxter, A. L. Glazyrin, G. E. Mann, K. J. Woad, C. O. Hogg, and R. Webb. 2000. Expression of mRNA encoding IGF-I, IGF-II and type 1 IGF receptor in bovine ovarian follicles. *J. Endocrinol.* 165:101–113.
- Armstrong, D. G., T. G. McEvoy, G. Baxter, J. J. Robinson, C. O. Hogg, K. J. Woad and R. Webb. 2001. Effect of dietary energy and protein on bovine follicular dynamics and embryo production in vitro: associations with the ovarian insulin-like growth factor system. *Biol. Reprod.* 64:1624–1632.
- Armstrong, D. G., and R. Webb. 1997. Ovarian follicular dominance: The role of intraovarian growth factors and novel proteins. *Rev. Reprod.* 2:139–146.
- Austin, E. J., M. Mihm, A. C. O. Evans, P. G. Knight, J. L. H. Ireland, J. J. Ireland, and J. F. Roche. 2001. Alterations in intrafollicular regulatory factors and apoptosis during selection of follicles in the first follicular wave of the bovine estrous cycle. *Biol. Reprod.* 64:839–848.
- Bao, B., and H. A. Garverick. 1998. Expression of steroidogenic enzymes and gonadotropin receptor genes in bovine follicles during ovarian follicular waves: a review. *J. Anim. Sci.* 76:1903–1921.
- Beam, S. W., and W. R. Butler. 1999. Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. *J. Reprod. Fertil. Suppl.* 4:411–424.

- Beg, M. A., C. Meira, D. R. Bergfelt, and O. J. Ginther. 2003. Role of oestradiol in growth of follicles and follicle deviation in heifers. *Reproduction* 125:847–854.
- Bergfeld, E. G. M., F. N. Kojima, A. S. Cupp, M. E. Wehrman, K. E. Peters, M. Garcia-Winder, and J. E. Kinder. 1994. Ovarian follicle development in prepubertal heifers is influenced by the level of dietary energy intake. *Biol. Reprod.* 51:1051–1057.
- Bleach, E. C. L., R. G. Glencross, S. A. Feist, N. P. Groome, and P. G. Knight. 2001. Plasma inhibin A in heifers: Relationship with follicle dynamics, gonadotropins and steroids during the estrous cycle and after treatment with bovine follicular fluid. *Biol. Reprod.* 64:743–752.
- Boland, M. P., P. Lonergan, and O'Callaghan. 2001. Effect of nutrition on endocrine parameters, ovarian physiology, and oocyte and embryo development. *Theriogenology* 55:1323–1340.
- Bossis, I., R. P. Wettemann, S. D. Welty, J. Vizcarra, and L. J. Spicer. 2000. Nutritionally induced anovulation in beef heifers: ovarian and endocrine function during realimentation and resumption of ovulation. *Biol. Reprod.* 62:1436–1444.
- Butler, W. R. 2000. Nutritional interactions with reproductive performance in dairy cattle. *Anim. Prod. Sci.* 60–61:49–459.
- Campbell, B. K., and D. T. Baird. 2001. Inhibin A is a follicle stimulating hormone-responsive marker of granulosa cell differentiation, which has both autocrine and paracrine actions in sheep. *J. Endocrinol.* 169:333–345.
- Campbell, B. K., R. J. Scaramuzzi, and R. Webb. 1995. Control of antral follicle development and selection in sheep and cattle. *Reproduction in Domestic Ruminants III. J. Reprod. Fertil. Suppl.* 49:335–350.
- Campbell, B. K., C. Souza, J. G. Gong, R. Webb, N. Kendall, P. Marsters, G. Robinson, A. Mitchell, E. E. Telfer, and D. T. Baird. 2003. Domestic ruminants as models for the elucidation of the mechanisms controlling ovarian follicle development in humans. *Reproduction in Domestic Ruminants V. Reproduction Suppl.* 61:429–443.
- Campbell, B. K., E. E. Telfer, R. Webb, and D. T. Baird. 2000. Ovarian autografts in sheep as a model for studying folliculogenesis. *Mol. Cell. Endocrinol.* 163:137–139.
- Clemmons, D. R., and L. E. Underwood. 1991. Nutritional regulation of IGF-I and IGF binding proteins. *Annu. Rev. Nutr.* 11:393–404.
- Crowe, M. A., P. Kelly, M. A. Draincourt, M. P. Boland, and J. F. Roche. 2001. Effects of follicle-stimulating hormone with and without luteinizing hormone on serum hormone concentrations, follicle growth, and intrafollicular estradiol and aromatase activity in gonadotropin-releasing hormone-immunised heifers. *Biol. Reprod.* 64:368–374.
- Delavaud, C. A., F. Ferlay, Y. Faulconnier, F. Bocquier, G. Kann and Y. Y. Chilliard. 2002. Plasma leptin concentrations in adult cattle: effects of breed, adiposity, feeding level, and meal intake. *J. Anim. Sci.* 80:1317–1328.
- Echternkamp, S. E., R. J. Howard, A. J. Roberts, J. Grizzle, and T. Wise. 1994. Relationships among concentrations of steroids, insulin-like growth factor-I and insulin-like growth factor binding proteins in ovarian follicular fluid of beef cattle. *Biol. Reprod.* 51:971–981.
- Ehrhardt, R. A., R. M. Slepatis, J. Siegal-Willot, M. E. Van Amburgh, A. W. Bell, and A. W. Boisclair. 2000. Development of specific radioimmunoassay to measure physiological changes of circulating leptin in cattle and sheep. *J. Endocrinol.* 166:519–528.
- Etherton, T. D., and D. E. Bauman. 1998. Biology of somatotropin in growth and lactation of domestic animals. *Physiol. Rev.* 78:745–761.
- Fortune, J. E. 1994. Ovarian follicular growth and development in mammals. *Biol. Reprod.* 50:225–232.
- Fortune, J. E., R. A. Cushman, C. M. Wahl, and W. S. Kito. 2000. The primordial to primary follicle transition. *Mol. Cell. Endocrinol.* 163:53–60.
- Galloway, S. M., K. P. McNatty, L. M. Cambridge, M. P. E. Laitinen, J. L. Juengel, T. S. Jokiranta, R. S. McLaren, K. Luiro, K. G. Dodds, G. W. Montgomery, A. E. Beattie, G. H. Davis, and O. Rivito. 2000. Mutations in an oocyte-derived growth factor gene (BMP 15) cause increased ovulation rate and infertility in a dosage sensitive manner. *Nature Genetics* 25:279–283.
- Garnsworthy, P. C., and R. Webb. 1999. The influence of nutrition on fertility in dairy cows. Pages 39–58 in *Recent Advances in Animal Nutrition*. P. C. Garnsworthy and J. Wiseman, ed. Nottingham University Press, Nottingham, U.K.
- Garverick, H. A., G. Baxter, J. Gong, D. G. Armstrong, B. K. Campbell, C. G. Gutierrez, and R. Webb. 2002. Regulation of expression of ovarian mRNA encoding hypogonadotrophic cattle. *Reproduction* 123:651–661.
- Gibbons, J. R., M. C. Wiltbank, and O. J. Ginther. 1997. Functional interrelationships between follicles greater than 4 mm and follicle-stimulating hormone surge in heifers. *Biol. Reprod.* 57:1066–1073.
- Ginther, O. J., M. A. Beg, D. R. Bergfelt, F. X. Donadeu, and K. Kot. 2001. Follicle selection in monovular species. *Biol. Reprod.* 65:638–647.
- Ginther, O. J., M. A. Beg, D. R. Bergfelt, and K. Kot. 2002a. Role of low circulating FSH concentrations in controlling the interval to emergence of the subsequent follicular wave in cattle. *Reproduction* 124:475–482.
- Ginther, O. J., M. A. Beg, D. R. Bergfelt, and K. Kot. 2002b. Activin A, estradiol and free insulin-like growth factor I in follicular fluid preceding the experimental assumption of follicle dominance in cattle. *Biol. Reprod.* 67:14–19.
- Glister, C., and P. G. Knight. 2002. Immunocytochemical evidence for a functional bone morphogenetic protein (BMP) signalling system in bovine antral follicles. *Reproduction Abstract Series* 29:5. (Abstr.)
- Glister, C., C. F. Kemp, and P. G. Knight. 2004. Bone morphogenetic protein (BMP) ligands and receptors in bovine ovarian follicle cells: Actions of BMP-4, -6, and -7 on granulosa cells and differential modulation of Smad-1 phosphorylation by follistatin. *Reproduction* 127:239–254.
- Gong, J. G., D. G. Armstrong, G. Baxter, C. O. Hogg, P. C. Garnsworthy, and R. Webb. 2002a. The effect of increased dietary intake on superovulatory response to FSH in heifers. *Theriogenology* 57:1591–1602.
- Gong, J. G., G. Baxter, T. A. Bramley, and R. Webb. 1997. Enhancement of ovarian follicle development in heifers by treatment with recombinant bovine somatotrophin: A dose response study. *J. Reprod. Fertil.* 110:91–97.
- Gong, J. G., T. A. Bramley, and R. Webb. 1991. The effect of recombinant bovine somatotropin on ovarian function in heifers: Follicular populations and peripheral hormones. *Biol. Reprod.* 45:941–949.
- Gong, J. G., T. A. Bramley, and R. Webb. 1993. The effect of recombinant bovine somatotrophin on ovarian follicular growth and development in heifers. *J. Reprod. Fertil.* 97:247–254.
- Gong, J. G., W. J. Lee, P. C. Garnsworthy, and R. Webb. 2002b. Effect of dietary-induced increases in circulating insulin concentrations during the early postpartum period on reproductive function in dairy cows. *Reproduction* 123:419–427.
- Gong, J. G., D. McBride, T. A. Bramley, and R. Webb. 1994. Effects of recombinant bovine somatotrophin, insulin-like growth factor-I and insulin on bovine granulosa cell steroidogenesis in vitro. *J. Endocrinol.* 143:157–164.
- Grigsby, M. E., and A. Trenkle. 1986. Plasma growth hormone, insulin, glucocorticoids and thyroid hormones in large, medium and small breeds of steers with and without an estradiol implant. *Domest. Anim. Endocrinol.* 3:261–267.
- Gutierrez, C. G., B. K. Campbell, and R. Webb. 1997b. Development of a long-term bovine granulosa cell culture system: induction and maintenance of estradiol production, response to follicle stimulating hormone and morphological characteristics. *Biol. Reprod.* 56:608–616.
- Gutierrez, C. G., A. L. Glazyrin, G. W. Robertson, B. K. Campbell, J. G. Gong, T. A. Bramley, and R. Webb. 1997a. Ultra-structural characteristics of bovine granulosa cells associated with maintenance of oestradiol production in vitro. *Mol. Cell. Endocrinol.* 134:51–58.

- Gutierrez, C. G., J. Oldham, T. A. Bramley, J. G. Gong, B. K. Campbell, and R. Webb. 1997c. The recruitment of ovarian follicles is enhanced by increased dietary intake in heifers. *J. Anim. Sci.* 75:1876–1884.
- Gutierrez, C. G., J. H. Ralph, E. E. Telfer, I. Wilmut, and R. Webb. 2000. Growth and antrum formation of bovine antral follicles in long-term culture in vitro. *Biol. Reprod.* 62:1322–1328.
- Harrison, L. M., and R. D. Randel. 1986. Influence of insulin and energy intake on ovulation rate, luteinizing hormone and progesterone in beef heifers. *J. Anim. Sci.* 63:1228–1235.
- Hulshof, S. C. J., J. R. Figueiredo, J. F. Becker, M. M. Severs, J. A. van der Donk, and R. van den Hurk. 1995. Effects of fetal bovine serum, FSH and 17 β -estradiol on the culture of bovine preantral follicles. *Theriogenology* 44:217–226.
- Ireland J. J., M. Mihm, E. Austin, M. G. Diskin, and J. F. Roche. 2000. Historical perspective of turnover of dominant follicles during the bovine estrous cycle: Key concepts, studies, advancements, and terms. *J. Dairy Sci.* 83:1648–1658.
- Jimenez-Krassel, F., and J. J. Ireland. 2002. Development and validation of a short-term, serum-free culture system for bovine granulosa cells: Evaluation of the effects of somatotropin and growth hormone-releasing factor as estradiol production. *J. Dairy Sci.* 85:68–78.
- Juengel, J. L., N. L. Hudson, D. A. Heath, P. Smith, K. L. Reader, S. B. Lawrence, A. R. O'Connell, M. P. E. Laitinen, M. Cranfield, N. P. Groome, O. Ritvos, and K. P. McNatty. 2002. Growth differential factor 9 and bone morphogenetic protein 15 are essential for ovarian follicular development in sheep. *Biol. Reprod.* 67:1777–1789.
- Kenny, D. A., M. P. Boland, M. G. Diskin, and J. M. Sreenan. 2002. Effect of rumen degradable protein with or without fermentable carbohydrate Suppl.ation on blood metabolites and embryo survival in cattle. *Anim. Sci. (Pencaitland)* 74:529–537.
- Keisler, D. H., J. A. Daniel, and C. D. Morrison 1999. The role of leptin in nutritional status and reproductive function. *J. Reprod. Fertil. Suppl.* 54:425–435.
- Knight, P. G., and C. Glistler. 2001. Potential local regulatory functions of inhibins, activins and follistatins in the ovary. *Reproduction* 121:503–512.
- Kobayashi, Y., C. K. Boyd, C. J. Bracken, W. R. Lamberson, D. H. Keisler, and M. C. Lucy. 1999. Reduced growth hormone receptor (GHR) messenger ribonucleic acid in liver of periparturient cattle is caused by a specific down regulation of GHR 1A that is associated with decreased insulin-like growth factor I. *Endocrinology* 140:3947–3954.
- Kobayashi, Y., C. K. Boyd, B. L. McCormack, and M. C. Lucy. 2002. Reduced insulin-like growth factor-I after acute feed restriction in lactating dairy cows is independent. *J. Dairy Sci.* 85:748–754.
- Kojima, F. N., E. G. M. Bergfeld, M. E. Wehrman, A. S. Cupp, K. E. Fike, D. V. Mariscal-Aguayo, T. Sanchez-Torres, M. Garcia-Winder, D. T. Clopton, A. J. Roberts, and J. E. Kinder. 2003. Frequency of hormone pulses in cattle influences duration of persistence of dominant ovarian follicles, follicular fluid concentration of steroids, and activity of insulin-like growth factor binding proteins. *Anim. Reprod. Sci.* 77:187–211.
- Kulick, L. J., K. Kot, M. C. Wiltbank, and O. J. Ginther. 1999. Follicular and hormonal dynamics during the first follicular wave in heifers. *Theriogenology* 52:913–921.
- Leeuwenberg, B. R., P. R. Hurst, and K. P. McNatty. 1995. Expression of IGF-I mRNA in the ovine ovary. *J. Mol. Endocrinol.* 15:251–258.
- Lucy, M. C. 2000. Regulation of ovarian follicular growth by somatotropins and insulin-like growth factors in cattle. *J. Dairy Sci.* 83:1635–1647.
- Lucy, M. C. 2003. Mechanisms linking nutrition and reproduction in postpartum cows. *Reproduction in Domestic Ruminants V. Reproduction Suppl.* 61:415–417.
- Lucy, M. C., C. R. Bilby, C. J. Kirby, W. Yuan, and C. K. Boyd. 1999. Role of growth hormone in the maintenance of follicles and corpora lutea. *J. Reprod. Fertil. Suppl.* 54:49–59.
- Mackey, D. R., J. M. Screenan, J. F. Roche, and M. G. Diskin. 1999. Effect of acute nutritional restriction on the incidence of anovulation and periovulatory estradiol and gonadotropin concentrations in beef heifers. *Biol. Reprod.* 61:1601–1607.
- Mann, G. E., M. P. Green, K. D. Sinclair, K. J. Demmers, M. D. Fray, C. G. Gutierrez, P. C. Garnsworthy, and R. Webb. 2003. Effects of circulating progesterone and insulin on embryo development in beef heifers. *Anim. Reprod. Sci.* 79:71–79.
- Mazerbourg, S., J. Zapf, R. S. Bar, D. R. Brigstock, and P. Monget. 2000. Insulin-like growth factor (IGF)-binding protein-4 proteolytic degradation in bovine, equine, and porcine preovulatory follicles: Regulation by IGF and heparin-binding domain-containing peptides. *Biol. Reprod.* 63:390–400.
- McCaffery, F. H., R. Leask, S. C. Riley, and E. E. Telfer. 2000. Culture of bovine preantral follicles in a serum-free system: markers for assessment of growth and development. *Biol. Reprod.* 63:267–273.
- McCann, J. P., and W. Hansel. 1986. Relationship between insulin and glucose metabolism and pituitary-ovarian function in fasted heifers. *Biol. Reprod.* 34:630–641.
- McEvoy, T. G., J. J. Robinson, R. P. Aitken, P. A. Findlay, R. M. Palmer, and I. S. Robinson. 1995. Dietary-induced suppression of preovulatory progesterone concentrations in superovulated ewes impairs subsequent in vivo an in vitro development of ova. *Anim. Reprod. Sci.* 39:89–107.
- McGuire, M. A., D. A. Dwyer, R. J. Harrell, and D. E. Bauman. 1995. Insulin regulates insulin-like growth factors and some of their binding proteins in lactating cows. *Am. J. Physiol. Endocrinol. Metab.* 269:E723–E730.
- McGuire, M. A., J. L. Vicini, D. Bauman, and J. J. Veenhuizen. 1992. Insulin-like growth factors and their binding proteins in ruminants and their nutritional regulation. *J. Anim. Sci.* 70:2901–2910.
- McNatty, K. P., D. A. Heath, F. Lindy, A. E. Fidler, L. Quirke, A. O'Connell, P. Smith, N. Groome, and D. J. Tisdall. 1999. Control of early ovarian follicular development. *J. Reprod. Fertil. Suppl.* 54:3–16.
- McNatty, K. P., J. L. Juengel, T. Wilson, S. M. Galloway, G. H. Davis, N. L. Hudson, C. C. Moeller, M. Cranfield, K. L. Reader, M. P. E. Laitinen, N. P. Groome, H. R. Sawyer, and O. Ritvos. 2003. Oocyte-derived growth factors and ovulation rate in sheep. *Reproduction in Domestic Ruminants V. Reproduction Suppl.* 61:339–351.
- Mihm, M., E. J. Austin, T. E. M. Good, J. L. H. Ireland, P. G. Knight, J. F. Roche, and J. J. Ireland. 2000. Identification of potential intrafollicular factors involved in selection of dominant follicles in heifers. *Biol. Reprod.* 63:811–819.
- Mihm, M., T. E. M. Good, J. L. H. Ireland, J. J. Ireland, P. Knight, and J. Roche. 1997. Decline in serum follicle-stimulating hormone concentrations alters key intrafollicular growth factors involved in selection of the dominant follicle in heifers. *Biol. Reprod.* 57:1328–1337.
- Molento, C. F. M., E. Block, R. I. Cue, and D. Peticlerc. 2002. Effects of insulin, recombinant bovine somatotropin, and their interaction on insulin-like growth factor-I secretion and milk production in dairy cows. *J. Dairy Sci.* 85:738–747.
- Monget, P., S. Fabre, P. Mulsant, F. Lecerf, J. M. Elsen, S. Mazerbourg, C. Pisselet, and D. Monniaux. 2002. Regulation of ovarian folliculogenesis by IGF and BMP system in domestic animals. *Domest. Anim. Endocrinol.* 23:139–154.
- Monget, P., and G. B. Martin. 1997. Involvement of insulin-like growth factors in the interactions between nutrition and reproduction in female mammals. *Human Reprod.* 12 (Suppl. 1):33–51.
- Monget P., S. Mazerbourg, T. Delpuech, M. C. Maurel, S. Maniere, J. Zapf, G. Lalmanach, C. Oxvig, and M. T. Overgaard. 2003. Pregnancy-associated plasma protein A is involved in insulin-like growth factor binding protein-2 (IGFBP-2) proteolytic degradation in bovine and porcine preovulatory follicles: identification of cleavage site and characterization of IGFBP-2 degradation. *Biol. Reprod.* 68:77–86.

- Montgomery, G. W., S. M. Galloway, G. H. Davis, and K. P. McNatty. 2001. Genes controlling ovulation rate in sheep. *Reproduction* 121:843–852.
- Morimoto, S., C. Fernandez-Mejia, G. Romerero-Navarro, N. Morales-Peza, and V. Diaz-Sanchez. 2001. Testosterone effect on insulin content, messenger ribonucleic acid levels, promoter activity, and secretion in rats. *Endocrinology* 142:1442–1447.
- Mulsant, P., F. Lecerf, S. Fabre, L. Bodin, J. Thimonier, P. Monget, I. Lanneluc, D. Monniaux, J. Teyssier, and J. M. Elsen. 2003. Prolificacy genes in heep: The French genetic program. *Reproduction in Domestic Ruminants V. Reproduction Suppl.* 61:353–359.
- Nicholas, B., R. K. Scougall, D. G. Armstrong, and R. Webb. 2002. Changes in insulin-like growth factor binding protein (IGFBP) isoforms during bovine follicular development. *Reproduction* 124:439–446.
- Nicholas, B. L., R. Webb, and D. G. Armstrong. 2000. Characterization of insulin-like growth factor binding protein-2 (IGFBP-2) protease activity in bovine theca cell conditioned media. *J. Reprod. Fertil. Abstr. Ser.* 25:52. (Abstr.)
- O'Callaghan, D., and M. P. Boland. 1999. Nutritional effects on ovulation, embryo development and the establishment of pregnancy in ruminants. *Anim. Sci. (Pencaitland)* 68:299–314.
- O'Callaghan, D., H. Yaakub, P. Hyttel, L. J. Spicer, and M. P. Boland. 2000. Effect of nutrition and superovulation on oocyte morphology, follicular fluid composition and systemic hormone concentrations in ewes. *J. Reprod. Fertil.* 118:303–313.
- Parker, K. I., D. M. Robertson, N. P. Groome, and K. L. Macmillan. 2003. Plasma concentrations of inhibin A and follicle stimulating hormone differ between cows and two or three waves of ovarian follicular development in a single estrous cycle. *Biol. Reprod.* 68:822–828.
- Perks, C. M., P. A. Denning-Kendall, R. S. Gilmour, and D. C. Wathes. 1995. Localization of messenger ribonucleic acids for insulin-like growth factor-I (IGF-I), IGF-II and the type 1 IGF receptor in the ovine ovary throughout the oestrous cycle. *J. Endocrinol.* 136:5266–5273.
- Perks, C. M., A. R. Peters, and D. C. Wathes. 1999. Follicular and luteal expression of insulin-like growth factor I and II and the type 1 IGF receptor in the bovine ovary. *J. Reprod. Fertil.* 116:157–165.
- Rausch, M. I., M. W. Tripp, K. E. Govoni, W. Zang, W. J. Weber, B. A. Crooker, T. A. Hoagland, and S. A. Zinn. 2002. The influence of level of feeding on growth and serum insulin-like growth factor I and insulin-like growth factor-binding proteins in growing beef cattle supplied with somatotropin. *J. Anim. Sci.* 80:94–100.
- Richards, M. W., R. P. Welleman, L. J. Spicer, and C. L. Morgan. 1991. Nutritional anestrus in beef cows: Effect of body condition and ovariectomy on serum luteinizing hormone and insulin-like growth factor-I. *Biol. Reprod.* 44:961–966.
- Rhodes, F. M., L. A. Fitzpatrick, K. W. Entwistle, and G. De'ath. 1995. Sequential changes in ovarian follicular dynamics in Bos indicus heifers before and after nutritional anoestrus. *J. Reprod. Fertil.* 104:41–49.
- Rivera, G. M., Y. A. Chandrasekher, A. C. O. Evans, L. C. Giudice, and J. E. Fortune. 2001. A potential role for insulin-like growth factor binding protein-4 proteolysis in the establishment of ovarian follicular dominance in cattle. *Biol. Reprod.* 65:102–111.
- Rivera, G. M., and J. E. Fortune. 2003a. Selection of the dominant follicle and insulin-like growth factor (IGF)-binding protein 5: evidence that pregnancy-associated plasma protein A contributes to proteolysis of IGF-binding protein 5 in bovine follicular fluid. *Endocrinology* 144:437–446.
- Rivera, G. M., and J. E. Fortune. 2003b. Proteolysis of insulin-like growth factor binding proteins-4 and -5 in bovine follicular fluid: implications for ovarian follicular selection and dominance. *Endocrinology* 144:2977–2987.
- Royal, M. D., A. O. Darwash, A. P. F. Flint, R. Webb, J. A. Woolliams, and G. E. Lamming. 2000. Declining fertility in dairy cattle: Changes in traditional and endocrine parameters of fertility. *Anim. Sci. (Pencaitland)* 70:487–502.
- Saha, S., M. Shimizu, M. Geshi, and Y. Izaike. 2000. In vitro culture of bovine preantral follicles. *Anim. Reprod. Sci.* 63:27–39.
- Schams, D., B. Bensha, M. Krosmann, W. M. Amselgruber. 2002. Expression and localization of IGF family members in bovine antral follicles during final growth and in luteal tissue during different stages of estrous cycle and pregnancy. *Domest. Anim. Endocrinol.* 22:51–72.
- Silva, K. M., and C. A. Price. 2001. Effect of follicle-stimulating hormone on steroid secretion and messenger ribonucleic acids encoding cytochromes P450 aromatase and cholesterol side-chain cleavage as bovine granulosa cells in vitro. *Biol. Reprod.* 62:186–191.
- Simpson, R. B., C. C. Chase, L. J. Spicer, J. A. Carroll, A. C. Hammond, and T. H. Welsh. 1997. Effect of exogenous estradiol on plasma concentrations of somatotropin, insulin-like growth factor-I, insulin-like growth factor binding protein activity, and metabolites in ovariectomized Angus and Brahman cows. *Domest. Anim. Endocrinol.* 14:367–380.
- Simpson, R. B., C. C. Chase, Jr., L. J. Spicer, R. K. Vernan, A. L. Hammond, and D. O. Rae. 1994. Effects of exogenous insulin on plasma and follicular insulin like growth factor I, insulin like growth factor binding activity, follicular oestradiol and progesterone and follicular growth in superovulated Angus and Brahman cows. *J. Reprod. Fertil.* 102:483–492.
- Sinclair, K. D., M. Kuran, F. E. Gebbie, R. Webb, and T. B. McEvoy. 2000. Nitrogen metabolism and fertility in cattle: II. Development of oocytes recovered from heifers offered diets differing in their rate of nitrogen release in the rumen. *J. Anim. Sci.* 78:2670–2689.
- Smitz, J. E., and R. G. Cortvindt. 2002. The earliest stages of folliculogenesis in vitro. *Reproduction* 123:185–202.
- Souza, C. J. H., B. K. Campbell, A. S. McNeilly, and D. T. Baird. 2002. Effect of bone morphogenetic protein 2 (BMP2) on oestradiol and inhibin A production by sheep granulosa cells, and localization of BMP receptors in the ovary by immunohistochemistry. *Reproduction* 123:363–369.
- Souza, C. J. H., B. K. Campbell, A. S. McNeilly, and D. T. Baird. 2003. What the known phenotypes of the Booroola mutation can teach us about its mechanism of action? *Reproduction in Domestic Ruminants V. Reproduction Suppl.* 61:361–370.
- Spicer, L. J. 2001. Leptin: A possible metabolic signal affecting reproduction. *Domest. Anim. Endocrinol.* 21:251–270.
- Spicer, L. J., E. Alpizar, and S. E. Echterkamp. 1993. Effects of insulin, insulin-like growth factor I, and gonadotropins on bovine granulosa cell proliferation, progesterone production, estradiol production and (or) insulin-like growth factor I production in vitro. *J. Anim. Sci.* 71:1232–1241.
- Spicer, L. J., and C. S. Chamberlain. 2000. Production of insulin-like growth factor-I by granulosa cells but not thecal cells is hormonally responsive in cattle. *J. Anim. Sci.* 38:2919–2926.
- Spicer, L. J., C. S. Chamberlain, and S. M. Maciel. 2002. Influence of gonadotropins on insulin and insulin-like growth factor-I (IGF-I)-induced steroid production by bovine granulosa cells. *Domest. Anim. Endocrinol.* 22:237–254.
- Spicer, L. J., G. L. Chamberlain, and G. L. Morgan. 2001. Proteolysis of insulin-like growth factor binding proteins during preovulatory follicular development in cattle. *Domest. Anim. Endocrinol.* 21:1–15.
- Spicer, L. J., and S. E. Echterkamp. 1995. The ovarian insulin and insulin-like growth factor system with an emphasis on domestic animals. *Domest. Anim. Endocrinol.* 12:223–245.
- Spicer, L. J., and C. C. Francisco. 1997. The adipose obese gene product, leptin: evidence of a direct inhibitory role in ovarian function. *Endocrinology* 138:3373–3379.
- Thissen, J. P., J. M. Ketelslegers, and L. E. Underwood. 1994. Nutritional regulation of the insulin-like growth-factors. *Endocr. Rev.* 15:80–101.
- Thomas, F. H., R. Leask, V. Sisen, S. C. Rileys, N. Spears, and E. E. Telfer. 2001. Effect of ascorbic acid on health and morphology of bovine preantral follicles during long-term cultures. *Reproduction* 122:487–495.

- Vandehaar, M. J., B. K. Sharma, and R. L. Fogwell. 1995. Effect of dietary energy restriction on the expression of insulin-like growth factor 1 in liver and corpus luteum of heifers. *J. Dairy Sci.* 78:832–841.
- Vestergaard M., S. Purup, P. Henckel, E. Tonner, D. J. Flint, L. R. Jensen, and K. Serjrsen. 1995. Effects of growth hormone and ovariectomy on performance, serum hormones, insulin-like growth factor binding proteins, and muscle fibre properties of prepubertal Friesian heifers. *J. Anim. Sci.* 73:3574–3584.
- Wandji, S.-A., G. Pelletier, and M.-A. Sirard. 1992. Ontogeny and cellular localization of ¹²⁵I-labelled insulin-like growth factor-1, ¹²⁵I-labelled follicle-stimulating hormone, and ¹²⁵I-labelled human chorionic gonadotropin binding sites in ovaries from bovine fetuses and neonatal calves. *Biol. Reprod.* 47:814–822.
- Webb, R., B. K. Campbell, H. A. Garverick, J. G. Gong, C. G. Gutierrez, and D. G. Armstrong. 1999a. Molecular mechanisms regulating follicular recruitment and selection. *Reproduction in Domestic Ruminants IV. J. Reprod. Fertil. Suppl.* 54:33–48.
- Webb, R., P. C. Garnsworthy, J. G. Gong, R. S. Robinson, and D. C. Wathes. 1999c. Consequences for reproductive function of metabolic adaptation to load Metabolic stress in dairy cows. Pages 99–112 in Occasional Publication No. 24. Brit. Soc. Anim. Sci., Pencaitland, U.K.
- Webb, R., R. G. Gosden, E. E. Telfer, and R. M. Moor. 1999b. Factors affecting folliculogenesis in ruminants. *Anim. Sci. (Pencaitland)* 68:257–284.
- Webb, R., B. Nicholas, J. G. Gong, B. K. Campbell, C. G. Gutierrez, H. A. Garverick, and D. G. Armstrong. 2003. Mechanism regulating follicular development and selection of the dominant follicle. *Reproduction in Domestic Ruminants V. Reproduction Suppl.* 61:71–90.
- Yuan, W., B. Bao, H. A. Garverick, R. S. Youngquist, and M. C. Lucy. 1998. Follicular dominance in cattle is associated with divergent patterns of ovarian gene expression for insulin-like growth factor (IGF)-I, IGF-II and IGF binding protein-2 in dominant and subordinate follicles. *Domest. Anim. Endocrinol.* 15:55–63.