Regulation of Ovarian Follicular Growth by Somatotropin and Insulin-Like Growth Factors in Cattle

M. C. Lucy
Department of Animal Sciences, University of Missouri, Columbia 65211

ABSTRACT

Somatotropin (ST), insulin-like growth factor (IGF)-I, and IGF-II affect animal growth and lactation as well as animal reproduction. Understanding the effects of ST and the IGF on reproduction is important because ST and IGF-I undergo dynamic changes prior to the postpartum breeding period. In addition, administration of recombinant bovine somatotropin (rbST) to lactating cows is a common practice that increases blood concentrations of ST and IGF-I during the breeding period. In vivo, administration of rbST caused greater ovarian follicular development. The effects of rbST may represent direct actions of ST because ST receptors are found within granulosa cells as well as oocytes. Alternatively, the actions of ST may be indirectly mediated by increased IGF-I and (or) nutrient partitioning that occurs after rbST. Both IGF-I and IGF-II are synthesized within the ovary. Ovarian IGF are, therefore, a composite of IGF from both endocrine (liver) and autocrine and paracrine (ovary) sources. The IGF stimulate ovarian function by acting synergistically with gonadotropins to promote growth and steroidogenesis of ovarian cells. Actions of IGF-I and -II are restrained by a series of IGF binding proteins (IGFBP) that either originate from the binding proteins or are synthesized locally within the follicle. Degradation and differential synthesis of IGFBP are important mechanisms regulating IGFBP amounts. The relative amounts of IGFBP may ultimately determine ovarian IGF action. Future studies of ST and IGFs should focus on the hormones, receptors, and binding proteins as well as the metabolic requirements for normal ovarian function in dairy cattle.

Key words: ovary, insulin-like growth factor, follicle, bovine

Abbreviation key: CL = corpus luteum, IGFBP = IGF binding protein, rbST = recombinant bST, ST = somatotropin.

INTRODUCTION

Somatotropin (ST) is a pituitary hormone that controls many aspects of animal growth and nutrient metabolism (34, 39). In addition to its diverse actions on animal growth and nutrition, ST also affects reproductive function of animals. A direct effect of ST on a variety of reproductive cells was suggested because mRNA for ST receptor was found within reproductive tissues (49, 61, 70). For example, hypothalamus, pituitary, corpus luteum (CL), ovarian follicle, oviduct, endometrium, myometrium, and placenta had ST receptor mRNA (49, 61, 70). If the mRNA for ST receptor is translated into protein then ST could potentially act directly upon nearly every tissue of the reproductive system. For the whole animal, the tissue with the greatest concentrations of ST receptor is the liver, and liver expression of ST receptor exceeds that of reproductive tissues by severalfold (70). Somatotropin binding within liver stimulates a series of metabolic changes that influence the growth and metabolism of dairy cattle (34). In addition to changing metabolism within the liver, ST causes an increase in the synthesis and secretion of IGF-I as well as the principal IGF carrier protein, IGF binding protein (IGFBP)-3 (56). Insulin-like growth factor-I is a pleotrophic growth factor that stimulates growth and development within a variety of cell types (56). Insulin-like growth factor-II is similar to IGF-I in structure and function but has lower potency, and ST does not control its secretion (100). An endocrine theory (the somatomedin hypothesis) proposes that some of the effects of ST on growth and reproduction are mediated by the release of IGF-I from liver (56). Once released from the liver, IGF-I travels via the blood and acts on distant tissues including those of the reproductive tract (6, 38, 92) (Figure 1).

ENDOCRINE IGF-I AND REPRODUCTION IN CATTLE

Changes in Blood ST and IGF-I in Postpartum Cattle

The blood concentrations of ST and IGF-I undergo dynamic changes during the periparturient period (18, 94). Relative to lactating cattle, prepartum cattle have low blood concentrations of ST and high blood concen-
The effects of ST are directly dependent on the number of ST receptors as well as the activity of the ST receptor second messenger system (16). For example, greater ST receptor amounts or greater function of the ST receptor second messenger system leads to greater concentrations of blood IGF-I (59, 79) (Figure 1). In a variety of species (including farm animals, humans, and laboratory animals) higher concentrations of blood IGF-I are found in young, well nourished, healthy individuals (56, 99). Animals that are old, diseased, or malnourished have low blood IGF-I concentrations that reflect a compromised state of tissue, organ, and cell function (56, 69, 76, 99). The changes in blood IGF-I can be directly linked to changes in ST receptor expression or ST receptor second messenger systems in liver (Figure 1).

There are at least three gene promoters that control ST receptor expression (54, 88). The relative activity of each promoter within a tissue determines the amount of ST receptor. The activity of the different ST receptor promoters leads to ST receptor mRNA variants because promoter-specific first exons are spliced onto exons 2 through 10 of the ST receptor (32). The first ST receptor promoter, P1, is liver-specific, developmentally regulated, and metabolically controlled (88). The P1 transcribes ST receptor 1A mRNA. The primary location for ST receptor 1A mRNA is the liver of adult animals where ST receptor 1A represents the bulk of liver ST receptor mRNA (70) (Figure 1). The ST receptor 1A mRNA in liver is unique from other types of ST receptor mRNA because of its sensitivity to external signals that lead to rapid up or down regulation of the amount of ST receptor 1A (62, 63). The existence of ST receptor 1A mRNA may be a mechanism for maintaining a high-level of ST receptor expression in liver where ST has multiple actions through the ST receptor (34). Liver-specific transcription factors probably control transcription from P1 and ultimately determine the amount of ST receptor 1A mRNA (28).

Acute changes in ST receptor 1A occur in cattle during the periparturient period. There is a rapid decline in ST receptor 1A mRNA at calving (day 0) that is completely reversed by 21 d postpartum (62). The decrease in ST receptor 1A mRNA was associated with a decrease in total liver ST receptor mRNA, a loss of IGF-I mRNA, and a decrease in blood IGF-I concentrations. Therefore, one potential mechanism for decreased IGF-I during the periparturient period is decreased liver ST receptor amount that leads to a decrease in secretion of IGF-I from liver.

What Role Does Liver ST Receptor Play in Controlling Blood IGF-I Concentrations?

The somatomedin hypothesis for somatotropin (ST) and IGF-I action on the ovary (see text for details). Somatotropin is synthesized by the pituitary and interacts with receptors located within the liver and the ovary (corpus luteum [CL] and follicles [F]). Within the liver, ST causes the synthesis and secretion of IGF-I. The primary ST receptor in liver is ST receptor 1A. Insulin-like growth factor-I can travel through the blood and affect ovarian function (endocrine IGF-I). Somatotropin can also act directly on the ovary through ovarian ST receptors (ST receptors 1B and 1C). The ovary produces IGF-I and IGF-II (ovarian IGF) that can complement the IGF-I from endocrine sources (primarily liver). The ovarian IGF is not controlled by ST in the bovine but is controlled by ST in other species. The somatomedin hypothesis states that the actions of IGF-I on the ovary are caused by both endocrine and ovarian IGF-I. Several physiological conditions (box) are associated with decreased IGF-I synthesis and secretion by liver and decreases in blood IGF-I can potentially influence ovarian function through an endocrine mechanism. Insulin-like growth factor-II is less responsive to the physiological conditions that are listed (box).

Figure 1. The somatomedin hypothesis for somatotropin (ST) and IGF-I action on the ovary (see text for details). Somatotropin is synthesized by the pituitary and interacts with receptors located within the liver and the ovary (corpus luteum [CL] and follicles [F]). Within the liver, ST causes the synthesis and secretion of IGF-I. The primary ST receptor in liver is ST receptor 1A. Insulin-like growth factor-I can travel through the blood and affect ovarian function (endocrine IGF-I). Somatotropin can also act directly on the ovary through ovarian ST receptors (ST receptors 1B and 1C). The ovary produces IGF-I and IGF-II (ovarian IGF) that can complement the IGF-I from endocrine sources (primarily liver). The ovarian IGF is not controlled by ST in the bovine but is controlled by ST in other species. The somatomedin hypothesis states that the actions of IGF-I on the ovary are caused by both endocrine and ovarian IGF-I. Several physiological conditions (box) are associated with decreased IGF-I synthesis and secretion by liver and decreases in blood IGF-I can potentially influence ovarian function through an endocrine mechanism. Insulin-like growth factor-II is less responsive to the physiological conditions that are listed (box).
What Are the Relationships Between ST Receptor, Blood IGF-I, and Reproduction?

Insulin-like growth factor-I is decreased in postpartum cattle when energy requirements exceed nutrient intake (10, 94). Cattle in poor body condition or cows failing to increase body condition during lactation also have low blood IGF-I (94). Several studies have established a correlation between blood IGF-I concentrations of postpartum cattle and reproductive function. For example, Thatcher et al. (98) showed that anestrus dairy cows had lower blood IGF-I compared with dairy cows that initiated estrous cyclicity earlier during the postpartum period. A similar relationship was reported for beef cattle; postpartum anestrus cows had lower IGF-I compared with cyclic cows (84). Blood IGF-I was correlated with follicular fluid IGF-I because the majority of IGF-I in follicular fluid was derived from blood (65) (Figure 1). Therefore, endocrine IGF-I under ST control influences ovarian function through its contribution to follicular fluid IGF-I. According to the somatomedin hypothesis, nutritionally induced changes in liver IGF-I secretion have a direct effect on the ovary through the endocrine actions of IGF-I. An association between IGF-I and ovulation in postpartum dairy cows has been established, but a causative relationship should not be extrapolated from these correlative studies.

Insulin-like growth factor-I and gonadotropins are synergistic for growth and differentiation of the follicle (1, 2, 38, 92). Follicular growth and steroidogenesis in postpartum cattle, therefore, should be correlated with greater LH secretion as well as greater blood IGF-I concentrations. A positive correlation between LH pulsatility and ovarian follicular development has been established for postpartum cows (10). Likewise, a correlation between plasma estradiol during the first postpartum follicular wave and serum IGF-I was found (10). Postpartum ovarian function probably depends on both LH pulsatility and blood IGF-I concentrations. The independent contribution of each hormone to normal function is difficult to establish, however, because both LH pulsatility and blood IGF-I concentrations increase during the postpartum period and increase when nutrition is improved.

Do the Associations Between Blood IGF-I and Reproduction Represent Cause and Effect?

The development of gene targeting (knockout) technology in mice has enabled a rigorous test of the relationships between ST, IGF-I, and reproductive function. There is probably no absolute requirement for ST in reproduction because mice with naturally occurring mutations in the ST gene (lit mice) can reproduce (33). Furthermore, women with inactivating ST receptor mutations (Laron dwarfs) (77) and cattle with abnormal ST receptor expression (17) are capable of reproduction. A knockout mouse for the ST receptor was also fertile and confirmed that neither ST nor the ST receptor is necessary for animal reproduction (108). Although reproduction is possible in animals with either a ST gene mutation or a ST receptor deletion, the efficiency of reproduction is low. For example, the ST receptor knockout mice conceived, but litter size averaged 2.7 in knockout females compared with 6.9 in contemporary control females (108). Age of vaginal opening was delayed in ST receptor knockout mice and the delay was partially reversed by systemic administration of IGF-I (23). Therefore, ST and IGF-I probably facilitate reproductive processes so that the efficiency of reproduction is improved.

The effects of an IGF-I gene deletion on reproduction are more significant than those of a ST or ST receptor gene deletion. The IGF-I gene knockout mice had severe postnatal growth retardation (101). Ovarian follicles within the IGF-I knockout mice grew to the preantral stage but failed to complete ovulation (7). The block to ovulation occurred in untreated mice as well as in mice treated with exogenous gonadotropins to induce ovulation. When extreme doses of gonadotropins were used, the IGF-I knockout mice ovulated but the oocytes were of poor quality and were not fertilized. The effect of systemic IGF-I administration on reproduction in IGF-I knockout mice is unknown at this time. The reproductive phenotype of IGF-II knockout mice was less severe. The IGF-II knockout mice also had postnatal growth retardation (25). However, unlike the IGF-I knockout mice, the IGF-II knockout mice were fertile. Therefore, the relative importance of IGF-I may be greater than that of IGF-II for reproduction.

The relationship between blood IGF-I and reproduction has been explored further by using the Cre/loxP system for creating a conditional knockout mouse (102). The Cre/loxP system causes the targeted deletion of a gene within a specific tissue. Mice were created with an IGF-I gene flanked by loxP and mated to transgenic mice with an albumin-Cre recombinase gene (102). Expression of the Cre recombinase exclusively in liver caused the targeted deletion of the IGF-I gene in liver. The targeting experiment ablated liver IGF-I gene expression but left IGF-I gene expression in other tissues intact. Tissues such as muscle and fat (and presumably ovary) maintained or slightly increased IGF-I gene expression. The loss of IGF-I gene expression specifically in liver caused a decrease in blood IGF-I concentrations in the conditional knockout mice (blood IGF-I in knockout mice was approximately one-third of control). The mice grew normally despite the loss of liver IGF-I and...
The bovine ovary can respond directly to ST because the ST receptor is found within ovarian cells. Therefore, the relatively high concentrations of ST found in postpartum cattle can potentially influence follicular growth, steroidogenesis, and oocyte health. In addition, IGF-I, IGF-II, and IGFBPs are synthesized within ovarian follicles. The synthesis of IGFs within follicles creates an autonomous IGF system (Figure 2). The factors that affect the IGF system in bovine follicles are poorly understood.

Ovarian ST Receptors

The ST receptor gene is controlled by several gene promoters that control transcription in a variety of ST-responsive cells. Two related ST receptor promoters (P2 and P3) control ST receptor gene expression within the ovary (49, 54). The P2 and P3 transcribe ST receptor 1B and 1C mRNA, respectively. Within the genome, the P3 is located approximately 800 bp downstream from P2 (54). The spatial relationship between P2 and P3 is important. The P2 contains an enhancer element that causes constitutive activity of the promoter. The basis for the constitutive activity in the enhancer is a GC box that may bind the ubiquitous transcription factor SP-1 (53). On the genomic level, the P3 is close to P2 so that the enhancer drives expression of P3 as well as P2 (54). Therefore, many aspects of expression of P2 and P3 are similar. The essential features of P2 and P3 are activity in a wide variety of tissues (including ovary) with little developmental or metabolic regulation (62).

In cattle, the ST receptor was localized initially to the large cell of the CL (71) (Figure 1). The initial studies demonstrated ST receptor by several methods, including immunohistochemistry (71), ribonuclease protection assay (71), and in situ hybridization (105). When follicles were tested, the ST receptor mRNA was found but the amount was extremely low relative to CL. The ST receptor protein was not found in follicles when immunohistochemistry was used, whereas the CL contained abundant ST receptor protein (71). A similar result was obtained when histological sections were examined for ST receptor mRNA (i.e., expression in CL but no expression in adjacent follicles) (105). Izadyar et al. (52) were able to detect ST receptor mRNA in

Figure 2. Mechanisms for somatotropin (ST) and IGF action within an ovarian cell (see text for details). The model shows receptors in the cell membrane, hypothetical actions of second messenger systems (dotted arrows), and effects on gene transcription (solid arrows originating from the inner circle (nucleus)). Actions of IGF binding protein (IGFBP) are shown as dashed lines. General mechanisms are presented that may or may not be proven in all ovarian cell types (granulosa and theca) or in all species. Some of the effects of ST are mediated directly by the ST receptor within the ovary. The ovarian response to ST may involve the synthesis and secretion of IGF-I by ovarian cells. Ovarian cells synthesize IGF-I (granulosa) and IGF-II (theca). The IGF are synergistic with gonadotropins (LH and FSH) for steroidogenesis. The synergy involves an IGF-mediated up-regulation of gonadotropin receptor expression and gonadotropin receptor second messenger systems. The gonadotropins increase ovarian IGF action by causing the synthesis of IGF-I and type I IGF receptor. Concentrations of IGF in follicular fluid may not change as follicles grow. However, IGFBP are produced by follicular cells of atretic follicles and can bind and inactivate IGF. The IGFBP may also interact directly with an IGFBP receptor that can mediate a direct inhibitory effect of IGFBP on cell growth. In healthy ovarian follicles, IGFBP concentrations are decreased through decreased IGFBP synthesis as well as greater activity of IGFBP proteases.
bovine granulosa cells, cumulus cells, and the oocyte by using reverse transcriptase polymerase chain reaction. The reverse transcriptase polymerase chain reaction technique is more sensitive than the techniques used in the previous studies (71, 105). Therefore, greater sensitivity may be required to detect ST receptor mRNA in bovine follicles. Kölle et al. (64) demonstrated ST receptor mRNA and protein in the oocyte as well as granulosa cells. Expression of ST receptor was greatest in oocytes of primordial and primary follicles. In antral follicles, the greatest amount of ST receptor expression was found in the cells of the cumulus oophorus. Different conclusions for ST receptor expression were reached by Kölle et al. (64) who showed ST receptor mRNA and protein in follicles and other authors (71, 105) who failed to show ST receptor mRNA or protein in follicles. The discrepancy may be explained by differences in the types of follicles that were analyzed in each study. Kölle et al. (64) found the greatest amount of ST receptor in primordial/primary follicles and cumulus oophorus cells and the least amount of ST receptor in granulosa cells of antral follicles. Lucy et al. (71) and Yuan and Lucy (105) studied granulosa cells of antral follicles; a location with minimal ST receptor. Differences in the types of follicles in the analysis and differences in the sensitivity of ST receptor localization, therefore, may explain the different conclusions from the two studies.

**Direct Actions of ST on the Bovine Follicle**

The ST receptor was detected in preantral follicles and cumulus cells. Therefore, ST may have an important function in follicular growth and (or) cumulus/oocyte development. Somatotropin increased in vitro maturation of bovine oocytes within cumulus oocyte complexes (52). The effect was dependent on the cumulus cells because denuded oocytes did not respond to ST. Furthermore, the actions of ST were independent of IGF-I because an IGF-I neutralizing antibody failed to block the ST effect. Izadyar et al. (51) used specific inhibitors for adenylate cyclase and tyrosine kinase to demonstrate that the stimulatory effects of ST were mediated by a cAMP second messenger pathway instead of the expected tyrosine kinase (Jak2) pathway. The involvement of cAMP in the ST response was somewhat unexpected because cAMP is not a traditional ST receptor second messenger (16). Nevertheless, the data agree conceptually with those of Kölle et al. (64) who showed ST receptor protein in oocytes and cumulus cells.

In large antral follicles, ST inhibited growth and proliferation of granulosa cells (43, 93). Whether or not ST promotes differentiation of large antral follicles is less clear because both inhibitory (93) and stimulatory (44) effects of ST on estradiol synthesis in granulosa cells have been reported. Somatotropin stimulated estradiol synthesis in humans and pigs, and the effect of ST on steroidogenesis was blocked by the addition of a neutralizing IGF-I antibody (8, 74, 78). The in vitro responses to ST in some species, therefore, may be secondary to an increase in IGF-I that occurs after ST treatment (Figure 2). Surprisingly, bovine theca cells (not believed to be a site of ST receptor mRNA or protein) responded by increasing androstenedione synthesis when treated with ST (93). The ST response, however, depended on the LH-responsiveness of the cells. Somatotropin increased androstenedione secretion in theca cells that responded well to LH. Theca cells that responded poorly to LH did not have increased androstenedione secretion after ST treatment. Short-term ST infusion did not affect estradiol or androstenedione secretion from autotransplanted ovaries of the ewe (87).

**Ovarian IGF System**

The results of in vitro studies indicate the potential for direct effects of ST on oocytes and granulosa cells. In humans and pigs, ST caused the synthesis of ovarian IGF-I and, therefore, some actions of ST may depend on ovarian IGF-I synthesis (8, 74, 78). As mentioned above, the relative importance of local ovarian IGF compared to endocrine IGF is an important question. The ovarian synthesis of IGF-I may be more important than endocrine IGF-I for ovarian follicular growth because IGF-I Cre/loxP conditional knockout mice had normal litter size (102). The relevance of the conditional knockout mouse model to bovine reproduction will need to be addressed in future studies.

The IGF system (receptors, ligands, and binding proteins for both IGF-I and IGF-II) is expressed within granulosa and theca cells (1, 2, 6, 38, 92) (Figure 2). The cell-specific pattern of IGF gene expression, however, depends on the developmental stage of the follicle as well as the species from which the follicle was collected (e.g., human, mouse, rat, pig, and cattle). Even closely related species have differences in the ovarian IGF system. For example, IGFBP-2 was expressed in granulosa cells of the mouse but in theca cells of the rat (3). The location and developmental pattern for IGF system gene expression within the bovine follicle are presented in Table 1. Follicular cells are sites for IGF synthesis and secretion (Figure 2). The granulosa synthesizes IGF-I and the theca synthesizes IGF-II (81, 104). The amount of IGF-I synthesis is somewhat controversial in cattle because some laboratories have failed to identify IGF-I protein synthesis and secretion by bovine granulosa cells (46). Data showing IGF-II mRNA in theca are more consistent perhaps because IGF-II expression...
Table 1. Expression of the somatotropin (ST) and IGF system genes in bovine ovarian follicles.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Product</th>
<th>Location</th>
<th>References</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST receptor</td>
<td>mRNA</td>
<td>Granulosa, oocyte</td>
<td>52, 64</td>
<td>Primarily located in primordial and primary follicles as well as cumulus cells of large follicles.</td>
</tr>
<tr>
<td>ST receptor</td>
<td>Protein</td>
<td>Granulosa, oocyte</td>
<td>52, 64</td>
<td>Pattern of expression similar to ST receptor mRNA.</td>
</tr>
<tr>
<td>IGF-I</td>
<td>mRNA</td>
<td>Granulosa</td>
<td>81, 104</td>
<td>Low expression compared to IGF-II mRNA.</td>
</tr>
<tr>
<td>IGF-I</td>
<td>Protein</td>
<td>Follicular fluid</td>
<td>27, 29, 36, 96</td>
<td>Concentrations probably do not change in growing follicles.</td>
</tr>
<tr>
<td>IGF-II</td>
<td>mRNA</td>
<td>Theca</td>
<td>81, 104</td>
<td>Greater expression compared to IGF-I mRNA.</td>
</tr>
<tr>
<td>IGF-2</td>
<td>Protein</td>
<td>Follicular Fluid</td>
<td>27, 96</td>
<td>Concentrations may be greater in small follicles (96).</td>
</tr>
<tr>
<td>Type I IGF receptor</td>
<td>mRNA</td>
<td>Granulosa</td>
<td>81</td>
<td>Three- to fivefold greater IGF-I binding in granulosa compared with theca.</td>
</tr>
<tr>
<td>Type I IGF receptor</td>
<td>Protein</td>
<td>Granulosa, theca</td>
<td>96</td>
<td>In the ovine, pattern of expression similar to receptor mRNA (97).</td>
</tr>
<tr>
<td>Type II IGF receptor</td>
<td>mRNA</td>
<td>Unknown for bovine</td>
<td></td>
<td>In the ovine, pattern of expression similar to receptor mRNA (97).</td>
</tr>
<tr>
<td>Type II IGF receptor</td>
<td>Protein</td>
<td>Unknown for bovine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGFBP&lt;sup&gt;1&lt;/sup&gt;-2</td>
<td>mRNA</td>
<td>Granulosa</td>
<td>5, 104</td>
<td>Signal decreased in large healthy follicles.</td>
</tr>
<tr>
<td>IGFBP&lt;sup&gt;2&lt;/sup&gt;-2</td>
<td>Protein</td>
<td>Follicular fluid, granulosa</td>
<td>5, 27, 29, 36, 96</td>
<td>Low concentrations in healthy estrogen active follicles. Increased concentrations in atretic follicles.</td>
</tr>
<tr>
<td>IGFBP-3</td>
<td>mRNA</td>
<td>Not detected in follicle</td>
<td>104</td>
<td>May not be produced locally within the follicle.</td>
</tr>
<tr>
<td>IGFBP-3</td>
<td>Protein</td>
<td>Follicular fluid</td>
<td>27, 29, 36, 96</td>
<td>Concentrations do not change during follicular growth. Primary source may be serum.</td>
</tr>
<tr>
<td>IGFBP-4</td>
<td>mRNA</td>
<td>Theca</td>
<td>5</td>
<td>No change during follicular growth.</td>
</tr>
<tr>
<td>IGFBP-4</td>
<td>Protein</td>
<td>Follicular fluid, granulosa, theca</td>
<td>5, 27, 29, 36</td>
<td></td>
</tr>
<tr>
<td>IGFBP-5</td>
<td>mRNA</td>
<td>Unknown for bovine</td>
<td></td>
<td>In the ovine, detected in the theca of healthy follicles and the granulosa of atretic follicles (11).</td>
</tr>
<tr>
<td>IGFBP-5</td>
<td>Protein</td>
<td>Follicular fluid</td>
<td>5, 27, 29, 36</td>
<td>Low concentrations in healthy estrogen active follicles. Increased concentrations in atretic follicles.</td>
</tr>
</tbody>
</table>

<sup>1</sup>IGF binding protein.
within theca is greater than that of IGF-I in granulosa (81).

The type I IGF receptor mediates most actions of IGF-I and -II (Figure 2). The receptor is a tetrameric molecule that acts through a classical growth factor signaling cascade involving the activation of the tyrosine kinase domain of the receptor (66, 95). The IGF second messenger pathways are probably active in ovarian cells, but specific studies with bovine granulosa or theca have not been completed. When the IGF were tested in vitro, IGF-I demonstrated greater potency for the type I receptor compared to IGF-II (66, 95). Nevertheless, there is intense local expression of IGF-II within the theca, and IGF-II from the theca may act upon the type I IGF receptor in granulosa. In addition to expressing the type I receptor in granulosa, ovarian follicles express the type II IGF receptor (specific for IGF-II) in granulosa and theca cells (97). The type II IGF receptor is different from the type I IGF receptor because it is a single-chain membrane-spanning receptor that does not have a clearly defined second messenger pathway. The type II IGF receptor does not activate the classically described IGF second messenger pathways that are believed to be involved in follicular growth. The primary function of the type II IGF receptor is to clear IGF-II from the cell surface by binding and internalizing IGF-II through receptor-mediated endocytosis. The IGF-II is then degraded by intracellular lysosomes (14). This unique activity of the type II IGF receptor may be required to prevent tumor formation in tissues that express IGF-II (14).

Insulin-like growth factors-I and -II cause growth, differentiation, and survival of follicular cells (1, 2, 38, 58). The most important actions of ovarian IGF are, however, observed when the IGF act synergistically with the gonadotropins (either FSH or LH). The synergistic relationship between the IGF and gonadotropins is seen for a variety of cellular functions including mitogenesis and steroidogenesis (47, 90). The synergism is caused by the ability of IGF to increase gonadotropin receptor numbers and increase the activity of gonadotropin receptor second messenger systems (1, 2, 38). At the same time, gonadotropins increase type I IGF receptor expression and may increase IGF-I synthesis in granulosa cells (92). The dependence of gonadotropin receptors on IGF-I may be greater, however, because IGF-I knockout mice underexpress ovarian FSH receptors, while FSH receptor knockout mice have normal ovarian IGF-I gene expression (107). Therefore, the amount of IGF-I gene expression determines the amount of FSH receptor gene expression.

In addition to being an active site of IGF-I and -II synthesis and secretion, the ovary also synthesizes, secretes, and degrades IGFBP (1, 2, 11, 12, 27, 29, 36, 96) (Figure 2). Indeed, the response of the ovary to the IGF may ultimately depend on the net effect of IGFBP rather than on the absolute concentration of IGF because the concentrations of IGF-I and -II in follicular fluid do not change markedly during follicular growth (27, 96). The amount of IGFBP in follicular fluid depends on follicular health; atretic follicles have the greatest concentrations of IGFBP (11, 27, 96). The IGFBP may prevent the IGF from binding to receptors located within granulosa cells and, therefore, prevent further growth or differentiation in follicles undergoing atresia. A direct inhibitory effect of IGFBP on the growth of ovarian cells is also possible through specific IGFBP receptors (35, 83) (Figure 2).

There is local synthesis of IGFBP-2, and -4 in granulosa and theca cells (respectively) of bovine follicles (5). The mRNA for IGFBP-3 was not detected in bovine follicles (104) and apparently does not arise from local ovarian synthesis (27). The amount of IGFBP is controlled at two levels during follicular growth. First, there is transcriptional control of IGFBP mRNA. For example, the IGFBP-2 mRNA and protein in granulosa cells decreased during follicular growth (5, 104). Changes in transcription did not, however, occur for all IGFBP because IGFBP-4 mRNA and protein remained constant in growing follicles (5). Second, IGFBP protein degrades. Proteases are present in the follicular fluid of estrogen active dominant follicles (12) (Figure 2). The IGFBP proteases degrade IGFBP and maintain a low concentration of IGFBP in follicular fluid. The IGFBP concentration theoretically enables greater IGF action in dominant follicles by creating a larger free IGF pool. When tested in vitro, IGFBP-3 inhibited the stimulatory effects of IGF-I on bovine granulosa cells (91). Therefore, decreased IGFBP may lead to greater IGF action.

Changes in IGF Gene Expression during Follicular Growth

The first wave dominant follicle in cattle has been used extensively as a model for gene expression during ovarian follicular growth. The amounts of IGF-I and IGF-II mRNA were greater in dominant follicles of the first follicular wave than in subordinate follicles collected at the same time (104). Differences in gene expression were present during both early-dominance (9-mm dominant follicle) and middominance (14- to 16-mm dominant follicle). At the same time, IGFBP-2 mRNA was greatest in subordinate follicles and nearly undetectable in dominant follicles. A similar study examining preovulatory follicles (i.e., second or third wave dominant follicles after luteolysis) has not been completed in cattle. In a study of follicles collected at specific
stages after weaning in sows, mRNA for ST receptor, IGF-I and type I IGF receptor did not change as follicles grew from 2 to 8 mm (preovulatory diameter) (68). The IGF-II mRNA, however, was increased as follicles grew to 6 mm and then decreased in 8 mm follicles. Type II IGF receptor mRNA (in granulosa) was greatest in 8-mm follicles. These data agree with data from pigs during an estrous cycle, in which IGF-I was similar in small antral and large antral follicles while IGF-II mRNA increased as follicles grew (106). The IGFBP-2 mRNA decreased in growing porcine follicles. Therefore, the general trend for increasing IGF mRNA (particularly IGF-II) and decreasing IGFBP-2 mRNA during follicular growth is conserved for cattle and pigs (5, 68, 104, 106).

**Does ST Control Ovarian IGF-I in Cattle?**

Ovarian follicles contain ST receptor mRNA and protein (52, 64, 71). In liver, ST acts on the ST receptor and causes an increase in hepatic IGF-I synthesis (56) (Figure 1). One important question is whether ST controls ovarian IGF-I synthesis. Somatotropin dependent, ovarian IGF-I synthesis has been shown in the rat (24), rabbit (103), pig (50), and sheep (57). In cattle, however, ST failed to increase ovarian IGF-I synthesis (61). In addition, immunization of heifers against somatotropin releasing factor decreased blood ST and IGF-I but did not change ovarian IGF-I mRNA concentration (19). Both bovine studies, however, measured IGF-I mRNA in whole ovaries. Therefore, subtle changes in IGF-I gene expression that may have occurred in ovarian follicles may not have been detected. Additional work using in situ hybridization is necessary to clarify the relative expression of IGF-I mRNA is specific ovarian cell types following manipulation of blood concentrations.

**FOLLICULAR RESPONSES TO RECOMBINANT BST IN THE WHOLE ANIMAL**

One method to test the effects of ST and (or) IGF-I on follicular growth is to administer recombinant bovine ST (rbST) or create a transgenic animal that overexpresses ST. These experiments have been completed in a variety of laboratory and farm animal species. Farm and laboratory animals with increased concentrations of ST have greater blood concentrations of IGF-I and greater ovarian follicular development (9, 26, 31, 40, 41, 42, 60). Data from cattle are consistent with most species because cattle had increased numbers of ovarian follicles after rbST administration. In addition, rbST changed patterns of ovarian follicular growth and development by causing premature loss of dominance in dominant follicles.

**Effect of rbST on the Number of Ovarian Follicles in Cattle**

Cattle have waves of follicular development during the estrous cycle (37). Early in a follicular wave, a cohort of follicles is recruited (recruitment phase). From the cohort, a single follicle is selected and continues development (deviation or selection phase). Once deviation has occurred, the single follicle remains dominant by suppressing the development of other smaller follicles and other members of the original cohort (dominance phase). The number of recruited follicles increased in cows or heifers that were either injected daily with rbST or treated with a sustained release formulation of rbST (26, 40, 41, 42, 60). Greater populations of recruited follicles could be maintained for at least 84 d and persisted for at least 21 d after termination of rbST treatment (60). In these original studies, rbST increased the number of recruited follicles and rbST-treated cattle did not have a greater number of dominant follicles (>10 mm diameter) or increased ovulation rate. Therefore, recruitment but not selection was sensitive to rbST stimulation. The method of rbST administration may be important to follicular responses, however, because in a recent study, increased numbers of dominant follicles and increased ovulation rate were found in dairy cattle infused for 63 d with pulsatile doses of rbST (55). Greater follicular growth and ovulation could increase twinning in dairy cattle, and rbST caused a higher proportion of twin births in some herds (20). The increase in twinning, however, was not replicated in later studies (21). The failure to observe a consistent effect of rbST on twinning in dairy cattle may reflect an interaction of ST with either genetic or environmental factors.

The increase in follicular development after rbST treatment may be a direct effect of rbST on the follicle (through receptors located in the cumulus cells). Alternatively, greater blood IGF-I concentrations or changes in nutrient partitioning that occur after rbST may alter patterns of follicular development. The ST receptor mRNA and protein were found within the follicle (52, 64) and, in laboratory species, ST increased preantral follicular growth in vitro (67). Bovine oocyte development in vitro was also improved by ST treatment (52). The level of ST receptor expression in antral follicles, however, is relatively low (71, 105). Therefore, the functional significance of ST for antral follicular growth remains to be established.

Several lines of evidence support the possibility that the increase in IGF-I in blood after rbST treatment mediates the effects of rbST. Heifers treated with increasing doses of ST failed to have greater growth of antral follicles when the ST dose was below the threshold for increased IGF-I (40). Miniature cattle with high
blood ST but low blood IGF-I concentrations had one-third the number of small antral follicles compared with control cattle in the same herd (17). Finally, cattle selected for multiple births had greater blood and follicular fluid IGF-I concentrations (30). Based on in vitro data, the most likely mechanism for increased follicular growth is the synergistic effect of IGF-I on expression or action of gonadotropin receptors. In vivo, however, ST increased follicular fluid IGF-I but did not increase gonadotropin binding sites in bovine follicles (4). The possibility that ST or IGF-I increases the in vivo activity of gonadotropin second messenger pathways without changing gonadotropin receptor number has not been addressed. An alternative possibility is that ST supplementation decreases atresia of growing follicles and leads to a greater number of healthy antral follicles. In nonruminant granulosa cells, ST and IGF-I decreased apoptosis (58) and, in cattle, rbST decreased follicular atresia following estradiol treatment (22). A mechanism involving decreased atresia, therefore, may partially explain greater follicular development in cattle with greater blood IGF-I concentrations.

There is a strong intuitive argument for an IGF-I-mediated mechanism for increased follicular growth in rbST-treated cows. The relationship between follicular growth and IGF-I may not, however, represent cause and effect. The Cre/loxP mice with a liver IGF-I gene knockout had normal reproductive despite blood IGF-I concentrations that were one-third of control (102). In addition, cattle fed twice maintenance had increased follicular development that was correlated with blood concentrations of insulin but not blood concentrations of IGF-I (48). The changes in follicular growth that occur after nutritional changes or rbST, therefore, are not necessarily caused by IGF-I but may be caused by changes in blood concentrations of other hormones (i.e., insulin) or metabolites (i.e., glucose or nonesterified fatty acids). Glucose is the primary energy source for the bovine ovary (82) and abomasal glucose infusion increased numbers of recruited follicles in lactating dairy cows (80). The follicular response to glucose was similar to that classically observed in rbST-treated cows. Whole body glucose partitioning, therefore, should be considered as a mechanism for increased follicular growth after rbST. Glucose metabolism within ovarian cells increased significantly in response to gonadotropins (86). The effect of glucose on ovarian follicular populations is a relatively new area that will require additional investigation.

**Effect of rbST on Dominant and Subordinate Follicles**

Cows treated with rbST had dominant follicles that were similar to control in maximum size (26, 60). Although the absolute size of the dominant follicle was not changed, the duration of the dominance phase in the first wave dominant follicle was shortened by about 2 d in rbST-treated cattle (60, 72). This led to an earlier emergence of the second wave dominant follicle. The shift toward earlier loss of dominance was associated with a shift in the timing of the midcycle blood FSH peak (60). Greater concentrations ST and IGF-I, therefore, did not necessarily prolong or improve the function of dominant follicles. Instead, greater ST and IGF-I may accelerate the series of events that lead to dominant follicle atresia and cause premature loss of dominance in midcycle dominant follicles. Dominant follicles from rbST-treated cattle had increased amounts of low-molecular weight binding proteins (55, 73). Therefore, a mechanism leading to premature loss of dominance in rbST-treated cows may involve the accumulation of IGFBP in the follicular fluid. Under control conditions, IGFBP are decreased in the follicular fluid of dominant follicles, and IGF availability is presumably increased. Atretic follicles have greater amounts of IGFBP (11, 27, 96). Therefore, the increase in IGFBP in rbST-treated cattle may cause premature atresia and loss of dominance. The mechanisms leading to increased IGFBP in rbST-treated cows remain undiscovered.

Direct effects of rbST and (or) IGF-I on the ovary probably cause the changes in follicular dynamics that were observed in rbST-treated cows. Other mechanisms, however, may also be involved. For example, rbST may cause a transient negative energy balance in some cows (34). The negative energy balance may indirectly lead to decreased LH pulsatility (15). A decrease in LH pulsatility could lead to shorter dominance phases (i.e., premature loss of dominance) and greater growth of subordinate follicles through less follicular dominance. Effects of rbST on follicular growth and dominance were detected in nonlactating heifers (40, 41, 42, 72) as well as lactating cows (26, 60) and this may suggest that changes in milk production or negative energy balance do not explain the follicular responses that were observed. Nevertheless, the relative importance of direct versus indirect effects of ST on reproduction should be addressed in future studies.

**Expectations for Reproduction in Dairy Herds Treated with rbST**

In a large study involving 28 dairy herds, rbST administration to primiparous cows caused a 16-d increase in days open but did not affect overall pregnancy rate (21). In the same study, days open in multiparous cows were not affected, but pregnancy rate of multiparous cows was decreased by 7 percentage points. Cattle treated with rbST have similar numbers of follicular...
waves and similar estrous cycle lengths but an earlier emergence of the second wave dominant follicle (13, 60, 72). The lifespan of the dominant follicle (time from emergence to ovulation) in rbST-treated cattle with two follicular waves, therefore, is theoretically increased. Roche et al. (85) showed that extending the lifespan of the dominant follicle (i.e., creating an aged follicle) caused a decrease in pregnancy rate. The decrease in pregnancy rate for rbST-treated cows may be explained by an earlier emergence of the second wave dominant follicles that leads to the ovulation of a more aged dominant follicle. Greater milk production and negative energy balance after rbST, however, may have a greater effect on herd reproductive efficiency than changes in follicular dynamics. McGrath et al. (75) showed that extending the voluntary waiting period from 60 to 165 d in primiparous rbST-treated cows led to pregnancy rates and intervals to conception that were similar to control (untreated) cows (75). Therefore, negative effects of rbST on reproduction were not observed when the breeding period was delayed until later lactation when milk production was less. One conclusion from these data is that there are no inherent negative effects of rbST on reproduction other than those associated with increased milk production and negative energy balance.

CONCLUSIONS

Somatotropin and the IGF are important hormones for ovarian follicular growth. Receptors for ST and IGF are present in follicular cells. In addition, the granulosa and theca cells of the follicle are sites of IGF-I and IGF-II synthesis (respectively). Endocrine sources (primarily liver) for IGF-I and IGF-II also exist but recent data from conditional knockout mice suggest that the local production of IGF by the ovary is more important than endocrine IGF-I. Somatotropin can increase ovarian IGF-I synthesis in some species but the response has not been demonstrated in cattle. The IGF are important for follicular growth because both IGF-I and IGF-II are synergistic with gonadotropins for growth and differentiation of ovarian follicles. Activities of IGF-I and IGF-II within the follicular fluid are influenced by IGFBP5 that can potentially bind and inactivate IGFs or have inhibitory actions that are separate from their interaction with IGF. Future studies should examine the potential direct effects of nutrient partitioning during early lactation or after rbST treatment on growth and development of follicles.

IMPLICATIONS

Somatotropin and IGF are hormones that control many aspects of growth and lactation in cattle. They are also directly involved in the nutritional control of reproduction because ST and IGF-I concentrations change in response to nutritional status (greater ST and less IGF-I during negative energy balance and less ST and greater IGF-I during positive energy balance). Changes in ST and IGF-I are especially dynamic in early lactation dairy cattle in negative energy balance when LH pulsatility is also decreased. A greater understanding of the effects of ST and IGFs on the ovary may lead to methods to correct infertility in early postpartum cattle by manipulating the GH and IGF system.

REFERENCES

13 Bilby, C. R., and M. C. Lucy. 1997. Reproductive responses to bovine somatotropin (bST) in primiparous (P) and multiparous (M) cows with or without corpora lutea (CL). J. Dairy Sci. 80(Suppl. 1):151(Abstr.).


minimal regulatory region containing a CAAT box and a GC box contains constitutive expression of the growth hormone receptor gene. Page 196 in Program and Abstracts of the 81st Mtg. the Endocrine Soc., San Diego, CA (Abstr.).


