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Role of growth hormone in development and maintenance of follicles and corpora lutea

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Growth hormone (GH) is a pituitary hormone that affects animal growth, metabolism, lactation, and reproduction. Many of the effects of GH are mediated by insulin-like growth factor I (IGF-I) which is synthesized in liver and ovary in response to GH. Insulin-like growth factor I synergizes with gonadotrophins (LH and FSH) to stimulate growth and differentiation of ovarian cells. There are species differences in the effects of GH in reproductive biology. In most species, ovarian follicles and corpora lutea are potential sites for GH action because the GH receptor is found within granulosal cells as well as corpora lutea. However, growth hormone does not control ovarian IGF-I in all species and, in ruminants, endocrine IGF-I from liver may be the principal mediator of GH action. In cattle, administration of GH increases the number of small antral ovarian follicles but does not increase the number of large antral (dominant) follicles. Growth hormone may antagonize some aspects of dominant follicular function because dominant follicles are shorter-lived in GH-treated cattle. The corpora lutea has increased growth and steroidogenesis in response to GH. Growth hormone-induced steroidogenesis in cultured granulosal and luteal cells depends on IGF-I release after GH treatment. Bovine and ovine granulosal cells do not release IGF-I in response to GH *in vitro* and, therefore, are less responsive to GH. These results demonstrate that GH is required for normal reproductive function in ruminant as well as nonruminant species.

Introduction

Growth hormone (GH) is a product of the pituitary somatotroph. As its name implies, GH is involved in animal growth. However, its actions are not confined to the growing animal and it is now clear that numerous metabolic and physiological processes (including reproduction) of the adult animal are controlled partially by GH. After its release from the pituitary, GH can act on a variety of tissues because cell-surface receptors for GH are widely distributed throughout the ruminant body (Lucy *et al.*, 1998; Fig. 1). Reproductive tissues that contain mRNA for GH receptor include hypothalamus, pituitary, corpus luteum, ovarian follicle, oviduct, endometrium, myometrium, and placenta (Kirby *et al.*, 1996; Lucy *et al.*, 1998). The highest amount of GH receptor is in the liver, where GH binding causes an increase in the synthesis and secretion of insulin-like growth factor I (IGF)-I. Insulin-like growth factor-I complexes with one of a series of IGF-binding proteins (IGFBP) and then travels as an endocrine hormone to stimulate several additional physiological and metabolic processes including those required for reproduction (Spicer and Echternkamp, 1995; Armstrong and Webb, 1997).

Ovarian IGF Physiology

The focus of this review is GH and ovarian function. A brief discussion of ovarian IGF-I physiology is necessary because many of the actions of GH are mediated by IGF-I. Insulin-like growth factor II

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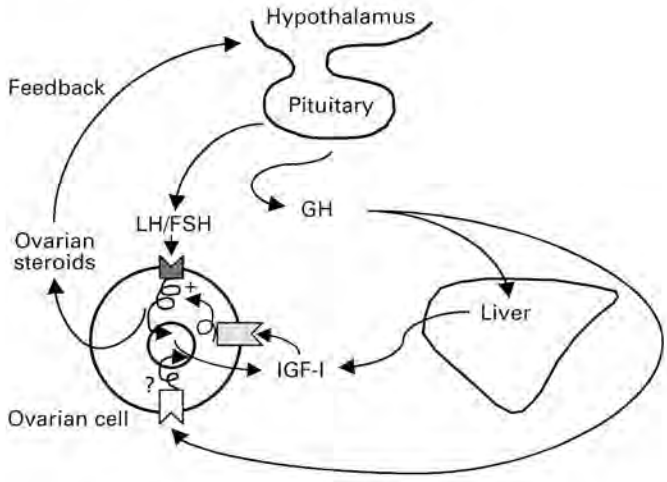

Fig. 1. Model for the actions of growth hormone (GH) and insulin-like growth factor I (IGF-I) on ovarian cells. The model represents general mechanisms that may or may not be proven in all ovarian cell types (thecal, granulosal and luteal) or in all species. Some of the effects of GH are mediated directly by GH at the ovary. The ovarian response may involve the synthesis and secretion of IGF-I by ovarian cells. An endocrine effect is also associated with GH because GH can bind to liver receptors and increase blood IGF-I that can affect ovarian function. Insulin-like growth factor I is synergistic with gonadotrophins for its effect on ovarian cells because gonadotrophins increase IGF-I and IGF receptor synthesis and IGF-I increases gonadotrophin receptor expression and second messenger systems. The synergy increases ovarian cell steroidogenesis. Ovarian function is linked to hypothalamic and pituitary function because oestradiol feeds back positively on GH secretion which can ultimately lead to increased concentrations of IGF-I reaching the ovary. In addition, nutritionally induced changes in liver function can alter ovarian function by modifying the amount of endocrine IGF-I.

has similar effects to IGF-I on ovarian cells but has lower potency than IGF-I and is not under GH control. Granulosal, thecal and luteal cells are sites for IGF action (Spicer and Echternkamp, 1995; Armstrong and Webb, 1997). Endocrine IGF-I (from liver) is not the only source of ovarian IGF-I because ovarian cells (granulosal and luteal cells) synthesize IGF-I and contribute to the total amount of IGF-I that reaches the ovary (endocrine plus local ovarian sources). Within the ruminant follicle, IGF-II produced by the theca may be the most important, locally produced IGF, because thecal IGF-II synthesis is greater than granulosal cell IGF-I synthesis (Yuan *et al.*, 1998). There is synergy between IGF-I and gonadotrophins (LH and FSH) that explains some of the actions of the IGF on ovarian cells. A maximal effect of IGF-I or IGF-II is only observed when cells are treated in combination with either FSH or LH (Spicer and Echternkamp, 1995). Gonadotrophins maintain IGF action by stimulating IGF-I and IGF receptor expression. Furthermore, IGF-I increases gonadotrophin responsiveness by stimulating the adenylate cyclase complex (Fig. 1).

Nutritional Regulation of GH and IGF-I

The release of IGF-I in response to GH within liver is regulated by nutritional status (energy and protein intake relative to requirements; McGuire *et al.*, 1992). The nutritional regulation of IGF-I

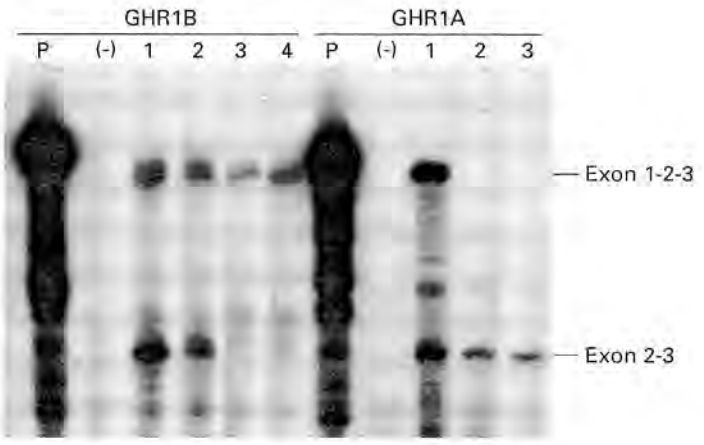

Fig. 2. Ribonuclease protection assay showing alternative splicing of GH receptor mRNA. The GH receptor mRNA is present in two forms (GHR1A and GHR1B) which are controlled by different promoters (1A and 1B). The GHR1B assay yields a full-length protected fragment (exon 1-2-3) for liver (lanes 1 and 2) and corpus luteum (lanes 3 and 4) showing that the 1B promoter is active in liver and corpora lutea. An exon 2-3 fragment is found in liver but not in corpora lutea. Therefore, other alternatively spliced forms of the GH receptor are found in liver but not in corpora lutea. The GHR1A assay yields a full-length protected fragment in liver (lane 1) but only the exon 2-3 fragment in corpora lutea (lanes 2 and 3). The 1A promoter, therefore, is active in liver but not corpora lutea. The exon 2-3 fragment represents GHR1B mRNA found in corpora lutea and liver. P = undigested probe; (-) = negative control.

should not be ignored when considering the effects of GH on reproduction because IGF-I is an important regulator of follicular and luteal function. Animals fed adequate nutrition have the highest concentration of blood IGF-I. Blood IGF-I is highly correlated with follicular fluid IGF-I because the majority of IGF-I in follicular fluid is derived from blood (Leeuwenberg *et al.*, 1996). Postpartum anoestrous cattle have lower blood IGF-I concentrations than cattle that have resumed oestrous cycles (Roberts *et al.*, 1997). Blood GH concentrations are correlated inversely with blood IGF-I because IGF-I is the primary negative feedback regulator of GH secretion. Therefore, animals with increased blood GH usually have low blood IGF-I. An exception to this relationship is found in animals treated with exogenous GH where both GH and IGF-I are increased. An example of the interplay between liver and ovary within the GH–IGF-I endocrine system is found in postpartum cattle. Insulin-like growth factor I is decreased in postpartum cattle because energy requirements exceed nutrient intake (McGuire *et al.*, 1992). Cattle in poor body condition or cows failing to improve body condition during lactation also have low blood IGF-I. However, blood GH concentrations are higher in postpartum cows with low concentrations of blood IGF-I. Later in the postpartum period, when nutritional deficiencies are corrected, blood IGF-I increases. Greater blood concentrations of IGF-I act on the hypothalamus and pituitary to decrease GH secretion. These changes in GH and IGF-I, which are a consequence of nutritionally induced changes in hepatic function, have a direct effect on the ovary by modifying the amounts GH and IGF-I in blood. Improved postpartum ovarian function is correlated with lower blood concentrations of GH and higher blood IGF-I (Roberts *et al.*, 1997).

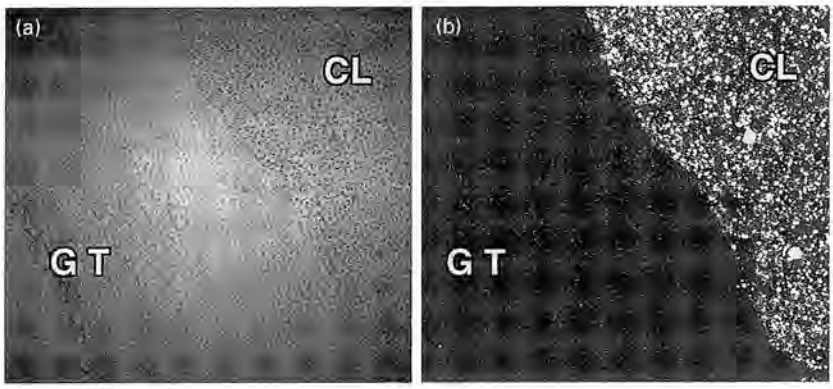

Fig. 3. Growth hormone receptor mRNA in bovine ovary measured by *in situ* hybridization. Photographs were taken with brightfield (a) and darkfield (b). The corpus luteum (CL) shows intense hybridization for GH receptor mRNA. Both granulosal cells (G) and thecal cells (T) of the follicle are negative for GH receptor mRNA. Original magnification × 42. Reprinted from Yuan and Lucy (1996) with permission from Elsevier Science.

Direct Actions of GH on the Ovary

Localization of GH receptors within the ovary

Growth hormone receptors are members of the cytokine–haematopoietic receptor superfamily that includes GH and prolactin receptors, as well as cytokine and haematopoietic hormone receptors. The GH receptor mediates the actions of GH by binding GH, dimerizing and transducing an intracellular signal via Janus Kinase (JAK) and signal transducers and activators of transcription (STAT). Other second messenger systems, including insulin receptor substrate (IRS-1), phosphatidylinositol-3-kinase, and mitogen-activated protein (MAP) kinase, may mediate the action of GH under different conditions or cell types. The GH receptor mRNA is almost identical in cattle and sheep and contains 4160 base pairs that encode 634 amino acids. Alternative exon 1 splicing of the GH receptor mRNA occurs in a variety of species including cattle and sheep. Two different promoters transcribe two GH receptor mRNAs with alternatively spliced exon 1 sequences. One promoter (bovine/ovine 1A) has liver-specific activity. A second promoter (bovine/ovine 1B) is active in adult liver but is also active within non-hepatic tissues including the reproductive tract (Lucy *et al.*, 1998; Fig. 2).

If GH has a direct effect on the ovary then the GH receptor should be present within ovarian cells. In cattle, the GH receptor was found within the large luteal cells (Lucy *et al.*, 1993). The presence of GH receptor was demonstrated by using several methods including immunohistochemistry (Lucy *et al.*, 1993), ribonuclease protection assay (Lucy *et al.*, 1993) and *in situ* hybridization (Yuan and Lucy, 1996). It is not known why the GH receptor was not localized specifically within large luteal cells in pigs (Yuan and Lucy, 1996).

There may be species differences for GH receptor expression in ovarian follicles. In humans (Sharara and Nieman, 1994) and rats (Carlsson *et al.*, 1993), GH receptor was detected in granulosal cells as well as corpora lutea. In cattle, GH receptor expression in follicles was approximately 20-fold lower when compared with corpora lutea (Lucy *et al.*, 1993). Furthermore, when histological sections containing both follicles and corpora lutea were examined, corpora lutea contained abundant GH receptor protein (Lucy *et al.*, 1993) or mRNA (Yuan and Lucy, 1996), whereas neighbouring follicles were negative for GH receptor (Fig. 3). However, when examined by reverse transcriptase PCR, the GH receptor was detected in bovine granulosal cells, cumulus cells and the oocyte (Izadyar *et al.*, 1997). In addition, the GH receptor was detected in the granulosal cells and oocytes of ovine small

follicles by *in situ* hybridization (Eckery *et al.*, 1997). These results indicate that ruminant follicles contain the GH receptor but the concentration of GH receptor in follicles may be considerably lower than in corpora lutea.

Does GH control ovarian IGF-I?

Both follicles and corpora lutea have GH receptor and IGF-I mRNA. In liver, GH controls hepatic IGF-I synthesis. One important question, therefore, is whether GH controls ovarian IGF-I synthesis. Growth hormone-dependent, ovarian IGF-I synthesis has been shown in rats, pigs, and rabbits (Spicer and Echternkamp, 1995). In hypophysectomized ewes, LH increased luteal GH receptor mRNA and GH increased luteal IGF-I mRNA (Juengel *et al.*, 1997). However, in other studies GH failed to increase ovarian IGF-I synthesis either *in vitro* (Wathes *et al.*, 1995) or in intact cattle (Kirby *et al.*, 1996). In addition, immunization of heifers against GH releasing hormone (GRF) decreased blood GH and IGF-I but did not change the ovarian IGF-I mRNA concentration (Cohick *et al.*, 1996). Data showing GH-dependent follicular IGF-I synthesis have not been reported for sheep or cattle. Therefore, follicular IGF-I is probably not locally controlled by GH. Instead, endocrine IGF-I, under GH control, influences ovarian function through its contribution to follicular fluid IGF-I (Leeuwenberg *et al.*, 1996). However, the physiological importance of locally produced (ovarian) IGF-I and endocrine IGF-I (hepatic, GH dependent) is debated because the availability of IGF-I from different sources may depend on the interaction of IGF-I with locally produced and serum-derived IGFBP (Yuan *et al.*, 1998).

In Vivo Effects of GH on Reproduction

There is probably no absolute requirement for GH in reproduction, because women with inactivating GH receptor mutations (Laron dwarfs; Menashe *et al.*, 1991) and cattle with abnormal GH receptor expression (Chase *et al.*, 1998) are capable of reproduction. A knockout mouse for the GH receptor was also fertile (Zhou *et al.*, 1997). Although reproduction is possible in each of these conditions, the efficiency of reproduction is low. Hence, there is a facilitatory but not obligatory role for GH in reproductive processes.

Confounding effects of GH and IGF-I in vivo

A limitation of all *in vivo* studies using exogenous GH is the confounding effects of increased blood IGF-I after GH treatment. Insulin-like growth factor I is a potent ovarian growth factor (Spicer and Echternkamp, 1995; Armstrong and Webb, 1997). Therefore, the increase in IGF-I that occurs after GH treatment confounds the direct effects of GH on the ovary. Furthermore, the increase in blood GH followed by the increase in blood IGF-I leads to an unphysiological relationship between blood GH and blood IGF-I concentrations. In untreated animals, blood GH and blood IGF-I are inversely correlated because IGF-I feeds back negatively on GH secretion. In GH-treated animals, blood GH and blood IGF-I are positively correlated (that is, GH-treated animals have high blood GH concentrations and high IGF-I). When examined in heifers fed high-energy diets, increased follicular growth was negatively correlated with serum GH because greater nutrient intake increased insulin and IGF-I but suppressed GH (Gutierrez *et al.*, 1997). Therefore, the results of *in vivo* studies using exogenous GH should be interpreted with caution. Effects of exogenous GH *in vivo* are either direct effects of GH or IGF-I or a combined effect of both hormones.

Number of ovarian follicles

Administration of exogenous GH increased IGF-I as well as the number of recruited follicles (2–9 mm in diameter; Gong *et al.*, 1997; Kirby *et al.*, 1997). The increased number of recruited follicles

can be maintained for at least 84 days and may persist for at least 21 days after GH treatment (Kirby *et al.*, 1997). The greater number of recruited follicles did not lead to increased numbers of selected follicles (> 10 mm diameter). Therefore, additional follicles in the recruited pool cannot proceed to larger size classes when stimulated with GH (selection process is unchanged). In controlled studies, ovulation rate was not changed in cattle treated with GH (Kirby *et al.*, 1997). Thus, ruminants are different from mice because exogenous GH (or a GH transgene) increases ovulation rate and litter size in mice (Cecim *et al.*, 1995). A higher proportion of twin births was reported in GH-treated cattle (Cole *et al.*, 1991). However, the twinning response varied across herds, and may reflect an interaction of GH with either genetic or environmental factors. Indeed, Bilby and Lucy (1997) found that other factors including parity and number of corpora lutea had a greater effect on follicular growth than did exogenous GH. Pigs are similar to cattle in this respect because GH increased the number of small follicles (Spicer *et al.*, 1992).

Hypophysectomized ewes did not develop preovulatory follicles unless GH and FSH were administered (Eckery *et al.*, 1997). This indicates that GH has a direct effect on small follicles. However, the study did not preclude an indirect effect of GH on the ovary through increased IGF-I after GH treatment. Most lines of evidence support an endocrine IGF-I effect rather than a direct GH effect on ruminant follicles. First, very little GH receptor mRNA or protein is found within ruminant follicles (Lucy *et al.*, 1993; Yuan and Lucy, 1996; Eckery *et al.*, 1997). Second, heifers treated with increasing doses of GH failed to have greater growth of antral follicles when the GH dose was below the threshold for increased IGF-I (Gong *et al.*, 1997). Third, cattle selected for multiple births have higher blood and follicular fluid IGF-I concentrations (Echternkamp *et al.*, 1990). Fourth, heifers immunized against GRF had low blood IGF-I, delayed puberty, fewer large antral follicles, but equivalent ovarian IGF-I mRNA compared with control heifers (Cohick *et al.*, 1996; Schoppee *et al.*, 1996). Finally, cattle with a liver GH receptor deficiency, causing high blood GH with low blood IGF-I, had one quarter of the number of small antral follicles compared with control cattle (Chase *et al.*, 1998).

The reason why the number of antral follicles is increased in animals supplemented with GH is not known. *In vitro*, IGF-I increases the number of gonadotrophin binding sites and the activity of gonadotrophin second messenger systems (Spicer and Echternkamp, 1995). Perhaps greater gonadotrophin action caused by GH or IGF-I can lead to an increase in follicular growth. *In vivo*, GH increased follicular fluid IGF-I but did not increase gonadotrophin binding sites in bovine follicles (Andrade *et al.*, 1996). Greater gonadotrophin receptor concentration, therefore, does not occur after GH treatment in cattle. The possibility that GH or IGF-I increases the activity of gonadotrophin second messenger pathways *in vivo* without changing the number of gonadotrophin receptors has not been addressed. An additional possibility is that GH supplementation decreases atresia of the growing pool and leads to a greater number of antral follicles. In nonruminant granulosal cells, GH and IGF-I decrease apoptosis (Kaipia and Hsueh, 1997). In cattle, a GH-mediated decrease in atresia (Cushman *et al.*, 1996) indicates that GH increases antral follicle populations by reducing atresia.

Dominant and subordinate follicles

Exogenous GH does not affect the growth rate or size of dominant follicles (Kirby *et al.*, 1997). However, the size of second largest follicles is increased in GH-treated cows. The increase in second largest follicle diameter is associated with greater development of the recruited pool of ovarian follicles (Gong *et al.*, 1997; Kirby *et al.*, 1997). 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 days in GH-treated cattle. This led to an earlier emergence of the second wave dominant follicle (Kirby *et al.*, 1997). The shift towards a reduced period of dominance was also associated with a shift in the timing of the mid-cycle peak in blood concentration of FSH (Kirby *et al.*, 1997). The reason for the faster turnover in dominant follicles of GH-treated cattle is unknown. Bovine dominant follicles are dependent on LH for the maintenance of dominance and GH treatment decreases LH secretion (Schemm *et al.*, 1990). In pigs, GH decreases LH/hCG binding sites within follicles (Spicer *et al.*, 1992) but a similar response was not observed in cattle (Andrade *et al.*, 1996). *In vitro*, IGF-I antagonized insulin-induced oestradiol synthesis (Spicer *et al.*, 1993). Increased concentrations of

GH and IGF-I, therefore, do not necessarily prolong or improve the function of dominant follicles. Instead, greater concentrations of GH and IGF-I may accelerate the series of events that lead to dominant follicle atresia and cause premature turnover of mid-cycle dominant follicles.

Puberty

Heifers immunized against GRF have lower blood concentrations of GH and IGF-I and reach puberty at an older age than control heifers (Cohick *et al.*, 1996; Schoppee *et al.*, 1996). The effect of GRF immunization on puberty occurred despite normal patterns of LH secretion (Schoppee *et al.*, 1996). The delay in the timing of puberty in GRF-immunized heifers was caused by inadequate follicular oestradiol production that failed to trigger an LH surge. A synergistic relationship between GH, IGF-I and LH for puberty was demonstrated, therefore, because heifers with normal LH failed to reach puberty when GH and IGF-I were inadequate. Nutrient-restricted heifers also had delayed puberty and low IGF-I. However, unlike the GRF-immunized heifers, blood GH was increased and blood LH was decreased by nutrient restriction (Schoppee *et al.*, 1996). Undernutrition, therefore, delays puberty through a combined effect of decreased IGF-I and LH. Treating heifers with GH increased body growth but did not decrease age at puberty or increase the number of small follicles (Hall *et al.*, 1994). These results suggest that the initiation of LH secretion in peripubertal heifers is the most important factor that determines age at puberty. Growth hormone and IGF-I may play a role in puberty but their effects are permissive to LH.

Growth and steroidogenesis of corpora lutea

Growth hormone is required for growth and development of the corpus luteum in ruminants because decreased corpora lutea weight in hypophysectomized ewes could be restored to near normal size with exogenous GH and LH (Juengel *et al.*, 1997). In addition to a direct effect of GH on the corpora lutea, there may also be a requirement for endocrine IGF-I in corpora lutea growth. Cattle with a liver GH receptor deficiency (high blood GH concentrations but low blood IGF-I concentrations) had smaller corpora lutea and shorter luteal phases (Chase *et al.*, 1998). Nutrient-restricted heifers had smaller corpora lutea, greater blood GH, and lower blood IGF-I concentrations (Vandehaar *et al.*, 1995). In these heifers, the IGF-I mRNA in corpora lutea was not changed while the amount of IGF-I mRNA in liver was decreased by undernutrition. An endocrine mechanism involving low blood IGF-I, therefore, explained reduced corpus luteum size in nutrient-restricted heifers (Vandehaar *et al.*, 1995). However, in other studies of nutrient-restricted heifers, exogenous GH restored blood IGF-I to control concentrations but failed to increase corpora lutea weight (Yung *et al.*, 1996). The effect of exogenous GH on the corpora lutea of intact or normal-fed cattle is equally unclear. The corpora lutea of dairy cattle treated with GH from days 1–17 of an oestrous cycle were 60% heavier than those of control cattle (Lucy *et al.*, 1995). The increase in corpora lutea weight occurred without a change in IGF-I mRNA concentration within the corpora lutea (Kirby *et al.*, 1996). Greater plasma progesterone concentrations were reported in dairy cows treated with GH (Schemm *et al.*, 1990; Gallo and Block, 1991). However, in other studies, GH tended to decrease plasma progesterone concentrations (Kirby *et al.*, 1997). The inconsistencies in corpora lutea responses to GH suggest that other physiological factors may over-ride any stimulatory effect of exogenous GH on the corpora lutea. One concern for studies of GH in lactating animals is the confounding effect of increased milk production and loss of body condition on corpora lutea function in GH-treated animals. In one study of lactating cows, a period of anoestrus occurred after GH treatment (Waterman *et al.*, 1993). Therefore, GH-induced changes in milk production may compromise corpora lutea function and confound reproductive effects of GH.

In Vitro Effects of GH on Ovarian Cells

One method to avoid the confounding effects of endocrine IGF-I in tests of GH action is to treat ovarian cells with GH *in vitro*. The *in vitro* treatments can be tested further with IGF-I neutralizing

antibodies to determine whether a GH-dependent IGF-I release is responsible for the effects of GH on reproductive cells. One detraction for most *in vitro* studies of GH is the supraphysiological doses (10^2–10^5 ng ml^{-1}) used to show an effect of GH. This is of great concern when pituitary GH is used because of the possible contamination of the GH preparation with either LH or FSH. There are no data that show the direct activation of the JAK-STAT pathway by GH in ovarian cells. One focus of future studies should be the elucidation of GH signalling pathways in ovarian cells cultured *in vitro*. Furthermore, a physiological dose of recombinant GH should be used.

Granulosal cells

Porcine granulosal cells increase progesterone secretion in response to GH (Spicer and Echternkamp, 1995). An effect of GH on oestradiol synthesis in cultured human granulosal cells has also been demonstrated (Barreca *et al.*, 1993). The effect of GH on steroidogenesis was blocked by the addition of a neutralizing IGF-I antibody. Therefore, the responses to GH *in vitro* may be secondary to an increase in IGF-I that occurs after GH treatment. There is no consensus for the effects of GH on ruminant granulosal cells. In bovine granulosal cells isolated from small or large follicles, GH inhibited oestradiol synthesis and inhibited proliferation of cells from large follicles (Spicer and Stewart, 1996). Other workers also reported an inhibitory effect of GH on the proliferation of granulosal cells from large follicles (Gong *et al.*, 1993) but showed a stimulatory effect of GH on oestradiol secretion (Gong *et al.*, 1994). The inhibitory effect of GH on cell proliferation may be explained partially by the inhibitory effect of GH on IGF-I mRNA (and perhaps protein) in bovine granulosal cells (Spicer *et al.*, 1993). Growth hormone did not affect progesterone secretion from granulosal cells isolated from large follicles but progesterone secretion and proliferation were increased when granulosal cells were isolated from small follicles and co-treated with insulin (Spicer and Stewart, 1996). In ovine granulosal cells co-treated with insulin, GH also increased progesterone secretion in long-term culture (Wathes *et al.*, 1995). One conclusion from these studies is that GH will increase steroidogenesis in cultured granulosal cells when GH causes the release of IGF-I (pig and human). The IGF-I may increase steroidogenesis itself or may be permissive to the effect of GH. An increase in steroidogenesis may not occur in ruminants because GH does not increase IGF-I in cultured granulosal cells. It may be necessary to supplement cell culture media with either insulin or IGF-I to detect any effect of GH on steroidogenesis in cultured ruminant granulosal cells.

Cumulus cells

Growth hormone increased *in vitro* maturation of bovine oocytes as well as cumulus expansion by an IGF-I independent mechanism (Izadyar *et al.*, 1997). Further analyses showed that the stimulatory effects of GH were not mediated by tyrosine kinase activation. Instead, a cAMP second messenger pathway was suggested (Izadyar *et al.*, 1997). This result was unexpected because cAMP is not a traditional GH receptor second messenger. Nevertheless, the data implicate GH in follicular control of oocyte development.

Thecal cells

Rat thecal cells increased androgen synthesis in response to GH (Apa *et al.*, 1996a). However, the response to GH was different from that of granulosal cells, because an IGF-I antibody could not neutralize the effect of GH. In rats, therefore, GH may act on thecal cells through an IGF-I-independent mechanism. The response of bovine thecal cells to GH depended on LH-responsiveness of the cells. Growth hormone increased androstenedione secretion in thecal cells that responded well to LH. Thecal cells that responded poorly to LH did not have increased androstenedione secretion after GH treatment (Spicer and Stewart, 1996).

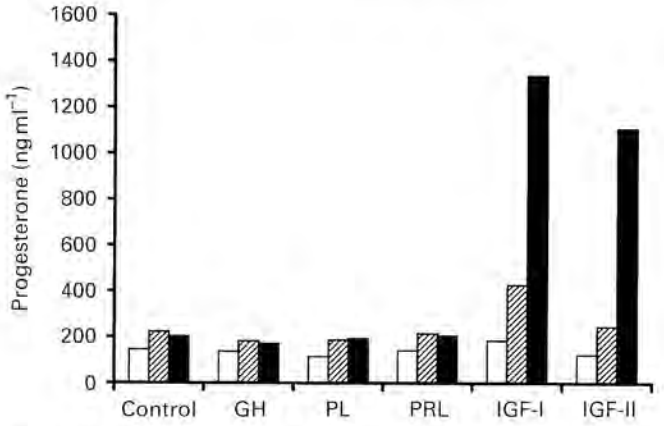

Fig. 4. Concentration of progesterone in tissue culture media from a mixed population of bovine luteal cells (oestrous cycle day 10) treated with 1 ng ml^{-1} (□), 10 ng ml^{-1} (▨) or 100 ng ml^{-1} (■) bovine LH as well as increasing dosages of control (no hormone) or recombinant hormones [bovine growth hormone (GH), bovine placental lactogen (PL), bovine prolactin (PRL), insulin-like growth factor I (IGF-I) or IGF-II]. Data represent secretion between 48 and 72 h of cell culture. Both IGF-I and IGF-II increase progesterone secretion with increasing dosage. Other hormones (GH, PL and PRL) have no effect on progesterone secretion. Data are means for duplicate wells from each of three heifers (pooled standard error = 89.5) (Lucy and Collier, unpublished).

Luteal cells

Bovine and ovine corpora lutea have increased secretion of progesterone in response to IGF-I in microdialysis systems (Sauerwein *et al.*, 1992; Khan-Dawood *et al.*, 1994). Furthermore, tyrosine kinase-mediated second messenger pathways for IGF-I and insulin were demonstrated for cultured bovine luteal cells (Chakravorty *et al.*, 1993). There is no equivalent demonstration of the GH receptor second messenger system in luteal cells from any species. Nevertheless, *in vitro* data suggest an effect of GH on luteal cell steroidogenesis and oxytocin secretion. Liebermann and Schamms (1994) increased progesterone secretion and caused oxytocin release by treating bovine corpora lutea with GH in a microdialysis system. In cultured cells, GH increased progesterone secretion in human luteal cells and the effect of GH could be blocked by an IGF-I neutralizing antibody (Apa *et al.*, 1996b). Therefore, it appears that the effects of GH on progesterone synthesis may depend on IGF-I synthesis. There is very little evidence that GH will increase steroidogenesis in cultured ruminant luteal cells. We were unable to show an effect of GH, prolactin, or placental lactogen on bovine luteal cells. At the same time, addition of IGF-I and IGF-II resulted in greater progesterone concentrations in luteal cell cultures (Fig. 4).

Conclusions

Growth hormone is involved in many aspects of ovarian physiology in ruminants. Most importantly, GH increases the growth and development of antral follicles and increases the growth and steroidogenesis of the corpora lutea. These actions of GH are usually synergistic with IGF-I and gonadotrophins (Fig. 1). An important issue that should be addressed is the relative importance of GH compared with IGF-I for ovarian function. Many of the perceived effects of GH on the ovary can

be explained by changes in blood IGF-I that occur when GH causes hepatic IGF-I synthesis and secretion. The ruminant may be different from pigs, humans, and laboratory animals in which GH has a direct effect on the ovary through the control of ovarian IGF-I. The presence of GH receptors in ruminant corpora lutea and follicles presumes a direct action for GH. The direct actions of GH on the ovary, however, may be less important than the ovarian actions of endocrine IGF-I that are ultimately under nutritional and GH control.

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