Growth hormone: roles in female reproduction

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Abstract

GH, as its name suggests, is obligatory for growth and development. It is, however, also involved in the processes of sexual differentiation and pubertal maturation and it participates in gonadal steroidogenesis, gametogenesis and ovulation. It also has additional roles in pregnancy and lactation. These actions may reflect direct endocrine actions of pituitary GH or be mediated by its induction of hepatic or local IGF-I production. However, as GH is also produced in gonadal, placental and mammary tissues, it may act in paracrine or autocrine ways to regulate local processes that are strategically regulated by pituitary GH. The concept that GH is an important modulator of female reproduction is the focus of this review.

Introduction

Although the somatogenic and gonadotrophic axes have long been known to be closely linked during growth and sexual maturation (Simpson et al. 1944), until recently the role of growth hormone (GH) in reproduction had been described as ‘more akin to fine tuning than that of a major player . . .’ (Ogilvy-Stuart & Shalet 1992). Experimental studies suggest, however, that this statement underestimates the importance of GH in reproductive function, since GH modulates steroidogenesis, gametogenesis and gonadal differentiation as well as gonadotrophin secretion and responsiveness (Zachmann 1992). Mammary and placental roles for GH have also been proposed (Mol et al. 1996, Alsat et al. 1997). Moreover, while these actions may reflect endocrine roles of pituitary GH, they may also reflect local autocrine or paracrine actions of GH produced in reproductive tissues (Fig. 1).

Puberty

GH is usually, but not always, required for the timing of sexual maturation, since delayed or absent puberty is often associated with GH-deficient or GH-resistant states and GH administration accelerates puberty. For instance, in a cohort of 60 GH-deficient women, puberty occurred normally in only 16 and was delayed in 10 others (de Boer et al. 1997). Puberty is similarly delayed in a large proportion of GH receptor (GHR)-knockout mice (Bartke et al. 1999), GH-resistant women (Laron 1984) and GH-releasing hormone (GHRH)-immunized cattle (Simpson et al. 1991). The importance of GH in sexual maturation is further demonstrated by the ability of exogenous GH to accelerate sexual maturation in GH-replete monkeys (Wilson et al. 1989) and GH-deficient children (Darendeliler et al. 1990, Stanhope et al. 1992). GH may accelerate puberty by activating the luteinizing hormone (LH)-releasing hormone pulse generator (Bartke et al. 1999) and/or by potentiating androgen action (Ilondo et al. 1982.

GH administration has not, however, been shown to accelerate pubertal development in pigs (Bryan et al. 1989, 1990, Andres et al. 1991). This may, however, reflect the reproductive state at the time of GH treatment, since GH appears to increase the rate of human sexual maturation only when a pubertal pattern of pituitary gonadotrophin secretion is established (Sharara & Giudice 1997). Moreover, since the implantation of bovine GH (bGH) in the median eminence of young female rats delays puberty (Advis et al. 1981), it is possible that GH exerts inhibitory effects on the hypothalamo–pituitary–gonadal axis at central sites, contrary to its stimulatory actions on pituitary gonadal function.

Ovarian Actions

Experimental studies performed in vivo suggest that GH acts on the ovary to affect gametogenesis and steroidogenesis. The interpretation of these in vivo studies is, however, complicated by the increase in circulating insulin-like growth factor-I (IGF-I) resulting from in vivo
GH administration and the proven ability of IGF-I to stimulate ovarian activity (Adashi et al. 1985). Indeed, the administration of exogenous GH results in an artificial situation in which both GH and IGF-I are elevated, whereas physiological increases in IGF-I normally result in a decrease in pituitary GH production. In vivo responses are thus likely to partially reflect the actions of GH-induced hepatic IGF-I. Numerous in vitro studies and the finding of GHR mRNA and protein in ovarian cells suggest, however, that direct ovarian actions of GH provide an important modulation of gonadotrophin-dependent and -independent functions.

**Folliculogenesis and gametogenesis**

The production of viable gametes and adequate steroid production requires a series of developmental events to occur in the follicle (Fig. 2) and within the oocyte. GH affects the maturation of the follicle and gamete and thereby plays a facilitatory role in fertility.

**Early folliculogenesis** GH may play a particularly important role in early, follicle-stimulating hormone (FSH)-independent follicular development (Fig. 2), since GH-binding activity peaks during early folliculogenesis in porcine follicles (Quesnel 1999) and fish ovarian...
homogenates (Gomez et al. 1998). Indeed, in vivo and in vitro studies suggest that GH stimulates growth and prevents atresia in small follicles, which develop and undergo atresia throughout the menstrual cycle. For instance, GH administration in vivo increases the number of small follicles in cattle (Gong et al. 1991, 1993) and horses (Cochran et al. 1999). In vitro studies in mice, accordingly, show a stimulatory effect of GH on preantral follicle development and follicular cell proliferation in immature mice that is synergistic with (and therefore independent of) IGF-I (Kumar et al. 1997, Liu et al. 1998, Kobayashi et al. 2000). Other studies in perfused rabbit ovaries, however, implicate IGF-I in early folliculogenesis, since follicular growth and intraovarian IGF-I increase in a coordinate fashion following GH administration (Yoshimura et al. 1993, 1994). Other ovarian growth factors, such as activin, may also mediate the actions of GH, since folliculostatin (which binds and inactivates activin) blocks the stimulatory effect of GH on murine preantral follicle growth (Liu et al. 1998). GH may thus be particularly important in the recruitment of follicles and initiation of oocyte growth, perhaps by matching nutritional status with the number of growing oocytes.

Late folliculogenesis and luteinization GH acts in conjunction with gonadotrophins to stimulate later stages of folliculogenesis and luteinization (Fig. 2), since both GH and gonadotrophins are required to prevent atresia of larger follicles (>2 mm) following hypophysectomy in sheep (Eckery et al. 1997). GH may play a role in follicle selection, since GH-binding sites in sow granulosa cells are lost in atretic follicles (Quesnel 1999) and the development of the dominant follicle is impaired in GHR-deficient cattle (Chase et al. 1998). GH administration in vivo similarly increases the number of large follicles in pigs (DeLaSota et al. 1993, Lucy et al. 1995) and GH-deficient dwarf rats (Ozawa et al. 1996) and the number of corpora lutea in cattle (Lucy et al. 1992) and GH transgenic rats (Danilovich et al. 2000). The stimulatory effect of GH on follicle number and size reflects increased cell proliferation, at least in luteinized human granulosa cells (Ovesen et al. 1994), but also is indicative of the suppressive effect of GH on apoptosis (Eisenhauer et al. 1995, Sirotkin & Makarevich 1999, Danilovich et al. 2000). GH may enhance follicular survival and cell proliferation by potentiating LH action, since GH deficiency is associated with decreased LH receptor gene expression and LH

Oocyte maturation An important role for GH in the development of the oocyte is indicated by the relationship between follicular GH concentrations and human oocyte maturity (Mendoza et al. 1999). Oocytes harvested from follicles with high antral fluid GH concentrations are more fertile than those from follicles with low GH concentrations, and amongst fertilized oocytes, intrafollicular GH concentration is inversely related to subsequent cleavage failures and morphological dysfunctions of the cleaved embryos (Mendoza et al. 1999). In contrast, prolactin, LH, interleukin-1 and tumour necrosis factor-α are unrelated to the development of competent oocytes (Mendoza et al. 1999). Mendoza et al. (1999) have therefore concluded that an early rise in the GH concentration in small antral follicles is beneficial for oocyte quality, by enhancing or acting in synergy with gonadotrophin–controlled developmental processes. Accordingly, the fertilization rate in bovine oocytes in vitro is increased by incubation with GH (Izadyar et al. 1998).

GH may enhance oocyte quality by accelerating and coordinating cytoplasmic and nuclear maturation, at least in bovine oocytes. This possibility is indicated by the greater proportion of bovine oocytes manifesting the markers of cytoplasmic maturation (for instance, the ability to decondense sperm chromatin and form sperm asters (Hytrel et al. 1989)) in GH-treated cell populations (Izadyar et al. 1997, 1998). Nuclear maturation is similarly enhanced by GH, since GH-treated bovine oocytes complete meiosis I faster and undergo zygote cleavage and blastocyst formation more frequently than untreated oocytes (Van der Westerlaken et al. 1994, Izadyar et al. 1996, 1998). This effect of GH is dependent upon cAMP and the presence of cumulus cells but is IGF-I–independent (Bevers et al. 1989, Zuelke & Brackett 1993, Izadyar et al. 1997, 1998, Kolle et al. 1998). GHR mRNA and protein are, moreover, not detected in oocytes from secondary or tertiary follicles of the bovine ovary (Kolle et al. 1998); thus, direct actions at the oocyte itself appear unlikely. GH also stimulates nuclear maturation of fox, rat, rabbit and pig oocytes; however, at least in rats, cumulus cell IGF-I appears to be the mediator and cAMP is not involved (Hagen & Graboski 1990, Yoshimura et al. 1993, 1994, Apa et al. 1994b, Kalous et al. 1998).

Ovulation H plays a non-essential but facilitatory role in ovulation. For instance, although GH alone fails to cause ovulation in sheep (Davis et al. 1990), pigs (Gilbertson et al. 1991) or rabbits (Yoshimura et al. 1993), gonadotrophin-induced ovulation in perfused rabbit ovaries is significantly improved by GH co-administration (Yoshimura et al. 1994). Moreover, fertility is reduced but not abolished in GHR–knockout mice (Bartke 1999) and egg production and fertility are not impaired in GH-resistant sex-linked dwarf chickens (Decuyper et al. 1991). In such cases, other GH-like hormones, particularly prolactin, may compensate for the lack of GH action (Bartke 1999). Indeed, unlike GHR–knockout mice, Snell and Ames mice are totally sterile, lacking GH, prolactin and thyrotrphin (Bartke 1999).

GH may facilitate ovulation by increasing sensitivity to gonadotrophins and by reducing the incidence of apoptosis in preovulatory ovarian follicles. The increased number of corpora lutea and reduced numbers of atretic follicles in the ovaries of mice transgenically expressing GH supports this view (Danilovich et al. 2000). The overexpression of GH in these mice has thus been correlated with an increase in the number of ova shed during each ovulation (Cecim et al. 1995, Danilovich et al. 2000). Since treatment with IGF-I also suppresses apoptotic DNA fragmentation in preovulatory follicles, this action of GH is thought to be IGF-I mediated (Danilovich et al. 2000). GH may also facilitate ovulation by increasing tissue plasminogen activator synthesis, which activates the serine protease required for rupture of the ovarian capsule (Politis et al. 1990).

The timing of ovulation may also be GH–dependent, since it is delayed in normal female mice paired with GHR–knockout mice (Bartke et al. 1999). A reduced secretion of the male pheromones responsible for synchronizing the ovulatory cycle in the female has been implicated (Bartke et al. 1999). In female GHR–knockout mice, the oestrous cycle is prolonged and irregular and the ovulation rate is reduced, resulting in smaller litters (Bartke et al. 1999, Danilovich et al. 1999).

Fertility: clinical studies Normal fertility does not always require a normal GH axis. Indeed, it is well established that a proportion of GH–deficient (de Boer et al. 1997) and GH-resistant (Menashe et al. 1991, Dor et al. 1992) women have normal menstrual cycles and conceive normally. However, many GH–deficient women require assisted reproductive technologies to conceive, principally for the induction of ovulation, and it has been hypothesized that other women with reproductive dysfunctions may be partially GH–deficient (de Boer et al. 1997). The possible use of GH as an adjunct to human menopausal gonadotrophin (hMG) to induce ovulation has thus been the focus of extensive research (Jacobs 1992, Shoham et al. 1992a, Homburg & Farhi 1995) and reviews (Katz et al. 1993, Homburg & Farhi 1995, Franks 1998). Clinical studies have shown that GH may be therapeutically useful in some, but not all, infertile women. In particular, GH administration to hypogonadotrophic anovulatory women significantly reduces the dosage and duration of hMG treatment required for ovulation induction (Blumenfeld & Lundenfeld 1989, Homburg et al. 1990, Volpe et al. 1990, Burger et al. 1991, Fowler & Templeton
1991, Jacobs 1992). Furthermore, a significant proportion of women who respond to GH fail to respond to hMG alone. GH administration also restores FSH responsiveness in women with Down’s syndrome, as determined by serum oestradiol concentrations (Cento et al. 1997).

GH therapy may similarly improve the success of in vitro fertilization techniques by enhancing the hyperovulatory response to hMG. Numerous clinical studies have demonstrated that the addition of GH to the hMG treatment regimen improves oocyte recovery and/or the rate of successful fertilizations and pregnancies (Volpe et al. 1989, Ibrahim et al. 1991, Jacobs 1992, Stone & Marrs 1992). Responsiveness to hMG in normogonadotrophic women with polycystic ovary syndrome is similarly improved by the administration of GH (Owen et al. 1991a,b). In initial studies that employed pharmacological doses of GH, the improvement in fertility was thought to be partially due to the lipolytic action of GH and a reduction in obesity (Morales et al. 1991, Tapanainen et al. 1992, Younis et al. 1989, Blumenfeld & Amit 1993). Conversely, GH therapy does not, however, enhance gonadotrophic responsiveness in all women with infertility (Shaker et al. 1992, Tapanainen et al. 1992, Younis et al. 1992). Most of the women that do respond to GH–hMG co-treatment have an impairment in their secretion of GH (Blumenfeld et al. 1991). The infertility in these women may thus result in part from relative GH deficiency, whereas other dysfunctions are likely to be causal in the infertility of women that fail to respond to GH–hMG co-treatment (Blumenfeld et al. 1991). GH responsiveness is also greatest in young, hypo-oestrogenic women who have been subjected to previous pituitary suppression (Shaker et al. 1992, Tapanainen et al. 1992, Younis et al. 1992, Blumenfeld & Amit 1993). Conversely, GH therapy is largely ineffective in hypergonadotrophic women (Homburg & Farhi 1995) or women experiencing incipient or current ovarian failure (Homburg et al. 1991). Thyroid and adrenal function, and lifestyle characteristics, such as smoking, may also be important determinants in GH treatment efficacy (Hillenjo & Bergh 1993). In vitro fertilization protocols using gonadotrophin–releasing hormone agonists in the treatment regimen usually report that GH improves fertilization rate but not oocyte retrieval (Bergh et al. 1994, Hughes et al. 1994). The lack of GH response in some patients may also reflect concomitant changes in follicular IGF-binding proteins (IGFBPs), which would negate the beneficial effect of GH on follicular IGF-I (Rabinovici et al. 1997).

Summary
A significant amount of literature supports a role for GH in the production of viable gametes, since GH modulates gonadotrophin-independent early folliculogenesis and gonadotrophin-dependent late folliculogenesis by increasing cell proliferation and inhibiting atresia. GH also increases oocyte fertility by enhancing nuclear and cytoplasmic maturation and facilitating ovulation. Ovarian and hepatic IGF-I appear to be involved in some, but not all, of these actions in some species. As a result of these gametogenic and folliculogenic actions, GH has been shown to influence fertility.

Steroidogenesis
The production of steroid hormones by ovarian cells is essential for follicular recruitment, oocyte maturation, ovulation, corpus luteal function, the maintenance and implantation of the blastocyst and the regulation of the hypothalamo–pituitary–gonadal axis. Steroidogenesis is therefore a prerequisite for successful reproduction. Although LH and FSH are the primary regulators of ovarian steroidogenesis, GH also modulates the production of ovarian steroids (Fig. 3).

In vitro studies
The ovarian actions of GH are partly mediated by changes in ovarian steroidogenesis. This is indicated by the partial progesterone deficiency in GHR-deficient cattle (Chase et al. 1998). Plasma oestradiol–17β levels in female killifish are, moreover, increased by exogenous GH, which also prevents the hypophysectomy-induced decline in gonadal weight (Singh et al. 1988). However, consistent changes in blood steroid concentrations in response to GH administration have been difficult to demonstrate. For instance, whereas Bryan et al. (1992) noted a stimulatory effect of GH administration on plasma oestradiol levels in pigs, Samaras et al. (1994) observed GH alone was ineffective and actually inhibited the gonadotrophin-induced rise in plasma oestradiol. Conversely, in GH-treated cattle, changes in progesterone but not oestradiol levels (Schemm et al. 1990, Gallo & Block 1991), or in oestradiol but not progesterone (Andrade et al. 1996), or in neither hormone (Yuan & Lucy 1996a) have been reported. GH administration also reduces plasma androgen concentrations in humans (Tapanainen et al. 1992, Volpe et al. 1992), but not in cattle (Andrade et al. 1996) or sheep (Scaramuzzi et al. 1999). This reduction may represent increased conversion of androgens into oestrogens, since GH enhances aromatase and 3β-hydroxysteroid dehydrogenase activity in women (Tapanainen et al. 1992). GH-induced changes in follicular steroid synthesis may be independent of IGF-I, since follicular fluid IGF-I levels can be increased without parallel increases in oestrogen or progesterone in pigs (Bryan et al. 1992, Samaras et al. 1994) or cows (Andrade et al. 1996).
enhances oestradiol secretion from human granulosa cells (Mason et al. 1990, Ovesen et al. 1994, Doldi et al. 1996), perifused rabbit ovaries (Yoshimura et al. 1993), goldfish follicles (Van der Kraak et al. 1990) and bovine follicles (Sirokin & Nitray 1994, Sirokin 1996, Sirokin & Makarevich 1999). Progesterone production from human (Lanzone et al. 1992, Doldi et al. 1996), rat (Jia et al. 1986, Hong & Herington 1991, Apa et al. 1994) and bovine follicles (Langhout et al. 1991, Spicer et al. 1993, Liebermann & Schams 1994) and sheep (Wathes et al. 1995) luteal cells is similarly enhanced by GH. The steroidogenic effect of GH, at least in fish, is equivalent to that of chorionic gonadotrophin (Singh & Thomas 1993). Inhibitory or insignificant effects of GH on progesterone (Ovesen et al. 1994) in human; Yoshimura et al. (1993) and Ando et al. (1994) in rabbit; Sirokin & Nitray (1994) and Lucy et al. (1999) in cow) or oestradiol (Jia et al. 1986) in rat; Rajkumar et al. (1993) and Spicer & Stewart (1996) in pig levels have, however, been documented in other studies. GH responsiveness may be dependent on follicle size, since the stimulatory effect of GH on oestradiol synthesis in porcine granulosa cells was only observed in cells from small follicles (Spicer & Echternkamp 1995).

GH may induce steroidogenesis by potentiating gonadotrophin action, since GH only stimulates oestradiol production from goldfish follicles (Van der Kraak et al. 1990) and progesterone production from rat follicles (Singh & Thomas 1993) if gonadotrophins are present. In addition, GH stimulates androgen synthesis in LH-responsive, but not LH-resistant, bovine thecal cells (Spicer & Stewart 1996). Other studies indicate that GH and gonadotrophins act synergistically to increase oestradiol synthesis in human granulosa cells (Carlsson et al. 1992, Lanzone et al. 1992) and progesterone synthesis in rat granulosa cells (Hsu & Hammond 1987). This synergy may reflect upregulation of gonadotrophin receptors by

Figure 3 Role of GH in steroidogenesis. Thecal and granulosa cells interact during the follicular phase to synthesize oestrogens, which are secreted. Following ovulation, granulosa cells become luteinized and acquire the ability to synthesize and secrete both oestrogens and progesterone. Experimental studies described in the text have demonstrated that GH increases (+) the activity of certain steroidogenic enzymes during the follicular and/or luteal phase, thereby increasing oestrogen or progesterone secretion. STAR is responsible for translocating cholesterol to the mitochondria for processing. The large, solid arrows indicate the release of newly synthesized steroids into interstitial fluid, from which they can access other ovarian cells or the bloodstream.
GH or the upregulation of GHRs by gonadotrophin-induced cAMP (Adashi et al. 1994). Indeed, at least in sheep, adequate ovarian GHR gene expression requires pituitary gonadotrophins (Juengel et al. 1997).

The stimulatory effects of GH on folliculogenesis and ovulation have obvious implications for steroidogenesis (Jia et al. 1986, Apa et al. 1994a). However, the steroidogenic effects of GH in some studies are too rapid to reflect cell differentiation and may instead reflect increased synthesis of steroidogenic enzymes (Fig. 3). For instance, GH stimulates steroidogenic acute regulatory protein (StAR) gene expression in the ovine corpus luteum (Juengel et al. 1995), facilitating the translocation of the cholesterol substrate to the mitochondrion for steroidogenic processing. GH also increases P450 side-chain cleavage (P450 scc) gene expression in porcine granulosa cells (Xu et al. 1997a), increasing the conversion of cholesterol to pregnenalone. GH similarly increases P450c17 mRNA in luteinized human granulosa cells, thereby increasing dehydroepiandrosterone production (Doldi et al. 1996). Basal and FSH-induced aromatase activity is also augmented by GH in human granulosa cells (Mason et al. 1990). GH also enhances aromatase activity and other unidentified steroidogenic enzymes involved in earlier stages of oestradiol synthesis in sea trout ovaries (Singh & Thomas 1993).

GH may stimulate steroid synthesis in humans by IGF-I-dependent and -independent mechanisms. The paucity of IGF-I in human follicles (Mason et al. 1990, Ovesen et al. 1994), even those treated with GH, would suggest that IGF-I does not mediate steroidogenic effects of GH in humans. Indeed, IGF-I independence is also indicated by the synergistic effect of GH and IGF-I/IGF-II on oestradiol production in porcine (Xu et al. 1997b) granulosa cells. The existence of distinct signalling mechanisms for GH and IGF-I is further supported by the opposite effects of cortisol and transferrin on GH- and IGF-I-induced progesterone synthesis (Hong & Herington 1991, Xu et al. 1995). This occurs by two distinct mechanisms, since cyanoketone treatment blocks bGH-induced oestradiol production but not bGH-induced conversion between testosterone and oestradiol (Singh & Thomas 1993). Aromatase activity, is, conversely, blocked by protein synthesis inhibitors and may thus be increased by IGF-I–dependent mechanisms (Singh & Thomas 1993).

Although GH signal transduction is normally mediated by the JAK2 cascade, cAMP may also be involved in GH-induced steroidogenesis. This possibility is suggested by the coincident increases in cAMP, IGF-I, and oestradiol in GH-treated bovine granulosa cells and the ability of protein kinase A blockers to inhibit these GH-induced changes (Sirotkin & Makarevich 1996, Sirotkin & Makarevich 1999). GH similarly induces cAMP production and subsequently steroid production in porcine (Sirotkin et al. 1998) and piscine (Van der Kraak et al. 1990) follicles and sea trout ovaries (Singh & Thomas

Table 1: The involvement of IGF-I in the reproductive actions of GH

<table>
<thead>
<tr>
<th>Dependent on local IGF-I</th>
<th>Independent of IGF-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH-induced progesterone secretion in rat granulosa cells</td>
<td>Progesterone synthesis in porcine granulosa cells</td>
</tr>
<tr>
<td>Oestradiol secretion in human granulosa cells</td>
<td>Nuclear maturation in bovine oocytes</td>
</tr>
<tr>
<td>Nuclear maturation in rat oocytes</td>
<td>Cell growth in human follicles</td>
</tr>
<tr>
<td>Follicle growth in rabbit ovaries</td>
<td>Cell growth in mice preantral follicles</td>
</tr>
</tbody>
</table>

Reference

Hutchinson et al. (1988) | Mondschein et al. (1989) |
Barreca et al. (1993) | Izadyar et al. (1997) |
Apa et al. (1994b) | Ovesen (1998) |
Yoshimura et al. (1994) | Liu et al. (1998) |
In pigs, this effect is dependent upon protein synthesis and acts both proximally and distally to the generation of cAMP (Xu et al. 1997b). Conversely, GH stimulates progesterone synthesis in gonadotrophin-primed rat granulosa cells independently of cAMP accumulation and de novo protein synthesis (Apa et al. 1994d).

Summary

GH has been shown to affect progesterone and oestrogen synthesis both in vivo and in vitro. The steroidogenic action of GH is associated with increased activity of several enzymes and may be partially responsible for the facilitatory effect of GH on folliculogenesis and gametogenesis.

The ovarian mini-hypophysis

**GH synthesis** Ovarian steroidogenesis and gametogenesis may be regulated by locally produced GH as well as pituitary GH (Fig. 1), since low levels of GH gene transcripts and proteins are present in the human ovary (Schwarzler et al. 1997). Granulosa cells and oocytes in particular may be regulated by local GH, since granulosa cells are avascular and separated from systemic circulation by the basal lamina (Lobie et al. 1992).

The synthesis of GH within the ovary may be regulated by locally produced GH secretagogues, since GHRH and GHRH receptors are abundantly present in ovarian tissues (Moretti et al. 1990b) (Table 2). Somatostatin (SRIF) immunoreactivity and SRIF receptors are similarly present (Mori et al. 1984, Baumeister et al. 1998) (Table 2). The importance of ovarian GHRH and/or SRIF in ovarian GH synthesis is uncertain, since GH synthesis in non-pituitary sites is often independent of traditional GH secretagogues (Harvey & Hull 1997). Moreover, these factors have been shown to have other local roles, unrelated to GH regulation (Scanes & Campbell 1995).

**GHRs** The possibility that ovarian GH may act at local sites is supported by the detection of high-affinity ($K_d=2.8-6.1 \times 10^{-9}$ M) low-capacity binding sites in ovarian membranes (e.g. in rabbits (Ando et al. 1994), swine (Quesnel 1999) and fish (Yao et al. 1991)) and in pig follicles (Quesnel 1999) (Table 3). In fish, this GH-binding activity is preferentially found in intracellular membranes, and, to a lesser extent, in plasma membranes (Yao 1993). GHR mRNA and immunoreactivity are also present in the ovary and other female reproductive tissues of numerous species (Table 1).

GHR gene expression is differentially regulated in ovarian and non-ovarian tissues, suggesting that ovarian GHRs serve an important role in regulating reproductive function. For instance, ovarian and hepatic GHR and GH-binding protein (GHBP) transcripts peak 14 or 20 days post-fertilization respectively (Sakaguchi et al. 1998). A transitory peak in both transcripts is also observed postnatally (day 8), corresponding to the lactation period (Sakaguchi et al. 1998). Intestinal and renal GHR and GHBP transcripts do not follow this pattern (Sakaguchi et al. 1998). GHR transcripts similarly increase in abundance during gestation in cows in the liver and corpora lutea, but not in adipose tissue (Hauser et al. 1990, Yuan & Lucy 1996b).

Differential regulation of ovarian and non-ovarian GHR transcripts may reflect the use of alternate promoters. For instance an alternate splice variant (1B) has been detected in the bovine ovary and corpora lutea that contains a different promoter from the cloned hepatic GHR mRNA (1A) (Spicer & Echternkamp 1995, Heap et al. 1996). The 1A variant is specific to adult bovine liver but the 1B mRNA variant is found in both hepatic and non-hepatic tissues (Lucy et al. 1998). In rats, the 1A and 1B variants are differentially regulated by GH and reproductive steroids (Baumbach & Bingham 1995); thus, GHR synthesis, hence GH responsiveness, may thus be differentially regulated in hepatic and reproductive tissues (Lucy et al. 1998, Gomez et al. 1999).

**IGFs** Many of the actions of GH may be induced by the local production of IGFs (Fig. 1, Table 1 and above text), since IGF-I (Geisthoevel et al. 1989) and IGF-I receptors (Poretsky et al. 1985) are usually detectable in ovarian tissues. Moreover, GH increases IGF-I in follicular fluid from humans (Volpe et al. 1992), cows (Sirotkin & Makarevich 1999), pigs (Sirotkin et al. 1998), and sheep (Juengel et al. 1997) and in perfused rabbit ovaries (Yoshimura et al. 1994). However, circulating GH and ovarian IGF-I are not always related, since GHRH immunization of heifers decreases plasma GH and IGF-I but

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**Table 2** GHRH and SRIF gene expression and immunoreactivity (IR) in female reproductive tissues. (R= receptor)

<table>
<thead>
<tr>
<th></th>
<th>Ovary</th>
<th>Placenta</th>
<th>Mammary gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>GHRHR mRNA/GHRHR-IR</td>
<td>Moretti et al. (1990a)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>SRIF mRNA</td>
<td>Mori et. al. (1984)</td>
<td>Lee et al. (1982)</td>
<td>—</td>
</tr>
<tr>
<td>SRIF-IR</td>
<td>Baumeister et al. (1998)</td>
<td>Caron et al. (1997)</td>
<td>—</td>
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<tr>
<td></td>
<td></td>
<td>Caron et al. (1997)</td>
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**References**

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Table 3 GHR/GHBP transcripts and immunoreactivity (IR) and GH-binding sites* in the female reproductive tract

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Species</th>
<th>GHR/GHBP-IR or GH-binding sites††</th>
<th>GHR/GHBP mRNA†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovary</td>
<td>Rabbit</td>
<td>Ando et al. (1994)</td>
<td></td>
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<td></td>
<td>Cattle</td>
<td>—</td>
<td>Lucy et al. (1998)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>Lobbie et al. (1990)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Pigs</td>
<td>Quesnel (1999)</td>
<td>*Yuan &amp; Lucy (1996b)</td>
</tr>
<tr>
<td></td>
<td>Cattle</td>
<td>Kolle et al. (1998)</td>
<td></td>
</tr>
<tr>
<td>Luteal cells</td>
<td>Human</td>
<td>Carlsson et al. (1993)</td>
<td>Carlsson et al. (1993), Sharara et al. (1994)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>Lobbie et al. (1990)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Pig</td>
<td>Yuan et al. (1996)</td>
<td>Scott et al. (1992), Lucy et al. (1993a), Yuan &amp; Lucy (1996b)</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>Yuan et al. (1996)</td>
<td>Juengel et al. (1997)</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Corpus albicans</td>
<td>Human</td>
<td>—</td>
<td>Sharara et al. (1994)</td>
</tr>
<tr>
<td></td>
<td>Oocyte</td>
<td>—</td>
<td>*Sharara et al. (1994)</td>
</tr>
<tr>
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*Not detected. †Extra-embryonic membrane.
††It should be noted that the presence of GHR mRNA is indicative but not proof of function. Similarly, the presence of GHR/GHBP-IR is indicative but not proof of local synthesis and/or function.
not have ovarian IGF-I (Cohick et al. 1996). Indeed, some investigators have failed to detect IGF-I and/or IGF-I mRNA in human granulosa cells (Ovesen et al. 1994) or bovine follicles (El-Roeiy et al. 1993). Ovarian IGF-I in the fish ovary is, moreover, significantly different in sequence from hepatic IGF-I (Kermouni et al. 1998). Basal IGF-I production is similarly at the lower limit of detection in isolated rabbit ovaries (Yoshimura et al. 1994). In vivo GH administration similarly does not stimulate the synthesis of IGFs or their receptors in pre-menopausal human ovaries (Penarrubia et al. 2000). In addition to its roles in reproduction, the local production of IGF-I in the ovary might provide negative feedback to regulate ovarian GH production, comparable to the regulation of pituitary GH secretion by peripheral IGF-I (Harvey 1995).

Summary

Components of the hypothalamo–pituitary–hepatic axis for GH are present in the ovary, including GH, GH secretagogues, GHRs, IGF-I and IGF-I receptors. The ovary may thus contain a GH ‘mini-hypophysis’, as has been previously described in immune tissues (Hull et al. 1997). This ‘mini-hypophysis’ may be concerned with short-term (‘emergency’) modulation of ovarian function in an autocrine or paracrine manner, whereas the traditional (‘strategic’) hypothalamo–pituitary–target organ axis may regulate more long-term functions, in an endocrine way.

Placental Actions

Roles of placental GH

In humans, it is well established that GH modulates maternal metabolism during human pregnancy to induce nutrient repartitioning for fetal development. Pituitary GH deficiency does not, however, abolish the normal pregnancy-induced increase in IGF-I (Eriksson 1989) and does not reduce fetal weight (Curran et al. 1998). Maternal IGF-I abundance is, in contrast, closely correlated with GH of placental origin (Beckers et al. 1990, Caufriez et al. 1993, Mirlesse et al. 1993) and intrauterine growth retardation results from a deficiency in placental GH–producing cells (Mirlesse et al. 1993, Chowen et al. 1996). Conversely, in animals without a placental GH variant, pituitary GH is closely linked with fetal weight. For instance, GH-deficient rats are characterized by low IGF-I, maternal weight gain, and litter size (Gargosky et al. 1993), and GH replacement results in heavier fetuses and faster postnatal growth of pups (Spencer et al. 1994). Litter size and fetal size are also reduced in female GHR–knockout mice, in comparison with normal females (Bartke et al. 1999). Enhanced GH secretion as a result of SRIF immunoneutralization similarly increases the mean birth weight of rat pups (Spencer et al. 1994). Fetal weight in pigs is similarly increased by maternal GH administration (Sterle et al. 1995).

GH may enhance fetal growth by increasing placental size rather than by acting directly on the fetus, since placental GH is not detected in fetal circulation (de Zegher et al. 1990) and maternal GH does not cross the placental barrier (Fholenhag et al. 1994). Indeed, exogenous GH stimulates placental growth in rats (Botero–Ruiz et al. 1997) and pigs (Sterle et al. 1995) and increases the weight of the myoendometrium and gravid uterus in ewes (Jenkinson et al. 1999). The stimulatory effect of GH on placental growth may be mediated at the placenta itself rather than by changes in maternal metabolism, since both pituitary and placental GH have mitogenic activity (Nickel et al. 1990, MacLeod et al. 1991). Moreover, placental GH also stimulates placental IGF-I production (Challier et al. 1991), and enhances endometrial cell growth (Strowitzki et al. 1991).

However, other investigators suggest that GH affects fetal growth independently of placental growth. For instance, Takeda & Hashimoto (1994) have shown in rats that maternal GH affects placental growth but not fetal growth, and fetal GH affects fetal but not placental growth. Placental GH may also alter the endocrine activity of the placenta, since human GH (hGH) stimulates production of placental lactogens, oestradiol and progesterone in vitro (Barnea et al. 1989, Di Simone et al. 1995). It also increases DNA synthesis and the growth of fetal tissues in the rat and sheep fetus during the latter part of pregnancy (Botero–Ruiz et al. 1997, Jenkinson et al. 1999).

The placental mini-hypophysis

The presence of GH or GH-like proteins in the placenta of primates (Alsat et al. 1998), ruminants (Anthony et al. 1995), rodents (Talamantes et al. 1988) and other mammals (Forsyth 1986) is well established. In primates, placental GH-like proteins largely result from expression of the hGH-V gene, which is at least 92% homologous to the hGH-N gene. GH-V gene expression is largely restricted to the syncytiotrophoblast layer of the placenta by proteins that are almost ubiquitous in other tissues and function to repress the hGH-V promoter (Scippo et al. 1993, Alsat et al. 1998). The hGH–V gene may, however, be weakly expressed in normal and tumorous pituitary glands (Scippo et al. 1991, Nickell & Cattini 1992) and the pituitary GH gene appears to be minimally expressed in human placental tissues (Lacroix et al. 1996).

Placental hGH is the same length (191 amino acids) as pituitary GH but contains 13 different amino acids and is more basic (Frankenne et al. 1988, 1990). Unlike pituitary GH, placental GH contains an N-linked glycosylation site; thus, placental GH isoforms corresponding to non-glycosylated (22 kDa) and glycosylated (25 kDa) proteins are produced (Frankenne et al. 1988, 1990). These small differences are thought to be responsible for the reduced
lactogenic activity of placental GH compared with pituitary GH (MacLeod et al. 1991). A poorly characterized GH-like protein is also produced from a splice variant of the hGH-V gene (hGH-V2) and may be membrane-bound (Cooke et al. 1988). This variant shows complete sequence divergence from hGH in the carboxy terminus. Two other transcripts of the hGH-V gene have also been described; one that predicts a truncated hGH-V isoform and one that predicts a novel 219 amino acid protein (hGH-V3) in which the first 124 amino acids are identical to placental GH (Boguszewski et al. 1998).

The GH-V gene is not present in the genomes of non-primates. GH variants are, however, present in the ovine (Lacroix et al. 1996) and rodent (Ogilvie et al. 1990) placentae, and GH mRNA has been detected in the ovine placenta, particularly in trophoectoderm and syncytiotrophoblast (Lacroix et al. 1999).

Placental GHs may act in an endocrine way to regulate extraplacental GH action, since hepatic GHR and GHBP mRNA are more abundant in mice with larger litters (Cramer et al. 1992), presumably because of the higher levels of placental lactogen. Placental GH and/or placental lactogens may also act in a paracrine or autocrine fashion to modulate placental function, since binding activity for placental and pituitary GH is abundantly present in the human placenta (Ray et al. 1990). The GH-binding activity in the placenta may reside in proteins identical to the cloned hepatic GHR, since GHR/GHBP mRNA and immuno-reactivity are abundantly present in trophoblastic cells of the human (Frankenne et al. 1990, Hill et al. 1992, Urbanek et al. 1993), bovine (Kolle et al. 1997), ovine (Lacroix et al. 1999) and rat (Barnard et al. 1994) placenta (Table 3).

In primates, GH-V binds the cloned hepatic GHR with high affinity, and is not completely displaced from placental membranes by hGH-N (Frankenne et al. 1992). A separate, placental hGH-V receptor may thus exist but has yet to be identified. A hGHR gene isoform (hGHRd3), lacking sequences encoded by exon 3, is also present in the chorion, amnion and decidua of the placenta and is the exclusive isoform in placental villi (Urbanek et al. 1992). The binding characteristics of this isoform are, however, identical to the full-length receptor (Urbanek et al. 1993).

Summary

In addition to its well-documented effect on maternal metabolism, GH stimulates fetal growth by binding to placental GHRs and increasing placental size. Autocrine and/or paracrine actions of GH may be particularly important in the primate placenta, since the placenta is the primary source of GH during pregnancy.

Uterine Actions

Uterine roles of GH

The uterus of pregnant and non-pregnant females is also a site of GH action. For instance, exogenous GH promotes uterine proliferation and cellular growth in rats with (Kennedy & Doktorcik 1988, Gunin 1997) or without (Miura & Koida 1970) the addition of oestrogen. Its induction of decidualization in the rat is partly mediated by an upregulation of oestriol receptors (Chilton & Daniel 1987, Bezeny et al. 1992) and by an increased production of uterine IGFBPs and prostaglandins (Kennedy & Doktorcik 1988, Yallampalli et al. 1993). The number of implantation sites is also increased in the uteri of rats transgenically overexpressing the GH gene (Danilovich et al. 2000). In sheep, GH increases the weight of the myoendometrium and the gravid uterus (Jenkinson et al. 1999), probably by stimulating uterine milk protein mRNA levels and stratum spongiosum gland density (Spencer et al. 1999). However, unlike in rats, the uterine effects of GH do not involve the upregulation of oestradiol receptors and may be mediated by interferon tau (Spencer et al. 1999).

The growth promoting actions of GH in uterine tissues have also been indicated by the very high incidence (81%) of leiomyomas in women with acromegaly (Cohen et al. 1998) and the prevalence of GHR mRNA in the nuclei and cytoplasm of leiomyomas and myometrial tissue (Sharara & Nieman 1995). A role for GH in the formation of uterine tumours is also indicated by their suppression in mice in which GH secretion is blocked by neonatal monosodium glutamate treatment (Nagasawa et al. 1985) and their increased incidence in mice with enhanced GH secretion (Singtripop et al. 1993). The induction of cystic endometrial hyperplasia in dogs may also be mediated by GH, since GH mRNA and immunoreactive GH are present in the cytoplasm of glandular epithelial cells (Schoenmakers et al. 1997). The uterus is thus also an extrapituitary site of GH synthesis and action, in which GH may have paracrine or autocrine actions to regulate reproductive function.

Uterine GHRs

GH-induced changes in uterine growth may be independent of hepatic IGF-I, since GHR mRNA is present throughout the uterine layers of numerous mammalian species (Table 3). For instance, the GHR gene is expressed in the myometrium of non-pregnant humans and mice and in the endometrium, glands and stroma of non-pregnant mice (Sharara et al. 1994, Sharara & Nieman 1995). A splice variant of the GHR gene has been shown to be preferentially expressed in the bovine endometrium and myometrium (Heap et al. 1996, Lucy et al. 1998). This variant, which is also present in the ovary and corpus luteum, contains an alternative promoter and may permit specific regulation of GHRs involved in reproduction (Heap et al. 1996, Lucy et al. 1998). For instance, hepatic, but not uterine, GHRs are dramatically upregulated at mid-pregnancy in rats (Sakaguchi et al. 1998). Bovine uterine GHR transcripts appear to be more responsive to
the endocrine changes of pregnancy, since GHR mRNA is not detected in endometrium from non-pregnant cows but is readily detectable in uterine epithelium, glands and blood vessels of pregnant cows, particularly 6 months post-fertilization (Kolle et al. 1997). Conversely, GHR mRNA is detected from day 8 to day 120 in pregnant sheep and day 4 to day 16 in cycling sheep (Lacroix et al. 1999). Species-specific differences in GHR mRNA regulation by gonadal steroids may be responsible for this extensive variation between species, since uterine GHR gene expression is downregulated by GH in cows (Kirby et al. 1996) but unaffected by gonadal steroids in mice (Sharara et al. 1994).

Placental and uterine GHRs may affect reproductive fitness by IGF-I-dependent mechanisms, since IGF-I has been shown to modulate endometrial function (Wang & Chard 1999). Indeed, IGF-I is present in bovine endometrium and myometrium (Kirby et al. 1996) and IGFBPs are present in the rat and bovine uterus (Yallampalli et al. 1992, Kirby et al. 1996). Uterine IGF-I and IGFBPs are not, however, regulated by GH in rat (Yallampalli et al. 1992) or cows (Kirby et al. 1996); thus, their relevance to uterine GH function remains uncertain.

Summary
GH stimulates mitogenesis and, at high levels, tumorigenesis in the uterus. The presence of GH and GHR mRNA in this organ suggests that GH may be acting in a paracrine or autocrine manner.

Oviduct Actions
The presence of GHR mRNA in the bovine oviduct (Kirby et al. 1996) indicates that it may also be a site of GH action. This is supported by the increased content of cAMP, prostaglandin F-α and IGF-I in cultured bovine epithelial cells incubated with GH (Makarevich & Sirotkin 1997). The increased shell thickness of eggs laid by GH-treated hens (Donoghue et al. 1990) suggests that the avian oviduct, particularly the shell gland, is a site of GH action. This possibility is supported by the presence of GHRs and GHR mRNA in these tissues (Hull et al. 1999). Furthermore, the presence of GH proteins in the chicken oviduct (Harvey et al. 1998) suggests that this may reflect an autocrine or paracrine action of GH produced locally.

Mammary Actions

Mammogenesis
GH is obligatory for normal pubertal mammary development. Although pubertal mammary development occurs in response to an increase in oestradiol, it cannot take place in the absence of the pituitary gland or GH in rats (Kleinberg et al. 1990, Feldman et al. 1993, Walden et al. 1998). Specifically, GH acts on mammary stromal and epithelial tissues in rats to induce the differentiation of ductal epithelia into terminal end buds (TEBs) and alveolar structures and promotes the morphogenesis of the TEBs in the mammary fat pad (Walden et al. 1998). GH administration to peri-pubertal heifers (Sejrsen et al. 1986, Sandle et al. 1987, Purup et al. 1993) and lambs (McFadden et al. 1990) similarly increases the amount of parenchymal tissue, although overall mammary size may not be increased, and immunization of heifers against GHRH similarly impairs pubertal mammary development (Sejrsen et al. 1999). Increased milk yield resulting from peripubertal GH administration has not, however, been observed in numerous studies in heifers (reviewed by Sejrsen et al. 1999).

The increased mammary growth induced by GH treatment in pregnant heifers (Stelwagen et al. 1991), ewes (Stelwagen et al. 1993) and goats (Knight et al. 1994) is, conversely, accompanied by increased milk yield. The increased mammogenesis appears to reflect hyperplasia during pregnancy, hypertrophy postnatally, and a postponement of mammary differentiation (Sejrsen et al. 1999). However, neither mammary growth nor milk yield is increased by GH treatment in pregnant sows (Farmer et al. 1997).

Expression of the hGH transgene in mice similarly stimulates normal and neoplastic growth of the mammary glands (Bchini et al. 1991, Nasagawa et al. 1993) and GH administration to ageing monkeys increases glandular size and epithelial proliferation of the mammary glands (Ng et al. 1997). GH also has growth-promoting effects on human mammary cancer cells in vitro (Benlot et al. 1997). The administration of GH does, nevertheless, prevent the age-related involution of the mammary gland that normally occurs in response to increased plasmin activity (Politis et al. 1990).

Galactopoiesis
GH is the major galactopoietic hormone in cows and is commonly used to increase milk yield in commercial dairy herds (Tauer & Knoblauch 1997, Etherton & Bauman 1998). Indeed, treatment of dairy cows with bGH increases milk yield by 10–40%, by affecting both mammogenesis and lactogenesis (Breier et al. 1991). The importance of GH in ruminant lactation is further shown by the correlation between GH gene polymorphisms (Lucy et al. 1993b, Lee et al. 1996, Yao et al. 1996), GHR gene polymorphisms (Falaki et al. 1996), plasma GH levels (Powell & Keisler 1995) and heritable patterns of GH secretion (Vasilatos & Wangsness 1981, Klinert 1988, Beerepoot et al. 1991) with milk production. GH treatment also increases milk yield in humans (Milsom et al. 1992) and milk yield and glucose concentration in goats


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Galactopoietic ewes increases milk yield for 8 weeks following the cessation of mammogenesis, since ovine GH treatment of 1988, Breier output and mammary blood ability in the mammary gland, since GH increases cardiac In addition, GH may selectively increase nutrient availability in adipose tissue but stimulates fatty acid synthase gene transcription (but not translation) in mammary gland tissue adipose tissue and mammary blood flow in cows (Davis et al. 1988, Breier et al. 1991) and goats (Mepham et al. 1984). Galactopoietic effects of GH may also reflect the stimulation of mammogenesis, since ovine GH treatment of ewes increases milk yield for 8 weeks following the treatment period (Kann et al. 1999).

Early studies hypothesized that systemic IGF-I mediates the effects of GH on the mammary gland in ruminants, since binding sites for IGF-I, but not GH, are easily detectable in mammary tissue and IGF-I, but not GH, exerts mammogenic effects in vitro (Akers 1985, Shamay et al. 1988, Cohick 1998). In addition, the mitogenic effect of serum relates more closely to the IGF-I concentration than the GH concentration (Sejrsen et al. 1999). An indirect mechanism of GH action is further supported by the lack of a lactational response to GH administered s.c. over the bovine mammary gland (Flint & Knight 1997).

GH may also directly affect mammary function, since GHR mRNA and protein have been detected in the mammary gland of ruminants and other species (Table 3). Experimental studies also suggest a direct effect, since GH administered through the teat canal in goats results in a small, but significant, increase in milk yield (Collier et al. 1993), although another study observed no change (Sejrsen & Knight 1994). Moreover, GH implants in the mammary gland of GH-deficient rats stimulates milk production in the treated, but not the contralateral gland (Flint & Gardner 1994). IGF-I of mammary, rather than hepatic, origin has also been implicated, since milk IGF-I immunoreactivity increases more rapidly than serum IGF-I immunoreactivity following GH administration in goats (Faulkner 1999). GH thus stimulates mammary IGF-I production more rapidly than hepatic IGF-I production. Accordingly, GH rapidly stimulates IGF-I synthesis in mammary fibroblasts (Mol et al. 1996, Van Garderen et al. 1997) and, synergistically with oestradiol, in the mammary fat pad (Ruan et al. 1995, Walden et al. 1998). GH-induced IGF-I may subsequently affect local function, since IGF-I receptors are present in mammary tissue (Purup et al. 1995, Bassett et al. 1998). Indeed, the galactopoietic response to GH in goats is correlated with a sustained increase in IGF-I in milk and mammary tissue (Prosser et al. 1991). GH may also facilitate actions of IGF-I on the mammary gland, since the synthesis of plasma IGFBP-3 is increased in GH-treated cows (Davis et al. 1988, Breier et al. 1991).

The mammary mini-hypophysis

The mammary gland is a site of GH production as well as action (Mol et al. 1996). GH is found in canine mammary secretions (particularly pre-partum and colostrum) at concentrations 100–1000 times those in plasma; however, milk GH concentrations are not correlated with fetal plasma GH and GH is not absorbed intact through the canine gastrointestinal tract (Selman et al. 1994, Schoenmakers et al. 1997). The role of ‘milk GH’ thus remains uncertain, although mammary GH production results in the recruitment and hyperplasia of stem cells, which differentiate into ductal epithelium by clonal expansion (Mol et al. 1996). Mammary GH also induces maturation of the duct system into TEBs and alveolar structures (Feldman et al. 1993).

Immunoreactive GH in milk and mammary glands reflects local synthesis more than the sequestering of pituitary GH, since GH mRNA is readily detectable in normal and neoplastic mammary glands of humans (Mol et al. 1995a) and of dogs and cats (Mol et al. 1995b). Mammary GH may also contribute to systemic GH concentrations, since GH immunoreactivity is more abundant in mammary veins than in mammary arteries (Selman et al. 1994). Systemic GH is generally considered to be entirely of pituitary origin, since GH is largely not detected in hypophysectomized animals. However, under particular conditions, mammary GH production noticeably alters systemic GH and could thus exert systemic effects. For instance, progestin-induced mammary GH can induce coat growth and increase serum IGF-I in pituitary GH-deficient German shepherd dogs (Kooistra et al. 1998).

The sequence of mammary GH is identical to its pituitary counterpart, but its secretory and regulatory patterns are significantly different (Mol et al. 1996). For instance, mammary GH secretion is non-pulsatile (Mol et al. 1996) and is not altered by SRIF or GHRH, despite the presence of both peptides in the mammary gland
(Table 2). This secretory pattern may be characteristic of non-pituitary GH production, since GH secretion from other extrapituitary sites (e.g. placenta) is similarly non-pulsatile and independent of traditional GH secretagogues, although GHRH and SRIF are usually present (Harvey 1995). The chronic, rather than pulsatile, pattern of mammary GH release can heighten tissue responses to GH, since progestin-induced GH production in hypophysectomized and pituitary-deficient dogs can result in acromegalic features despite non-pathophysiological levels of circulating GH (Selman et al. 1994, Kooistra et al. 1998).

GH regulation in non-pituitary sites usually reflects site-specific influences. For instance, the primary regulator of mammary GH synthesis is likely to be progestins (Loveridge & Farquhharson 1993). This possibility is suggested by the symptoms of mammary GH excess that accompany progestin administration in cats and dogs, which include mammary hyperplasia and cellular proliferation (Selman et al. 1994, Mol et al. 1995b, 1996). Indeed, the non-pathological increase in progestins during the luteal phase is sufficient to stimulate mammary GH production and breast development (Rijnberk & Mol 1997). The mechanism by which progestins stimulate GH production is still uncertain. Progesterone receptors have been detected on all normal GH-secreting mammary cells, although not on all GH-secreting tumorous mammary cells (Mol et al. 1995a, Van Garderen et al. 1997). Although the sequence of mammary GH cDNAs is identical to pituitary GH cDNA in both coding and non-coding regions, mammary GH gene expression may be pit-1-independent (Lantinga-van Leeuwen et al. 1999). This possibility is suggested by the low or absent pit-1 gene expression in the mammary glands of dogs with progestin-induced mammary GH excess (Lantinga-van Leeuwen et al. 1999).

GH-binding sites are detectable in bovine mammary glands (Newbold et al. 1997) but are apparently absent from rabbit mammary gland membranes (Zebrowska et al. 1997). It is, nevertheless, well accepted that the mammary gland expresses GHRs and is thus a GH-target site, because GHR mRNA and/or immunoreactivity are readily observable in the mammary glands of numerous species (Table 3). The receptor is present in both mammary epithelium and stromal tissue, although it is much more abundant in the stroma (Ilkbahar et al. 1999). The importance of these receptors in mammary function in rabbits is suggested by the upregulation of GHR gene expression and immunoreactivity during epithelial proliferation and lactation (Jamnes et al. 1991, Lincoln et al. 1995). In contrast, expression of both GHR and GHBP mRNA gradually decreases throughout pregnancy in rats and mice and is further reduced during lactation, reaching an overall 7-fold reduction by day 6 of lactation in comparison with mammary tissue of virgin animals (Ilkbahar et al. 1995, Sakaguchi et al. 1998, Ilkbahar et al. 1999).

GHR immunoreactivity and mRNA are also found in the epithelial and stromal cells of the human mammary gland (Mertani et al. 1998). However, unlike rabbits, the level of expression is equivalent in inactive, proliferating, and tumorous mammary glands (Mertani et al. 1998). The sub-cellular distribution of GHR immunoreactivity suggests that both GHRs and GHBP are synthesized, since the plasma membrane, cytoplasm and nucleus are all immunoreactive (Lincoln et al. 1995). Indeed, GHBP with similar binding characteristics to serum GHBP have been detected in milk from numerous mammalian species (Postel-Vinay et al. 1991, Amit et al. 1997). An additional GHBP has been identified in human milk which binds hGH and hPRL with equal affinity, but it is immunologically unrelated to the GHR (Mercado & Baumann 1994, Amit et al. 1997). The promoter of the bovine mammary GHR transcript differs from that of the well-characterized hepatic transcript (Lucy et al. 1998); thus, mammary and hepatic GH-responsiveness may be differentially regulated.

**Summary**

The stimulatory effect of GH on mammogenesis and lactogenesis has been well established by numerous *in vitro* and *in vivo* studies. The involvement of hepatic and local IGF-I in these actions appears to be species-dependent. The mammary gland is also a site of GH synthesis. Mammary GH probably acts locally by autocrine/paracrine mechanisms but also, at least in dogs, contributes to circulating GH.

**Hepatic Actions**

**Vitellogenesis**

Many non-mammalian vertebrates (birds, reptiles, amphibia and fish) produce eggs with substantial quantities of yolk. Yolk is deposited into the ovum in the ovary, although the yolk precursors are largely synthesized in the liver. The synthesis of these precursors, vitellogenesis, is primarily controlled by oestradiol, but GH plays an important role. Indeed, in the absence of GH, oestradiol-induced vitellogenesis is severely impaired in hypophysectomized birds (pigeons [Harvey et al. 1978]), reptiles (lizards [Callard et al. 1990], turtles [Ho et al. 1982]) and fish (silver eels [Peyon et al. 1996]) and restored by exogenous GH administration. The action of oestradiol is dependent upon GH, since GH induces and/or maintains hepatic oestrogen receptor abundance (Scanes & Harvey 1995). The interaction of GH with oestradiol in the induction of vitellogenesis is, however, synergistic rather than permissive (Paolucci 1989). In the absence of oestradiol, GH itself can promote vitellogenesis in liver explants or hepatocytes *in vitro* (Carnevali & Mosconi 1992, Peyon et al. 1998).
et al. 1996). This may be a physiological role, since pituitary somatotrophs have greater secretory activity during vitellogenesis stages of reproductive activity (Young & Ball 1983).

**Conclusion**

GH has numerous gonadotrophic roles in female reproduction and is additionally gestational, mammogenic and galactopoietic. The actions of GH are generally gonadotrophic at physiological concentrations and angionadal at pharmacological concentrations and in pathophysiological excess. These actions are thought to reflect endocrine roles of pituitary GH and complementary autocrine or paracrine roles of GH produced within reproductive tissues. The local production of GH within these tissues may thus reflect an ‘emergency’ mechanism to rapidly regulate or ‘fine-tune’ cellular functions that are normally regulated in a ‘strategic’ way by pituitary GH. GH is thus an important regulator of reproduction. However, as the GH axis is not always required for fertility and as GH-resistant patients often have normal pubertal development, it is a modulator of reproduction rather than a bona fide gonadotrophin or primary regulator.

**References**


Bartke A 1999 Role of growth hormone and prolactin in the control of reproduction: what are we learning from transgenic and knockout animals? *Steroids* 64 598–604.


Baumbach WR & Bingham B 1995 One class of growth hormone (GH) receptor and binding protein messenger ribonuclear acid in rat liver, GHR1, is sexually dimorphic and regulated by GH. *Endocrinology* 136 749–760.


Cooke NE, Ray J, Watson MA, Estes PA, Kuo BA & Liebhauber SA 1988 Two distinct species of human growth hormone variant


Flint DJ & Gardner M 1994 Evidence that growth hormone stimulates milk synthesis by direct action on the mammary gland and that prolactin exerts effects on milk secretion by maintenance of mammary deoxynucleic acid content and tight junction status. *Endocrinology* **135** 1119–1124.


Jueggel J, Nett TM, Anthony RV & Niswender GD 1997 Effects of luteotrophic and luteolytic hormones on expression of mRNA


www.endocrinology.org


Owen Ej, Shoham Z, Mason BA, Otsgaard H & Jacobs HS 1991a Cotreatment with growth hormone after pituitary


Ray J, Okamura H, Kelly PA, Cooke NE & Liebhaber SA 1990 Human growth hormone variant demonstrates a receptor binding profile distinct from that of normal pituitary growth hormone. \textit{Journal of Biological Chemistry} \textbf{265} 7939–7944.


Sandles JK, Peel C & Temple-Smith PD 1987 Mammary development and first lactation milk yields of identical twin heifers following prepuberal administration of bovine growth hormone. \textit{Animal Production} \textbf{45} 349–357.


Scheunemann MK, Deaver DR, Griel LC & Muller D 1990 Effects of recombinant bovine somatotropin on luteinizing hormone and ovarian function in lactating dairy cows. \textit{Biological of Reproduction} \textbf{42} 815–821.


Sejrsen K & Knight CH 1994 Unilateral infusion of growth hormone does not support a local galactopoietic action of growth hormone. \textit{Proceedings of the Nutrition Society} \textbf{52} 278A.


www.endocrinology.org


Singhrop T, Mori T, Shiraiishi K, Park MK & Kawashima S 1993 Age-related changes in gonadotropin, prolactin and growth hormone levels with reference to the development of uterine adenomyosis in female SHN mice. In vivo 7 147–150.


Stone BA & Marn RP 1992 Ovarian responses to menopausal gonadotropins in groups of patients with differing basal growth hormone levels. Fertility and Sterility 58 32–36.


Urbanek M, MacLeod JN, Cooke NE & Liebhaber SA 1992 Expression of a human growth hormone (hGH) receptor isoform is