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Patterns of Ovarian Growth and Development in Cattle with a Growth Hormone Receptor Deficiency

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ABSTRACT: Nutrionally induced changes in growth hormone (GH) and IGF-I are associated with decreased ovarian function and may partially explain infertility and anestrus in undernourished cattle. The reproductive importance of GH and IGF-I was tested in cattle with a GH receptor deficiency (GHRD) that have reduced blood IGF-I. Blood was collected daily for plasma, and ovaries were examined daily by ultrasonography for 3 wk during an estrous cycle (estrus = d 0) in GHRD (n = 8) and control (n = 8) cattle. On d 18, blood samples were collected every 10 min for 6 h to measure LH. The GHRD cattle had fewer small antral ovarian follicles (2 to 5 mm, P < .01). After estrous cycle d 5, the first-wave dominant follicle stopped growing in GHRD but continued growing in controls (P < .001). Size of the CL was equivalent for GHRD and controls until d 5, after which CL development slowed in GHRD (P < .01). Likewise, plasma progesterone concentrations were less in GHRD (P < .001). During the luteal phase, GHRD cattle failed to develop follicles greater than 10 mm in diameter (endocrine status × day, P < .05). Size and rate of growth of preovulatory follicles, plasma estradiol, plasma FSH, and plasma LH (d 18 bleed) were similar in GHRD and controls. In conclusion, an important role for GH, GH receptor, and IGF-I in ovarian function was supported because GHRD cattle had distinctly different patterns of ovarian development compared with control cattle.

Key Words: Somatotropin, Insulin-Like Growth Factor, Ovaries, Bovidae

Introduction

Changes in growth hormone (GH) and IGF-I can affect ovarian function through a variety of mechanisms (Webb et al., 1994; Monniaux et al., 1997). In ruminants, a direct effect of GH is possible because the GH receptor is found within the large cells of the corpus luteum (CL) (Lucy et al., 1993a; Yuan and Lucy, 1996). A second mechanism for GH action does not involve a direct effect of GH on the ovary. Instead, GH acts in an indirect (endocrine) manner to cause hepatic IGF-I secretion that stimulates ovarian development (Armstrong and Benoit, 1996). Liver and ovary synthesize IGF-I (Spicer and Echtenkamp, 1995). Therefore, ovarian IGF-I is a summation of endocrine and paracrine/autocrine components. Endocrine IGF-I, however, may represent the predominant source of follicular fluid IGF-I (Leeuwenberg et al., 1996).

Function of ovarian gonadotropin receptors is dependent on IGF-I (Giudice, 1992; Spicer and Echtenkamp, 1995). One hypothesis for decreased fertility and anestrus in undernourished cattle, therefore, is that decreased blood IGF-I concentrations caused by undernutrition lead to decreased ovarian sensitivity to gonadotropins (Armstrong and Benoit, 1996). Indeed, follicles in cattle with low IGF-I develop more slowly (Beam and Butler, 1997; Burns et al., 1997), and CL in undernourished cattle are subfunctional (Gombe...
and Hansel, 1973; Yung et al., 1996). To establish the role that GH, GH receptor, and IGF-I play in reproductive function, we studied a line of cattle with a GH receptor deficiency (GHRD) that leads to severely reduced blood IGF-I. Reproductive function was monitored during an estrous cycle using daily ovarian ultrasonography and blood collection. We hypothesized that if a functional GH/GH receptor/IGF-I axis was necessary for normal reproduction, patterns of follicular and luteal development would be compromised in GHRD cattle.

Materials and Methods

Animals. Cattle were selected from the Brahman herd at USDA, ARS, Subtropical Agricultural Research Station (STARS) located near Brooksville, Florida. The GHRD in cattle is associated with a miniature condition resulting in mature cattle of approximately 70% height and weight of normal frame-size Brahman. The GHRD cattle are homozygous for the recessive trait. Individuals with GHRD are diagnosed based on pedigree analyses, birth weight, mature size, and plasma GH:IGF-I ratio. Sixteen GHRD (n = 8; 4.6 ± 0.9 yr of age) and control (n = 8; 5.4 ± 1.1 yr of age) female cattle (age- and parity-matched, mature cows and heifers) were selected for study and fed peanut hay with ad libitum access. Cows and heifers were used because the GHRD herd is small (approximately 10 mature females). The GHRD condition was not associated with excessive body fat because the amount of external body fat (evaluated by body condition scoring) averaged 67 and 82% of controls, respectively (Table 1).

Blood Collection and Assays. Blood samples were collected by jugular venipuncture once daily just prior to ultrasound examination. Blood (18 mL) was collected into two 9-mL blood collection tubes containing EDTA (Monovette®, Sarstedt, Newton, NC), placed on ice, and centrifuged (3,000 × g, 15 min) for collection of plasma. Plasma was stored at −20°C until analyses.

Plasma concentrations of progesterone were measured in daily samples with a solid-phase RIA (Coat-A-Count®; Diagnostic Products, Los Angeles, CA) validated for bovine plasma (Kirby et al., 1997). Intra- and interassay CV for progesterone were 7 and 2%, respectively. Plasma concentrations of estradiol were measured on the 3 d before estrus by using a single antibody RIA (Kirby et al., 1997). Intraassay CV was 14%. Plasma concentrations of FSH (daily sample), GH (alternate days), and IGF-I (weekly sample) were measured by using a double antibody RIA (Kirby et al., 1997). Intraassay CV were 15, 10, and 8% for FSH, GH, and IGF-I, respectively.

Venous blood samples were collected via jugular catheters during a 6-h window every 10 min on d 18 from GHRD (n = 6) and control (n = 6) cattle. Blood samples were stored on ice and centrifuged for collection of plasma (described above). Plasma concentrations of LH were analyzed with a double antibody RIA as described (Zaied et al., 1980). Intraassay and interassay CV were 13 and 12%, respectively. Number of LH peaks, LH peak height, and LH baseline were determined from Cluster program analyses (Veldhuis and Johnson, 1986).

Statistical Analyses. Ovarian ultrasound and plasma hormone data were analyzed by using the GLM procedure of SAS (SAS, 1985). The analyses accounted for repeated measurements by using a statistical model with endocrine status (GHRD or control), animal nested within endocrine status (error term for endocrine status), day of the estrous cycle, the endocrine status × day interaction, and residual. Data consisting of single observations per animal (i.e., estrous cycle length, number of LH peaks, etc.) were analyzed by using a statistical model that included endocrine status (GHRD or control). Data are presented as least squares means (lsmean) ± SEM.

Results

Physical and Endocrine Characteristics of GHRD and Control Cattle. The weight and height of GHRD cattle averaged 67 and 82% of controls, respectively (Table 1). The GHRD condition was not associated with excessive body fat because the amount of external body fat (evaluated by body condition scoring) was similar for GHRD and control cattle (Table 1). Plasma IGF-I concentrations were decreased in GHRD
Table 1. Body weight, hip height, and body condition of growth hormone receptor deficient (GHRD) and control cattle (least squares means ± SEM)

<table>
<thead>
<tr>
<th>Item</th>
<th>GHRD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg(^a)</td>
<td>314 ± 13</td>
<td>472 ± 13</td>
</tr>
<tr>
<td>Hip height, mm(^b)</td>
<td>1,157 ± 15</td>
<td>1,407 ± 15</td>
</tr>
<tr>
<td>Body condition score(^b)</td>
<td>5.7 ± .4</td>
<td>5.4 ± .4</td>
</tr>
</tbody>
</table>

\(^a\)P < .001, GHRD vs control.  
\(^b\)1 = thin to 9 = obese.

Table 2. Plasma concentrations of insulin-like growth factor (IGF)-I (weekly), growth hormone (GH) (alternate days), follicle-stimulating hormone (FSH) (daily), estradiol (3 d preceding estrus), and luteinizing hormone (LH) (estrous cycle d 18) in GH receptor-deficient (GHRD) and control cattle (least squares means ± SEM)

<table>
<thead>
<tr>
<th>Item</th>
<th>GHRD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-I, ng/mL(^a)</td>
<td>10.3 ± 7.2</td>
<td>78.8 ± 7.2</td>
</tr>
<tr>
<td>GH, ng/mL(^a)</td>
<td>103.8 ± 6.4</td>
<td>14.4 ± 6.4</td>
</tr>
<tr>
<td>FSH, ng/mL</td>
<td>.81 ± .07</td>
<td>.71 ± .06</td>
</tr>
<tr>
<td>Estradiol, pg/mL</td>
<td>6.6 ± .7</td>
<td>5.2 ± .7</td>
</tr>
<tr>
<td>LH</td>
<td>5.4 ± .6</td>
<td>4.0 ± .5</td>
</tr>
<tr>
<td>Number of peaks/6 h(^b)</td>
<td>9.0 ± .32</td>
<td>1.00 ± .27</td>
</tr>
<tr>
<td>Peak height, ng/mL</td>
<td>1.83 ± .35</td>
<td>1.71 ± .30</td>
</tr>
</tbody>
</table>

\(^a\)P < .001, GHRD vs control.  
\(^b\)P < .01, GHRD vs control.

while, at the same time, plasma GH concentrations were elevated in GHRD (Table 2).

**Number of Ovarian Follicles During the Estrous Cycle.** The number of class 1 ovarian follicles was decreased (P < .01) in GHRD compared with control cattle (Figure 1A). Average numbers of class 1 follicles were 22.4 ± 1.1 and 63.1 ± 9.9 follicles/animal for GHRD and control, respectively. The effects of day and endocrine status × day were not significant for class 1 follicles (P > .10). Across all days, the numbers of class 2 follicles were similar for GHRD and control (1.7 ± .2 and 2.0 ± .2 follicles/animal for GHRD and control, respectively; Figure 1B). There was an endocrine status × day interaction (P < .01), however, for the number of class 2 follicles, because the pattern of class 2 follicle development differed between GHRD and control cattle. Early in the estrous cycle (first follicular wave), the number of class 2 follicles was greater in control than in GHRD cattle. A similar difference was observed late during the estrous cycle (d 16 to 20). There was an effect of endocrine status (P < .01) for the number of class 3 ovarian follicles (.3 ± .1 and .7 ± .1 follicles/animal for GHRD and control, respectively; Figure 1C) and a tendency for an endocrine status × day interaction (P < .10). Beginning on d 6 of the estrous cycle, the GHRD cattle had fewer class 3 follicles compared with controls. This difference was maintained until late in the estrous cycle (d 17) when GHRD cattle demonstrated recruitment and selection of a class 3 dominant follicle.

**Dominant Follicle Growth and Development.** Estrous cycle length was similar for GHRD (20.4 ± .6 d) and...
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Figure 2. Diameter (least squares means and SEM) of dominant ovarian follicles for GH receptor-deficient (GHRD) and control cattle determined by once-daily transrectal ultrasonography. A, first wave dominant follicle; B, preovulatory follicle.

control (20.6 ± .6 d). Cattle had two or three follicular waves (periods of recruitment leading to an increase in class 2 follicles) within each estrous cycle. The percentage of two and three follicular wave estrous cycles was similar for GHRD (29 and 71%, respectively) and control (12 and 88%, respectively). The initiation of the first and third follicular waves occurred at a similar time (estrus cycle d 2.8 ± .1 and 16.6 ± .6, respectively) for GHRD and control. The second follicular wave, however, was initiated earlier (P < .02) for GHRD (estrus cycle d 8.5 ± .5) than for control (estrus cycle d 10.6 ± .5).

During the first 5 d of the estrous cycle, size of the first wave dominant follicle was identical for GHRD and control (Figure 2A). After d 5, however, the first wave dominant follicle in GHRD arrested development and reached an average mature size of 9.8 ± .5 mm on d 7 (endocrine status × day, P < .001). For control cattle, the first wave dominant follicle continued development to an average mature size of 12.0 ± .5 mm on d 10.

Analyses of the preovulatory follicle detected no differences between GHRD and control (P > .10, Figure 2B). Average sizes of the preovulatory follicle on the day of estrus were 12.4 ± .6 and 10.4 ± .8 mm for GHRD and control, respectively. Concentrations of estradiol in plasma on the 3 d preceding estrus were also similar (P > .10, Table 2).

Corpus Luteum Size and Plasma Progesterone Concentrations. The average size of the CL was smaller for GHRD than for control (Figure 3A; 168.5 ± 5.2 vs 215.1 ± 5.3 mm2; P < .01). There was a tendency (P < .10) for an endocrine status × day interaction for CL size because CL size was similar for GHRD and control until d 5 of the estrous cycle. After d 5, the CL grew more slowly in GHRD than in control. Although the GHRD and control CL initiated growth at approximately the same time (d 2 to 5), the decline in CL size (associated with luteolysis) occurred about 2 d earlier in GHRD compared with control. Concentrations of plasma progesterone paralleled the observed changes in CL size (Figure 3B). There was an endocrine status × day interaction (P < .001) for plasma progesterone concentration. Concentrations of progesterone increased an equivalent amount for GHRD and controls up to d 10 of the estrous cycle. After d 10, plasma progesterone concentration continued to increase in controls and reached a maximum of 3.8 ± .3 ng/mL on d 12. For GHRD, plasma progesterone concentration reached a maximum of 3.0 ± .3 ng/mL on d 13. Decline in progesterone concentration seemed to occur earlier in GHRD than in control. Using a definition of serum progesterone concentration < 1 ng/mL, however, luteolysis occurred at a similar time for GHRD (d 17.2 ± .7) and control (d 18.8 ± .7, P > .10).

Follicle-stimulating Hormone and Luteinizing Hormone. Concentrations of plasma FSH were measured once-daily during the estrous cycle. There were no differences for FSH concentration for GHRD compared with control (Table 2). The concentration and dynamics of LH secretion were measured during a 6-h LH window bleed on d 18 of the estrous cycle. There was a tendency for a greater number of LH peaks per 6 h in GHRD compared with control (Table 2, P < .10). Other measures of LH (i.e., peak height and baseline) were similar for GHRD and control (Table 2).

Discussion

Growth hormone and IGF-I are endocrine regulators of growth and differentiation within multiple
Figure 3. Size of the corpus luteum (CL) and plasma progesterone concentrations in GH receptor-deficient (GHRD) and control cattle. A, Cross-sectional area of the CL (least squares means and SEM) determined by once-daily transrectal ultrasonography. B, Plasma progesterone concentrations (least squares means and SEM).

because IGF-I is the primary negative feedback regulator of GH (Berelowitz et al., 1981). A GHRD leads to a failure of GH-stimulated IGF-I release and a loss of IGF-I negative feedback on GH secretion (Rosenfeld et al., 1994). Therefore, as expected for GHRD, plasma GH concentrations were increased and plasma IGF-I concentrations were reduced compared with control. In separate studies, exogenous GH failed to elicit a normal IGF-I response in this line of GHRD cattle (Elsasser et al., 1990). Therefore, the GHRD cattle have a deficiency in either GH receptor amount or GH receptor signal transduction leading to IGF-I release. Our preliminary analyses show that the predicted GH receptor protein is identical in GHRD and control (data not shown), but for an unknown reason, the GH receptor mRNA is underepressed in the liver (Lucy et al., 1993b).

Several important reproductive observations were made when GHRD cattle were compared with control. First, the GHRD cattle had fewer small antral ovarian follicles (class 1, Figure 1A) and decreased numbers of recruited follicles (class 2, Figure 1B) during the first follicular wave. Reduced follicular growth in GHRD cattle supported a hypothesis that changes in serum IGF-I affect numbers of small antral and recruited follicles. Cattle with severely reduced IGF-I (GHRD) had decreased follicular populations and decreased recruitment (Figure 1B). When normal cattle were supplemented with GH and had increased IGF-I concentrations, antral follicular populations increased (Gong et al., 1993a; Kirby et al., 1997). The mechanisms through which GH or IGF-I changes the patterns of follicular development are largely unknown. Insulin-like growth factor-I increases the sensitivity of follicular cells to LH and FSH (Spicer and Echternkamp, 1995). When tested in cultured bovine granulosa cells, IGF-I, but not GH, acted synergistically with LH and FSH to stimulate cell proliferation (Gong et al., 1993b). Therefore, changes in follicular growth associated with IGF-I may be related directly to ovarian gonadotropin response. Increased ovarian gonadotropin response during periods of high IGF-I leads to an increase in follicular growth. Conversely, decreased ovarian gonadotropin response during periods of low IGF-I (GHRD) leads to a decrease in follicular growth.

The patterns of dominant follicle development in GHRD cattle also suggested a change in gonadotropin action. The first wave dominant follicle in GHRD cattle grew to approximately 9 mm and then decreased in size. Contemporary control cattle had dominant follicles that grew to 12 mm. The LH receptor mRNA is expressed initially in theca interna cells of cattle (Xu et al., 1995). At a larger follicular diameter (>9 mm), granulosa cells expressed the LH receptor (Xu et al., 1995). Changes in LH receptor mRNA amounts (Xu et al., 1995) were confirmed by studies of $^{[125]}$IhCG binding in granulosa
and theca cells from first wave dominant follicles (Stewart et al., 1996). The timing of follicular regression in GHRD cattle was an indication of a failure in gonadotropin support. We speculate that low serum IGF-I led to inadequate LH receptor function or expression within the granulosa cell layer of the first wave dominant follicle of GHRD cattle. Possibly, reduced LH receptor expression combined with decreasing LH pulse frequency (shown for the early period of the estrous cycle by Kinder et al., 1996) led to a premature follicular arrest. Development of large ovarian follicles in GHRD cattle did not resume until after luteolysis.

A period of follicular growth occurred after luteolysis in GHRD cattle. This led to an increase in the average number of class 3 follicles for GHRD. A preovulatory follicle was subsequently formed that achieved equivalent size and steroidogenic capacity for GHRD and control cattle. Apparently, follicular growth was restored in GHRD cattle by greater pulsatile LH secretion that occurred after luteolysis. These data suggest that in the presence of low IGF-I, highly pulsatile LH secretion can drive follicular growth. Perhaps greater LH supersedes the minimum requirements for IGF-I within the follicle. A dominant role for LH in preovulatory follicular development is also shown by McShane and Keisler (1991), who restored follicular growth in nutritionally anestrous ewes by using pulsatile administration of LH. Luteinizining hormone will increase IGF-I synthesis by in vitro-cultured granulosa cells (Hsu and Hammond, 1987). Therefore, a second possibility is that the additional LH increased the follicular expression of IGF-I, amplified LH action, and restored dominant follicle development.

 Corpus luteum development differed for GHRD and control cattle. This difference was most clearly observed during the mid- and late-luteal phase of the estrous cycle. The differences in size of the CL were paralleled by plasma progesterone. We conclude that GH and IGF-I are required for normal CL formation and function. Growth hormone and IGF-I may affect the follicle before ovulation and/or the luteal cells after ovulation. A stimulatory effect of GH on ovarian progesterone synthesis was demonstrated in luteinized granulosa cells (Wathes et al., 1995) and microdialysed bovine CL (Liebermann and Schams, 1993). The actions of GH in these in vitro systems did not depend on GH-stimulated IGF-I synthesis (Wathes et al., 1995). In vivo, the ruminant CL makes little IGF-I (Kirby et al., 1996), and luteal IGF-I synthesis was not changed by negative energy balance (VandeHaar et al., 1995), administration of exogenous GH (Kirby et al., 1996), or blocking endogenous GH by GHRH immunization (Cohick et al., 1996). The role that decreased plasma IGF-I played in the slower CL development in GHRD cattle should not be overlooked because IGF-I increased progesterone synthesis in cultured luteal cells (McAr-dle and Holter, 1989; Constantino et al., 1991; Sauerwein et al., 1992). Administration of GH (and increased IGF-I), however, failed to improve CL development in heifers in negative energy balance (Yung et al., 1996). Therefore, other factors, including health and number of cells within the preovulatory follicle, should be considered as mediators of abnormal CL function.

In a previous study of this GHRD line, plasma glucose was equivalent but insulin was lower in GHRD compared with control (Hammond et al., 1991). Therefore, plasma insulin concentration may be a confounding factor in studies of GHRD cattle. Physiological concentrations of insulin will stimulate granulosa cell steroidogenesis (Spicer et al., 1993), and insulin is equal to or more potent than IGF-I for stimulating estradiol synthesis in cultured granulosa cells from cattle (Spicer and Echternkamp, 1995). However, when administered to beef cattle, insulin failed to increase follicular populations (Simpson et al., 1994). Therefore, insulin differences between GHRD and control may not have contributed to differences in follicular growth. Insulin was probably not a factor for CL development because insulin potency is less than IGF-I for CL growth and steroidogenesis (Constantino et al., 1991; Sauerwein et al., 1992; Chakravorty et al., 1993).

One unresolved question is whether the ovarian expression of IGF-I or IGF binding proteins (IGFBP) was changed in the GHRD cattle. The ovarian synthesis of IGF-I and IGFBP is tightly regulated and controls many aspects of follicular growth and differentiation (Spicer and Echternkamp, 1995). During follicular maturation in cattle, follicular fluid IGF-I remains constant but the amount of IGFBP changes and increases IGF-I availability (Echternkamp et al., 1994; Stewart et al., 1996). Changes in the relationship between IGF-I and IGFBP within GHRD could influence the responses that we observed. Studies of GHRD in chickens (sex linked dwarf, SLD) demonstrated GH-independent IGF-I mRNA expression in all tissues except liver and testes (Tanka et al., 1996). As expected, liver had decreased IGF-I mRNA in SLD. Testes, however, had increased IGF-I in SLD. Increased IGF-I mRNA in SLD testes suggests that reproductive tissues may compensate partially for decreased blood IGF-I. When muscle IGF-I mRNA was assayed in GHRD bulls, we found equivalent IGF-I mRNA in GHRD and controls (data not shown). The possibility that ovarian IGF-I is greater in GHRD should be investigated further.

In summary, patterns of reproduction were distinctly different for GHRD and control cattle. The GHRD cattle failed to maintain follicular growth during the midluteal phase of the estrous cycle. Follicular growth resumed following luteolysis when LH secretion increased. Function of the CL was
compromised by GHRD. This line of GHRD cattle demonstrates unique patterns of ovarian function. These data argue for an essential role of a functional GH/GH receptor/IGF-I axis in normal patterns of ovarian growth and development in cattle.

Implications

Growth hormone (GH) and insulin-like growth factor (IGF-I) are regulated by nutrition and have direct effects on ovarian function. Therefore, the GH and IGF-I systems are an endocrine link between nutrition and reproduction in farm animals. Cattle with GH receptor deficiency (RD) have abnormally high GH and abnormally low IGF-I. Gonadotropin concentrations are normal in GHRD, but GHRD demonstrates unique patterns of ovarian function. Therefore, the GH factor (IGF)-I are regulated by nutrition and have direct effects on ovarian function. Therefore, the GH and IGF-I systems are an endocrine link between nutrition and reproduction in farm animals. Cattle with GH receptor deficiency (RD) have abnormally high GH and abnormally low IGF-I. Gonadotropin concentrations are normal in GHRD, but GHRD compromised by GHRD. This line of GHRD cattle demonstrates unique patterns of ovarian function. These data argue for an essential role of a functional GH/GH receptor/IGF-I axis in normal patterns of ovarian growth and development in cattle.

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