ACTH treatment disrupts ovarian IGF-I and steroid hormone production

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Abstract

Hyper-adrenal activity and increased glucocorticoid hormone release are associated with disruptions in reproductive function and adverse effects on the ovary. The aim of this investigation was to determine whether elevated glucocorticoid hormone levels can influence ovarian IGF-I synthesis and action *in vivo*. To elevate endogenous glucocorticoid levels, gilts were treated with ACTH during the luteal phase of the oestrous cycle (days 9–13) while the control group received saline. The gilts were subsequently ovariectomized on either day 14 or day 18 of the oestrous cycle. Follicular fluid (FF) was collected from individual follicles; IGF-I and steroid hormone concentrations were determined by radioimmunoassay, and IGF-binding protein (IGFBP) expression was assessed by Western ligand blotting. Granulosa cells were also recovered and placed in culture to determine IGF-I, progesterone (P_4) and oestradiol-17 β (E_2) production levels. The cells were cultured in serum-free medium for 5 days and

supplemented with: (a) media alone, (b) IGF-I, (c) FSH and androstenedione (A_4), or (d) IGF-I with FSH and A_4 . The FF from ACTH-treated gilts was characterized by elevated ($P<0.05$) cortisol levels on day 14 and lower ($P<0.05$) E_2 values on both day 14 and day 18. Lower ($P<0.05$) IGF-I concentrations were also measured in the FF of ACTH-treated gilts collected on day 18. This altered hormone profile in FF was associated with impaired IGF-I and steroid hormone synthesis by granulosa cells. IGF-stimulated P_4 production ($P<0.01$) by cells recovered from ACTH-treated gilts on day 14 was lower ($P<0.05$). By day 18, IGF-I, P_4 and E_2 production by cells from the ACTH group were all significantly ($P<0.05$) lower. These results demonstrate that increased glucocorticoid concentrations can disrupt subsequent ovarian IGF-I synthesis and IGF action *in vivo* and can, potentially, impair follicle maturation.

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Introduction

Hyper-adrenal activity and stress-associated stimulation of the hypothalamic–pituitary–adrenal axis have been reported to disrupt reproductive function (Moberg 1987). While the mechanism of action is not well understood, previous research suggests that increased adrenal glucocorticoid hormone release may mediate the adverse effects on the reproductive process. In the pig, poor follicle development, reduced ovulation rates and ovarian cyst formation have been reported in association with elevated glucocorticoid concentrations (Liptrap 1970, 1993). Exposure to high glucocorticoid levels during the late follicular phase of the oestrous cycle can disrupt gonadotrophin release (Barb *et al.* 1982, Li & Wagner 1983, Fonda *et al.* 1984). However, administration of glucocorticoids or adrenocorticotrophic hormone (ACTH) to sows during the luteal phase does not significantly alter the pulsatile secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), yet lowers both plasma and follicular fluid (FF) oestrogen levels (Frautschy

& Liptrap 1988). In addition, an increased percentage of follicles fail to reach ovulatory size (Frautschy & Liptrap 1988) and exhibit morphological signs of degeneration (Gee *et al.* 1991), thus indicating a possible direct glucocorticoid influence on ovarian function.

The expression of glucocorticoid receptors in rat ovarian cells (Schreiber *et al.* 1982) and the detection of cortisol binding globulin in FF (Mahajan *et al.* 1980) supports the possibility of direct glucocorticoid action in the ovary. Glucocorticoid hormone metabolism by ovarian cells is also indicated by the expression of 11 β -hydroxysteroid dehydrogenase mRNA (Tetsuka *et al.* 1997) and enzyme activity in human granulosa cells (Michael & Cooke 1994). Furthermore, data from *in vitro* studies demonstrate cortisol-mediated disruptions in steroid hormone synthesis. Exposure to high glucocorticoid concentrations during culture lowers FSH-stimulated oestradiol-17 β (E_2) synthesis, but promotes progesterone (P_4) production by murine (Hsueh & Erickson 1978, Adashi *et al.* 1981, Schoonmaker & Erickson 1983), porcine (Danisova *et al.* 1987), and bovine granulosa cells (Kawate *et al.* 1993).

Recent work also demonstrated that porcine granulosa cells cultured with high levels of cortisol respond with lower insulin-like growth factor (IGF)-I synthesis and reduced IGF-I-stimulated P_4 production (Viveiros & Liptrap 1999).

Significant research over the last decade supports the existence of an 'intra-ovarian insulin-like growth factor system' in women, rodents and a number of domestic species including the pig (Adashi *et al.* 1985a, Guidice 1992, Hammond *et al.* 1993). Both IGF-I and IGF-II, as well as IGF receptors and various IGF-binding proteins (IGFBPs) are expressed in the porcine ovary (Hammond *et al.* 1993, Spicer & Echternkamp 1995). IGF-I is secreted by granulosa cells in culture, and IGF-I mRNA expression has been detected in both granulosa and luteal cells (Gadsby *et al.* 1996, Yuan *et al.* 1996). Several IGFBPs have also been isolated from FF (Mondschein *et al.* 1991, Howard & Ford 1992) and are specific products of the ovary, with mRNA expression confirmed in granulosa and luteal cells (Samaras *et al.* 1993, Gadsby *et al.* 1996, Yuan *et al.* 1996). Functionally, IGF-I can regulate ovarian steroidogenesis and is thought to play an important role in follicle development (Guidice 1992, Hammond *et al.* 1993).

If high glucocorticoid hormone levels disrupt ovarian IGF-I production and/or IGF-I action *in vivo*, as demonstrated with granulosa cell culture (Viveiros & Liptrap 1999), follicle development may be adversely affected. To assess this possibility, endogenous glucocorticoid concentrations were elevated by ACTH administration to gilts during the luteal phase of the oestrous cycle (days 9–13). The treatment regime used significantly elevates both plasma and FF cortisol levels (Montgomery *et al.* 1997) without disrupting gonadotrophin secretion (Frautschy & Liptrap 1988). The ovaries were subsequently recovered on day 14 and day 18 to assess granulosa cell IGF-I and steroid hormone production, as well as FF hormone levels.

Materials and Methods

Animals and experimental procedure

In this investigation, sexually mature Yorkshire-Landrace gilts, which ranged in age from 5 to 6.5 months and had an approximate bodyweight of 125 kg, were used. All animals were maintained according to the Guidelines of the Canadian Council on Animal Care; they were housed in individual pens, subjected to controlled light conditions (14 h light, 10 h darkness) and fed a commercial sow ration (16% protein) with water freely available. Timing of the oestrous cycle for each gilt was determined by daily observation, with day 0 of the cycle denoting the first day of characteristic oestrous behaviour including a positive response to back pressure and to mounting by a boar. Two successive cycles were observed for each animal prior to random allocation to either the control or the treatment

group. Treated gilts received intra-muscular injections (0.5 IU/kg bodyweight) of a long acting preparation of ACTH (ACTHAR GEL, Rorer Canada Inc., Bramalea, ON, Canada) at 12-h intervals for 5 days (from day 9 to day 13), while control gilts received an equivalent volume of physiological saline under the same conditions. This treatment effectively raises both plasma and follicular fluid cortisol concentrations to mean values of 80 and 30 ng/ml respectively (Montgomery *et al.* 1997).

Follicular fluid and granulosa cell collection

The gilts were ovariectomized by mid-ventral laparotomy on day 14, approximately 12 h after the final ACTH injection (control $n=5$, ACTH-treated $n=5$), or on day 18, 5 days after treatment was discontinued (control $n=5$, ACTH-treated $n=5$). Upon recovery, the ovaries were rinsed with phosphate buffered saline (PBS) and the 7 largest follicles (with a minimum diameter of 3 mm) per ovary were dissected from the interstitial tissue. Follicular fluid was collected by aspiration from individual follicles and stored at -20°C for subsequent assessment of IGF-I, IGFBP and steroid hormone concentrations. To recover granulosa cells for culture, the follicles were bisected, flushed with PBS and gently scraped with a rubber-tipped spatula. Cells collected from both ovaries were pooled, washed twice and dispersed in 5 ml Dulbecco's Modified Eagle's Medium Nutrient Mixture/Ham F-12 (DMEM/F-12) (Gibco BRL, Burlington, ON, Canada). The cells were counted on a haemocytometer and cell viability was determined using a trypan blue dye exclusion test.

Cell culture

Granulosa cells were cultured in 24-well plates, at a density of 0.5×10^6 viable cells per well, in 1 ml DMEM/F-12 supplemented with 0.1 mM non-essential amino acids (Gibco BRL), and an antibiotic-antimycotic solution (Gibco BRL) containing penicillin (100 U), streptomycin (10 µg/ml) and amphotericin B (0.025 µg/ml). At the onset of culture the cells were treated with different hormone supplements (3 replicates for each treatment) which included: (1) medium alone to assess basal production, (2) 10 ng/ml IGF-I (Boehringer Mannheim, Laval, PQ, Canada), (3) 10 ng/ml porcine FSH (100 × the NIH-FSH-P1 standard, Cedarlane Laboratories, Hornby, ON, Canada) + 100 ng/ml androstenedione (A_4) as an aromatase substrate, or (4) 10 ng/ml IGF-I with FSH (10 ng/ml) and A_4 (100 ng/ml). Culture was carried out under standard conditions of 95% air and 5% CO_2 at 37°C for 5 days. A sample (150 µl) of medium was collected after 12 h and 24 h of culture and then subsequently at 24-h intervals, with a media change at 48 h. The samples were stored at -20°C for future radioimmunoassay (RIA) analysis to determine E_2 , P_4 and IGF-I levels. Hormone

levels are expressed as 'cumulative hormone concentrations' to indicate that concentrations reported at 72 h and beyond have the 48 h value added. The experiment was replicated 5 times for control and ACTH-treated gilts, with ovaries recovered on both day 14 and day 18.

Insulin-like growth factor-I measurement

Insulin-like growth factor concentrations in the FF and culture media were determined using a specific radioimmunoassay (Houseknecht *et al.* 1988). Prior to assay, the IGFBPs were separated from the IGF ligand by acidification of the samples with 0.2 M glycylglycine hydrochloride for 24 h at room temperature. An IGF-I (Boehringer Mannheim) standard curve was prepared by serial dilution, and ranged from 0.01–1.0 ng. The samples and standards were incubated with rabbit anti-human IGF-I (UKB 487; distributed for research by the Hormone Distribution Program of NIDDK, National Institutes of Health, Baltimore, MD, USA) for 1 h at room temperature. Subsequently, ^{125}I -labelled IGF-I ($\sim 10\,000$ c.p.m./100 μl , Amersham Canada Ltd, Oakville, ON, Canada) was added and the samples were maintained for 48 h at 4 °C. Goat anti-rabbit serum (Daymar Laboratories, Toronto, ON, Canada) was used to separate the bound and free fractions. The inter- and intra-assay coefficients of variation were 13.9% and 8.6% respectively. The anti-IGF-I is reported to cross-react 0.5% with IGF-II and minimally with insulin at 10^{-6} M.

Insulin-like growth factor binding protein expression

The IGFBP profile in FF from individual follicles was determined by Western ligand blotting procedures (Hossenlopp *et al.* 1986). Follicular fluid samples were initially separated by electrophoresis using SDS-PAGE under non-reducing conditions for 1.5 h and then transferred onto a nitrocellulose membrane by electroblotting for 1 h at 100 volts. The membrane was hybridized overnight with approximately 0.25 μCi ^{125}I -labelled IGF-I (Amersham). The following day, the membrane was taken through a series of washes with 0.15 M NaCl supplemented with 0.1% Tween 20, then air dried and exposed to film (Amersham) for 14 days at 4 °C. The autoradiographs were analysed by the NIH image analysis system. The IGFBP band(s) intensity in FF samples was expressed as a percentage of the corresponding IGFBP expressed in a porcine serum standard which was run in parallel on the same gel (Cataldo & Guidice 1992).

Steroid hormone measurement

Specific radioimmunoassays, previously validated in the laboratory (Liptrap & Cummings 1991), were used to determine cortisol, P_4 , E_2 , and A_4 levels in FF, as well as P_4 and E_2 concentrations in the culture media. Each

sample was assayed in duplicate after extraction with diethyl ether (Caledon Laboratories Ltd, Georgetown, ON, Canada). All radioisotopically labelled steroids were purchased from Amersham, and all non-radioactive steroid standards from Steranti Research Ltd (St Albans, Herts, UK). The cortisol antiserum was obtained from Sigma Chemical Co. (St Louis, MO, USA) while the A_4 and P_4 antisera were purchased from Cedarlane Laboratories. The E_2 antiserum was generously provided by Dr J Pratt (Isotopenlaboratorium Academisch, Groningen, The Netherlands). The intra-assay variation was 4.9% for cortisol, 4.6% for P_4 , 6.7% for E_2 and 8.3% for A_4 , while the interassay variation was 9.8%, 10.3%, 9.6% and 10.9% respectively.

Statistical analysis

All values are reported as a mean $\pm$ standard error (S.E.). Analysis of variance (ANOVA) procedures, by the Statistical Analysis System (1985), were used for the comparison among means. The effect of ACTH treatment and day of the oestrous cycle on follicle size and FF hormone levels was determined. The effect of ACTH treatment and specific hormone supplementation during culture on granulosa cell IGF-I, E_2 and P_4 production was also determined. Pair-wise comparison between means was determined using the Student-Newman-Keuls test and the experiment-wise level of significance ($\alpha=0.05$) was controlled using Bonferroni's adjustment.

Results

Individual follicles ($n=70$ per group) were recovered from control and ACTH-treated gilts for evaluation on day 14 and day 18 of the oestrous cycle. On day 14, the mean follicle diameter (mm $\pm$ S.E.) was similar in the ACTH and the control group (3.8 ± 0.17 vs 4.2 ± 0.22). However, on day 18 the mean diameter of follicles from the ACTH-treated group (5.2 ± 0.25) was smaller ($P<0.05$) than the control group (7.6 ± 0.35).

IGF-I and IGFBP profiles in follicular fluid

Mean IGF-I levels (ng/ml $\pm$ S.E.) measured in control follicles were higher ($P<0.05$) on day 18 compared with day 14. In contrast, the mean IGF-I values in follicles from ACTH-treated gilts increased to a lesser extent by day 18, and were lower ($P<0.05$) than same day control follicles (Fig. 1).

A representative profile of IGFBP expression in individual FF samples (Fig. 2) demonstrates an upper 'doublet' with an approximate molecular mass of 44 and 40 kDa corresponding to IGFBP-3, and a somewhat smaller band (34 kDa) corresponding to IGFBP-2 (Mondschein *et al.* 1991, Howard & Ford 1992). The level of IGFBP

Figure 1 Mean IGF-I concentration (ng/ml $\pm$ S.E.) in the follicular fluid of individual follicles ($n=70$ for each group) recovered from control (open bars) and ACTH-treated (hatched bars) gilts on day 14 and day 18 of the oestrous cycle. Different superscripts denote statistical differences at a $P<0.05$ level of significance.

Figure 2 Western ligand blot demonstrating the IGFBP profile in the follicular fluid of individual follicles recovered from control and ACTH-treated (ACTH-Trt) gilts on day 14 and day 18 of the oestrous cycle. SS, serum standard.

expression was calculated as a percentage of the porcine serum standard. In the ACTH-treated group, both IGFBP-2 ($P=0.055$) and IGFBP-3 ($P=0.066$) expression tended to be lower on day 14 relative to the same day control group, but the difference did not reach significance (Fig. 3).

Follicular fluid steroid hormone levels

Mean cortisol concentrations (ng/ml $\pm$ S.E.) were significantly ($P<0.05$) elevated in the FF of ACTH-treated gilts

Figure 3 Mean levels (% porcine serum standard $\pm$ S.E.) of (a) IGFBP-2 and (b) IGFBP-3 in the follicular fluid of individual follicles recovered from control (open bars) and ACTH-treated (hatched bars) gilts on day 14 and day 18 of the oestrous cycle.

on day 14 of the cycle; however, by day 18 cortisol values were similar in the control and ACTH-treated follicles (Fig. 4a). Progesterone, E_2 and A_4 concentrations were each higher ($P<0.05$) on day 18, relative to day 14 follicles, in both the control and ACTH group

Figure 4 Mean (a) cortisol, (b) progesterone, (c) oestradiol-17 β and (d) androstenedione concentrations (ng/ml $\pm$ S.E.) in the follicular fluid of individual follicles ($n=70$ per group) recovered from control (open bars) and ACTH-treated (hatched bars) gilts on days 14 and 18 of the oestrous cycle. Different superscripts denote statistical differences at a $P<0.05$ level of significance.

(Fig. 4b,c,d). Follicles from the ACTH gilts were characterized by significantly ($P<0.05$) lower E_2 concentrations on both day 14 and day 18 (Fig. 4c), while P_4 and A_4 values did not differ significantly (Fig. 4b,d).

Granulosa cell IGF-I production

On day 14, there was no difference between the control and ACTH-treated group in either basal (Fig. 5a) or FSH-stimulated (Fig. 5b) IGF-I production (ng/ml) by granulosa cells. In contrast, by day 18 both basal (Fig. 5a) and FSH-stimulated (Fig. 5b) IGF-I production by cells from ACTH-treated gilts were significantly ($P<0.05$) lower.

Progesterone and oestradiol-17 β production

Progesterone production (ng/ml $\pm$ S.E.) by granulosa cells was dependent on the day of the oestrous cycle as well as on gonadotrophin and IGF-I stimulation. Both FSH and IGF-I treatment stimulated P_4 production ($P<0.05$) by day 14 granulosa cells, and no difference was observed between the control and ACTH group in either basal or FSH-mediated P_4 levels (Fig. 6a). However, when the cells were stimulated with IGF-I alone or IGF-I together with FSH, P_4 production by cells from the ACTH-treated gilts was significantly lower ($P<0.05$) (Fig. 6a). Granulosa cells recovered on day 18 also responded to FSH and IGF-I stimulation with increased P_4 production (Fig. 6b).

Figure 5 Mean cumulative IGF-I production (ng/ml $\pm$ S.E.) by granulosa cells recovered from control ($n=5$, open bars) and ACTH-treated ($n=5$, hatched bars) gilts on day 14 and day 18 of the oestrous cycle. The cells were cultured serum free for 5 days in (a) media alone or (b) with FSH (10 ng/ml) and androstenedione (A_4 , 100 ng/ml). Different superscripts denote statistical differences at a $P<0.05$ level of significance.

However, overall basal, FSH-, as well as FSH+IGF-I-stimulated P_4 production by cells recovered from the ACTH group on day 18 was significantly lower ($P<0.05$) compared with the same day controls (Fig. 6b).

Oestradiol-17 β production (ng/ml $\pm$ S.E.) was also influenced by the day of the oestrous cycle as well as by gonadotrophin stimulation (Fig. 7). Stimulation with FSH, either alone or together with IGF-I, increased ($P<0.05$) E_2 production by both day 14 and day 18 cells, while stimulation with IGF-I alone did not promote a significant rise in E_2 . Oestradiol-17 β production did not differ

Figure 6 Mean cumulative progesterone production (ng/ml $\pm$ S.E.) by granulosa cells recovered from control ($n=5$, open bars) and ACTH-treated ($n=5$, hatched bars) gilts on (a) day 14, and (b) day 18 of the oestrous cycle. The cells were cultured serum free for 5 days in media alone, IGF-I (10 ng/ml), FSH (10 ng/ml) and androstenedione (A_4 , 100 ng/ml), or IGF-I with FSH and A_4 . Different superscripts denote statistical differences at a $P<0.05$ level of significance.

between the control and ACTH group on day 14 (Fig. 7a). However, overall basal, FSH- and IGF-I-stimulated E_2 production by cells recovered from the ACTH group on day 18 was significantly lower ($P<0.05$) relative to the same day controls (Fig. 7b).

Discussion

In this study, we demonstrate that ACTH administered during the luteal phase of the oestrous cycle to elevate

Figure 7 Mean cumulative oestradiol-17 β production (ng/ml $\pm$ S.E.) by granulosa cells recovered from control ($n=5$, open bars) and ACTH-treated ($n=5$, hatched bars) gilts on (a) day 14, and (b) day 18 of the oestrous cycle. The cells were cultured serum free for 5 days in media alone, IGF-I (10 ng/ml), FSH (10 ng/ml) and androstenedione (A_4 , 100 ng/ml), or IGF-I with FSH and A_4 . Different superscripts denote statistical differences at a $P<0.05$ level of significance.

endogenous glucocorticoid levels, can alter ovarian IGF-I and steroid hormone production and potentially impede follicle maturation. Cortisol-mediated disruptions in steroid hormone production by granulosa cells have previously been demonstrated in a number of *in vitro* studies. Treatment with high glucocorticoid concentrations during culture lowers FSH-stimulated E_2 synthesis, but enhances P_4 production by murine, porcine and bovine granulosa cells (Schoonmaker & Erickson 1983, Danisova *et al.* 1987, Kawate *et al.* 1993). Lower IGF-I production, and IGF-

stimulated steroid hormone synthesis by porcine granulosa cells, were also observed in response to culture with high levels of cortisol (Viveiros & Liptrap 1999). However, it was not apparent whether marked elevations of glucocorticoid hormones would elicit similar disruptions *in vivo*. Hence, in the current study an increase in endogenous glucocorticoid levels was induced by ACTH administration. This treatment raises both plasma and FF cortisol concentrations. However, the subsequent decline in cortisol levels was considerably slower in FF after cessation of treatment (Montgomery *et al.* 1997), indicating that follicular cells may be exposed to high glucocorticoid concentrations for a longer period than that suggested by plasma levels.

Follicles collected from the ovaries of ACTH-treated gilts were characterized by disruptions in IGF-I and steroid hormone levels in FF. Immediately after ACTH treatment was discontinued, lower E_2 levels were evident, and by day 18 (5 days after treatment was discontinued) IGF-I concentrations were also significantly attenuated in the FF of ACTH-treated gilts. The reduced IGF-I levels were attributed to a decrease in granulosa cell IGF-I synthesis. Granulosa cells collected 5 days post ACTH-treatment produced significantly less IGF-I when placed in culture, with or without FSH stimulation. Attenuation of local IGF-I synthesis in the ovary may impair subsequent follicle development. Ovarian steroid hormone production (Veldhuis *et al.* 1986, Urban *et al.* 1990), gonadotrophin receptor expression, and cell replication are all affected by IGF-I stimulation (Adashi *et al.* 1985b, Maruo *et al.* 1988). Higher IGF-I concentrations are also reported in larger porcine follicles and following gonadotrophin treatment (Hammond *et al.* 1988, Samaras *et al.* 1994), suggesting that IGF-I levels in FF may be developmentally regulated.

The adverse effects observed with ACTH treatment are thought to be mediated by subsequently elevated glucocorticoid hormone levels. High cortisol concentrations were measured in FF following ACTH treatment. Receptors for cortisol have been identified in rat granulosa cells (Schreiber *et al.* 1982), and previous studies report glucocorticoid-mediated suppression of IGF-I synthesis in a number of different cell or tissue types in culture. Exposure to cortisol has been shown to reduce IGF-I levels in the culture medium of liver cells (Binoux *et al.* 1982), and to attenuate IGF-I mRNA expression as well as IGF-I protein secretion by rat osteoblast and fibroblast cultures (McCarthy *et al.* 1990). Glucocorticoid analogues, such as dexamethasone, also specifically lower IGF-I mRNA expression in neuronal and glial cells within hours of culture with no change in total DNA content (Adamo *et al.* 1989, McCarthy *et al.* 1990), thus suggesting an effect of cortisol at the transcriptional level. However, it should be noted that glucocorticoids can influence post-transcriptional events by affecting mRNA stability (Frost *et al.* 1993).

Glucocorticoids can also suppress IGFBP synthesis by different cell types in culture. For example, cortisol lowers IGFBP production by rat (Chen *et al.* 1991) and human osteoblast cells by attenuating specific IGFBP mRNA expression (Okazaki *et al.* 1994). The IGFBPs are thought to regulate IGF-I availability and delivery to target tissues (Clemmons 1992), and hence disruptions in binding protein levels may influence IGF-I action. Evaluation of the IGFBP profile in control and ACTH-treated gilts confirmed the presence of IGFBP-3 and IGFBP-2 in the FF of individual follicles. Both of these proteins have previously been detected in porcine FF and are known products of granulosa and luteal cells (Howard & Ford 1992, Grimes *et al.* 1994). IGFBP-4 and -5 have also been measured in porcine FF, but were not detected in the current study, indicating that their concentrations are too low (in individual follicles) to be discerned with this method. However, significant shifts in IGFBP expression in FF were not observed post ACTH treatment and this result suggests that elevated glucocorticoid hormone levels in the ovary may be less detrimental to binding protein synthesis than to IGF-I.

Treatment with ACTH also disrupted IGF-stimulated steroid hormone synthesis. On day 14, IGF-stimulated P_4 production by granulosa cells recovered from the ACTH-treated group was significantly lower. In contrast, both basal and FSH-stimulated P_4 synthesis were unaffected, and indicate a specific effect on IGF-I action rather than an overall suppression of cell function. Similar findings were observed previously when granulosa cells were directly exposed to high levels of cortisol *in vitro* (Viveiros & Liptrap 1999). By day 18 (5 days post ACTH treatment) both overall P_4 and E_2 synthesis by granulosa cells were lower in the ACTH group. In addition, lower E_2 concentrations were evident in the FF of ACTH-treated gilts on both days 14 and 18. Previous studies have reported lower oestrogen levels in FF recovered from gilts treated with dexamethasone or ACTH during the luteal phase of the cycle (Fraustchy & Liptrap 1988, Liptrap & Cummings 1991). In this investigation, the decrease in IGF-I and steroid production observed 5 days after ACTH treatment was discontinued suggests that the effects of cortisol, at the earlier stage of the cycle, may have hindered subsequent follicle development or, potentially, promoted follicle atresia. Follicles from the ACTH-treated group were smaller in diameter on day 18; moreover, both IGF-I and steroid hormone levels in FF were significantly lower and suggestive of follicles at an earlier stage of maturation.

The mechanism(s) whereby glucocorticoid hormones influence IGF-I action is not well understood. Granulosa cells are normally responsive to IGF-I, as follicles develop with sustained IGF receptor expression (Maruo *et al.* 1988, Hylka *et al.* 1989). In the current study, there was a suppressive effect on IGF-I, but not FSH action,

indicating a possible influence on IGF-I receptor expression and/or binding affinity. Results from previous studies that addressed this were variable; in one investigation dexamethasone lowered IGF-I receptor mRNA expression in primary rat granulosa cell culture (Botero *et al.* 1993), while in another study dexamethasone treatment prevented IGF-I receptor down regulation in porcine granulosa cells (Urban *et al.* 1994). Further analysis is, therefore, needed to determine the mechanism whereby glucocorticoids influence IGF-I action, and to ascertain what step(s) may be affected in the steroidogenic pathway. IGF-I can promote ovarian steroidogenesis through a number of different ways, ranging from enhanced cholesterol substrate availability to a direct increase in $P450_{SCC}$ enzyme activity and mRNA expression (Veldhuis *et al.* 1986, 1987, Urban *et al.* 1990).

In summary, the results of this study demonstrate that high levels of glucocorticoids can detrimentally influence the porcine ovarian IGF-I system *in vivo*. Exposure to elevated glucocorticoid concentrations following ACTH treatment reduced IGF-I synthesis by porcine granulosa cells resulting in lower IGF-I concentrations in FF. Elevated glucocorticoid levels also suppressed IGF-stimulated P_4 production by granulosa cells, but did not impair gonadotrophin stimulation, indicating a possible effect on IGF-I receptor expression and/or binding affinity. A reduction in ovarian IGF-I synthesis may have important implications for follicle maturation, as IGF-I plays a vital role in regulating ovarian cell proliferation and steroid hormone synthesis (Guidice 1992, Hammond *et al.* 1993). Follicles recovered 5 days post ACTH treatment appeared to be less mature; the follicles were smaller in size and were characterized by lower IGF-I and steroid hormone concentrations in FF, as well as lower steroid production by granulosa cells.

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