Effects of fibroblast growth factor 9 on steroidogenesis and control of FGFR2IIIc mRNA in porcine granulosa cells

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ABSTRACT: The objectives of this study were to investigate the effects of fibroblast growth factor 9 (FGF9) on hormone-stimulated porcine granulosa cell proliferation and steroid production and to further elucidate the hormonal and developmental control of FGFR2IIIc gene expression in granulosa cells. Porcine ovaries were collected from a local slaughterhouse and granulosa cells were collected from small to medium (1 to 5 mm) follicles for 5 in vitro studies that were conducted. Cells were cultured for 48 h in 5% fetal calf serum plus 5% porcine serum and then treated with various combinations of FSH, IGF-I, FGF9, Sonic hedgehog (SHH), cortisol, PGE2, and/or wingless-type mouse mammary tumor virus integration site family member 5A (WNT5A) in serum-free medium for an additional 24 or 48 h. Medium was collected for analysis of steroid concentration via RIA, or RNA was collected for gene expression analysis of FGFR2IIIc via quantitative reverse transcription PCR. Fibroblast growth factor 9 stimulated (P < 0.05) IGF-I-induced estradiol production in the presence of FSH and testosterone. However, FGF9 had inconsistent effects on progesterone production, stimulating progesterone production in the presence of FSH and testosterone but inhibiting progesterone production in the presence of IGF-I, FSH, and testosterone. Cell numbers were increased (P < 0.05) by FGF9 in the presence of IGF-I and FSH but not in the presence of FSH and absence of IGF-I. For FGFR2IIIc mRNA studies, granulosa cells were treated with FSH, IGF-I, FGF9, SHH, cortisol, PGE2, or WNT5A. Follicle-stimulating hormone alone had no effect (P > 0.10) whereas IGF-I increased (P < 0.05) FGFR2IIIc mRNA abundance. Cortisol, PGE2, SHH, and WNT5A had no effect (P > 0.10) on FGFR2IIIc gene expression whereas FGF9 in the presence of FSH and IGF-I inhibited (P < 0.05) FGFR2IIIc gene expression. In an in vivo study, granulosa cells from large (7 to 14 mm) follicles had greater (P < 0.05) abundance of FGFR2IIIc mRNA than small (1 to 3 mm) or medium (4 to 6 mm) follicles. In conclusion, IGF-I-induced FGFR2IIIc mRNA may be a mechanism for increased responses to FGF9 in FSH plus IGF-I-treated granulosa cells. Fibroblast growth factor 9 and IGF-I may work together as amplifiers of follicular growth and granulosa cell differentiation by stimulating estradiol production and concomitantly stimulating granulosa cell growth in pigs.

Key words: fibroblast growth factor 9, fibroblast growth factor receptor 2IIIc, granulosa cell, insulin-like growth factor I, pigs

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INTRODUCTION

Fibroblast growth factor 9 (FGF9) is part of the fibroblast growth factor (FGF) family of hormones consisting of 22 members (Moroni et al., 2002; Chaves et al., 2012). A role for FGF9 in ovarian function was first reported by Drummond et al. (2007) who showed that FGF9 mRNA and protein are localized in rat granulosa and theca cells, receptors exist for FGF9 including fibroblast growth factor receptor (FGFR) 2 and FGFR3, and FGF9 stimulates granulosa cell progesterone production. More recently, it
was discovered that FGF9 acts as a dedifferentiation factor inhibiting IGF-I-induced granulosa and theca cell steroidogenesis while stimulating cell proliferation in cattle (Schreiber and Spicer, 2012; Schreiber et al., 2012). Using microarray technology, FGF9 mRNA was found to be downregulated in cystic follicles as compared to noncystic follicles of cattle (Grado-Ahuir et al., 2011). In another study, gene expression of 1 of the receptors for FGF9, FGFR2IIIC, was upregulated by IGF-I in FSH-treated porcine granulosa cells (Grado-Ahuir et al., 2009). What other hormones regulate FGFR2IIIC mRNA in granulosa cells is unknown. We hypothesized that hormones in addition to IGF-I may be regulating FGFR2IIIC mRNA in porcine granulosa cells, and therefore we evaluated the effects of FSH, PGE₂, Sonic hedgehog (SHH), wingless-type mouse mammary tumor virus integration site family member 5A (WNT5A), and cortisol and PGE2 from Sigma-Aldrich Chemical Co. Amino acid sequence homology between human and porcine IGF-I, FGF9, SHH, and WNT5A is 98, 99, 91, and 99%, respectively.

The reagents used for radioimmunoassays were [125I]iodo-progesterone (MP Biomedicals, Solon, OH), anti-progesterone rabbit antiserum (X-16) provided by Dr. P. Natasha Rao (Southwestern Foundation for Research Education, San Antonio, TX), and normal rabbit serum (Invitrogen Corp., Carlsbad, CA). 125I-estradiol (MP Biomedicals), anti-estradiol rabbit antibody (Lilly Research Laboratories, Indianapolis, IN), and goat anti-rabbit antibody (Equitech-Bio, Inc., Kerrville, TX) as previously described (Spicer and Enright, 1991).

**Cell Culture**

Ovaries from nonpregnant gilts were collected from a local abattoir and transported to the lab in 0.9% saline with 1% streptomycin/penicillin on ice. Follicular fluid was aspirated from small to medium (1 to 5 mm) follicles and granulosa cells isolated as previously described (Ranzenigo et al., 2008). The small to medium size category selected was based on previous studies (Garmey et al., 2000; Ranzenigo et al., 2008). Granulosa cells were washed twice in short-term medium (1:1 DMEM and Ham’s F12, sodium bicarbonate, and gentamicin) and resuspended in serum-free medium containing collagenase and deoxyribonuclease (Sigma-Aldrich Chemical Co.) at 1.25 and 0.5 mg/mL, respectively, to prevent clumping. Viability of granulosa cells was determined by trypan blue exclusion method on a 0.1 mm deep hemocytometer (American Optical Corporation, Buffalo, NY). Viability of the various pools of porcine granulosa cells used for cell culture averaged 40.6 ± 3.5%.

On average, 3.0 × 10⁵ viable cells per well were plated on 24-well Falcon multiwell plates (number 3047; Becton Dickinson, Lincoln Park, NJ) in 1 mL of medium composed of a mixture of 1:1 DMEM and Ham’s F12 containing 2.0 mM of glutamine, 0.12 mM of gentamicin, 38.5 mM sodium bicarbonate, 5% FCS, and 5% PS (Ranzenigo et al., 2008). As previously described by Ranzenigo et al. (2008), cells were cultured in an environment of 5% CO₂ and 95% air at 38.5°C for the first 48 h with a medium change at 24 h. Cells were then washed twice with 0.5 mL of serum-free medium followed by the addition of different hormonal treatments applied in serum-free medium for either 24 or 48 h.

**Ribonucleic Acid Extraction and Real-Time PCR**

At the end of the treatment period, medium was either aspirated or collected from each well depending on the experiment and cells from 2 replicate wells were lysed in 0.5 mL of TRI reagent solution (Life Technolo-
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Ribonucleic acid was quantitated by spectrophotometry at 260 nm using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE). Aliquots of 1.5 μL of RNA were used to determine the concentration in nanograms per microliter as well as the purity given as a ratio of 260:280 nm where values between 1.8 and 2.2 were acceptable. Ribonucleic acid was then diluted to 10 ng/μL in DEPC-treated water.

Relative abundance of FGFR2IIIc mRNA was determined by fluorescent quantitative real-time PCR using an ABI Prism 7500 sequence detection system (Applied Biosystems, Foster City, CA). Primer and probe sequences used were previously described in Grado-Ahuir et al. (2009). The dual labeled probes (FAM-TAMRA) were obtained from Applied Biosystems as well as the 18S primers and probe (VIC) for TaqMan polymerase as previously described (Ranzenigo et al., 2008; Grado-Ahuir et al., 2009). For all RT-PCR runs, a no template control and a no reverse transcriptase control were included to insure the lack of contaminants in the master mix and the absence of any genomic DNA contamination, respectively. Furthermore, the RT-PCR products were run on agarose gels to verify the length and size of the expected target genes, and the same RT-PCR cDNA samples were sequenced to verify the amplified product. Target gene expression was normalized to constitutively expressed 18S rRNA and relative quantity of FGFR2IIIc mRNA was expressed as \(2^{-\Delta\Delta Ct}\) using the relative comparative threshold cycle (CT) method as described previously (Lagaly et al., 2008; Grado-Ahuir et al., 2009, 2011).

Radioimmunoassay and Cell Counting

A double antibody progesterone RIA was conducted as previously described (Baraño and Hammond, 1985; Ranzenigo et al., 2008). Intra-assay CV averaged 5.9%. A double antibody estradiol RIA was conducted as previously described (Spicer and Enright, 1991; Ranzenigo et al., 2008). Intra-assay CV averaged 6.8%.

To determine cell numbers, culture medium was aspirated and all wells were washed twice with 0.5 mL saline (0.9%). Trypsin (0.25% solution; 0.5 mL) was added and allowed to incubate for 20 min at room temperature. Wells were then scraped, aspirated, washed an additional time, and diluted 1:10 in saline as previously described (Ranzenigo et al., 2008). Cell number was determined using a Z2 Coulter Particle Counter and Size Analyzer (Beckman Coulter, Hialeah, FL).

Experimental Design

Experiment 1 was designed to evaluate the effect of IGF-I on the dose response of FGF9 on steroidogenesis and cell numbers in FSH- and testosterone-treated granulosa cells. Cells were cultured as described above and treatments applied in triplicate culture wells for 48 h as follows (all treatments included 30 ng/mL of FSH and 500 ng/mL of testosterone): 0, 3, 10, or 30 ng/mL of FGF9 with or without 30 ng/mL of IGF-I. After 48 h of treatment, medium was collected for estradiol and progesterone determinations and cells were counted. Medium was changed 24 h after initial treatment.

Experiment 2 was designed to evaluate the effect of FSH, IGF-I, and PGE2 on abundance of FGFR2IIIc mRNA in granulosa cells. Cells were cultured as described above in serum-containing medium for 48 h and then washed and cultured for 24 h in serum-free medium containing no additions (control), 30 ng/mL FSH, or 30 ng/mL IGF-I with or without PGE2 (300 ng/mL). Doses of FSH, IGF-I, and PGE2 were selected based on previous studies (Kage et al., 1999; Lagaly et al., 2008; Ranzenigo et al., 2008; Grado-Ahuir et al., 2009). Prostaglandin E2 (PGE2) was evaluated because it has been shown to interact with the FGF system (Kage et al., 1999; Finetti et al., 2009).

Experiment 3 was designed to evaluate the effect of WNT5A and cortisol on FGFR2IIIc mRNA abundance in FSH plus IGF-I-treated granulosa cells. Cells were cultured as described in Exp. 2 and then cultured for an additional 24 h in serum-free medium as follows: control (30 ng/mL FSH plus 30 ng/mL IGF-I) with and without WNT5A (300 ng/mL) or cortisol (300 ng/mL). Doses of FSH, IGF-I, WNT5A, and cortisol were selected based on previous studies showing that these doses significantly alter granulosa cell function (Spicer and Chamberlain, 1998; Spicer et al., 2002, 2009; Grado-Ahuir et al., 2009) and that these hormones interact with the FGF system (Li et al., 2002; Salaria et al., 2006; Pond et al., 2010; Schreiber and Spicer, 2012).

Experiment 4 was designed to evaluate the effect of SHH and FSH on abundance of FGFR2IIIc mRNA in IGF-I-treated granulosa cells. Cells were cultured as described in Exp. 2 and then cultured for an additional 48 h in serum-free medium as follows (all treatments included 500 ng/mL testosterone): 30 ng/mL IGF-I and 30 ng/mL FSH plus IGF-I with or without SHH (500 ng/mL). Medium was changed 24 h after initial treatment. Doses of FSH, IGF-I, and SHH were selected based on previous studies (Spicer et al., 2002, 2009; Grado-Ahuir et al., 2009; Lagaly et al., 2008). Sonic hedgehog was evaluated because previous studies indicated that hedge-
RESULTS

Experiment 1: Effect of Fibroblast Growth Factor 9 and IGF-I on Steroidogenesis and Numbers of Granulosa Cells

Fibroblast growth factor 9 increased \((P < 0.05)\) FSH-induced estradiol production in a dose-dependent manner with or without IGF-I in porcine granulosa cells (Fig. 1A). Maximal stimulation by 30 ng/mL of FGF9 increased estradiol production by 4.6-fold and 8.6-fold in the presence and absence of IGF-I, respectively. The estimated effective dose \((ED_{50})\) of FGF9 stimulating 50% of the maximal aromatase response (calculated from stimulation curves that were linearized using a semi-log plot) averaged 13 ng/mL in IGF-I-treated cells. Fibroblast growth factor 9 at 3 and 30 ng/mL had no effect on progesterone production in the presence of IGF-I plus FSH, but FGF9 at 30 ng/mL weakly increased \((P < 0.05)\) progesterone production (by 20%) in the presence of FSH and testosterone (Fig. 1B). At 10 ng/mL, FGF9 decreased \((P < 0.05)\) progesterone production by 20% in the presence of FSH, IGF-I, and testosterone (Fig. 1B). Insulin-like growth factor-I inhibited \((P < 0.05)\) progesterone production by 18 and 33% in the presence of 10 and 30 ng/mL of FGF9, respectively, but had no effect in the presence of 0 or 3 ng/mL of FGF9 (Fig. 1B). In the presence of IGF-I and FSH, FGF9 at 10 and 30 ng/mL stimulated \((P < 0.05)\) granulosa cell numbers by 85 and 76%, respectively, but had no significant effect at 3 ng/mL \((ED_{50}\) of 8 ng/mL in IGF-I-treated cells; Fig. 2). In the presence of FSH but absence of IGF-I, FGF9 had no significant effect on granulosa cell numbers (Fig. 2).

Experiment 2: Effect of FSH, IGF-I, and PGE_2 on Abundance of FGFR2IIIC mRNA in Granulosa Cells

Treatment of granulosa cells with PGE2, FSH, or both had no significant \((P > 0.10)\) effect on \(FGFR2IIIC\) mRNA abundance (Fig. 3A). However, treatment of granulosa cells with IGF-I increased \((P < 0.05)\) \(FGFR2IIIC\) mRNA abundance by 1.8-fold (Fig. 3A) in the presence and absence of PGE2.

Experiment 3: Effect of Wingless-Type Mouse Mammary Tumor Virus Integration Site Family Member 5A and Cortisol on mRNA Abundance of FGFR2IIIC in FSH plus IGF-I-Treated Granulosa Cells

Treatment of granulosa cells with either WNT5A or cortisol had no effect \((P > 0.10)\) on \(FGFR2IIIC\) mRNA abundance in FSH plus IGF-I-treated granulosa cells. Relative abundance of \(FGFR2IIIC\) mRNA in control, WNT5A, and cortisol treated cultures averaged 2.03, 2.20, and 1.82 \pm 0.26, respectively.

Statistical Analyses

For Exp. 1 to 5, 3 different pools of porcine granulosa cells were used as experimental replicates. Each pool of cells originated from a volume of 1 to 2 mL of follicular fluid (from 2 to 4 gilts) per pool within each experimental replicate. At least 1 of the replicate experiments was conducted using cells collected from a separate trip to the slaughterhouse. Ovaries from a total of 21 gilts were used for these experiments. Steroid production was expressed as nanograms or picograms/\(10^5\) cells per 24 h, and cell numbers determined at the end of the experiment were used for this calculation. For RNA experiments, medium was applied to 4 wells and duplicate samples for each pool of cells were derived by combining RNA from 2 wells. The treatment effects of the dependent variables (e.g., steroid production, \(FGFR2IIIC\) mRNA abundance) were determined using ANOVA and the general linear models (GLM) procedure of SAS for Windows (version 9.2; SAS Inst. Inc., Cary, NY). Data from Exp. 1 were analyzed as a 2 \times 4 factorial ANOVA whereas Exp. 2 data were analyzed as a 2 \times 3 factorial ANOVA and Exp. 4 data were analyzed as a 2 \times 2 factorial ANOVA. Data from Exp. 3, 5, and 6 were analyzed as a 1-way ANOVA. Mean differences were determined by Fisher’s protected least significant differences test (Ott, 1977) if significant main effects \((i.e., P < 0.05)\) in the ANOVA were detected. Data were presented as the least square means \pm\ SEM.
Experiment 4: Effect of Sonic Hedgehog and FSH on Abundance of FGFR2IIIc mRNA in IGF-I-Treated Granulosa Cells

Treatment of granulosa cells with 500 ng/mL of SHH with IGF-I or IGF-I plus FSH did not affect \( (P > 0.10) \) FGFR2IIIc mRNA abundance. Furthermore, FSH did not influence \( (P > 0.10) \) abundance of FGFR2IIIc mRNA in the presence of IGF-I or IGF-I plus SHH. Relative abundance of FGFR2IIIc mRNA in IGF-I and IGF-I plus FSH treated cultures averaged 1.47 and 1.50 ± 0.12, respectively, in the absence of SHH and 1.55 and 1.85 ± 0.12, respectively, in the presence of SHH.

Experiment 5: Effect of Fibroblast Growth Factor 9 on Abundance of FGFR2IIIc mRNA in Granulosa Cells

Treatment of granulosa cells with FGF9 in the presence of FSH plus IGF-I decreased \( (P < 0.05) \) abundance of FGFR2IIIc mRNA by 44% compared to treatment of FSH plus IGF-I without FGF9 (Fig. 3B).

Experiment 6: Changes in FGFR2IIIc Gene Expression during Follicle Development

Abundance of FGFR2IIIc mRNA did not differ \( (P > 0.10) \) between small- and medium-sized follicles but was 100-fold greater \( (P < 0.05) \) in large follicles as compared to either small or medium follicles (Fig. 4).

DISCUSSION

The effects of FGF9 on ovarian cell steroidogenesis in pigs have not been studied previously. We found a dramatic dose-dependent increase in estradiol production induced by FGF9 in the presence of IGF-I in granulosa cells treated with FSH and testosterone. To a much lesser extent, FGF9 at 30 ng/mL stimulated progesterone production in the absence of IGF-I and is in agreement with Drummond et al. (2007) who found that treatment of cultured rat granulosa cells with 1 to 50 ng/mL of FGF9 increases basal and FSH-induced progesterone production. At 5 and 50 ng/mL, FGF9 also stimulates basal but not hCG-induced testosterone production by mouse Leydig cells (Lin et al., 2010) and a related FGF, FGF2 (at 0.64 to 10 ng/mL), stimulates both basal and hCG-induced testosterone production in cultured porcine Leydig cells treated with insulin (Sordoi-llet et al., 1992). In contrast, FGF9 (at 10 and 30 ng/mL) is...
a potent inhibitor of IGF-I-induced steroidogenesis in gonadotropin-treated bovine granulosa (Schreiber and Spicer, 2012) and theca (Schreiber et al., 2012) cells. Why FGF9 stimulates progesterone production in the absence of IGF-I but inhibits it in the presence of IGF-I is unclear but may be related to the increase in cell numbers induced by FSH plus IGF-I. Previously, bovine granulosa cell proliferation and progesterone production has been shown to require de novo cholesterol synthesis (Spicer et al., 1996). Perhaps granulosa cells’ priority is to use cholesterol for cell proliferation rather than progesterone production. Interestingly, FGF2 inhibits steroidogenesis in porcine (Biswas et al., 1988) and bovine (Vernon and Spicer, 1994) granulosa cells. Because multiple receptors for FGF exist, a specific cell type and species response to a specific FGF is likely determined by the cadre of FGFR that are present in that cell type. For the 22 FGF ligands, there are a total of 7 FGFR receptors (which includes splice variants of 3 of the 4 main FGF receptor subtypes) each of which bind some but not all of the FGF ligands (Ornitz et al., 1996; Moroni et al., 2002; Chaves et al., 2012). The preferred receptors for FGF9 are FGFR2c, FGFR3c, FGFR3b, and FGFR4 (Santos-Ocampo et al., 1996; Kinkl et al., 2003).

In the present study, abundance of FGFR2IIIc mRNA did not differ between small and medium-sized follicles but large follicles had 100-fold greater abundance of FGFR2IIIc mRNA than either small or medium follicles. Similarly, in cattle, granulosa cells from large follicles have 3-fold greater FGFR2IIIb mRNA abundance than do those from small or medium follicles (Parrott and Skinner, 1998). In contrast, FGFR2IIIc mRNA abundance in bovine theca and granulosa cells did not differ among follicles with various estradiol levels (Berisha et al., 2004) or change between 0 and 60 h post-GnRH (Berisha et al., 2006). However, FGFR3 mRNA increased with follicle estradiol content in cattle (Buratini et al., 2005). Therefore, the specific FGFR that is present and physiologically regulated within follicles may depend upon species and cell type. In support of the idea that abundance of FGF receptors changes during follicle growth in pigs, FGFR2IIIc and FGFR2IIIb mRNA abundance in theca cells increased during follicle growth but neither changed in porcine granulosa cells (Schams et al., 2009). Why FGFR2IIIc mRNA was greater in large vs. medium and small follicles in the present study and not in a previous study (Schams et al., 2009) is unclear but may be due to methodological differences. In vitro, FGF2 inhibits progesterone production in porcine granulosa cells from small foli-
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Similar to previous studies in nonovarian tissue (Cohen and Chandross, 2000), we found that FGF9 downregulated abundance of mRNA for 1 of its own receptors, FGFR2IIIc, suggesting that an autocrine FGF9-FGFR2IIIC signaling loop may exist in porcine granulosa cells. This autocrine feedback inhibition would presumably operate to prevent overstimulation by FGF9. A similar effect has been reported during teratocarcinoma cell differentiation, further suggesting that cell surface FGF receptors are downregulated as an autocrine response to FGF ligand binding (Moscatelli, 1994). Consistent with the present study, IGF-I increased FGFR2IIIE mRNA in FSH-treated porcine granulosa cells (Grado-Ahuir et al., 2009), and based on results of the present study, FSH does not appear to influence the stimulatory effect of IGF-I on FGFR2IIIE gene expression. However, FSH and IGF-I synergistically stimulate steroidogenesis and mitogenesis in porcine granulosa cells (Barañó and Hammond, 1985; Ranzenigo et al., 2008). Therefore, as follicles grow, stimulation of FGFR2IIIE gene expression by IGF-I would enhance FGF9 signaling, further promoting the differentiation effects of FGF9 as well as stimulating angiogenesis (Gómez-Raposo et al., 2009; Sakurai and Kudo, 2011), an important event as follicles grow larger (Jiang et al., 2004; Martelli et al., 2009; Chaves et al., 2012). In contrast, FGF9 has been proposed as a dedifferentiation factor in bovine follicles as FGF9 is a potent inhibitor of IGF-I-induced steroidogenesis of both granulosa and theca cells in cattle (Schreiber and Spicer, 2012; Schreiber et al., 2012).

The present studies also indicate that WNT5A and SHH have no regulatory effect on FGFR2IIIE gene expression in porcine granulosa cells although wingless-related integration site proteins control cell differentiation and proliferation in mouse chondrocytes (Yang et al., 2003) and regulate bovine granulosa cell steroidogenesis (Castañón et al., 2012) while hedgehog proteins (SHH or Indian hedgehog) control proliferation of granulosa (Russell et al., 2007) and theca (Spicer et al., 2009) cells. Both cortisol (Spicer and Chamberlain, 1998; Viveiros and Liptrap, 1999) and PGE2 (Tsang and Carnegie, 1984; Moon et al., 1986) stimulate granulosa cell steroidogenesis, but like WNT5A and SHH, neither cortisol nor PGE2 affected FGFR2IIIE gene expression. In contrast to the present study, dexamethasone significantly decreased FGFR2 and FGFR1 mRNA expression in rat gastric epithelial cells (Luo et al., 2008), supporting the idea that species and/or cell-type differences likely exist in terms of the hormonal control of FGFR2IIIE gene expression.

Similar to what we have reported for bovine granulosa cells (Schreiber and Spicer, 2012), we found that FGF9 stimulated cell numbers in a dose-dependent manner in FSH- plus testosterone-treated granulosa cells. In the presence of IGF-I and FSH, FGF9 had an ED$_{50}$ of 8 ng/mL, which is similar to its ED$_{50}$ of 6 ng/mL reported for bovine granulosa cells (Schreiber and Spicer, 2012) and further suggests that the FGF9 effect in bovine and porcine granulosa cells is being mediated by high affinity receptors because these values approximate the dissociation constant ($K_d$) values obtained for FGF9 and FGF2 binding to their respective receptors (Neufeld and Gospodarowicz, 1985; Hecht et al., 1995). Unfortunately, concentrations of FGF9 in serum or follicular fluid have not been reported. Therefore, FGF9 may dualy support follicular development in pigs by stimulating granulosa cell proliferation and differentiation. Previous evidence indicated that FGF9 acts as a potential embryonic growth factor during placental attachment in pregnant sows (Østrup et al., 2010), as an endometrial stromal growth factor in humans (Tsai et al., 2002), and as an embryonic stem cell growth factor in mice (Peterslund and Serup, 2011). In the absence of IGF-I, FGF9 had no significant effect on granulosa cell numbers, suggesting that IGF-I may increase the sensitivity of granulosa cells to the mitogenic effects of FGF9. Indeed, the present experiments evaluating FGFR2IIIE mRNA indicated that IGF-I may increase FGF9 sensitivity by increasing numbers and/or sensitivity of 1 of the FGF9 receptors in porcine granulosa cells. Studies using rat skeletal muscle satellite cells indicate IGF-I can induce FGFR1 mRNA (Sheehan and Allen, 1999). Previously, FGF2 has been shown to be a potent stimulus of granulosa cell proliferation in pigs (Gospodarowicz and Bialecki, 1979), rats (Anderson and Lee, 1993), and cattle (Hoshi et al., 1995; Rodgers et al., 1996), suggesting multiple FGF may stimulate proliferation of granulosa cells in vertebrates.

Our results show that FGF9 upregulates estradiol production and cell numbers induced by IGF-I in FSH- plus testosterone-treated porcine granulosa cells. We also show developmental and hormone (i.e., IGF-I and FGF9) sensitive changes in FGFR2IIIE mRNA abundance in porcine granulosa cells that are consistent with the notion that IGF-I may enhance FGF9-induced granulosa cell differentiation via increased FGFR2IIIE gene expression. More research is needed to elucidate the role of the various types of FGF receptors and their ligands during follicular growth in pigs. An understanding of FGF9’s mechanism of action and its receptor regulation may also lead to a better understanding of the difference in follicular development between monovular and polyovular species.
LITERATURE CITED


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