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# Insulin-like growth factor I receptor mRNA and protein expression in pig corpora lutea

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Insulin-like growth factor I (IGF-I) is believed to play a luteotrophic role in the pig corpus luteum during the oestrous cycle. Since the actions of IGF-I in target tissues are mediated by the type I IGF receptor, the concentrations of IGF-I receptor mRNA and protein were examined in pig corpora lutea at different stages of the oestrous cycle. Corpora lutea were collected from normally cyclic gilts on days 4, 7, 10, 13, 15 and 16 of the oestrous cycle ($n = 4$ animals per day). Corpora lutea on days 7, 10 and 13 were dissociated with collagenase, and large and small luteal cell sub-populations were separated by elutriation. Northern and slot blots were used to examine mRNA, and western blots were used to measure the concentrations of IGF-I receptor protein in the pig corpus luteum. On northern blots, luteal IGF-I receptor mRNA was present as a single 11 kb transcript. The slot blots showed that the steady state expression of IGF-I receptor mRNA increased significantly ($P < 0.05$) from its lowest value on day 4, to reach a maximum on days 13–16. IGF-I receptor mRNA was also expressed to a greater extent in large compared with small luteal cells ($P < 0.05$). On western blots, IGF-I receptor appeared as a 95 kDa protein band (β-subunit) and IGF-I receptor protein concentrations were significantly higher ($P < 0.05$) on days 4–10 than on days 13–16. Finally, large luteal cells appeared to contain more IGF-I receptor protein than the small luteal cells. In conclusion, since IGF-I receptor was detected in the pig corpus luteum, it is a likely target tissue for IGF-I, especially during the early luteal phase. Furthermore, IGF-I receptor was localized primarily on large luteal cells, thus it is hypothesized that IGF-I may play a paracrine role in the pig corpus luteum.

## Introduction

IGF-I has mitogenic, differentiative and metabolic effects on the cells of many tissues in a number of species (Stewart and Rotwein, 1996). In particular, the IGF-I system, comprising IGF-I, IGF-binding proteins (IGFBPs) and the IGF type I receptor, plays an important role in regulating ovarian follicular development in a variety of species, including pigs (Giudice, 1992). mRNAs and proteins for most of the IGF system components have been found in pig follicles (Hammond *et al.*, 1991; Zhou *et al.*, 1996; Quesnel, 1999), and IGF-I stimulates granulosa cell proliferation, aromatase activity and progesterone biosynthesis in pig granulosa cells (Giudice, 1992). Thus, IGF-I is believed to play an autocrine and a paracrine role in the control of pig follicular development (Hammond *et al.*, 1991; Zhou *et al.*, 1996).

Much less is known about the role of the IGF system in the development and function of the corpus luteum. IGF-I treatment of luteal tissue or cells *in vitro* or of corpora lutea *in vivo* increases the secretion of either progesterone, oxytocin or relaxin (depending on the species) indicating that this peptide has luteotrophic potential in several species, including pigs (McArdle and Holtorf, 1989; Einspanier *et al.*, 1990; Constantino *et al.*, 1991; Sauerwein *et al.*, 1992; Apa *et al.*, 1996; Yuan and Lucy, 1996). Other studies have indicated that pig corpora lutea express mRNAs for IGF-I and IGFBP-2, -3, -4 and -5, indicating that IGF-I system components are produced locally and thus may have autocrine or paracrine roles in regulating luteal function (Gadsby *et al.*, 1996; Zhou *et al.*, 1996). In addition, IGF-I mRNA was found to be expressed primarily in small compared with large luteal cells, and to a greater extent in small luteal cells collected on days 4–10 compared with days 12–16 of the oestrous cycle (Gadsby *et al.*, 1996). Pig corpora lutea can develop autonomously without gonadotrophic support for approximately the first 12 days of the oestrous cycle (Anderson and Melampy, 1967), thus it was proposed that IGF-I plays an important luteotrophic role during this period.

The IGF-I receptor is the primary mediator of the action of

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IGF-I on its target cells (LeRoith *et al.*, 1995) and thus evidence of IGF-I receptors within the corpus luteum in various species provides strong support for the notion that the corpus luteum is a target tissue for IGF-I. IGF-I receptor protein or mRNA expression has been demonstrated in bovine (Sauerwein *et al.*, 1992), rat (Parmer *et al.*, 1991), ovine (Perks *et al.*, 1995) and human corpora lutea (Johnson *et al.*, 1996). To date, the only data on IGF-I receptor expression in the pig corpus luteum have been obtained using histochemical approaches (mRNA, *in situ* hybridization: Zhou *et al.*, 1996; protein, topical labelling: Quesnel, 1999), which have not generated adequate quantification of signal or a comprehensive examination of the entire oestrous cycle.

The objectives of this study were to examine the steady state expression of IGF-I receptor mRNA and protein in the pig corpus luteum, and to determine the relative concentrations of IGF-I receptor mRNA and protein in small and large luteal cells at different stages of the oestrous cycle. Overall, the aim was to generate data that would begin to address the following hypotheses: (i) that the IGF-I receptor is an important mediator of the luteotrophic actions of IGF-I in the pig corpus lutem; and (ii) that IGF-I (from small luteal cells) acts in a paracrine manner (via the IGF-I receptor on large luteal cells) to regulate the pig corpus luteum.

## Materials and Methods

### *Materials*

The following materials and reagents were used for this study: Hank's balanced salt solution (HBSS) without calcium and magnesium, Hepes, DNase (type I from bovine pancreas), hyaluronidase (type V from ovine testis), BSA (fraction V), penicillin–streptomycin, gentamicin, aprotinin, leupeptin, phenylmethylsulphonyl fluoride (PMSF), Triton-X100, Tri®-reagent, Tween 20 (Sigma Chemical Co., St Louis, MO); collagenase (type IV, Worthington Biochemical Corp., Freehold, NJ); SuperScript™ II RNase $H^-$ reverse transcriptase, RTS Radprime DNA labelling system (Gibco, Grand Island, NY); Taq DNA polymerase, agarose gel DNA extraction kit, RNase free DNase (Boehringer Mannheim, Indianapolis, IN); pGEM®-T-easy vector, JM109 competent cells, RNase inhibitor, RNA molecular mass marker (Promega, Madison, WI); Zeta-Probe+ nylon and nitrocellulose membranes (Bio-Rad, Richmond, CA); NucTrap Probe purification column (Stratagene, Menasha, WI); 4°C stable [$\alpha$-$^{32}$P]dCTP (ICN, Costa Mesa, CA); T7 Sequenase kit, horseradish peroxide (HRP)-linked donkey anti-rabbit IgG (secondary antibody), ECL kit reagents, Rainbow (protein) molecular mass (14–220 $M_r \times 10^{-3}$) markers (Amersham Pharmacia Biotech, Amersham); RT–PCR primers (5′-ATGCTGTTTGAACTGATGCGCA-3′, exon 20 and 5′-CCGCTCGTTCTTGCGGCCCCCG-3′, exon 21) designed from the human IGF-I receptor cDNA sequence were synthesized by Genosys Biotechnologies, Inc. (The Woodlands, TX); BCA protein assay kit (Pierce, Rockford, IL); anti-IGF-I receptor β-subunit polyclonal IgG, sc-713 (Santa Cruz Biotechnology, Santa Cruz, CA); polyvinylidene difluoride (PVDF) membrane (Pall Gelman Laboratory, Ann Arbor, MI); XAR-5 and X-OMAT-AR films (Eastman Kodak, Rochester, NY).

### *Animals and surgery*

The experimental protocol involving animal care and use was performed in accordance with the *Guiding Principles for the Care and Use of Research Animals* promulgated by the Society for the Study of Reproduction and with the approval of the North Carolina State University Institutional Animal Care and Use Committee.

Naturally cyclic pubertal gilts (White Landrace × Black line hybrid) were checked for oestrous behaviour once a day using a mature boar. Day 0 of the oestrous cycle was considered the first day on which oestrus was observed. On days 4, 7, 10, 13, 15 and 16 ($n = 4$ per day) of the oestrous cycle, animals were ovariectomized surgically as described by Gadsby *et al.* (1990). All corpora lutea from days 4, 15 and 16 of the oestrous cycle, and two corpora lutea from each gilt on days 7, 10 and 13 of the oestrous cycle, were bisected and removed from the ovaries, immediately snap-frozen in liquid nitrogen and stored at –70°C.

### *Dissociation and elutriation of corpora lutea*

The remaining corpora lutea from gilts on days 7, 10 and 13 of the oestrous cycle were dissected from the ovaries and dissociated by collagenase and hyaluronidase to obtain dispersed luteal cells as reported by Gadsby *et al.* (1994). The viability of the dissociated luteal cells was > 80% based on trypan blue exclusion. Immediately after cell dispersal, small and large luteal cells were isolated by elutriation (Gadsby *et al.*, 1994). The small luteal cell fraction was found to contain > 95% small cells, of which approximately 50% were steroidogenic, and the viability, as determined by trypan blue exclusion, was 60–80%. The large luteal cell fraction was found to contain approximately 70% large (steroidogenic) cells (approximately 80% viability), and the remaining proportion was made up of clumps of small non-steroidogenic cells (Gadsby *et al.*, 1994; current study). Small and large luteal cells were each diluted to a concentration of $1 \times 10^6$ cells $ml^{-1}$ in HBSS, aliquotted (approximately $1 \times 10^6$ cells per tube) into cryovials, snap-frozen in liquid nitrogen and stored at –80°C until processing.

### *PCR cloning and sequence analysis*

An IGF-I receptor cDNA was isolated and subcloned from pig corpus luteum for use as a homologous probe. Total cellular RNA from a pig corpus luteum tissue pool obtained on day 7 of the oestrous cycle was used for first-strand cDNA synthesis using reverse transcriptase in the presence of oligo $d(T)_{15}$ primers. The primers used for PCR amplification of the pig IGF-I receptor cDNA were derived from the human IGF-I receptor cDNA sequence as described by Green *et al.* (1995). The amplification product representing a putative pig IGF-I receptor cDNA was purified from agarose gels by extraction

and ligated into the pGEM®-T-easy vector. Competent JM109 cells were transformed with ligated plasmid using standard protocols. Positive clones were selected, the plasmids were harvested, and the plasmid DNA was sequenced (Sanger *et al.*, 1977). Sequence data were analysed and compared with known sequences in GenBank using SEQUENCHER 3 software (Gene Code Corp., Ann Arbor, MI). The sequence reported for IGF-I receptor cDNA isolated from pig corpus luteum has been deposited in GenBank and given the accession number AF127087.

### *RNA analysis*

Total cellular RNA was extracted from two corpora lutea from each animal at each stage of the oestrous cycle as described by Nicholson *et al.* (1999). Briefly, frozen corpora lutea were pulverized in liquid nitrogen and homogenized in 10% (w/v) Tri-reagent. After chloroform extraction, RNA was precipitated with isopropanol. RNA pellets were dissolved in diethylpyrocarbonate (DEPC)-treated water and stored at –80°C.

Northern blots were prepared as described by Nicholson *et al.* (1999). Thirty micrograms of total cellular RNA was used per corpus luteum. Blots were prehybridized at 65°C for 3 h in 0.25 mol $Na_2HPO_4$ $l^{-1}$ and 7% SDS (w/v) (pH 7.2), hybridized with $1 \times 10^8$ c.p.m. $ml^{-1}$ [$^{32}$P]cDNA probe in prehybridization buffer for at least 24 h and then analysed. cDNAs for pig IGF-I receptor and pig 3-phosphoglyceraldehyde dehydrogenase (pGAD), (gift from J. Hammond, Pennsylvania State University, Hershey, PA) were radiolabelled with [$^{32}$P]dCTP by random priming to a specific activity of $1–3 \times 10^9$ c.p.m. $\mu g^{-1}$.

Slot blots were performed as described by Nicholson *et al.* (1999). Total cellular RNA (2.5 μg per corpus luteum) was treated with RNase-free DNase before application to nylon membranes (slot blots). After preparation of the slot blots, hybridization and washing were similar to that described for northern blots.

Northern and slot blots were hybridized with IGF-I receptor cDNA probe, and exposed to XAR-5 film for 3 days at –80°C with intensifying screen (northern blots), or for 24 h in a phosphorimager cassette (Molecular Dynamics, Sunnyvale, CA; slot blots). Subsequently, northern and slot blots were stripped (by incubation twice in 0.1 × SSC–0.5% SDS (w/v) for 20 min at 95°C), re-probed with the p-GAD cDNA probe and exposed as described above. Quantification of hybridization signals from the slot blots was performed by phosphorimager analysis (Molecular Dynamics).

### *IGF-I receptor western blot analysis*

IGF-I receptor proteins were extracted and western blot analysis was performed as described by Nicholson *et al.* (1999). Briefly, corpus luteum tissue or small and large luteal cell fractions were homogenized in cold buffer containing 1% (v/v) Triton-X100, 2 mmol EGTA $l^{-1}$, 2 mmol EDTA $l^{-1}$, aprotinin (20 μg $ml^{-1}$), leupeptin (20 μg $ml^{-1}$), 1 mmol phenylmethylsulphonyl fluoride (PMSF) $l^{-1}$, in 20 mmol Hepes $l^{-1}$. Corpus luteum homogenates were centrifuged (14 000 ***g*** at 4°C for 10 min, followed by 100 000 ***g*** at 4°C for 1 h) and the supernatants were used for western blotting. Protein samples were subjected to 3–10% (w/v) gradient SDS-PAGE under reducing conditions (300 μg corpus luteum or 200 μg of small and large luteal cell protein extracts) and then blotted onto PVDF membranes using a wet-gel transfer system (Bio-Rad). Blots were incubated with a 1:1000 dilution of the IGF-I receptor β-subunit polyclonal antibody overnight at 4°C, followed by a peroxidase-labelled secondary antibody. The concentration of IGF-I receptor protein was detected using ECL chemiluminescence reagents. Blots were exposed to Kodak X-OMAT-AR film and quantified using densitometric analysis.

### *Statistical analysis*

Slot blot analysis data from IGF-I receptor mRNA were expressed as a ratio of IGF-I receptor:pGAD and are presented as mean ± SEM for each day of the oestrous cycle examined. Data for IGF-I receptor protein were expressed as arbitrary units relative to the value of a 'corpus luteum pool' (that is, protein extract from a pool of corpora lutea collected on day 7 of the oestrous cycle), which was run on each blot, and are presented as mean ± SEM for each day of the oestrous cycle examined. The variances of each data group were found to be homogeneous. Comparison of IGF-I receptor mRNA and protein concentrations at the different stages of the oestrous cycle was carried out by one-way ANOVA using the GLM procedures of SAS (1988). When a significant $F$ statistic was obtained, means were separated by Duncan's multiple-range test. Data for IGF-I receptor mRNA concentrations from large and small luteal cells collected at different stages of the oestrous cycle were analysed by two-way ANOVA; when a significant effect was detected, differences between large and small luteal cells were compared within each day using LSD (least significant difference), and $P$ values $< 0.05$ were considered as significant.

## Results

### *Subcloning and sequencing of IGF-I receptor cDNA*

The cDNA product of PCR amplification of the IGF-I receptor from pig corpus luteum was 354 base pairs (bp) in length and was isolated from plasmid by EcoRI digestion (data not shown). The authenticity of the PCR products was confirmed by sequence analysis. The isolated cDNA was located at exons 20–21, which codes for the C-terminal end of the cytoplasmic portion of the IGF-I receptor β-subunit (data not shown). The nucleotide sequence of the pig luteal IGF-I receptor cDNA is identical to the IGF-I receptor sequence subcloned from pig conceptus (Green *et al.*, 1995), except for a single base substitution (C instead of T) at 87 bp from the 5′ end. However, the deduced amino acid sequence was not changed because both GAT and GAC code for the same amino acid (D-Asp).

### Northern blot analysis

From northern hybridization of a $^{32}$P-labelled pig IGF-I receptor cDNA probe to 30 μg total cellular RNA prepared from pig corpora lutea at different stages of the oestrous cycle, a predominant hybridizing transcript of approximately 11 kb was identified (Fig. 1). This transcript was present in luteal samples from all stages of the oestrous cycle examined and its size remained constant throughout the oestrous cycle. Hybridization for pGAD mRNA resulted in the identification of a single transcript of 1.5 kb, the size of which did not vary with the stage of the oestrous cycle (Fig. 1).

### Slot blot analysis

Slot blot analysis was used to detect changes in the steady state expression of IGF-I receptor mRNA throughout the oestrous cycle (Fig. 2) and in small and large luteal cells (Fig. 3a). IGF-I receptor mRNA increased steadily as the oestrous cycle progressed, with the lowest concentrations recorded on day 4 and maximum values recorded on days 13–16. Statistical comparisons using Duncan's multiple-range test revealed that the concentration of IGF-I receptor mRNA on day 4 was significantly ($P < 0.05$) lower than on days 7–16; in addition, IGF-I receptor mRNA on days 4–10 was significantly lower than on days 13–16 ($P < 0.05$) (Fig. 2). IGF-I receptor mRNA was expressed in both large and small luteal cells, but was significantly higher in large compared with small luteal cells ($P < 0.05$) on all days examined (Fig. 3a). Expression of IGF-I receptor mRNA did not change significantly between day 7 and day 13 for either small or large luteal cells.

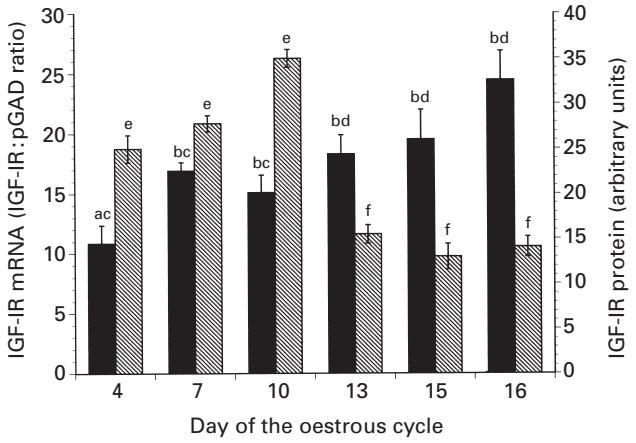


**Fig. 2.** IGF-I receptor (IGF-IR) mRNA (■) and protein (▨) expression in pig corpora lutea. At different stages of the oestrous cycle, IGF-I receptor mRNA was examined by slot blot analysis and data were expressed as a ratio of IGF-I receptor:pig 3-phosphoglyceraldehyde dehydrogenase (pGAD), and IGF-I receptor protein was measured by western blot analysis and data were expressed as densitometry units. Data are expressed as mean ± SEM ($n = 4$ animals per day). For mRNA, different superscript letters represent statistically significant differences (ANOVA; $P < 0.05$, Duncan's multiple-range test) between day 4 and all other stages (a versus b), and between days 4–10 and days 13–16 (c versus d), respectively. For protein, different superscript letters represent statistically significant differences (ANOVA; $P < 0.05$, Duncan's multiple-range test) between days 4–10 (e) and days 13–16 (f).

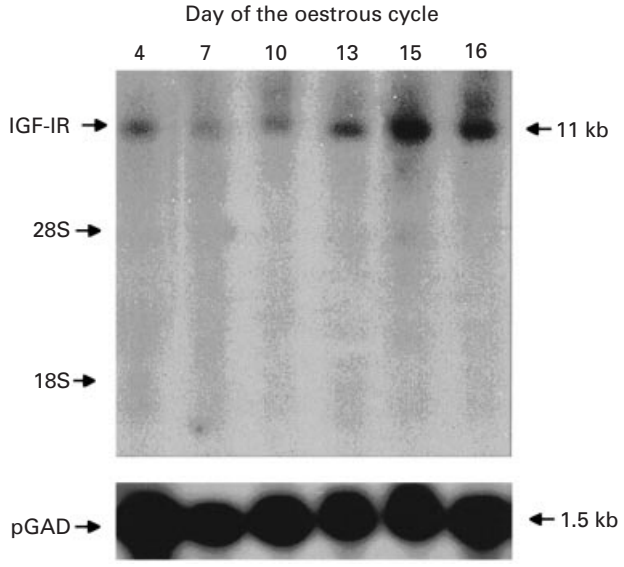


**Fig. 1.** Northern blot of IGF-I receptor (IGF-IR) mRNA in pig corpus luteum. Representative corpora lutea from a single animal per day of the oestrous cycle (days 4–16) are presented. IGF-I receptor mRNA was expressed in the pig corpus luteum as a single 11 kb transcript. Also presented is the luteal 3-phosphoglyceraldehyde dehydrogenase (pGAD) transcript of 1.5 kb.

### Western blot analysis

Western blots of intact corpus luteum extracts demonstrated an immunoreactive protein band of approximately 95 kDa, representing the β-subunit of IGF-I receptor (data not shown). In elutriated large and small luteal cells, the IGF-I receptor immunoreactive proteins resolved into two protein bands of similar molecular mass (approximately 95 kDa; exact molecular masses could not be calculated on the basis of these gels) (Fig. 3b). All protein bands could be eliminated by pre-incubating the IGF-I receptor antibody with the IGF-I receptor peptides that were used to generate this antibody, indicating that they represent authentic IGF-I receptor proteins (data not shown).

The IGF-I receptor protein concentrations in pig corpora lutea changed significantly during the oestrous cycle ($P < 0.05$) and were significantly higher on days 4–10 than on days 13–16 (Fig. 2). Protein samples from the large and small luteal cells of individual animals were also examined using western blots (Fig. 3b) and showed that large luteal cells expressed IGF-I receptor protein to a greater extent than did small luteal cells at each stage of the oestrous cycle examined (days 7–13). The densitometric analysis of the immunoreactive bands (Fig. 3b) indicated that the large luteal cells appeared to contain approximately 3–85-fold more IGF-I receptor than the small luteal cells.

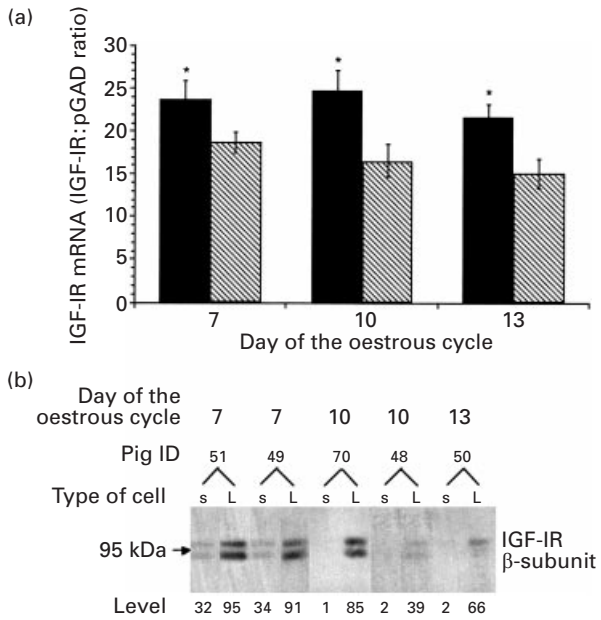


**Fig. 3.** IGF-I receptor (IGF-IR) mRNA and protein concentrations in large and small luteal cells isolated at different stages of the oestrous cycle. (a) IGF-I receptor mRNA expression (slot blots) in large (■) and small (▨) pig luteal cells on days 7, 10 and 13 of the oestrous cycle. Data represent mean ± SEM ($n = 4$ animals per day per type of cell). *Indicates a significant difference (ANOVA; $P < 0.05$, LSD) in IGF-I receptor mRNA between large and small luteal cells on days 7, 10 and 13. (b) IGF-I receptor protein concentrations in large (L) and small (S) luteal cells from individual animals taken on days 7, 10 and 13 of the oestrous cycle (the animal IDs indicate individual gilts). Numerical values (level) represent densitometric analysis of the blot. The position of the IGF-I receptor β-subunit at 95 kDa is indicated.

## Discussion

The present study confirms the presence of the IGF-I receptor (both mRNA and protein) in the pig corpus luteum, which strongly supports the hypothesis that the corpus luteum is a target tissue for IGF-I in this species.

IGF-I receptor mRNA in pig corpus luteum was represented as a single transcript of 11 kb and the size of the transcript did not appear to change during the oestrous cycle. The IGF-I receptor mRNA transcript in the pig corpus luteum is identical in size to that found in human placenta, rat placenta, mouse fibroblasts and rat corpus luteum (Ullrich *et al.*, 1986; Parmer *et al.*, 1991). In the human placenta, a minor band for IGF-I receptor mRNA of 7 kb was also detected (Ullrich *et al.*, 1986), although this was not observed in the present study on the pig corpus luteum. The sequence of the IGF-I receptor cDNA isolated and subcloned from the pig corpus luteum was virtually identical to that of IGF-I receptor cDNA isolated from the pig conceptus (Green *et al.*, 1995). The only significant difference was at a single base (C instead of T) located 87 bp from the 5′ end, which does not affect the amino acid sequence. IGF-I receptor mRNA was expressed in the pig corpus luteum at each stage of the oestrous cycle. The steady state concentrations increased as the oestrous cycle progressed and were lowest during corpus luteum development (days 4–10) and reached maximum values between day 13 and day 16 of the oestrous cycle, when corpora lutea are undergoing regression.

Western blot analysis using an antibody that recognizes the β-subunit of IGF-I receptor demonstrated the presence of immunoreactive IGF-I receptor proteins of 95 kDa within the pig corpus luteum. This finding is consistent with other reports on IGF-I receptors, which indicate that the β-subunit of IGF-I receptor has a molecular mass of 95 kDa (Ullrich *et al.*, 1986). In the large and small luteal cell extracts in the present study, two immunoreactive protein bands were detected; however, the exact individual molecular masses could not be ascertained from the gels, but appeared to be approximately 95 kDa. In mice (uterus and mammary gland), IGF-I receptor also displays two molecular mass variants of similar size, which may represent different degrees of phosphorylation (Richards *et al.*, 1996; R. G. Richards, personal communication). In addition to characterizing the molecular mass of the IGF-I receptor, western blots were used to quantify IGF-I receptor at different times of the oestrous cycle, and some trends in IGF-I receptor protein concentrations were detected. Luteal tissue concentrations of IGF-I receptor were significantly higher on days 4–10, during the period of luteal development, compared with days 13–16, the period encompassing luteal regression. Thus, since IGF-I receptor protein is involved directly in mediating the actions of IGF-I, the concentrations of IGF-I receptor protein in the pig corpus luteum appear to align with the proposed luteotrophic role of IGF-I (reviewed above) in this tissue. In support of this point, preliminary studies *in vitro* have indicated that luteal cells obtained on day 7, but not those obtained on day 13, displayed a dose-dependent increase in progesterone secretion in response to IGF-I (E. A. Miller and J. E. Gadsby, unpublished), which correlates with the IGF-I receptor protein concentrations found in the present study. Further studies in which luteal IGF-I responsiveness is being examined *in vivo* are in progress and should provide additional clarification of the physiological role of IGF-I receptor within the pig corpus luteum.

Concerning the possible regulation of IGF-I receptor within the pig corpus luteum, several growth factors including IGF-I, IGF-II and steroid hormones (oestrogen and progesterone) have been shown to regulate IGF-I receptor mRNA and protein expression in other tissues and species (Thissen *et al.*, 1994; LeRoith *et al.*, 1995). Since the pattern of IGF-I receptor protein concentrations in pig corpora lutea showed the inverse trend to that seen for mRNA, it is probable that transcription (including processing and degradation of mRNA) and translation of the IGF-I receptor are differentially regulated, making it difficult to explain such discrepant patterns with any single control mechanism.

IGF-I receptor mRNA and protein expression was detected in large and small luteal cells in the pig corpus luteum, although IGF-I receptor was present at significantly higher concentrations in large compared with small luteal cells. These observations are consistent with other studies in pigs (Yuan and Lucy, 1996; J. E. Gadsby, J. A. Lovdal and D. M. Plotner, unpublished), demonstrating that progesterone secretion by large but not small luteal cells was increased in a dose-dependent manner in response to exogenous IGF-I. Thus, large luteal cells appear to be the major site of the IGF-I

receptor and of the action of IGF-I in the pig corpus luteum. Nevertheless, the presence of IGF-I receptor (mRNA and protein) in small luteal cells, coupled with a minimal steroidogenic response of this cell sub-population to IGF-I *in vitro* (Yuan and Lucy, 1996; J. E. Gadsby, J. A. Lovdal and D. M. Plotner, unpublished), indicates that IGF-I receptor expression is confined to the non-steroidogenic small luteal cell sub-population, which can form 50–60% of the small luteal cell fraction (Gadsby *et al.*, 1994). Alternatively, if IGF-I receptor is located on small steroidogenic luteal cells, it is probable that the action of IGF-I on these cells influences cellular functions other than steroidogenesis. Studies designed to examine more precisely the cellular location of IGF-I receptor mRNA and protein by immunocytochemistry and *in situ* hybridization are currently in progress, and should enable clarification of the role of IGF-I receptor in cellular interactions within the pig corpus luteum.

In summary, IGF-I receptor mRNA was expressed in the pig corpus luteum and was present as a single transcript of 11 kb. In the intact corpus luteum, steady state concentrations of IGF-I receptor mRNA increased as the oestrous cycle progressed and reached maximum values during the late luteal phase (days 13–16). In addition, IGF-I receptor protein was detected (as the β-subunit of 95 kDa) within pig corpora lutea, and was present at the highest concentration during the early luteal phase (days 4–10). These data indicate that IGF-I receptor is produced in the pig corpus luteum and that this tissue is a target for IGF-I. Furthermore, these data are consistent with the hypothesis that IGF-I receptor is an important mediator of the luteotrophic actions, particularly during the early stages of corpus luteum development. Finally, both IGF-I receptor mRNA and protein were expressed preferentially in large luteal cells. These observations, taken together with data demonstrating that IGF mRNA is located primarily in small luteal cells (Gadsby *et al.*, 1996), support the hypothesis that IGF-I acts via a paracrine pathway to regulate the function of the steroidogenic types of luteal cell. Studies are in progress to examine the physiological importance of the changing patterns of IGF-I receptor in the pig corpus luteum.

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