Synergistic effects of insulin-like growth factor I and gonadotrophins on relaxin and progesterone secretion by ageing corpora lutea of pigs*

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Summary. Insulin-like growth factor I (IGF-I) is involved in paracrine/autocrine regulation of gonadal steroidogenesis and peptide hormone biosynthesis. This study was designed to determine whether IGF-I alone, or an interaction of IGF-I, is involved in augmenting the actions of luteinizing hormone (LH) and prolactin in controlling relaxin and progesterone secretion from ageing corpora lutea of hysterectomized gilts at days 110, 113 and 116 after oestrus. Luteal tissue slices were incubated for 8 h with IGF-I (0, 50, 300 ng ml⁻¹), LH (0, 100, 1000 ng ml⁻¹), and prolactin (0, 100, 1000 ng ml⁻¹) alone or in combination. Progesterone and relaxin concentrations were determined by radioimmunoassay of spent medium and of homogenates from luteal tissue slices before and after incubation. Porcine luteal tissue from day 110 had a net output of 25 ng progesterone and 26 ng relaxin in the control and of 65 ng progesterone and 2125 ng relaxin in the combined IGF-I, LH and prolactin treatment mg⁻¹ of luteal tissue, respectively. IGF-I, LH and prolactin alone or in combination significantly increased ($P < 0.01$) progesterone production by luteal tissue from day 110, but they were partially effective at day 113 and ineffective at day 116. By contrast, the same hormone treatments increased relaxin production by luteal tissue from days 110 and 113. Even at day 116, prolactin alone or with LH or IGF-I continued to stimulate relaxin production. In conclusion, IGF-I augments the ability of prolactin and LH to increase relaxin production by ageing corpora lutea; however, a decrease in progesterone secretion and an increase in relaxin secretion at day 113 indicate that different mechanisms control progesterone and relaxin secretion in pigs.

Keywords: IGF-I; gonadotrophins; relaxin; progesterone; ageing luteal cells; pig

Introduction

Relaxin and progesterone are produced by corpora lutea of pigs during pregnancy and after hysterectomy (Belt *et al.*, 1970, 1971; Anderson *et al.*, 1973, 1983). The corpora lutea from oestrous cycles of about 21 days secrete progesterone and small amounts of relaxin (Masuda *et al.*, 1967; Anderson *et al.*, 1973; Sherwood & Rutherford, 1981). During a normal pregnancy of 114 days, circulating progesterone concentrations peak by day 8 and remain high until they decrease just before parturition. Relaxin accumulates in luteal tissue and increases gradually during pregnancy, and it is released into the blood in peak quantities just before parturition (Belt *et al.*, 1971; Sherwood *et al.*, 1981; Felder *et al.*, 1986; Li *et al.*, 1989). After hysterectomy of unmated gilts, the corpora lutea are maintained beyond the duration of the normal pregnancy; these ageing corpora lutea continue to secrete relaxin and progesterone. The profiles of decreased progesterone secretion

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and simultaneous increase in relaxin secretion from these ageing corpora lutea at day 113 were similar to those on the same day just preceding normal parturition (Felder *et al.*, 1986; Li *et al.*, 1989; Adair *et al.*, 1989). Thus, hysterectomized pigs serve as an appropriate model to investigate the lifespan and hormonal secretion of corpora lutea of pigs. Relaxin and progesterone secretion from ageing corpora lutea of hysterectomized gilts are increased by prolactin and luteinizing hormone (LH) *in vitro* (Huang *et al.*, 1991) and *in vivo* (Felder *et al.*, 1988; Li *et al.*, 1989). However, whether the increased relaxin secretion is caused by relaxin release from a preformed hormone pool or requires biosynthesis of the hormone is unknown. Furthermore, the interaction of prolactin with LH may play a role in steroidogenesis because physiological concentrations of LH increase the number of prolactin receptors (Chen *et al.*, 1981), and prolactin can increase the number of LH receptors (Richards, 1978). Insulin-like growth factor I (IGF-I) is involved in gonadal steroidogenesis and peptide biosynthesis in an autocrine or paracrine manner (Adashi *et al.*, 1985a; Kasson & Hsueh, 1987; Caubo *et al.*, 1989).

This study was designed to determine the roles of IGF-I, LH and prolactin alone and in combination on the regulation of relaxin and progesterone secretion from ageing corpora lutea of hysterectomized gilts during a period equivalent to late pregnancy and normal parturition in this species.

Materials and Methods

Chemicals

The following reagents were obtained from Sigma Chemical Co., St Louis, MO: Dulbecco's Modified Eagle's Medium (DMEM); bovine transferrin; low-density lipoprotein (LDL, from human plasma) and porcine insulin. Insulin-like growth factor I (IGF-I) was obtained from IMCERA Bioproducts Inc. (Terre Haute, IN). Porcine prolactin (USDA-pPRL-B-1) and porcine luteinizing hormone (USDA-pLH-B-1) were provided by D. J. Bolt, Reproduction Laboratory, USDA, Beltsville, MD, and S. Raiti, National Hormone and Pituitary Program, NIH, Baltimore, MD.

Animals

All ovaries were obtained by ovariectomy of purebred Yorkshire gilts previously hysterectomized at day 8 (oestrus = day 0), at days 110 ($n = 5$), 113 ($n = 5$) and 116 ($n = 5$) after oestrus. Anaesthesia was induced by i.v. injection of thiamylal sodium (0.8–1.0 g; Surital, Parke-Davis, Morris Plains, NJ) and maintained by a closed circuit system of halothane (4–9%; Ayerst Laboratories, Rouses Point, NY) and O₂ (500–1000 ml min⁻¹). Each corpus luteum was marked with a loop of black silk suture at hysterectomy for later identification. Corpora lutea from both ovaries of each of the 15 pigs were used for one incubation and for electron microscopy.

Tissue incubation

The corpora lutea were dissected from the ovarian stroma and trimmed immediately. Luteal slices averaging 0.4 mm thickness (each weighing about 50–60 mg) were cut with a microtome. Three luteal slices from each pig were saved to determine hormone storage at time zero. One tissue slice per well in a 24-well, flat-bottom culture plate with cover lid (Falcon 3847, Becton Dickinson Labware, Lincoln Park, NJ) was incubated for 8 h in a Dubnoff Metabolic Shaking Incubator at 37°C, 5% CO₂, 95% O₂ with an oscillating speed of 60 cycles min⁻¹. Hormone treatments (each in triplicate) added to the incubation media were 0, 50, and 300 ng ml⁻¹ IGF-I; 0, 100, and 1000 ng ml⁻¹ LH; and 0, 100, and 1000 ng ml⁻¹ prolactin. The greatest concentration of each hormone was used in two- and three-factorial combinations to test hormonal interactions. The incubation medium (1 ml well⁻¹) was DMEM supplemented with LDL (10 µg ml⁻¹), insulin (5 µg ml⁻¹) and transferrin (5 µg ml⁻¹). Appropriate controls were included in luteal tissue incubations for each series of hormone treatments. Relaxin and progesterone concentrations were determined by radioimmunoassay of spent medium from each incubation well. Each luteal tissue slice was blotted, weighed and homogenized for the hormone assays. The incubation procedure for the hormone treatments described here was carried out independently for each of the 15 animals in the study.

Electron microscopy

Fixation and dehydration of the luteal tissue were performed at 2°C. Surgical specimens were immediately immersed in fixative on dental wax. The fixative contained two aldehyde forms. Paraformaldehyde (4%) was included

because it is a smaller molecule that penetrates more rapidly. Because the methylene bridges formed when formaldehyde reacts with proteins require two successive reactions (addition and condensation) and because the small size of the formaldehyde molecule cannot form bridges between some of the reactive groups on proteins, we also included glutaraldehyde, a dialdehyde that is a potent protein cross-linker. The tissue was cut into cubes < 1 mm on a side and fixed for 3 h in 2.5% glutaraldehyde in Millonig's phosphate buffer, pH 7.25. Silver sections (70–80 nm) were cut on a glass knife with an LKB Ultratome III microtome (LKB Instruments, Rockville, MD). The thin sections were double-stained, first with 2% uranyl acetate in methanol and then with lead citrate, and examined in a JEOL-100CXII electron microscope (Adair *et al.*, 1989). From each of the 15 gilts in this study, 21 blocks of luteal tissue were fixed of which two blocks were randomly selected for electron microscopy. If the fine structure of those two samples differed, another two blocks were randomly selected for electron microscopy to ensure that samples were representative of the luteal tissue. Six micrographs were prepared from each block for selection of photomicrographs representative of luteal cells from days 110, 113 and 116.

Radioimmunoassay of progesterone

The procedures for radioimmunoassay of progesterone in the incubation medium and luteal tissue homogenates were similar to those described by Felder *et al.* (1986). Progesterone antibody (GDN 337) was generously supplied by G. D. Niswender (Department of Physiology and Biophysics, Colorado State University, Fort Collins). Briefly, progesterone concentrations in the incubation medium were assayed in duplicate 50 μ l aliquots of a 1:50 dilution. Assay sensitivity for the incubation medium was 50 pg per tube (range 50–10 000 pg). To determine the precision and accuracy of this assay, we added 0, 50, 100, 250, 500, 1000, 2500, 5000 and 10 000 pg progesterone in quadruplicate aliquots of 50 μ l incubation medium. These standard samples were assayed; blank values were subtracted; and the concentrations of progesterone averaged ($\pm$ SEM) 0, 46 ± 5 , 100 ± 8 , 226 ± 15 , 547 ± 15 , 1027 ± 44 , 2835 ± 96 , 5027 ± 111 and 9179 ± 118 pg (50 μ l)⁻¹, respectively. Replicate samples were included in each assay for determination of intra- and interassay variances which were 7.5% ($n = 9$) and 9.6% ($n = 9$), respectively. Nonspecific binding was 3.7% and the maximal antibody binding was 34.3%. Progesterone contents in luteal tissue slices (i.e. 50–60 mg) were quantified in duplicate 50 μ l samples of a 1:100 dilution of the homogenate of fresh tissue (i.e. equivalent to 0.5 mg ml⁻¹ 0.01 mol PBS l⁻¹). The assay sensitivity was 50 pg per tube (range 50–10 000 pg). The intra- and interassay variances were 7.8% and 11.5%, respectively. Nonspecific binding was 2.8% and the maximal antibody binding was 34.8%.

Radioimmunoassay of relaxin

The procedures for radioimmunoassay of relaxin in the incubation medium and luteal tissue homogenates were similar to those described by Felder *et al.* (1986). Monotyrosyl relaxin for radiolabelling with ¹²⁵I was generously provided by C. Schwabe (Department of Biochemistry, Medical University of South Carolina, Charleston). Briefly, relaxin concentrations in the incubation medium were assayed in duplicate 50 μ l aliquots of a 1:2000 dilution. Assay sensitivity for the incubation medium was 50 pg per tube (range 50–5000 pg). To determine the precision and accuracy of this assay, we added 0, 50, 100, 1000, 2000, 4000, and 5000 pg purified porcine relaxin to quadruplicate aliquots of 50 μ l incubation medium. The standard used for the relaxin radioimmunoassay was porcine relaxin (CM-B, 3000 units mg⁻¹) and antibody was R6 antibody against porcine relaxin from B. G. Steinetz (New York University Medical Center, Tuxedo) and was previously described (O'Byrne & Steinetz, 1976). These standard samples were assayed, blank values were subtracted and the concentrations of relaxin averaged ($\pm$ SEM) 0, 54 ± 13 , 95 ± 13 , 1228 ± 44 , 2261 ± 85 , 3499 ± 74 , and 5003 ± 70 pg in 50 μ l, respectively. Replicate samples were included in each assay for determination of intra- and interassay variances which were 6.7% ($n = 9$) and 9.0% ($n = 9$), respectively. Nonspecific binding was 3.4% and maximal antibody binding was 34.2%. Relaxin contents in luteal tissue slices (i.e. 50–60 mg) were quantified in duplicate 50 μ l samples of a 1:4000 dilution of the homogenate of fresh tissue (i.e. equivalent to 12.5 μ g ml⁻¹ of 0.01 mol PBS l⁻¹). The assay sensitivity was 50 pg (range 50–5000 pg). The intra- and interassay variances were 6.3% ($n = 9$) and 12.8% ($n = 9$), respectively. Nonspecific binding was 2.8% and the maximal antibody binding was 34.3%.

Protein determination

The protein content in the extract of each luteal slice was determined spectrophotometrically using an acidic solution of Coomassie Brilliant Blue G-250 (500–600, Bio-Rad Protein Assay, Bio-Rad Chemical Division, Richmond, CA) and measuring the absorbance at 595 nm (Bradford, 1976). The assay standard was crystalline BSA (Intergen Co., Purchase, NY).

Statistical analysis

Data were analysed by least square analysis of variance using the General Linear Models procedure of the Statistical Analysis System (SAS, 1985). The experimental units are luteal tissue slices in the culture wells. The effects

of treatments were tested by pooling the data within a day and the least significant difference was applied using day $\times$ treatment as error term to compare treatment means with the control value within that day (Snedecor & Cochran, 1989).

Results

Without incubation, progesterone and relaxin contents were greater ($P < 0.01$, $P < 0.05$) at day 110 than those at days 113 and 116 (Table 1). After 8 h incubation, the output of progesterone in the medium and in the luteal tissue was greater than that in tissue without incubation (within a day). However, incubation for 8 h did not increase the output of relaxin secretion on days 110, 113 and 116 (Table 1).

Table 1. Mean progesterone and relaxin contents in incubation medium and in luteal tissues from hysterectomized pigs before and after 8 h incubation at days 110, 113 and 116 (hysterectomy performed at day 8, oestrus = day 0)

Hormones in tissue + medium	Progesterone (ng mg ⁻¹ tissue)			Relaxin (ng mg ⁻¹ tissue)		
	day 110	day 113	day 116	day 110	day 113	day 116
Before incubation	153 $\pm$ 14 ^a	129 $\pm$ 14 ^b	134 $\pm$ 7 ^b	3475 $\pm$ 74 ^c	3140 $\pm$ 118 ^b	3176 $\pm$ 75 ^b
After incubation	179 $\pm$ 16 ^b	161 $\pm$ 13 ^a	166 $\pm$ 13 ^a	3501 $\pm$ 208 ^c	3144 $\pm$ 186 ^b	3087 $\pm$ 237 ^b

Values are means and SEM, each from 15 samples of 5 pigs (only data from control incubations were included).

Means within columns and rows with different superscript letters are significantly different from each other: a:b at $P < 0.01$; b:c at $P < 0.05$.

At day 110, IGF-I at a concentration of 50 or 300 ng ml⁻¹ alone did not increase progesterone secretion (Fig. 1). Luteinizing hormone at 100 or 1000 ng ml⁻¹, and prolactin at 100 ng ml⁻¹ alone stimulated a significant ($P < 0.05$) progesterone release into the medium compared with that in the control. Luteinizing hormone and prolactin in combination induced a greater ($P < 0.05$) release of progesterone than LH alone. At day 113, only LH at a concentration of 100 ng ml⁻¹, LH plus prolactin, or LH plus prolactin and IGF-I stimulated ($P < 0.05$) progesterone secretion. At day 116, the capacity of these ageing luteal tissues to respond to trophic stimulation was lost (Fig. 1). Relaxin secretion followed a somewhat different pattern. At day 110, IGF-I at a concentration of 50 ng ml⁻¹ alone or in the presence of LH or prolactin induced a significant release of relaxin compared with that in the control (Fig. 2). At day 113, IGF-I at 300 ng ml⁻¹ alone or in the presence of LH with or without prolactin, LH at 100 ng ml⁻¹, and prolactin at 1000 ng ml⁻¹ stimulated ($P < 0.05$) relaxin secretion. At day 116, only IGF-I at a concentration of 50 or 300 ng ml⁻¹ induced a greater release of relaxin than in the controls.

Net progesterone and relaxin production was defined as the difference in hormone concentration in luteal tissue and medium after 8 h compared with 0 h incubation (Figs 3 and 4). This net hormone production resulted from the biosynthesis of progesterone and relaxin by luteal tissue in response to different hormone challenges during the 8 h incubation. All hormone treatments at day 110 increased net progesterone biosynthesis (Fig. 3). At day 113, only LH alone or combined with prolactin significantly stimulated progesterone biosynthesis. By day 116, all hormone treatments were ineffective, but net progesterone production remained unchanged from that of the controls at days 110 and 113 (Fig. 3). Over all treatments, net progesterone concentration at day 110 averaged 48 ± 4 ng mg⁻¹ luteal tissue, which was significantly greater ($P < 0.05$) than 36 ± 3 ng mg⁻¹ at day 113, and 34 ± 1 ng mg⁻¹ at day 116.

Fig. 1. Progesterone release into the medium after 8 h incubation by ageing luteal tissue from hysterectomized pigs at days 110, 113 and 116 (hysterectomy performed at day 8, oestrus = day 0). Treatments are insulin-like growth factor-I (IGF, 0, 50, 300 ng ml⁻¹), luteinizing hormone (LH, 0, 100, 1000 ng ml⁻¹) and prolactin (PRL, 0, 100, 1000 ng ml⁻¹); the greatest dose of each tested hormone was used in two- or three-factor combination. Values are means $\pm$ SEM. Comparison with control (^a P < 0.05; ^b P < 0.01) was made within a day (n = 5 animals; treatment replication = 3 within animal and day).

Fig. 2. Relaxin release into the medium after 8 h incubation of ageing luteal tissue from hysterectomized pigs at days 110, 113 and 116 (hysterectomy performed at day 8, oestrus = day 0). Treatments are insulin-like growth factor I (IGF, 0, 50, 300 ng ml⁻¹), luteinizing hormone (LH, 0, 100, 1000 ng ml⁻¹) and prolactin (PRL, 0, 100, 1000 ng ml⁻¹); the greatest dose of each tested hormone was used in two- or three-factor combinations. Values are means $\pm$ SEM. Comparison with control (^a P < 0.05; ^b P < 0.01) was made within a day (n = 5 animals; treatment replication = 3 within animal and day).

Fig. 3. Net progesterone release during 8 h incubation by ageing luteal tissue from hysterectomized pigs at days 110, 113 and 116 (hysterectomy performed at day 8, oestrus = day 0). Treatments are insulin-like growth factor I (IGF, 0, 50, 300 ng ml⁻¹), luteinizing hormone (LH, 0, 100, 1000 ng ml⁻¹) and prolactin (PRL, 0, 100, 1000 ng ml⁻¹); the greatest dose of each tested hormone was used in two- or three-factor combinations. Values are means $\pm$ SEM. Comparison with control (^a $P < 0.05$; ^b $P < 0.01$) was made within a day ($n = 5$ animals; treatment replication = 3 within animal and day).

Fig. 4. Net relaxin release during 8 h incubation by ageing luteal tissue from hysterectomized pigs at days 110, 113 and 116 (hysterectomy performed at day 8, oestrus = day 0). Treatments are insulin-like growth factor I (IGF, 0, 50, 300 ng ml⁻¹), luteinizing hormone (LH, 0, 100, 1000 ng ml⁻¹) and prolactin (PRL, 0, 100, 1000 ng ml⁻¹); the greatest dose of each tested hormone was used in two- or three-factor combinations. Values are means $\pm$ SEM. Comparison with control (^a $P < 0.05$; ^b $P < 0.01$) was made within a day ($n = 5$ animals; treatment replication = 3 within animal and day).

At day 110, prolactin alone and combined with LH or IGF-I stimulated relaxin biosynthesis (Fig. 4). The greatest increase ($P < 0.001$ compared with control; $P < 0.01$ compared with other treatments at day 110) in relaxin biosynthesis resulted when IGF-I was combined with prolactin and LH at this time. A similar response to gonadotrophin treatments was seen at day 113, but the greatest net production of relaxin occurred when IGF-I was combined with LH or prolactin. Only prolactin alone or combined with LH or IGF-I increased ($P < 0.05$) relaxin biosynthesis at day 116. The negative value for the controls at day 116 may reflect relaxin degradation with little or no hormone biosynthesis during the 8 h incubation. Over all treatments, relaxin concentration at day 110 was $547 \pm 165 \text{ ng mg}^{-1}$ luteal tissue, which was similar to $496 \pm 110 \text{ ng mg}^{-1}$ at day 113 and

Fig. 5. (a) Luteal tissues removed at day 110 after hysterectomy contain abundant electron-dense cytoplasmic granules (GR). Vesicles (V) and mitochondria (M) of various sizes are prominent. (b) Luteal tissues removed at day 113 after hysterectomy also contain a considerable number of cytoplasmic granules (GR); more vesicles (V) were seen. (c) Luteal tissues removed at day 116 of hysterectomy contain fewer cytoplasmic granules (GR). An invasion of collagen between the luteal cells was seen, and there are enlarged mitochondria (M) that had fewer fenestrated lamelliiform cristae. Bar = 1 μm .

greater ($P < 0.05$) than $200 \pm 48 \text{ ng mg}^{-1}$ seen at day 116. There was a high correlation ($r = 0.93$ for progesterone, 0.95 for relaxin, $P < 0.01$) between hormone concentration expressed as ng mg^{-1} luteal tissue and ng mg^{-1} protein; thus, the results were expressed as ng mg^{-1} luteal tissue.

Luteal tissue from these hysterectomized gilts at day 110 contained maximum numbers of electron-dense granules in the cytoplasm, which were distributed in clusters among abundant large mitochondria (Fig. 5a). Vesicles were interspersed among the Golgi complexes. A granular or smooth endoplasmic reticulum (SER) and granular or rough endoplasmic reticulum were present at day 110 as well as at days 113 and 116, but SER was far more abundant. By day 113, there were slightly fewer cytoplasmic granules than at day 110, and more vesicles appeared (Fig. 5b). Fewer cytoplasmic granules and more small vesicles were seen by day 116 (Fig. 5c). An invasion of collagen between the luteal cells was evident at this time. Mitochondria were enlarged and had fewer fenestrated lamelliform cristae. Large lipid bodies and multilamellar bodies were also frequently seen in day 116 samples (data not shown).

Discussion

The results from this study show that IGF-I augments relaxin production and release by ageing luteal tissue removed from hysterectomized gilts at days 110 and 113 after oestrus when co-incubated for 8 h with LH and prolactin. By day 116, however, relaxin production in response to these hormones was marginal. Progesterone production and release was increased by IGF-I alone and further increased when prolactin with or without LH were added to the incubation at day 110, but not at days 113 and 116. Thus these trophic hormone responses of ageing luteal tissues to IGF-I and gonadotrophins depend on day of treatment and there is a distinct difference between relaxin and progesterone secretion. Although these results from luteal slice incubations were similar to those from dispersed monolayer culture of porcine luteal cells at the same stages (Huang *et al.*, 1991), IGF-I distinctly increased relaxin production at days 110 and 113 when added to the incubation with gonadotrophins. These patterns of relaxin and progesterone secretion by incubated luteal slices parallel the relaxin surge release that is coincident with decreased progesterone concentrations in the peripheral blood at the same stages of pregnant and hysterectomized gilts (Felder *et al.*, 1986, 1988; Li *et al.*, 1989). These results suggest that ageing corpora lutea may be programmed to have an inherent lifespan of approximately 113 days.

Prolactin is luteotrophic for ageing corpora lutea in this species (Felder *et al.*, 1988; Li *et al.*, 1989). Administration of prolactin or LH *in vivo* sustained or increased relaxin and progesterone concentrations in the peripheral blood, which maintained or increased luteal weight. Whether the high hormone concentrations result from an increased rate of secretion *in vivo* is unresolved. Results from dispersed monolayers of luteal cells in culture indicate that prolactin and LH increase the rates of relaxin and progesterone secretion (Grinwich *et al.*, 1983; Huang *et al.*, 1991). Porcine luteal cells have prolactin-specific binding receptors (Rolland *et al.*, 1976; Bramley & Menzies, 1987) and LH can stimulate progesterone secretion by these cells from the oestrous cycle and early pregnancy (Lemon & Loir, 1977; Hunter, 1981; Kineman & Rampacek, 1987), which is consistent with the present findings with ageing corpora lutea.

In the present study, IGF-I or LH alone did not increase relaxin production, whereas a synergistic effect occurred when IGF-I was combined with LH with and without prolactin. This synergistic action between IGF-I and gonadotrophin or the combination of the three hormones indicates similar mechanisms, or at least some overlap, in their pathways to cause progesterone and relaxin synthesis and release. It has been shown that porcine granulosa cells may be sites of IGF-I production (Mondschein & Hammond, 1988), receptors (Baranao & Hammond, 1984) and hormone action (Veldhuis *et al.*, 1986, 1987). Circulating concentrations of IGF-I are unrelated to the stage of ovarian function during the oestrous cycle in heifers, but this has not been determined in pigs (J. D. Armstrong, personal communication). It has been reported that IGF-I alone

stimulated or potentiated gonadotrophin-induced steroidogenesis in porcine thecal cells (Caubo *et al.*, 1989), bovine luteal cells (McArdle & Holtorf, 1989) and rat granulosa cells (Adashi *et al.*, 1985a, b). IGF-I binds the membrane receptor that has tyrosine kinase activity. Its action may be partly mediated by cAMP because IGF-I can increase adenylyl cyclase activity and stimulate cAMP-induced testosterone and progesterone biosynthesis (Lin *et al.*, 1987). Dibutryl cAMP has been demonstrated to be a potent stimulator of relaxin and progesterone secretion from dispersed ageing luteal cells in culture (Huang *et al.*, 1991). Alternatively, IGF-I may increase the numbers of LH receptors and LDL receptors in gonadal tissue. This contention is consistent with the report that IGF-I synergized with FSH in the induction of progesterone synthesis by an increase in numbers of LH receptors (Adashi *et al.*, 1985b) and of LDL receptors in porcine granulosa cells with no change in their affinity (Veldhuis *et al.*, 1987).

With the use of a serum-free chemically defined medium, insulin and transferrin are frequently included as important supplements to enhance the responses of the cell to hormone secretion. During steroidogenesis, IGF-I or insulin is required for the maintenance of this specific function of adrenal cells (Penhoat *et al.*, 1988). In this study, addition of insulin at $5 \mu\text{g ml}^{-1}$ to the medium may not produce significant effects to mask those of IGF-I because the affinity of insulin to IGF-I receptor is 700 times less than that of IGF-I to its own receptors (Nissley & Rechler, 1984; Davoren *et al.*, 1986) and insulin at $10 \mu\text{g ml}^{-1}$ plus IGF-I at 50 ng ml^{-1} produced the same effect as IGF-I at 50 ng ml^{-1} alone in corticosterone secretion by the isolated adrenal cells (Penhoat *et al.*, 1988). Accordingly, the addition of $5 \mu\text{g insulin ml}^{-1}$ to the incubation medium may produce a potency equivalent to IGF-I at a concentration of 7 ng ml^{-1} , and thus the added insulin in the present study may partly mask the effect of IGF-I when added at a concentration of 50 ng ml^{-1} to the incubation medium.

We determined whether relaxin in the medium is released from previous storage (including release from cell death) or from *de novo* synthesis. The hormone content at the beginning of the incubation was measured and subtracted from the total hormone content (both in the medium and in the tissue) after 8 h incubation. The results clearly indicate that the capacity of ageing luteal tissue to synthesize hormones is greatly reduced or lost at day 116 for relaxin, and at days 113 and 116 for progesterone. The different regulatory mechanisms between relaxin and progesterone secretion revealed in the present study are consistent with distinct intracellular pathways for relaxin (stored in cytoplasmic granule before release) and progesterone production and release.

The results of the present study indicate that ageing luteal tissues from hysterectomized gilts increase relaxin and progesterone production in response to incubation with IGF-I, LH and prolactin depending on the day of treatment. By day 116, an inability to increase progesterone and relaxin secretion signalled the occurrence of regression in luteal tissues, which was supported by morphological evidence in the fine structure of these cells at days 110, 113 and 116. Taken together, the distinct shifts in steroid and peptide hormone secretion and production patterns in luteal tissues during this brief period indicate that luteal tissue from hysterectomized gilts can mimic the events that occur in pregnant gilts at corresponding stages. This implies that incubated luteal tissues express their own species-specific lifespan of approximately 113 days.

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