Isolated porcine ovarian follicles as a model for the study of hormone and growth factor action on ovarian secretory activity

A V Sirotkin, A V Makarevich, J Kotwica¹, P-G Marnet²,
H B Kwon³ and L Hetenyi

Research Institute of Animal Production, 949 92 Nitra, Slovak Republic, ¹Institute of Animal Reproduction and Food Research, 10–718 Olsztyn-Kortowo, Poland, ²Laboratoire de Recherches sur la Traite, INRA/ENSAR, 35042 Rennes Cedex, France and ³Department of Biology, Chonnam National University, Kwangju 500–757, Korea

(Requests for offprints should be addressed to A V Sirotkin)

Abstract

The aim of our *in vitro* experiments with isolated porcine ovarian follicles was to study the effects of gonadotropins, GH, IGF-I and oxytocin (OT) on release of ovarian steroid, OT, IGF-I, insulin-like growth factor-binding protein-3 (IGFBP-3), prostaglandin F (PGF), prostaglandin E (PGE) and cAMP.

It was found that quarters of ovarian follicles cultured for 8 days produced significant amounts of progesterone, estradiol-17 beta, OT and IGFBP-3 with peaks of accumulation from the 3rd to the 8th day of culture. Addition of serum promoted progesterone, estradiol and OT release, whilst accumulation of IGFBP-3 was maintained to a greater extent in serum-free medium.

GH (10 ng/ml or above) was able to inhibit androstenedione, OT, PGF and IGFBP-3, to stimulate IGF-I and cAMP, and to alter testosterone and PGE release by follicles cultured in serum-supplemented and/or serum-free medium. IGF-I (10 ng/ml or more) inhibited androstenedione and PGF secretion, stimulated testosterone, estradiol, OT and cAMP production, but did not influence progesterone, IGFBP-3 or PGE output in these condi-

tions. OT (100 ng/ml) was able to inhibit androstenedione and to stimulate testosterone, IGF-I, PGF and PGE, but not estradiol or IGFBP-3 release. A stimulatory effect of LH on progesterone and OT and an inhibitory influence of LH on estradiol secretion in the serum-supplemented medium were observed. FSH in these conditions stimulated OT, but not progesterone or estradiol secretion.

The use of this experimental model suggests the involvement of gonadotropins, OT, GH and IGF-I in the control of ovarian steroid and nonapeptide hormone, growth factor, growth factor-binding protein, prostaglandin and cyclic nucleotide production. The stimulatory effect of GH on IGF-I, and the stimulatory influence of IGF-I on OT, as well as coincidence of the majority of effects of IGF-I and OT, suggest the existence of a GH-IGF-I-OT axis. On the other hand, the different patterns of action of GH and IGF-I on OT, estrogen and IGFBP-3 suggest that part of the GH effect on ovarian cells is IGF-I independent.

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Introduction

There is a growing body of evidence that ovarian function is controlled not only by gonadotropins (Hillier 1991, Sirotkin & Nitray 1994b, Sirotkin *et al.* 1994, Erickson 1995, Erickson & Danforth 1995), but also by other substances of hypophyseal or extra-hypophyseal origin. In particular, growth hormone (GH) (Jia *et al.* 1986, Sirotkin & Nitray 1994a, Sirotkin & Schaeffer 1995, Sirotkin 1996a), insulin-like growth factor-I (IGF-I) (Giudice 1992, Paton & Collins 1992, Spicer & Echternkamp 1995, Sirotkin & Makarevich 1996), oxytocin (OT) (Wathes 1989, Schaeffer & Sirotkin 1995, Sirotkin 1995, 1996b, Sirotkin *et al.* 1996) and prostaglandin (Dennfors *et al.* 1983, Satoh *et al.* 1984, Michael *et al.* 1994) can regulate gonadotropin receptors, secretion of growth factors, steroid

and nonapeptide hormones, prostaglandin F₂-alpha (PGF) and cyclic nucleotides by rodent, bovine and human ovarian cells.

In pigs, effects of gonadotropin (Hillier 1991, Sirotkin & Nitray 1994b, Sirotkin *et al.* 1994, Erickson 1995, Erickson & Danforth 1995), OT (Schaeffer & Sirotkin 1995, Sirotkin 1995, 1996b, Sirotkin *et al.* 1996) and prostaglandin (Dodson & Watson 1979, Michael *et al.* 1994) on ovarian secretions have been reported. On the other hand, there is very little information on the effects of GH and IGF-I on ovarian secretion in this species. Only an increase in IGF-I production after GH treatment, as well as a stimulatory influence of both GH and IGF-I on porcine ovarian steroidogenesis, has been reported (Giudice 1992, Spicer & Echternkamp 1995). These data suggest indirectly that IGF-I is a mediator of GH action on

ovarian steroid secretion. On the other hand, the influence of GH and IGF-I on porcine ovarian growth factor-binding protein, OT, prostaglandin and cyclic nucleotides, which may be also mediators of hormone and growth factor action, has not yet been studied.

To understand the role of various peptide hormones and related substances in the control of ovarian secretory activity, as well as to obtain indirect evidence on their possible inter-relationships, a comparison of their effects on the secretion of various substances by porcine ovarian cells is necessary.

Most *in vitro* studies of the ovary have been performed on cultured granulosa cells. It is known, however, that the complete, long-term production of ovarian secretions requires functional integrity of the theca and granulosa compartments of the follicle (Hillier 1991, Erickson 1995). In an attempt to find an adequate model for the study of ovarian steroidogenesis during follicular development and after gonadotropin treatment, several procedures for the culture of whole mouse (Boland *et al.* 1993, Spears *et al.* 1996), bovine (Staigmiller *et al.* 1982, Kruip & Dieleman 1989), sheep (Terlou *et al.* 1988, Mann *et al.* 1992) and porcine (Dodson & Watson 1979) ovarian follicles have been developed. In these systems, short-term (3 h to 2 days) perfusion with oxygenated serum-supplemented medium is usual. These perfusion systems have been used to study inter-relationships between gonadotropin, steroid hormones and prostaglandins within the ovary. Follicles do not appear to have been used previously for the study of GH, growth factors and their binding proteins, OT or intracellular messengers.

The aims of our study were: (1) to determine the value of isolated quarters of porcine ovarian follicles for long-term culture in serum-free or serum-supplemented medium and the production of hormones and other biologically active substances, and (2) to study the influence of GH, IGF-I, OT, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) on growth factor, growth factor-binding protein, steroid and nonapeptide hormones, eicosanoid and cyclic nucleotide secretion by these follicles.

Materials and Methods

Isolation and culture of ovarian follicles

Ovaries from non-cycling, Slovakian white gilts, 180 days old, without visible reproductive abnormalities, were obtained at a local slaughterhouse. Ovarian follicles were collected and processed according to Golubev & Zavertjajev (1989) with several modifications. Briefly, 1 h after slaughter the tissues surrounding the ovary were removed and the ovaries opened with scissors at the site of entry of blood vessels. The connective tissues inside the opened ovary were gradually broken with large surgical forceps in order to access the follicles. Follicles 3 mm in

diameter without visible signs of atresia were pressed down and separated from the surrounding connective tissues with small forceps. They were collected in a Petri dish and washed four times in sterile Dulbecco's modified Eagle's medium/F-12 1:1 mixture (Sigma, St Louis, MO, USA) supplemented with 10% heat-inactivated fetal calf serum (FCS, University of Veterinary Medicine, Brno, Czech Republic), and 1% antibiotic-antimycotic solution (Sigma). The follicles were then cut with small scissors into four equal parts, and washed gently, avoiding detachment of granulosa cells from the follicular wall. Quarters of follicles were placed individually in 24-well plates (Beckton Dickinson GmbH, Heidelberg, Germany) with 2 ml medium. In the first series of experiments (studies of hormone accumulation in follicle-conditioned medium) follicles were cultured for 8 days with or without FCS at 37.5 °C in humidified air. The incubation medium was collected on days 0, 1, 2, 3, 4, 6 and 8 of culture. Microscopical examination of the cultures after 8 days showed that granulosa cells remained attached to the follicular wall; only insignificant numbers of cells started to grow away separately. In the second series of experiments (studies of hormone and growth factor effects on secretory activity) follicles were cultured for 4 days in serum-supplemented and, in some cases, also in serum-free medium in the presence of recombinant porcine GH (rpSTH, Research and Development, Pittman-Moore Inc., Terre Haute, USA; 0, 10 or 0, 10, 100 or 1000 ng/ml), recombinant IGF-I (Calbiochem, Lucerne, Switzerland; 0, 10 or 0, 1, 10 or 100 ng/ml), porcine LH (National Hormone and Pituitary Program, Ogden BioService Corp., Rockville, USA; 0 or 10 ng/ml), porcine FSH (SPOFA, Prague, Czech Republic; 0 or 10 ng/ml) or synthetic OT (Sigma; 0 or 100 ng/ml). The blank control was represented by medium cultured without follicular tissue. After culture, the follicle quarters were weighed and the medium stored at -20 °C until analysis.

Immunoassay

Hormone, growth factor-binding protein and prostaglandin concentrations in 25–100 µl incubation medium were determined in duplicate without extraction. Progesterone, androstenedione, testosterone, estradiol and insulin-like growth factor-binding protein-3 (IGFBP-3) were analyzed using commercial IRMA kits from DSL (Webster, TX, USA). The RIA kit for cAMP assay was from Immunotech (Marseille, France). PGF and prostaglandin E (PGE) were determined using an RIA kit from the Institute of Isotopes (Budapest, Hungary) or the RIA described previously (Cetta & Goetz 1982, Chang *et al.* 1995). IGF-I was measured according to the method of Furlanetto *et al.* (1977). OT was determined using our RIA or EIA described previously (Kotwica & Skarzynski 1993, Marnet *et al.* 1994). The characteristics of these assays are presented in Table 1.

Table 1 Characteristics of immunoassays used in experiments

Assay	Specificity of assay (cross-reactivity of antiserum used)	Sensitivity of assay (ng/ml)	Coefficient of variation	
			Intra- assay	Inter- assay
Progesterone	<0.001% to cortisol, corticosterone, danazol, cortisol, androstenediol, pregnenolone, estradiol, testosterone	0.12	<8.0	<1
Androstenedione	<0.33% to androsterone, hydroxyprogesterone, isoandrosterone, cortisone, <0.1% to deoxycortisone, progesterone, corticosterone, cortisol, <0.001% to estrone, estradiol	0.03	<9.8	<5
Testosterone	<5.8% to hydrotestosterone, androstenedione, hydroandrostenedione, androstanedione, <0.14% to androstanediol, androstenediol, <0.001% to progesterone, estradiol, estrone, estriol	0.08	<9.6	<9.1
Estradiol	<0.01% to dehydroepiandrosterone, progesterone, cortisol, danazol, androsterone, testosterone, androstenedione, corticosterone, cortisone	6.5	<9.4	<19
PGF	<0.01% to PGA-1, PGA-2, PGB-1, PGB-2, <0.1% to PGE-1, PGE-2, 66.0% to PGF-1, 100% to PGF-2	0.005	<7.7	<4.3
PGE	<28.0% to PGA-1, <7.0% to PGA-2, <0.6% to PGB-1, <1.4% to PGB-2, <5.0% to PGF-1, <1.5% to PGF-2, 165% to PGE-1, 100% to PGE-2	0.015	<7.5	<4.0
OT	<0.4% to arg-vasopressin, lys-vasopressin, isotocin, tocinioc acid, PGF-2, pFSH, <12.5% to Tyr-Pro-Leu-Gly-NH ₂	0.0015	<14.0	<7.6
IGF-I	<0.0-1% to insulin, proinsulin, epidermal growth factor, OT, pGH, pLH, pFSH, 1.9% to IGF-II	0.0014	<3.4	<8.2
IGFBP-3	<0.03% to IGFBP-I, IGFBP-2, IGFBP-4, IGFBP-5, IGFBP-6, IGF-I, IGF-II, pGH, pLH, pFSH, OT	0.5	<3.9	<1.9
cAMP	<0.4% to cGMP, <0.7% to 5'AMP, <7.0% to ATP, <0.2% to ScGMP, 100% to ScAMP, <1500% to cAMP	0.2	<16.0	<11

Statistics

Each experimental group was represented by six cultures of follicles. The data shown are means obtained in three experiments using separate pools of ovaries. In each experiment the value of the blank control was subtracted from the values determined in follicle-conditioned medium to exclude non-specific background. Substance levels in the medium in the first series of experiments were expressed per ml medium. The rates of substance production in the second series of experiments were calculated per mg tissue/day. Significant differences between the experiments were evaluated using two-way ANOVA. When ANOVA indicated effects of treatments, experimental and control groups were compared by Duncan's multiple range test.

Results

Secretory activity of ovarian follicles cultured in serum-free and serum-supplemented medium

Accumulation of progesterone (Fig. 1a), estradiol (Fig. 1b), OT (Fig. 1c) and IGFBP-3 (Fig. 1d) in the medium from

follicle incubation was observed. Peaks of steroid hormones in media without and with serum occurred at 3 and 4 days of culture respectively. The amounts of progesterone and estrogen which accumulated in serum-supplemented medium were approximately 2.5- and 2-fold higher respectively than in serum-free medium. A 4- to 5-fold increase in the ratio of progesterone to estrogen in both media from day 1 to days 6-8 of incubation was noted (not shown).

OT concentration in the medium increased to day 8 of culture. The amount of OT which accumulated in serum-supplemented medium was 3.2-fold higher than that in serum-free medium.

IGFBP-3 level in the serum-supplemented medium increased, reaching a plateau at 3-6 days of culture, and decreased at the 8th day of culture. The increase in IGFBP-3 concentration in serum-free medium continued after 3 days of culture, reaching maximal value at the end of the experiment (day 8). At this time the IGFBP-3 level was 2.7 times higher in the serum-free medium than in the presence of serum.

In contrast to progesterone, estradiol and OT, daily changes of concentration of other substances during culture were not observed. Accumulation of IGF-I,

Figure 1 Accumulation of hormones in serum-supplemented (black bars) and serum-free (hatched bars) medium during culture of porcine ovarian follicles: (a) progesterone, (b) estradiol, (c) OT, (d) IGFBP-3. Values are means $\pm$ S.E.M.

androstenedione, testosterone, PGF and cAMP were seen, however; their concentration increased, from zero levels before culture, up to day 4 of culture (Figs 2 and 3, Tables 2 and 3).

Effects of GH, IGF-I, LH, FSH and OT on secretory activity of porcine ovarian follicles

GH (10 or 100 ng/ml) was able to inhibit androstenedione (in the serum-supplemented, but not in the serum-free medium, Fig. 2a, Table 2), OT (in the serum-supplemented medium, Table 3), IGFBP-3 (in the

serum-supplemented, but not in the serum-free medium, Table 3), PGF (both in the serum-free and serum-supplemented medium, Table 3). GH given in these concentrations was also able to stimulate IGF-I (in the serum-supplemented, but not in serum-free medium, Table 3), and cAMP (in the serum-supplemented medium, Fig. 2b). GH increased testosterone release in the serum-free, but decreased it in the serum-supplemented medium (Table 2). No influence of GH on PGE release (either in the serum-free or serum-supplemented medium) or progesterone secretion (in the serum-supplemented medium) was observed (Tables 2 and 3).

Figure 2 Effect of porcine GH on (a) androstenedione and (b) cAMP production by cultured porcine ovarian follicles. Values are means $\pm$ S.E.M. *Significant ($P < 0.05$) difference compared with control (medium without GH).

IGF-I (1 ng/ml or above) inhibited output of androstenedione and PGF (both in the serum-free and serum-supplemented medium, Table 3, Fig. 3a). Furthermore, IGF-I stimulated testosterone, estradiol (both in the serum-free and in the serum-supplemented medium, Table 2), OT (in the serum-supplemented medium, Table 3), and cAMP (in the serum-supplemented medium, Fig. 3b). No effect of IGF-I on IGFBP-3, PGE

(either in the presence or absence of serum, Table 3) or progesterone (in the presence of serum, Table 2) production was observed.

OT (100 ng/ml) was able to inhibit androstenedione and to stimulate testosterone and IGF-I, but not estradiol or IGFBP-3 release by follicles cultured either in the serum-free or serum-supplemented medium. Furthermore, OT stimulated both PGF and PGE production in

Figure 3 Effect of IGF-I on (a) androstenedione and (b) cAMP production by cultured porcine ovarian follicles. Values are means $\pm$ S.E.M. *Significant ($P < 0.05$) difference compared with control (medium without IGF-I).

Table 2 Effect of hormones and IGF-I on progesterone, androstenedione, testosterone and estradiol release by porcine ovarian follicles cultured in serum-free and serum-supplemented medium

Treatment	Presence of serum	Production (ng/mg tissue per day $\pm$ S.E.M.)			
		Progesterone	Androstenedione	Testosterone	Estradiol
None (control)	—	—	0.6 $\pm$ 0.1	1.2 $\pm$ 0.3	0.5 $\pm$ 0.1
	+	1.0 $\pm$ 0.1	0.6 $\pm$ 0.1	2.8 $\pm$ 0.3	1.3 $\pm$ 0.4
GH (10 ng/ml)	—	—	0.5 $\pm$ 0.1	2.2 $\pm$ 0.7*	1.3 $\pm$ 0.2*
	+	1.0 $\pm$ 0.2	0.3 $\pm$ 0.1*	2.2 $\pm$ 0.3*	1.5 $\pm$ 0.4
IGF-I (10 ng/ml)	—	—	0.1 $\pm$ 0.0*	2.4 $\pm$ 0.6*	0.7 $\pm$ 0.1*
	+	1.2 $\pm$ 0.8	0.2 $\pm$ 0.1*	3.5 $\pm$ 1.2*	1.3 $\pm$ 0.4
LH (10 ng/ml)	—	—	—	—	—
	+	1.9 $\pm$ 0.7*	—	—	0.9 $\pm$ 0.2*
FSH (10 ng/ml)	—	—	—	—	—
	+	1.1 $\pm$ 0.4	—	—	1.0 $\pm$ 0.1
OT (100 ng/ml)	—	—	0.3 $\pm$ 0.1*	3.5 $\pm$ 0.8*	0.9 $\pm$ 0.4
	+	—	0.5 $\pm$ 0.1	4.6 $\pm$ 1.7*	0.9 $\pm$ 0.6

* $P < 0.05$ compared with control.

the serum-free medium, but not in the presence of serum (Tables 2 and 3).

LH stimulated progesterone and OT, while it inhibited estradiol secretion in the presence of serum. FSH in these conditions stimulated OT, but not progesterone or estradiol secretion (Tables 2 and 3).

Discussion

The present paper appears to be the first report of the long-term culture of isolated porcine ovarian follicles in a static system. It was demonstrated that relatively long culture of follicle quarters in a static system in both serum-free and serum-supplemented medium without oxygenation can be used for studies of ovarian secretion.

Under our conditions follicles were able to survive and to maintain the granulosa cell layer and good secretory activity for several days. The use of quarters provides good contact for inner follicular cells with the medium. Consequently, they do not require the mobile, oxygenated medium, used for cultures of undisturbed follicles. Furthermore, our culture system allows the complete exclusion of serum, which may contain substances which interfere with treatments. On the other hand, serum-supplemented medium in all cases (except IGFBP-3 release) markedly promoted follicular secretion in our culture. In particular, the levels of accumulated progesterone, estrogen and OT in this medium were much higher, and were maintained longer, than those in serum-free medium. The ratio of progesterone to estradiol also increased during culture. No differences in this ratio

Table 3 Effect of hormones and IGF-I on (OT), IGF-I, IGFBP-3, PGF and PGE release by porcine ovarian follicles cultured in serum-free and serum-supplemented medium

Treatment	Presence of serum	Production (ng/mg tissue per day $\pm$ S.E.M.)				
		OT	IGF-I	IGFBP-3	PGF	PGE
None (control)	—	—	21.4 $\pm$ 4.6	11.5 $\pm$ 1.9	0.34 $\pm$ 0.02	1.94 $\pm$ 0.04
	+	0.5 $\pm$ 0.1	6.7 $\pm$ 1.8	9.0 $\pm$ 0.5	0.31 $\pm$ 0.02	1.03 $\pm$ 0.14
GH (10 ng/ml)	—	—	21.2 $\pm$ 6.7	10.0 $\pm$ 1.9	0.22 $\pm$ 0.03*	1.82 $\pm$ 0.34
	+	0.3 $\pm$ 0.1*	8.6 $\pm$ 1.8*	8.1 $\pm$ 0.6*	0.23 $\pm$ 0.03*	1.09 $\pm$ 0.24
IGF-I (10 ng/ml)	—	—	—	12.0 $\pm$ 1.4	0.22 $\pm$ 0.03*	1.63 $\pm$ 0.46
	+	0.7 $\pm$ 0.1*	—	8.5 $\pm$ 0.7	0.37 $\pm$ 0.04*	0.89 $\pm$ 0.09
LH (10 ng/ml)	—	—	—	—	—	—
	+	4.5 $\pm$ 0.4*	—	—	—	—
FSH (10 ng/ml)	—	—	—	—	—	—
	+	2.6 $\pm$ 0.5*	—	—	—	—
OT (100 ng/ml)	—	—	55.5 $\pm$ 12.9*	12.2 $\pm$ 2.9	0.43 $\pm$ 0.06*	2.20 $\pm$ 0.40*
	+	—	6.0 $\pm$ 1.9	9.2 $\pm$ 1.7	0.25 $\pm$ 0.04	1.12 $\pm$ 0.15

* $P < 0.05$ compared with control.

between serum-free and serum-supplemented medium were found. This suggests that in both culture systems ovarian cells cannot avoid the spontaneous luteinization which is characterized by a decrease in estrogen and an increase in progesterone production (Hillier 1991, Paton & Collins 1992). On the other hand, for the study of follicular secretion of IGFBP-3 the serum-free medium appears more suitable than the medium with serum. In the serum-free system IGFBP-I secretion was maintained longer than in the serum-supplemented medium (Fig. 1d). Furthermore, without serum IGFBP-3 and IGF-I were released in approximately equimolar ratios (similar to *in vivo* conditions, Giudice 1992), while the presence of serum promoted IGF-I, but not IGFBP-3 output (Table 3).

Our observations support available data on steroid (Hillier 1991, Paton & Collins 1992), nonapeptide hormone (Wathes 1989), IGF-I, IGFBP-3 (Giudice 1992, Spicer & Echternkamp 1995), prostaglandin (Dodson & Watson 1979, Ainsworth *et al.* 1984, Tsang *et al.* 1988, Paton & Collins 1992) and cyclic nucleotide (Paton & Collins 1992, Sirotkin *et al.* 1994, Sirotkin 1996b) production by porcine ovarian cells.

Furthermore, our data on isolated porcine follicles correspond with previous reports on gonadotropins (Hillier 1991, Sirotkin & Nitray 1994b, Sirotkin *et al.* 1994, Erickson 1995, Erickson & Danforth 1995) and OT (Wathes 1989, Schaeffer & Sirotkin 1995, Sirotkin 1995, 1996b, Sirotkin *et al.* 1996) influence on steroid, nonapeptide hormone, IGF-I and cyclic nucleotide production by porcine and bovine granulosa cells. The pattern of response and its expression in isolated follicles in our experiments were comparable with those reported previously for porcine granulosa cells. This is the first report on OT influence on ovarian PGE.

Furthermore, our present observations on isolated follicles confirm the available reports of an involvement of GH (Jia *et al.* 1986, Sirotkin & Nitray 1994a, Sirotkin & Schaeffer 1995, Sirotkin 1996a) and IGF-I (Giudice 1992, Paton & Collins 1992, Spicer & Echternkamp 1995, Sirotkin & Makarevich 1996), in the control of growth factor, growth factor-binding protein and steroid production by porcine granulosa cells. In species other than pigs, effects of both GH (Sirotkin & Nitray 1994a, Sirotkin & Schaeffer 1995, Sirotkin 1996) and IGF-I (Schams 1987, Makarevich & Sirotkin 1996, Makarevich *et al.* 1996, Sirotkin & Makarevich 1996) on other ovarian substances (nonapeptide hormones, prostaglandin, cyclic nucleotides) have been reported. Our present data are the first demonstration of an involvement of GH and IGF-I in control of ovarian OT, prostaglandin and cAMP in pigs.

In particular it was found that in pigs, in contrast to other species, GH can inhibit ovarian OT, IGFBP-3 and PGF output. Inhibitory influences of GH on IGFBP-3 production suggest that GH can influence ovarian cells not

only through stimulation of local IGF-I release, but also through reduction of ovarian IGF-I binding by IGFBP.

Since OT is a known regulator of steroid, peptide hormones and cyclic nucleotides in ruminant, human and porcine ovaries (Wathes 1989, Schaeffer & Sirotkin 1995, Sirotkin 1995, 1996b, Sirotkin *et al.* 1996), it is possible that OT is the third messenger of GH action on the ovary. The inhibitory influence of GH on porcine follicular OT release (which was observed previously on porcine granulosa cells (Marnet *et al.* 1997), differs from the stimulatory effect of GH on ovarian OT in other species. It suggests the existence of species-specific differences in the control of ovarian nonapeptide hormone release.

The inhibitory influence of GH on ovarian PGF (but not on PGE), which is a known regulator of ovarian steroidogenesis, ovulation and luteogenesis (Dennfors *et al.* 1983, Satoh *et al.* 1984, Michael *et al.* 1994), suggests that PGF can also mediate GH action on the ovary.

The stimulatory action of GH on ovarian cAMP output, which is an important regulator of various ovarian functions, and which mediates effects of gonadotropins on the ovary (Hillier 1991, Paton & Collins 1992) suggests also that cAMP may be a further mediator of GH effects on the ovary.

The evidence of a stimulatory action of GH on IGF-I release in our experiments supports the widely accepted hypothesis (Giudice 1992, Spicer & Echternkamp 1995) that growth factors are the main mediators of GH action.

IGF-I in our experiments, as in experiments on ovarian cells of other animals (Adashi *et al.* 1986, Schams 1987, Makarevich & Sirotkin 1996, Makarevich *et al.* 1996, Sirotkin & Makarevich 1996), stimulated OT and cAMP output. The inhibitory influence of IGF-I on PGF release is the first observation of a growth factor effect on prostaglandin release. OT, PGF and cAMP may be potential mediators of IGF-I on the ovary.

In contrast to granulosa cells of pigs and other species (Giudice 1992, Spicer & Echternkamp 1995), the follicles in our experiments responded to IGF-I with a reduced steroid hormone output. These data suggest that nonapeptide hormones, PGF, cAMP and steroid hormones can be involved in IGF-I action on the ovary and that theca as well as granulosa cells may be involved in the IGF-I action on ovarian steroidogenesis. It is possible that IGF-I induces production of some unknown factor(s) within the theca which can inhibit steroidogenesis in theca and/or granulosa cells through paracrine or autocrine mechanisms.

The present observations of an OT effect on steroid and IGF-I release by porcine follicles confirm previous reports of an OT action on the granulosa cells of pigs and other species (Wathes 1989, Schaeffer & Sirotkin 1995, Sirotkin 1995, 1996b, Sirotkin *et al.* 1996). On the other hand, this is the first report of an OT influence on porcine IGF-I, IGFBP-3, PGF and PGE. These substances may be mediators of OT action on the ovarian cells.

The effect of gonadotropins on steroid release is in agreement with similar observations on this and other species (Hillier 1991, Sirotkin & Nitray 1994b, Sirotkin *et al.* 1994, Erickson 1995, Erickson & Danforth 1995), while the stimulatory action of LH and FSH on ovarian OT secretion has previously only been demonstrated on bovine granulosa cells (Schams 1987, Wathes 1989). Since OT production increases during luteinization, and since it is a potent regulator of steroidogenesis (Wathes 1989), these data confirm the involvement of the gonadotropin–OT axis in the regulation of these processes.

The use of our experimental model suggests the involvement of gonadotropins, OT, GH and IGF-I in the control of ovarian steroid and nonapeptide hormones, growth factor, growth factor-binding protein, prostaglandin and cyclic nucleotide production. The interrelationships between these substances require further investigation. On the other hand, a comparison of the observations in our experiments and those in other laboratories may provide some indirect evidence. For example, the coincidence of effects of GH and IGF-I on PGF and cAMP observed in our experiments, as well as the influence of both PGF, (Dennfors *et al.* 1983, Satoh *et al.* 1984, Michael *et al.* 1994) and cAMP and related substances (Wathes 1989, Sirotkin 1996b) on steroid and nonapeptide hormone production suggest that effects of both GH and IGF-I on ovarian cells may be mediated through PGF- and/or cAMP-dependent intracellular mechanisms. Similarly, the stimulatory effect of GH on IGF-I, the stimulatory influence of IGF-I on OT, together with the convergence of the majority of effects of IGF-I and OT in our experiments suggest the existence of a GH–IGF-I–OT axis, which is involved in control of ovarian steroid and possibly prostaglandin and cAMP release. The stimulatory effect of gonadotropins on ovarian IGF-I (Adashi *et al.* 1986, Erickson 1995, Erickson & Danforth 1995) and OT (Schams 1987, Wathes 1989, Table 3) suggest the existence of some cross-talk between the GH–IGF-I–OT and gonadotropin–IGF-I–OT systems. On the other hand, different patterns of action of GH and IGF-I on OT, estrogen and IGFBP-3 suggest that at least the part of GH effect on ovarian cells is IGF-I independent.

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