Effect of Estradiol-17 β on Basal and hCG Stimulated Progesterone Secretion by Porcine Luteal Cells Isolated in Various Stages of the Luteal Phase

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Abstract. To explore the direct effect of estradiol-17 β on pig luteal cells and its ability to counteract a gonadotropic stimulus, luteal cells were incubated *in vitro* in the presence of estradiol alone, human chorionic gonadotrophin (hCG) alone or estradiol combined with hCG. Luteal cells were collected at three different stages of the luteal phase (0–3 days after ovulation, early; 8–10 days after ovulation, mid; and 14–16 days after ovulation, late). Estradiol alone did not exert any effect on progesterone production by any of the 3 types of luteal cells.

Progesterone production by cells in the mid-luteal phase was enhanced by hCG in a dose dependent manner. High doses of estradiol combined with hCG significantly diminished production of progesterone by cells collected during the early luteal phase. The strongest inhibitory effect of estradiol on hCG stimulated progesterone production was observed in cultures of luteal cells in the midluteal phase. In contrast, 100 ng of estradiol and the 10 and 100 ng/ml concentrations of hCG increased progesterone secretion over basal control values in cultured luteal cells in the late luteal phase. Differences in the production of progesterone in response to estradiol at different stage of the luteal phase suggest that different cellular mechanisms must be triggered in each luteal phase investigated. These data support a physiological role for estrogen in the regulation of the pig corpus luteum during its life span in the non-fertile cycle.

Key words: Estradiol, Pig, Corpus luteum, Progesterone secretion

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THE ROLE OF estradiol-17 β (E₂) in the luteal function of domestic animals is still unclear. Estradiol has been considered luteotropic in the sow on the basis of evidence that exogenous E₂ administered either via systemic injection or intrauterine infusion can prolong the life span of the corpus luteum [1–3]. On the other hand injection of antisera to E₂ or estrone on day 10–21 of pregnancy had no effect on the maintenance of the corpora lutea or the initiation of placental attachment in the pig. [4]. In all *in vivo* studies purporting to demonstrate a direct luteotropic effect of E₂, the possibility of an indi-

rect effect cannot be ruled out. Recent evidence suggests that estradiol produced by pig blastocysts during early pregnancy is an antiluteolytic rather than a luteotropic factor since it decreases and changes the direction and secretion of prostaglandin F_{2 α} (PGF_{2 α}) secretion by the endometrium [2]. However, Geisert *et al.* [5, 6] showed that in addition to E₂ other biologically active substances such as prostaglandin E₂ (PGE₂) are involved in the maintenance of the corpus luteum in the pig. Van Meulen *et al.* [7] indicated that administration of a small physiological or supraphysiological dose of E₂ into the lumen of the uterus did not result in the maintenance of CL in non-pregnant gilts. Furthermore Ford & Christensen [8] demonstrated a direct effect of PGE₂, but not of E₂ in protecting the CL against luteolysis induced by PGF_{2 α} .

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Our preliminary studies [9] showed that the administration of testosterone to the culture media of luteal cells collected in the mid-luteal phase caused an increase in estradiol secretion, which indicated the capacity of the corpora lutea to aromatize testosterone. On the other hand Gregoraszczuk [10] showed a decrease in the tissue concentration of progesterone and androgens and a simultaneous increase in estradiol in regressing corpora lutea which was suggested a signal for luteolysis. In addition, LH and FSH further stimulated estradiol secretion by luteal cells collected from the regressing corpora lutea [11].

To determine a possible direct effect of E_2 on luteal function in pig, the *in vitro* action of E_2 on progesterone secretion by luteal cells isolated during the early, middle and late luteal phases was investigated.

In order to assess the potential luteotropic or luteolytic effect of estradiol, luteal cells were incubated with various combinations of estradiol and hCG.

Materials and Methods

Animals

Mature, cycling, slaughterhouse pigs were the source of ovaries. The stage of the cycle was determined with help of the tables by Schilling [12]. Luteal cells were collected from newly forming corpora lutea of the ovaries in the early luteal phase (day 0–3), from mature corpora lutea (day 8–10) and from regressing corpora lutea (day 14–16).

Short term incubation

Dissected corpora lutea were enzymatically dissociated according to the technique of Gregoraszczuk [13]. The cells were suspended in medium M 199 supplemented with 10% of calf serum at a concentration of $3.5\text{--}5.0 \times 10^5$ cells/ml medium. They were incubated in multiwell plates (Corning) for 6 h. Control cultures were grown in medium alone while other cultures were supplemented with:

- 1) estradiol (E_2) in doses of either 1 ng/ml, 10 ng/ml or 100 ng/ml,
- 2) hCG in concentration either 1 ng/ml, 10 ng/ml or 100 ng/ml,
- 3) estradiol combined with hCG in the same

doses as above.

The cultures were incubated in quadruplicate in each of the 3 experiments.

After 6 h incubation, all cultures were terminated and the media were frozen at -20°C for further steroid analysis.

Reagents

Medium M199, calf serum, trypsin (0.25% solution in calcium and magnesium free PBS) were the products of the Laboratory of Sera and Vaccines (Lublin, Poland).

Penicilin, streptomycin and mycostatin were obtained from GIBCO (USA), and standards crystalline estradiol 17β and hCG (SIGMA).

Steroid analysis

Progesterone was analysed by the radioimmunoassay according to Abraham *et al.* [14]. A high specific antibody raised in sheep against 11α -hydroxyprogesterone, hemisuccinate coupled to bovine serum albumin was used. The cross-reaction with pregnenolone was 2.9%. All other steroids that were tested showed less than a 1% cross-reaction. $[1, 2, 6, 7\text{-}^3\text{H}]$ progesterone, Radiochemical Center, Amersham, England, sp. act 80 Ci/mmol was used as the tracer. The sensitivity of the assay was 50 pg. The coefficients of variation within and between assays were less than 2.5% and 15% respectively.

The data for progesterone were computed in nanograms per 10^5 cells per 6 h of incubation. The statistical difference between control and experimental cultures was calculated by student's *t*-test.

Results

The effect of exogenous E_2 on progesterone production in vitro

Under basal *in vitro* conditions, progesterone production by early luteal phase cells isolated on days 0–3 was significantly greater than that of mid and late luteal phase cells (800 ng/ 10^5 cells/6 h, 300 ng/ 10^5 cells/6 h, 22.7 ng/ 10^5 cells/6 h respectively) (Fig. 1). There was no response by three investigated types of luteal cells in terms of progesterone secretion to exogenous E_2 as shown in Fig. 1.

The effect of exogenous hCG on progesterone production

Progesterone production by mid-luteal phase cells was significantly enhanced by the presence of

1 ng, 10 ng and 100 ng hCG ($P<0.05$, $P<0.01$, $P<0.001$, respectively) (Fig. 2). In contrast progesterone production by early and late luteal phase cells was not stimulated by the three doses of hCG used (Fig. 2).

Fig. 1. Secretion of progesterone by luteal cells collected from pig during early (CL 1), mid (CL 2) and late (CL 3) luteal phases in the presence of various concentrations of exogenous estradiol. Production is expressed as ng progesterone released per 10⁵ cells during 6 h incubation. Values are the means for 3 experiments.

Fig. 2. Influence of various doses of hCG on progesterone production by pig luteal cells obtained during early (CL 1), mid (CL 2) and late luteal phases (CL 3). Production is expressed as ng progesterone released per 10⁵ cells during 6 h incubation. Values are the means for 3 experiments. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

The effect of E_2 and hCG on stimulated progesterone production

The effect of various concentrations of estradiol on hCG stimulated progesterone production by early luteal phase cells is shown in Fig. 3a.

Although luteal cells collected from early corpora lutea did not respond to the doses of hCG used with increased production of progesterone, high doses of hCG combined with E_2 significantly diminished secretion of progesterone ($P < 0.05$).

The stimulatory effects of 1 ng, 10 ng and 100 ng/ml concentrations of hCG on progesterone secretion were completely blocked by 1, 10 and 100 ng/ml concentrations of estradiol (Fig. 3b).

The effect of estradiol on hCG stimulated progesterone production by late luteal phase cells is summarized in Fig. 3c. The combinations of 100 ng of estradiol and the 10 and 100 ng/ml concentrations of hCG increased progesterone secretion over control values. In contrast, the 1 and 10 ng/ml concentrations of estradiol had no significant effect at any concentration of hCG.

Discussion

The first evidence showing that, in pig during the luteal phase, estradiol can originate from the corpora lutea has been obtained by Weiss *et al.* [15], who demonstrated that the porcine luteal tissue *in vitro* is capable of synthesizing estradiol. This was confirmed by Gregoraszczyk [13, 16, 17]. Gregoraszczyk [10] showed that the concentration of estradiol in homogenates of corpora lutea in the early luteal phase was very low (100 pg/ml). It was higher in homogenates of mature corpora lutea (340 pg/ml) and very high in regressing corpora lutea (50 ng/ml). Cultured luteal cells are able to secrete large basal amounts of estradiol not only when isolated during corpus luteum regression but also when collected from partially developed (5–8 days after ovulation) and mature corpora lutea (8–10 days after ovulation) [10]. They also clearly show that *in vitro* steroid production by different types of corpora lutea does not entirely reflect the *in vivo* situation. This was apparently related to the luteinization and differentiation of cells in the course of tissue culture. In order

to see whether estradiol can directly influence pig luteal function, the *in vitro* effect of estradiol on progesterone production by cells isolated during early, middle and late luteal phase was evaluated.

Fig. 3. Influence of estradiol on hCG stimulated progesterone production by pig luteal cells isolated during early middle and late luteal phases. Cells were incubated in the presence of hCG plus 0 (control), 1, 10 and 100 ng E_2 /ml. Values are the means for 3 experiments. *, $P < 0.05$; **, $P < 0.01$.

In addition, to study the ability of estradiol to counteract a luteotropic stimulus, luteal cells were studied with cultures treated with hCG. In the current study three distinct effects on progesterone production by luteal cells as a function of the estrogen concentration present during incubation were observed: firstly estradiol did not exert any effect on progesterone production by any of the 3 types of luteal cells investigated. So the increased estrogen synthesis during the late luteal phase is not a prerequisite for spontaneous luteolysis in the pig. Secondly, increased progesterone production under the influence of exogenous gonadotropin (hCG) was sensitive to estradiol inhibition in cultured luteal cells collected from mature corpora lutea. This is in accordance with the findings of Tekpetey and Armstrong [18] who observed that in the pig, E_2 decreased LH-stimulated P_4 production by small luteal cells and basal P_4 production by both cell types, particularly at 72 h incubation, but increased P_5 accumulation, suggesting an inhibitory effect on the enzyme 3β -hydroxysteroid dehydrogenase. We must remember that *in vivo* the estradiol content in CL during midluteal phase is still low. It was postulated that high circulating LH levels protect elevated estradiol secretion [9]. This was not related to the absence of substrate. High amount of androgens was observed in homogenates of the CL isolated during the midluteal phase [10]. Thirdly, however low estrogen doses (1 and 10 ng/ml) consistently antagonized the stimulatory effect of hCG during regression, while high estrogen doses (100 ng/ml) had a visible stimulatory effect on progesterone secretion during the late luteal phase.

It is still difficult to conclude that there is a stimulatory effect of estradiol combined with hCG on progesterone production by cells collected during the late luteal phase, keeping in mind that hCG alone and estradiol alone had no effect on progesterone production and the fact that in this stage of luteal phase the basal progesterone level is very low. The data of Ford & Christenson [8] suggest that the major antiluteolytic effect of E_2 on estrogen-induced pseudopregnant gilts is to decrease uterine release of $PGF_{2\alpha}$ into the peripheral circulation, rather than to have a direct protective effect at the level of the CL.

Would it be a great heresy to speculate that this might be an intraluteal luteotropic mechanism which could be started in cases when pregnancy occurs, taking into consideration the data of Rosenkrans *et al.* [19] indicating that catechol estrogens and E_2 may inhibit prostaglandin synthesis and modify the PGE: $PGF_{2\alpha}$ during the establishment of pregnancy in pigs?

Further experiments are necessary to answer this question.

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