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Local Control Mechanisms in Human and Porcine Ovaries

R. Denkova, V. Bourneva, K. Baleva, E. Yaneva, B. Nikolov, I. Ivanov, K. Simeonov**

Institute of Experimental Morphology and Anthropology, Bulgarian Academy of Sciences, Sofia
**Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Sofia*

The regulation of human and porcine ovarian granulosa and granulosa luteal cell (GCs; GLCs) morphology and steroid output was investigated. The in vitro application of some locally produced substances demonstrated that arginine-8-vasopressin, relaxin and oxytocin in physiological doses enhanced basal progesterone and estradiol production of GCs and GLCs and were additive in increasing gonadotropin (Gn)-stimulated steroid output. Endothelin-1 and chondroitin-4-sulphate treatment reduced basal and Gn-stimulated production of progesterone and estradiol. The ultrastructural changes in the cells reflected the alterations in the steroidogenic machinery and were parallel to the changes of the steroidogenic activity.

Key words: human and porcine ovaries; granulosa/ granulosa - luteal cells; morphology; local factors; ovarian steroids

Introduction

The growth, development and function of the different cellular components of the ovary follicles is an integrated process encompassing both extraovarian signals (gonadotropins and metabolic hormones) and intraovarian factors [9]. The ovarian follicle and corpus luteum are the complex tissues (organoids) composed of parenchymal (steroidogenic cells) and nonparenchymal (fibroblasts vascular smooth muscle,pericytes and endothelial) cells [7]. There are many local factors influencing the specific functions of these cells nesessary for the reproduction. These factors could be classified as: 1.factors participating in the selection of follicles, which will continue to develop or will become atretic ones (ovarian insulin growth factor, transformig growth factor, fibroblast-like growth factor, epidermal growth factor, activin/inhibin systems etc.); 2. factors,provoking the stimulation or inhibition of steroidogenesis (leptin etc.); 3.local factors effecting the formation and the involution of corpus luteum, an exceptionally dynamic organ, which plays a central role in the reproductive process [10]; 4. extracellular matrix proteins, interacting directly with cell surface receptors and in this manner actively participating in ovarian functions [6].

The current study examine the possibility that selected bioactive nonsteroidal factors produced locally in the ovarian follicles participate in the follicular development and maturation. To characterize such possible effect a monolayer culture system of porcine and human ovarian granulosa and granulosa luteal cells (GCs and GLCs) was used.

Material and Methods

Granulosa and granulosa luteal cells were harvested from porcine antral follicles (4-10 month old pigs) as well as from ovaries of young (aged 25-30 years) and pre-, post- and menopausal women, as previously described [1]. The cells were then innoculated at a density of 1×10^{-6} viable cells and incubated at 37 °C in a humidified atmosphere of 5% CO_2 in air in DMEM and 5% fetal calf serum (FCS). The plating efficiency was approximately 45%. The cells were cultured for 3 or 4 days with medium change and addition of the bioactive substances (chondroitin-4-sulphate /C-4-S/-3 mg/ml; arginine-8 vasopressin /AVP/-5 μg/ml; oxytocin /OT/-5 mIU/ml; relaxin /RLX/ 3μg/ml; endothelin-1 /ET-1/-10^{-7}M) with or without gonadotropins (FSH-100ng/ml and LH-200ng/ml). At the end of each experiment media were collected, centrifuged and supernatants were stored at —20 °C until steroids were assayed. The steroid contain of the culture medium was determined by RIA [4].

Results and Discussion

The action of some locally produced factors on porcine and human ovarian morphology and steroidogenesis was studied. Using phase-contrast microscopy untreated granulosa cells appeared as a monolayer of flattened fibroblast-like cells. Upon two day exposure to oxytocin (50 mIU) and LH (200 ng/ml) aggregates of rounded, epitheloid-shaped cells were observed. Granulosa cells cultured 2 days in

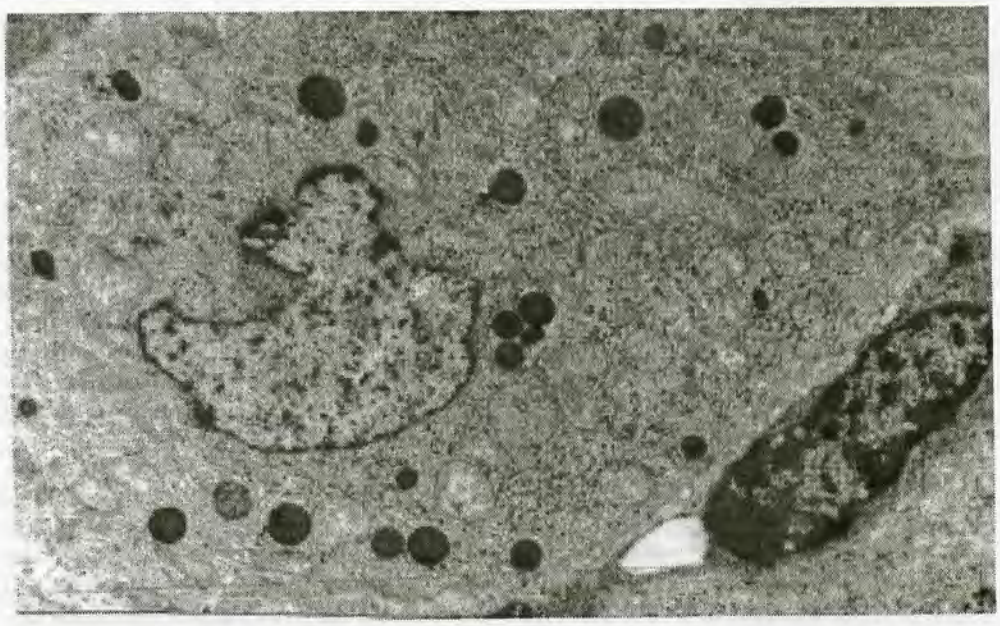

Fig. 1. Fine structure of cultured porcine granulosa cells - control (×6000)

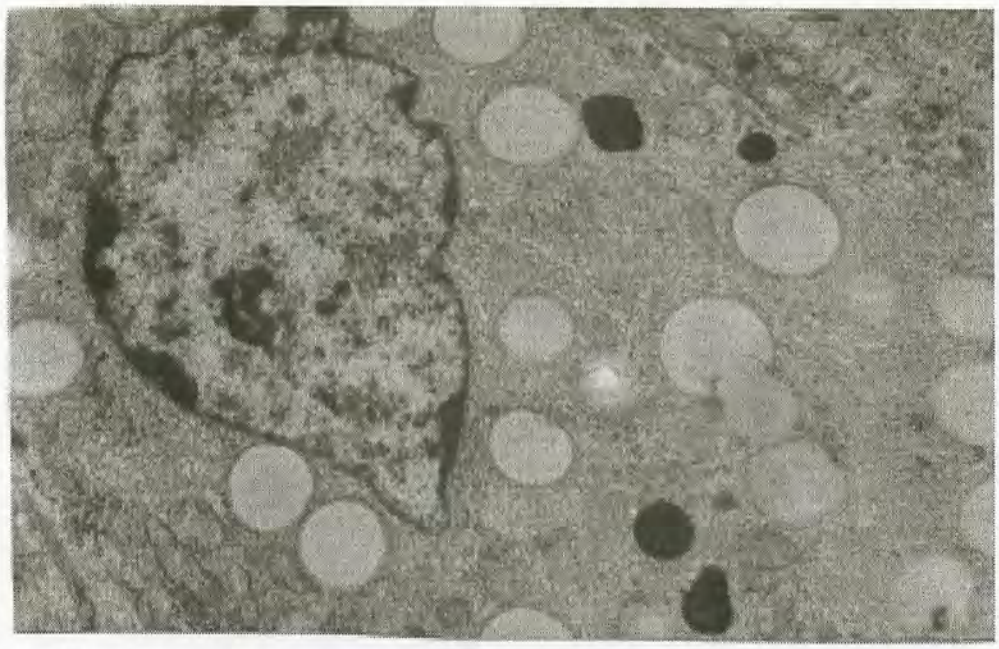

Fig. 2. Fine structure of cultured porcine granulosa cells, treated with OT (5mIU) and LH (200ng/ml) - increased number of lipid droplets, whorled smooth endoplasmic reticulum and mitochondria with tubular cristae (×6000)

media with OT and LH exhibited increased size, number and volume of lipid droplets, whorled smooth endoplasmic reticulum (SER) and mitochondria with tubular cristae as compared with controls (figs 1 and 2). The addition of FSH to media supplemented with OT minimized the changes induced by OT.

The nonapeptide hormone oxytocin alone increased progesterone secretion of granulosa and granulosa luteal cells. Over the course of GC culture, the production of progesterone was greatest in the presence of OT-5 (mIU) and LH (200 ng/ml). FSH had inhibitory effect on progesterone secretion in culture with OT. The nonapeptide arginine-8-vasopressin (5 μg/ml) and the polypeptide hormone relaxin (0,1 μg/ml) also stimulated basal progesterone and estradiol output and were additive in enhancing Gn-stimulated steroids (Fig. 3 — a, b). Incubation of GCs in media supplemented with these bioactive substances resulted in increased lipid bodies, mitochondria with tubular cristae, smooth endoplasmic reticulum and decreased rough endoplasmic reticulum content as compared with controls (Fig. 4).

The glycosoaminoglycan chondroitin-4 sulphate (3 mg/ml) inhibited basal and Gn-stimulated steroid production. Morphological analysis did not demonstrate changes in GC structure.

When grown in media with the vasoconstrictive peptide endothelin-1 (10^{-7} M) granulosa cells from human or porcine ovary (which in controls are epitheloid) became fusiform. In vitro ultrastructural analysis showed short undilated cisternae of granular endoplasmic reticulum, mitochondria with a limited number of cristae, free ribosomes and lysosomes. In vitro addition of ET-1 to the culture medium was followed by a marked inhibition of progesterone production in porcine GCs. Treatment with ET-1 and FSH failed to increase progesterone production over that seen with FSH

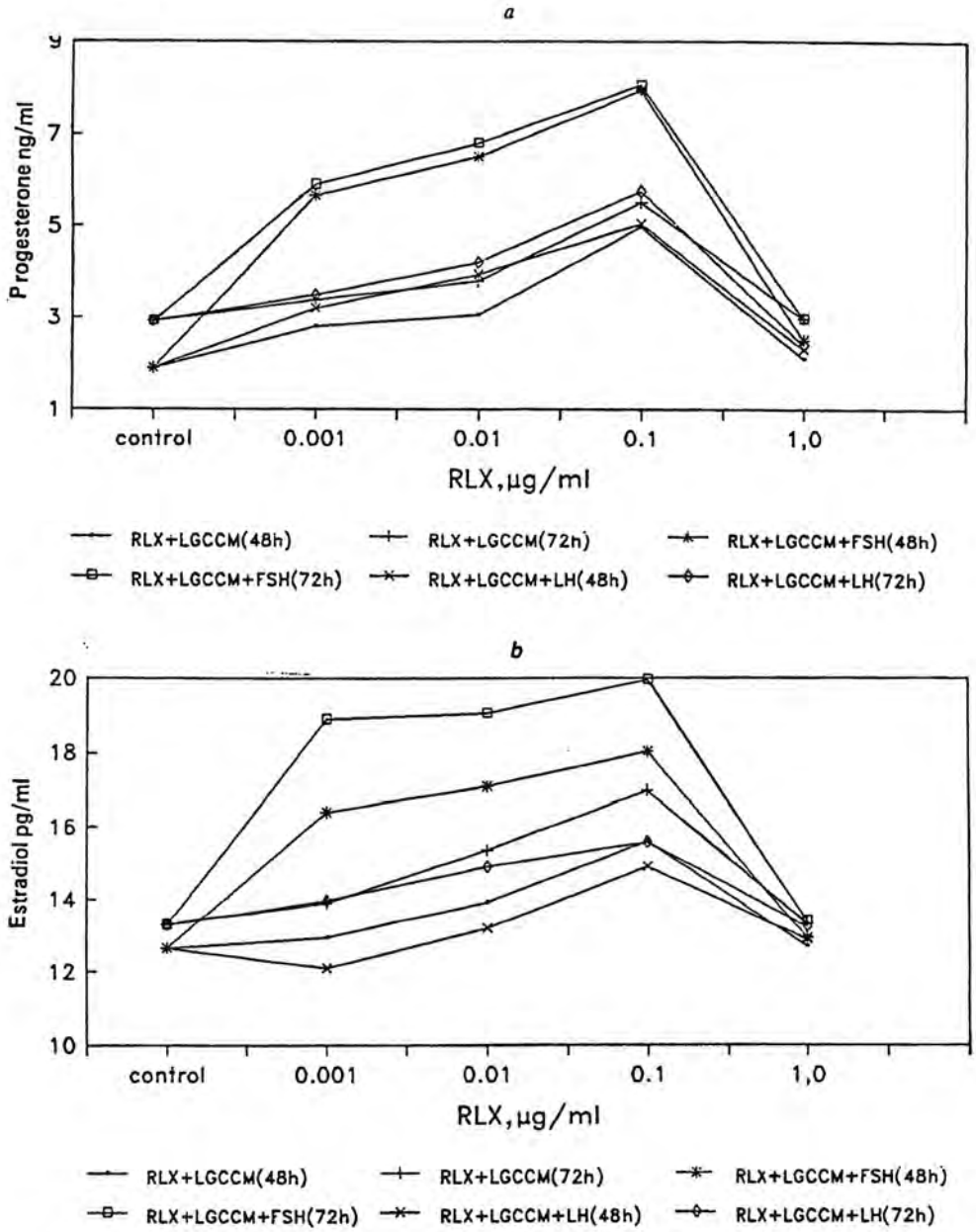

Fig. 3. In vitro effect of RLX and granulosa cell conditioned media on progesterone (*a*) and estradiol (*b*) production by porcine granulosa cells. The values represent the mean ±SE of 12 cutures from 3 experiments

only. Decrease of progesterone production was also observed when ET-1 was applied in cultured GCs and GLCs of human ovary (young patients) (Fig. 5) The progesterone biosynthesis was considerably reduced in the presence of FSH. Significant effect of the peptide on estradiol output was not seen. ET-1 application decreased basal and

FSH-stimulated progesterone production in GCs and GLCs of premenopausal women.

In response to the sequential stimulation by Gns, ovarian follicles follow a course of functional maturation that leads to ovulation and subsequent corpus luteum formation. One of the major features of follicular maturation is the differentiation and eventual luteinization of granulosa cells, a major constituent of an ovarian follicle. There is now increasing evidence suggesting that the gonadotropin regulation of GC differentiation is modulated by intraovarian, nonsteroidal regulators [3]. It is the microenvironment rather than the peripheral blood concentration of these intra-ovarian bioactive substances that modulates several aspects of GC function, morphological criteria related to steroidogenic machinery and Gn receptor number [1, 2]. In

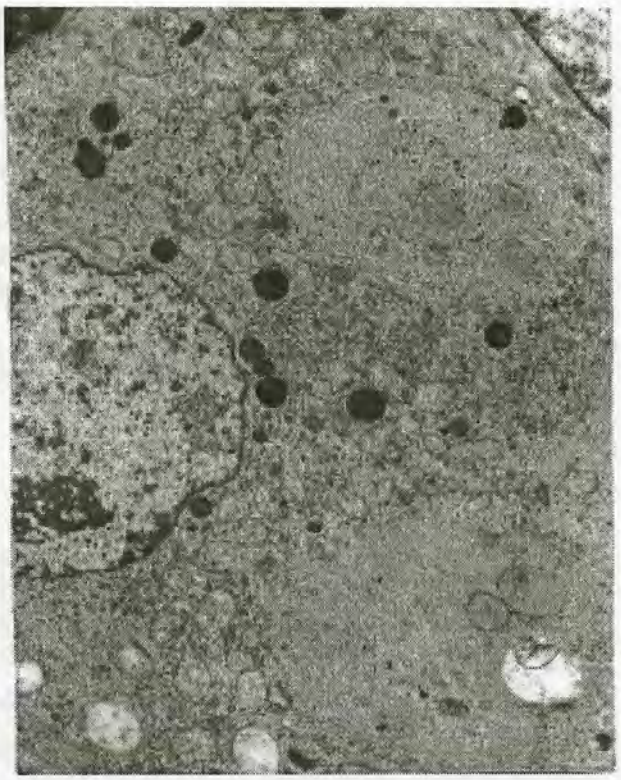

Fig. 4. Fine structure of cultured human GCs, treated with arginine-8-vasopressin (5μg/ml) — whorls of SER, mitochondria with tubular cristea and lipid droplets (×6000)

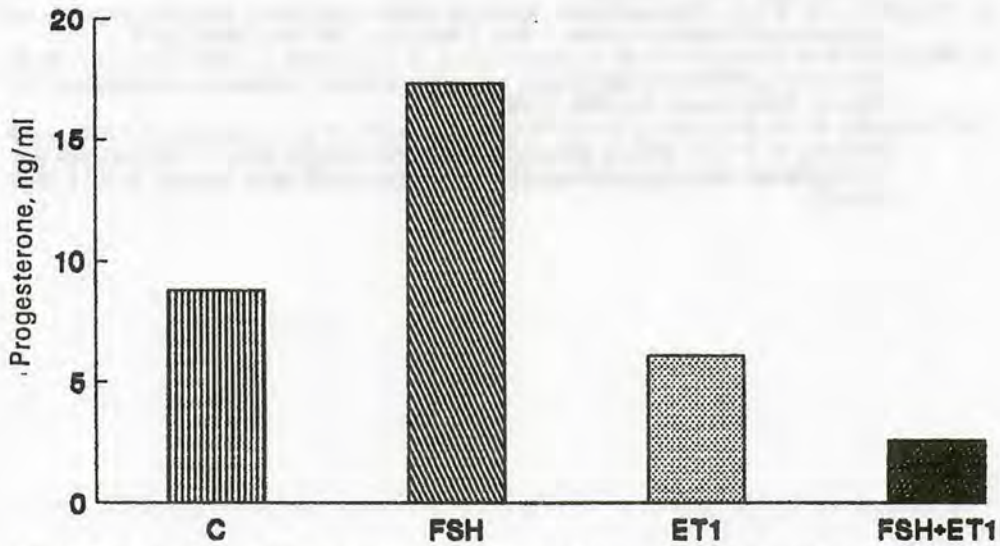

Fig. 5. Effect of ET-1 on the progesterone production by human granulosa luteal cells. The values represent the mean ±SE of 12 cultures from 3 experiments

this context, we reported the effect of some exogenously administered substances on GC and GLC steroid production and morphology. The findings of the modulatory action exerted by some local factors on the in vitro enhancement or reducement of progesterone and estradiol production is consistent with the literature [9, 7, 8] Controversial data that have been reported for some actions on GCs and GLCs and the discrepancy may be due to differences in the experimental models used. In conclusion, we may speculate that many of locally produced in human and porcine ovaries factors may play an important autocrine or paracrine role(s) at or adjacent to their sites of synthesis, thereby participating in the process of differentiation of GCs and GLCs.

During the past two decades nitric oxide signalling has been one of the most rapidly growing areas in biology [5].

Further studies could elucidate the role of nitric oxide (NO)/ nitric oxide synthase (NOS) system as a mediator involved in various ovarian processes (ovulation, luteolysis etc.).

References

1. Denkova, R. T., I. G. Ivanov, L. N. Kanchev. Porcine granulosa cell conditioned media as autocrine regulator of progesterone secretion. — Reprod. fertil. Dev., **5**, 1993, 95-102.
2. Denkova, R. T., B. Nikolov, A. Russinova. Porcine granulosa cells produce a progesterone secretion inhibitory activity. — Endocr. Reg., **33**, 1999, 33-37.
3. Hillier, S. G. Intragonadal regulation of male and female reproduction. — Ann. D Endocrinol., **60**, 1999, No 2, 111-117.
4. Kanchev, L. N., H. Dobson, W. R. Ward and R.J. Fitzpatrick. Concentration of steroids in bovine peripheral plasma during the oestrous cycle. — J. Reprod. Fertil., 4B,1976, 341-345.
5. Murad, F. Cellular signalling with nitric oxide and cyclic GMP. — Brasil. J. Med. a Biol. Res., **32**, 1999, No 11, 1317-1327.
6. Oksioki, S. Sallinen, E. Vuorio, L. Anttila. Cyclic expression of mRNA transcripts for connective tissue components in the mouse ovary. — Moll. Hum. Reprod., **5**, 1999, No 9, 803-808.
7. Reynolds, L. P., D. A. Redmer. Growth and development of the corpus luteum. — J. Reprod. Fertil., Suppl. **54**, 1999,181-191.
8. Tahei, A., H. Kogo. Dexamethasone increases follicle- stimulating hormone secretion via suppression of inhibition in rates. — Eur. J. Pharmac., **386**, No 1, 2000, 69-74.
9. Webb, R., B. K. Campbell, H. A. Garverick, J. G. Gong, C. G. Gutierrez, D. G. Amstrong. Molecular mechanisms regulating follicular recruitment and selection. — J. Reprod. Fertil., Suppl. **54**, 1999, 33-48.
10. Yamada, S., H. Fujivara, T. Honda, T. Higuchi, T. Nakayama, T. Inoue, M. Maeda, S. Fujii. Human granulosa cells express integrin alpha 2 and collagen type IV:possible involvment in granulosa cell luteinization — Moll. Hum. Reprod., **5**, No 7, 1999, 607-617.