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The Role of Neopterin in Regulating Human Ovarian Granulosa Cells Growth (Proliferation/Differentation) and Steroidogenesis in vitro[1]

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The in vitro influence of neopterin (NeoPt) on the human ovarian granulosa cells' (hOGCs') growth (proliferation/differentiation) and steroidogenesis has been studied in conditions of hOGCs' cultures. It was established that the biologically active substance NeoPt, at the doses 12,5 — 25 μg/ml culture media, acts as hOGCs' proliferative and colony-stimulating factor (h-OGCs-CSF), probably via activation of the nuclear proliferative (transcription) factor — NF-κB. It has been also concluded that exogenously added neopterin attenuated estradiol- (E2-) and stimulated progesterone (P4) secretion / accumulation in the hOGCs'culture media and that the luteinizing activity of NeoPt in vitro — as a probable luteotrophic factor, seems to depend on the degree of hOGCs' differentiation / luteinization.

Key words: neopterin (NeoPt), human ovarian granulosa cells (hOGCs), hOGCs'proliferation / differentiation and steroidogenesis in vitro, hOGCs'colony-stimulative factor (hOGCs-CSF), hOGCs' luteotrophic factor, nuclear proliferative (transcription) factor — NF-κB.

Ovarian follicular/granulosa cell (OGC) proliferation and differentiation as a hormonally regulated process depends not only on a peptide and steroid hormones, but also on some cell growth factors and local biologically active substances — autocrine/paracrine regulators [1 — 5; 7]. The effects of neopterin (NeoPt) on human ovarian ganulosa cells' (h-OGCs') growth in vitro have not yet been examined.

The aim of the present study is to investigate the influence of NeoPt on the in vitro growth (proliferation / differentiation) and steroidogenesis of hOGCs.

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Material and Methods

The investigations were carried out on ovarian follicular (granulosa) cells obtained from follicules of 6 young women (aged 27-31 years) as well as from follicules of three women (aged 31-39 years) undergoing follicular aspiration for the purposes of fertilisation in vitro (IVF). In the last case, granulosa cells containing follicular fluids were obtained from ovarian follicules during oocyte retrieval — as part of IVF.

HOGCs were isolated from antral follicles by the non-enzymatic needle puncture method and were *cultured* in Dulbecco's minimal essential medium (DMEM) in the presence of 10% fetal calf serum (FCS), as described by D e n k o v a et al. [1 — 3].

The modulatory action of NeoPt (Schircks Laboratories - Jona, Switzerland) on the proliferation/differentiation of hOGCs and its steroid secretion has been studied using freshly isolated hOGCs, cultured in vitro in the presence of exogenously added NeoPt at doses 10-25 μg/ml culture medium.

Cellular DNP, RNP and basic (cationic) proteins have been studied in the conditions of h-OGCs' monolayer cultures after the application of the cytochemical method of Zvetkova and Jelinek [8].

Concentrations of estradiol (E2) and progesterone (P4) in the culture condition media were determined by commercial radioimmunoassay kits: all the procedures have being conducted at the Clinical Laboratory of the Vth town Hospital "Klementinska" — Sofia.

Results and Discussion

Significant stimulatory effect of NeoPt on the cell proliferation/differentiation (luteinization) of the cultured H-OGCs has been observed. In the conditions of monolayer cultures — in the presence of exogenously added NeoPt at doses 10-25 μg/culture medium, hOGCs formed abundant cell colonies and clusters (Fig. 1, 2), containing cells in different state of proliferation/differentiation (luteinization):

♣ Cell groups, containing undifferentiated granulosa cells, with localized in its periphery more differentiated hOGCs, have been well presented in the cultures at 24th h of cultivation (Fig. 1). In this case one could see also bridges of differentiated cells — localized between hOGCs colonies and clusters.

♣ At the 48th-72th of cultivation, the differentiated hOGCs were prevalent (Fig. 2) — with very well expressed cytoplasmic protrusions, perinuclear hallo stained positively for basic proteins, and some of them with abundant — distributed in the whole cytoplasms diffusely staining and / or granules of basic (cationic) proteins, as a cytochemical sign of cell differentiation and/or luteinization/steroidogenesis (2) (Fig.2).

The levels of estradiol (E2) and progesterone (P4) in the culture media — from the protocol (at the 72 h of

Table 1. The levels of Estradiol (E2) and Progesterone (P4) in the culture media:

— **K1** and **K2** — *controls* (OGCs' cultivation without NeoPt in the cultures);
— **N1, N1,1** — *E2 in the ml/culture media* of NeoPt-treated hOGCs' cultures;
— **N2, N2,2** — *P4 in the ml/culture media* of the hOGCs' cultures, treated with exogenously added NeoPt.

Estradiol ***(pmol/l)***	**K1**	**230**
	N1	**80**
	N1,1	**140**
Progesterone ***(nmol/l)***	**K2**	**84**
	N2	**162**
	N2,2	**212**

hOGCs cultivation), have been presented (Table 1). The lower estradiol levels and the very higher ones of progesterone have been obtained — under influence of exogenously added NeoPt in hOGCs cultures. The data from the measurements of E2 and P4 in the hOGCs culture media seems to depend on the degree of the hOGCs in vitro differentiation/luteinization, which is activated in the presence of the exogenously added NeoPt. It has been concluded that NeoPt attenuated E2 and stimulated P4 secretion of hOGCs in vitro and that steroidogenesis (E2 and P4 accumulation in culture media) depends also on the time of cultivation – being very well expressed at the 72 h of in vitro cell growth and differentiation.

On the basis of the results obtained could be concluded that the biologically active substance NeoPt acts in vitro as ***hOGCs proliferative*** and ***colony-stimulating factor (hOGCs-CSF),*** probably via activation of the *nuclear proliferative (transcription) factor* — **NF-κB** [6, 9].

Conclusions

Biologically active substance NeoPt acts in vitro as ***H-OGCs proliferative*** and ***colony stimulating factor (hOGCs-CSF),*** probably via activation of the *nuclear proliferative (transcription) factor* — NF-κB.

From the measurements of **estradiol (E2)** and ***progesterone*** **(P4)** in the culture media, it has been concluded that *NeoPt attenuated E2 and stimulated P4 accumulation in cultures* and that this ***steroidogenic/luteinizing NeoPt activity*** seems to depend on the degree of hOGCs' differentiation/luteinization in vitro.

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