UDK 577 + 619 (05) YU SSN 0567—8315

ACTA VETERINARIA

FACULTY OF VETERINARY MEDICINE BEOGRAD, YUGOSLAVIA

Vol. 41 No. 4 Pp. 155—156 Beograd 1991

RESPONSE OF THE HYPOTHALAMIC-GONADAL SYSTEM IN JUVENILE RATS AFTER ADMINISTRATION OF PROGESTERONE TO PREGNANT MOTHERS

ODGOVOR HIPOTALAMO-GONADNOG SISTEMA JUVENILNIH PACOVA PO APLIKACIJI PROGESTERONA GRAVIDNIM MAJKAMA

NADA STOŠIĆ-BOGDANOVIĆ and ANITA RADOVANOVIĆ

Acta Veterinaria (Beograd), Vol. 41. No. 4, 155-166, 1991

UDK 619:612.018.2:577.175.32

RESPONSE OF THE HYPOTHALAMIC-GONADAL SYSTEM IN JUVENILE RATS AFTER ADMINISTRATION OF PROGESTERONE TO PREGNANT MOTHERS

NADA STOŠIĆ-BOGDANOVIĆ and ANITA RADOVANOVIĆ

Department of Histology and Embriology, Faculty of Veterinary medicine, Bulevar JNA 18, 11000 Beograd, Yugoslavia

(Received, 15 March 1991)

The effect of progesterone, when administered to rats during pregnancy, was studied in the litters (The hypothalamic paraventricular nuclei, the germ epithelum of the testis and on the process of folliculogenesis). Mature rats of the AO strain were mated and progestrone was injected into the females on days 3, 5 and 7 during pregnancy. After weaning, 30 day old litters of intact and treated rats were sacrificed. The cytological features of neurosecretory cells of the paraventricular nuclei (NPV) in the offspring were analysed as well as germ epithelium cell associations in the testis and different follicles of the ovary. Progestrone administered to pregnant rats altered the cytological properties of neurosecretory cells of the hypothalamic NPV. The NPV contained individual light-active cells, while dark-low active cells with accumulated granules in the cytoplasm were dominant in the progeny. In the testis progesterone suppressed spermatogenesis, due to regression of certain cells of the germ epithelium. Normal development of folliculogenesis was altered in the ovaries of the progeny.

INTRODUCTION

Steroid hormones, including progesterone, have key roles in the control of animal reproduction, especially secretion by the pituitary cells. In certain regions of the hypothalamus, biochemical methods have been used to identify progestrone receptors, which were later determined immunocytochemically in many animals using antibodies for receptor molecules (birds, guineapigs etc.). During differentiation of cerebral neurones, genes for steroid hormone receptors are expressed (Martinez-Vargas et al., 1974, 1975). progestrone immunoreactive cells have been identified in the paraventricular and periventricular region of the anterior hypothalamus, Organon vasculosum and lamina terminalis, during the early stage of embryonic development in chicks (Warembourg, 1986; Sterling et al., 1987). Gonadotrophin releasing hormone (GnRH) is detected in the brain

of the human foetus, stimulating gonadotrophin cells in the pituitary gland at an early stage (Aubert et al., 1985). Androgen and estrogen target cells are differentiated in the central nervous system during embryonic development of chicks (Reid et al., 1981; Weil, 1986) and the gonadotrophic effect of the putiutary gland is active before laying commences. Gonadotrophic cells have been discovered in rat foefus at 17-18 days of pregnancy (Bégeot et al., 1981). Gonadotrophin LH was determined by radioimmunoassay on the 17th day of pregnancy in the pituitary gland and plasma (Smets et al., 1989). Foetal gonads are dependent on the secretion of pituitary glands of the foetus during their development, especially in mid- and late gestation (Bela, 1978). In birds, testosterone, alpha-DHT, eostradiol and progesterone were detected as early as day 9 after incubation, in both male and female embryos (Schumacher et al., 1987).

Despite extensive research, the exact role of progesterone in the regulation of reproduction remains unsettled. There is little information of the effect of progesterone on the hypothalamic-gonadal system of litters, where the mothers were treated with progesterone during pregnancy. Consequently, the purpose of this study was to determine the specificity of response of hypothalamatic paraventricular neurosecretory cells, testes and ovaries of litters, after administration of progesterone to pregnant rats.

MATERIAL AND METHODS

Mature rats, Albert Oxford (AO) strain, were adapted to laboratory conditions. The males were separated from the females. All experimental animals were kept under identical conditions of humidity, temperature and feeding. Vaginal smears were taken from isolated females between 8-10 a. m. during a two week period. Females were mated and received progesterone (RO "Galenika"), i/m, in doses of 3.1 mg/per animal, on the third, fifth and seventh day of pregnancy. After partus, 30 day old litters (40 animals) and treated mothers were weighed and sacrificed. Testes and ovaries were isolated and weighed. They were then fixed in Bouin solution and stained with Weigert – haematoxylin and eosin and azan. Isolated hypothalamus was fixed in. Bouin solution and stained with paraldehyde fuchsin, according to the method of Gabe-Gomory. The relative frequency of cell associations of the germ epithelium of the testis was determined on cross sections of the seminiferous tubules. Sperm production per rat was determined from the number of spermatids. The number of primary, growth, preantral and antral follicles was calculated on successive sections of the ovary. Body mass, testis, ovary and pituitary mass, as well as, the number of individual cell associations of germ epithelium of the testis and certain elements of folliculogenesis of the ovary, were statistically analysed by means of Student's T-test for attributive properties.

RESULTS

When progesterone was injected into pregnant rats, it produced a significant decrease of body mass in male offspring, testis, ovary and pituitary mass ($p < 0.001$). There were no significant changes in the body mass of female offsprinng (Table 1; Figure 1).

Table 1. Body, testis, ovary and pituitary mass of control litters (C) and those from mothers treated with progesterone (Pr)

Tissue	Sex	C	Pr
Body mass (g)	M	67.67 +2.21	43.25 ±6.38***
Body mass (g)	F	43.20 +3.06	41.75 ±2.28
Testis mass (g)	M	0.64 +0.05	0.36 ±0.008***
Ovary mass (mg)	F	0.041+0.002	0.031±0.005**
Pituitary mass (mg)	M	3.14 +0.55	1.89 ±0.30***
Pituitary mass (mg)	F	2.7 +0.08	1.81 ±0.06

Values are mean ± S.D.
*** significantly different ($p<0.001$)
** significantly different ($p<0.01$)

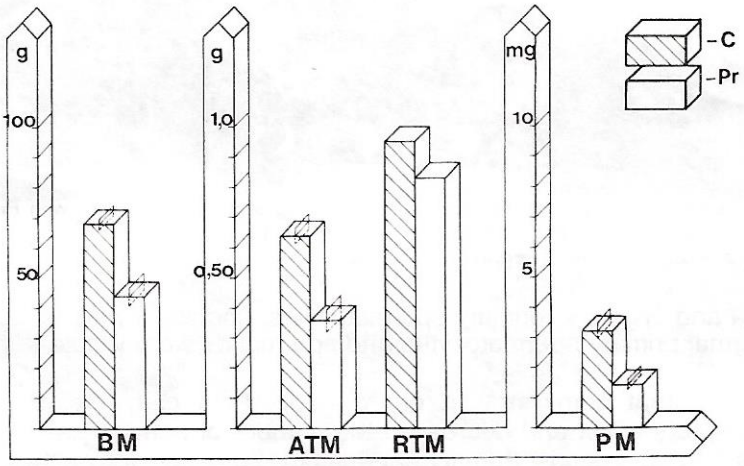


Fig. 1
Figure 1. Body mass (BM). absolute (ATM) and relative (RTM) testis mass and pituitary mass (PM) in control litters (C) and in litters from mothers treated with progesterone (Pr).

Our cytochemical analysis showed inhibition of synthetic proceses in *hypothalamic paraventricular* neurosecretory cells of male offspring originating from mothers injected with progesterone. Evident changes in appearance were noted in light (L-type) and Golgi type (G-type) cells, representing the active cells, and D-type or low active cells and regressive (R-type) cells in the paraventricular nuclei. A decrease of L-type and G-type cells was accompanied by a marked increase in D-type, or low active cells having numerous neurosecretory granules in the basophilic cytoplasm and the appearance of regressive forms. Tha same changes were noticed in the periventricular parts of the paraventricular nuclei (Figure 2).

In the *testis* of offspring whose dams had received progesterone, there was suppression of spermatogenic activity and in germ epithelium a row of

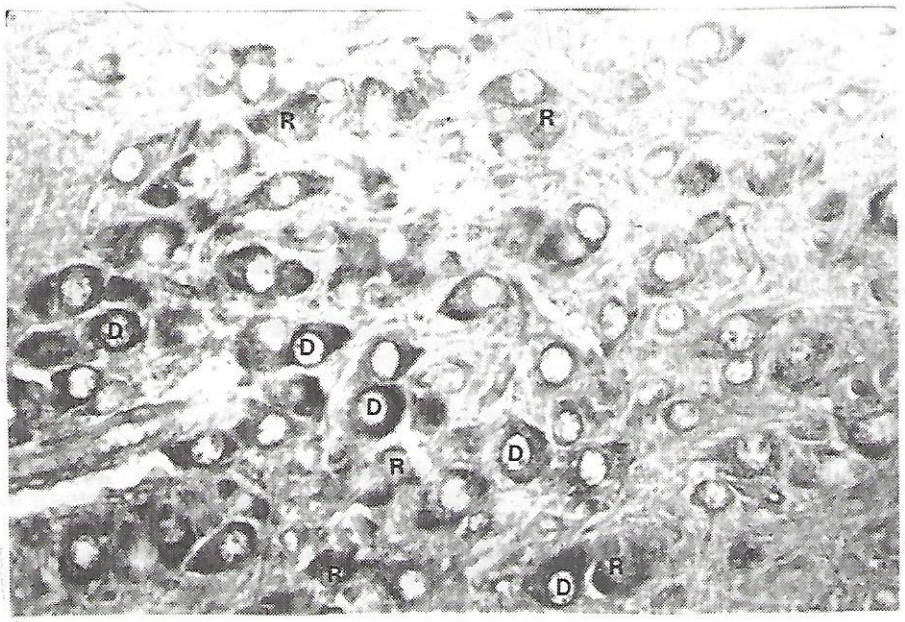


Figure 2. Hypothalamic nucleus paraventricularis in the offspring of a mother treated with Pr.
D — accumulation of neurosecretory granules in the cytoplasm of dark-low active cell;
R — neurosecretory cells in regression.

spermatogonia and zygotene primary spermatocytes, showed persistence. No pachytenic normal primary spermatocytes and spermatids were visible (Figure 3).

It is evident that administration of Pr induced an early meiotic and postmeiotic inhibitory effect and decreased the number of primary pachytenic spermatocytes and spermatids. Primary spermatocytes, especially pachytenic, were reduced and increasingly degenerated during meiotic division. Degenerative changes were followed by progressive condensation of nuclear chromatin granules which coalesced into several pycnotic masses. As the cells became necrotic, they formed spherules which contained a single condensed nuclear mass (Figure 4).

The pachytenic spermatocytes were reduced by 70% ($p < 0.001$). The percentage of spermatids significantly decreased the male offspring of treated mothers (0.6%) compared to control progeny (35.8%) (Figure 5).

The results show that the orderly progression of the first generation of spermatogenesis and early spermiogenesis was disrupted.

In the *ovaries* of 30 day old female offspring, progesterone caused inhibition of follicle development, while growth and antral follicles were very sensitive. There were marked differences in the histological appearance of granulosa cells from normal and regressed follicles. Granulosa cells from regressed follicles were heterogenous, with general destruction of the cytoplasm and pycnotic nuclei (Figure 6 A, B).

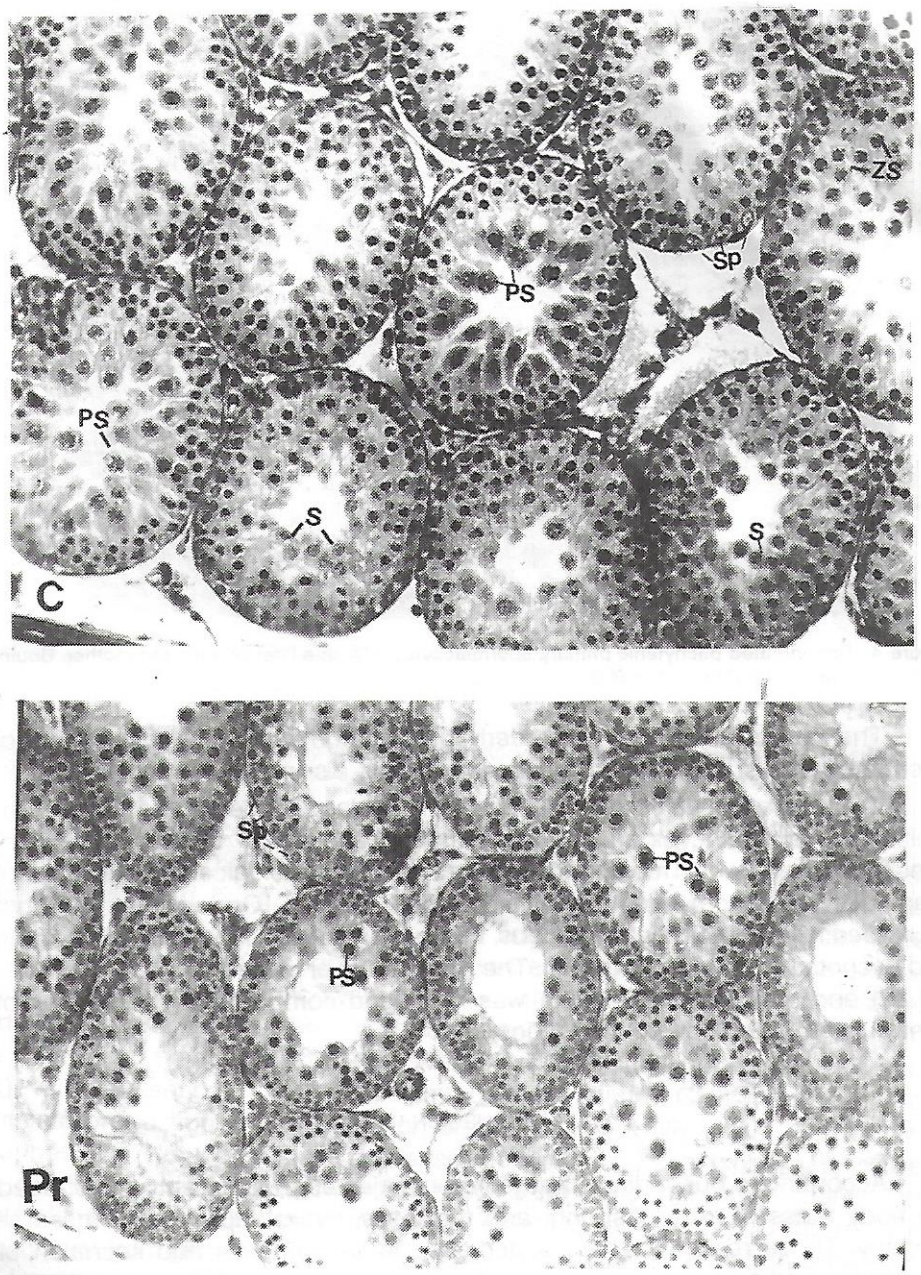


Figure 3. Seminiferous tubules in the testis of control offspring (C) and the progeny of treated mother (Pr). Bouin. Haematoxylin-eosin. x 560 (C), x 460 (Pr).
Sp — spermatogonia, ZS — zytogene primary spermatocytes, PS — pachytene primary spermatocytes, S — spermatids.

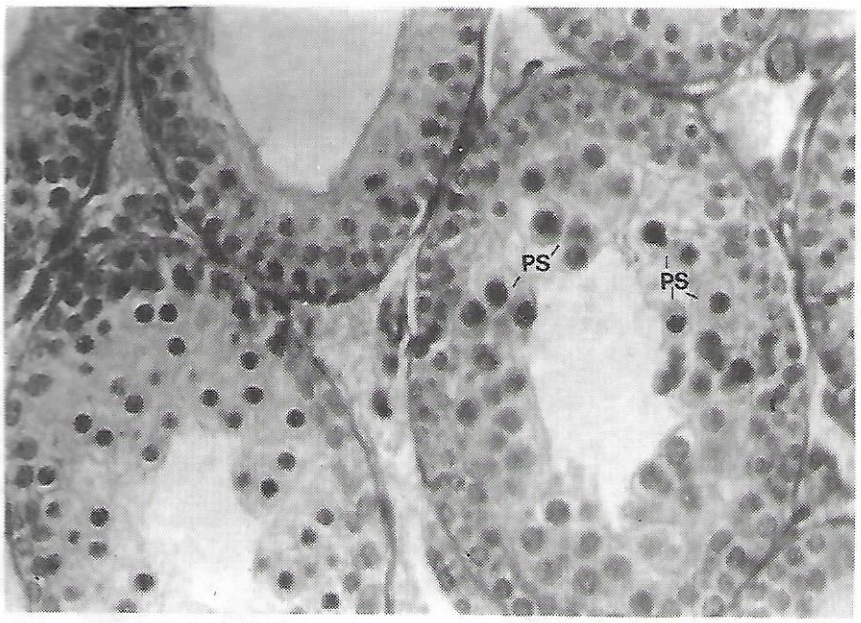


Figure 4. Degenerated pachytenic primary spermatocytes (PS) in a litter of a treated mother. Bouin. Haemathoxylineosin. x 900;

The results show that the orderly progression of the first generation of spermatogenesis and early spermiogenesis was disrupted.

In the *ovaries* of 30 day old female offspring, progesterone caused inhibition of follicle development, while growth and antral follicles were very sensitive. There were marked differences in the histological appearance of granulosa cells from normal and regressed follicles. Granulosa cells from regressed follicles were heterogenous, with general destruction of the cytoplasm and pycnotic nuclei (Figure 6 A, B). The percentage of regressed growth follicles (41.22) and antral follicles (54.55) was increased compared to the follicles of control litters (28.57 and 34.61) (Figure 7).

DISCUSSION

According to our findings, progesterone injected to pregnant rats reduced the body mass of male offspring, and the testis, ovary and pituitary mass all progeny. These results indicate a decrease in biosynthesis and secretion of the corresponding hormones, especially growth hormone (GH) and prolactine (PRL), which are, according to Chandola and Thapliyal (1972), directly or indirectly involved in the growth and development of the mentioned organs and the entire body.

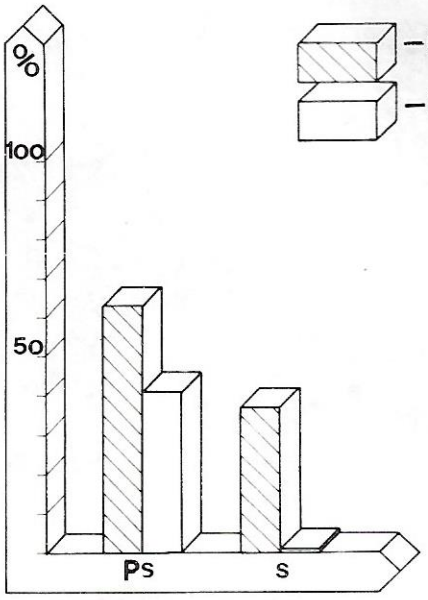
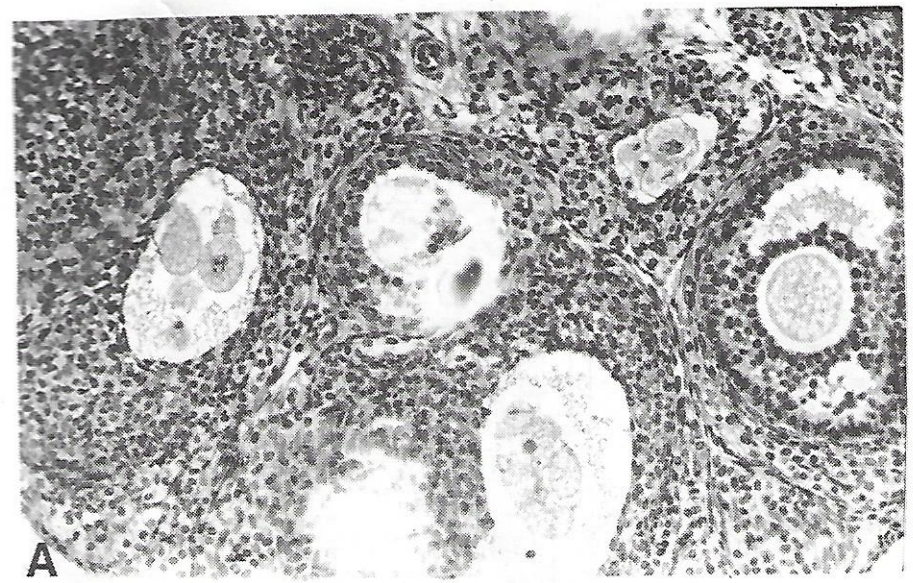


Figure 5. Percentage of pachytenic primary spermatocytes (PS) and spermatids (S) in litters of mothers treated with Pr compared to the control group (C).

Our cytochemical analysis has shown inhibition of synthetic and releasing processes in NVP. The decrease of L- and G-type, active neurosecretory cells, was accompanied by a marked increased in low activity cells, as well as regression in the paraventricular nuclei. At first, Sawechenko et al. (1983) and later Wilson et al (1986), suggested that secretion of GH may depend on the maturity of the serotonergic system (5HT) within the central nervous system, particulary in the NPV region. There are serotonergic neurones originationg in the midbrain which innervate PVN, while 5HT stimulates release of GH and ACTH. The presence of a critical plasma concentration of GH is neccessary to stimulate production and/or secretion of a fully biologically active form of LH. Changes in PVN suggest that a decrease in the production of biologically active LH results in the reduction of ATP and cAMP and thus the quantity of proteinkinases essential for aromatization of androstenedione and the release of estrogen from growing follicles. An insufficient quantity of estrogen eliminates the local reactions of estrogen on granulosa cells which leads to degeneration of the same cells. Under our experimental conditions, in the ovaries of litters from dams treated with progesterone, the percentage of regressed follicles in growth and antral follicles was elevated. This may be due to an insufficient



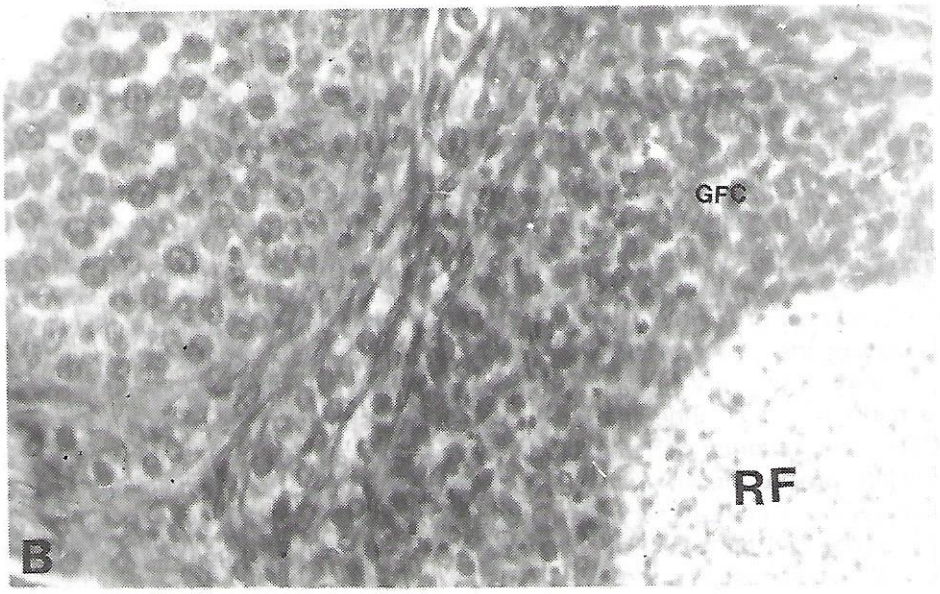


Figure 6 A. Regression of follicles (RF) in early stages of folliculogenesis in a litter of a mother treated with Pr;
B. General destruction of cytoplasm with pycnotic nucleus of granulosa follicle cells (GFC) in the same litter.
Bouin. Haematoxylin-eosin. x 560 (A), x 900 (B).

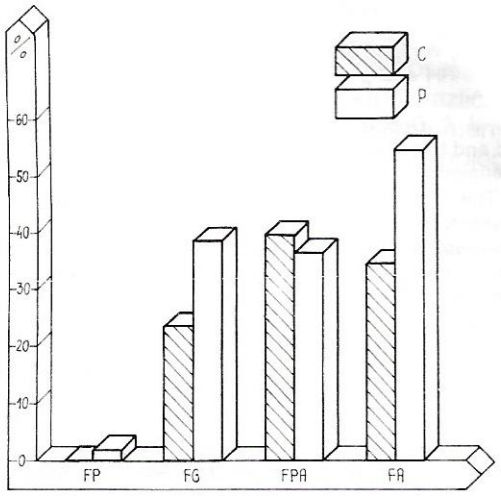


Figure 7. Percentage of altered follicles compared to all follicles in certain stages of folliculogenesis in control offspring (C) and litters of treated mothers (Pr). Significant increase in number of growth (FG) and antral follicles (FA).
FP — primary follicles, FG — growth follicles, FPA — preantral follicles, FA — antral follicles.

concentration of cortisol from the adrenal glands which altered cytological properties of granulosa cells, since the findings of Ben-Rafael et al. (1988) suggest that, in addition to pituitary and ovary hormones, cortisol may also be directly involved in granulosa cell function.

When progesterone was injected into pregnant rats, it supressed spermatogenesis and reduced the number of primary pachytenic spermatocytes and spermatids in the offspring. The longterm effect of progesterone is inhibition of control mechanisms of androgen secretion which are essential for normal development of the meiotic and postmeiotic phase of spermatogenesis. Decreased concentrations of LH and FSH presumably inhibit testis stimulation which is required to stimulate androgen secretion essential for maintaining spermatogenesis.

Research carried out so far indicates that changes in the neurosecretory cells of NVP are brought about by altered maturity of the hypothalamic-pituitary-gonadal system. The longterm action of progesterone affects mechanisms of biosynthesis and androgen and estrogen secretion, which are required for normal development of the first generation of spermatogenesis and folliculogenesis.

Futher investigation will provide additional data on the longterm effect of progesterone on the hypothalamic-pituitary-gonadal system of litters from dams treated with Pr.

REFERENCES

1. *Aubert, M. L., Bégeot, M., Winiger, B. P., Morel, G., Sizonenko, P. C., Dubois, P. M.* 1985. Ontogeny of hypothalamic GnRH and pituitary GnRH receptors in fetal and neontal rats. *Endocrinol., 116*, 1565-1576.
2. *Bégeot, M., Dupuoy, J. P., Dubois, M. P., Dubois, P. M.* 1981. Immunocytological determination of gonadotropic and thyrotropic cells in fetal rat anterior pituitary during normal development and under experimental conditions. *Neuroendocrinol., 32*, 285-294.
3. *Bela, J. G.* 1978. The hypothalamo-hypophysial-gonadal system in foetal rhesus monkeys. *Research in Reproduction, 5*, 3-4.
4. *Ben-Rafael, Z., Benadiva, A. C., Garcia, J. C., Flickinger, L. G.* 1988. Cortisol stimulation of estradiol and progesterone secretion by human granulosa cells is independent of follicle-stimulating hormone effects. *Fertility and sterility, 49*, 813-816.
5. *Chandola, A. and Thapliyal, J. P.* 1972. Effect of growth hormone and prolactin on the body weight and thyroid activity of Spotted Munia. *Annales d'Endocrinol., 33*, 583-591.
6. Martinez-Vargas, M.G., Sar, M. and Stumpf, W. E. 1974. Brain targets for androgens in dove (Streptopelia Risoria). Am. Zool., 14, 1285
7. *Martinez-Vargas, M. G., Gibson, D. B., Sar, M. and Stumpf, W. E.* 1975. Estrogen target sites in the brain of chick embryo. *Science, 190*, 1307-1308.
8. *Reid, F. A., Gasc, J. M., Stumpf, W. E. and Sar, M.* 1981. Androgen target cells in spinal cord, spinal ganglia and glycogen body of chick embryos. *Exp Brain Rec. 44*, 243-248.
9. *Sawchenko, D. E., Swanson, L. W., Steinbusch, H. W. M. and Verhoofstad, A. A. J.* 1983. The distribution and cells of origin of serotonergic inputs of the paraventricular and supraoptic nuclei of the rat. *Brain Res., 277*, 355-360.
10. *Smets, G., Velkeniers, B., Herregodts, P., Vanhaelst, L., Gepts, W. and Hooghe-Peters, E.* 1989. Ontogeny of hormone-secreting cells of the rat pituitary gland: an immunocytochemical study on dissociated cells. *Histochemic. Journal, 21*, 337-342.
11. *Sterling, R. G., Gasc, J. M., Sharp, P. J., Renoir, J. M., Tuohimaa, P. and Baulieu, E. E.* 1987. The distribution of nuclear progesterone receptor in the hypothalamus and forebrain of the domestic hen. *Cell. Tiss. Res., 248*, 201-251.
12. *Schumacher, M., Sulon, J. and Balthazart, R.* 1987. Changes in serum concentrations of steroids during embryonic and posthatching development of male and female Japanese quail (Coturnix coturnix Japonica). *J. Endocrinol., 118*, 127-134.
13. *Warembourg, M., Logeat, F. and Milgrom, E.* 1986. Immunocytochemical localization of progesterone receptor in the guinea pig central nervous system. *Brain Res., 384*, 121-131.
14. *Weill, C.* 1986. Characterization of androgen receptors in embryonic chick spinal cord. *Dev. Brain Res., 24*, 127-132.
15. *Wilson, C. A., Mc Neilly, A. S. and Yeo, T.* 1986. Mechanism by which 5HT stimulates ovulation in the immature rat treated with pregnant mare serum gonadotrophin. *Acta Endocrinol., 111*, 253-240.

ODGOVOR HIPOTALAMO-GONADNOG SISTEMA JUVENILNIH PACOVA PO APLIKACIJI PROGESTERONA GRAVIDNIM MAJKAMA

NADA STOŠIĆ-BOGDANOVIĆ i ANITA RADOVANOVIĆ

SADRŽAJ

Izučavano je delovanje progesterona, aplikovanog majkama u toku graviditeta, na paraventrikularno jedro hipotalamusa (NPV), germinativni epitel semenika i proces folikulogeneze jajnika mladunčadi. Polno zreli pacovi, AO

rase, su pareni i ženkama je injiciran progesteron (Pr) 3, 5 i 7. dana graviditeta. Posle odvajanja od majki, 30 dana stari mladunci kontrolnih i tretiranih majki su žrtvovani. Analizirane su citološke odlike neurosekretornih ćelija NPV, asocijacija ćelija germinativnog epitela semenika i različitih folikula jajnika mladunčadi. Progesteron aplikovan gravidnim majkama menja citološke odlike neurosekretornih ćelija NPV hipotalamusa. NPV sadrži pojedinačne svetle-aktivne ćelije, a preovladavaju tamne-malo aktivne ćelije sa akumuliranim granulama u citoplazmi. U semeniku Pr supresira spermatogenezu regresijom pojedinih ćelija germinativnog epitela. U jajniku mladunčadi promenjen je normalan razvoj procesa folikulogeneze.