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Morphofunctional Changes in Guinea Pig Ovaries in Experimental Chronic Herpesvirus Infection

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The ovaries of adult guinea pigs infected with herpesvirus-2 were examined by light microscopy under conditions of latent infection and relapse of infectious process. Morphofunctional changes in the ovarian follicles at all stages of folliculogenesis and in ovarian stroma were caused by the negative effects of type 2 herpes simplex virus.

Key Words: *ovaries; follicles; herpesvirus; granulosa*

According to the WHO data, about 90% of the population of the Earth are chronic carriers of *Herpesviridae* family viruses, one of which is type 2 herpes simplex virus (HSV-2) [2,4].

Infectious process caused by HSV-2 runs an asymptomatic and relapsing course [4]. Disorders in the neuroimmunoendocrine regulation of homeostasis caused by various stress factors, concomitant diseases, dyshormonal disorders, or pregnancy provoke activation of the virus and hence, a relapse of herpesvirus infection.

The incidence of HSV-2 infection in the Russian Federation is 13 per 100,000 population, in Moscow 50 per 100,000 population [3].

The virus is detected mainly in women of reproductive age (due to obligatory checkups of pregnant women and women suffering from sterility). Detection of HSV-2 in pregnant women is a prognostically unfavorable factor suggesting possible infection of the fetus during pregnancy and labor and subsequent disorders in the postnatal development of the child.

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The morphofunctional changes in the female reproductive system under conditions of chronic genital herpesvirus infection remain little studied, though these studies are practically important.

We studied morphofunctional changes in the ovaries of guinea pigs with experimental chronic genital herpesvirus infection during remission and exacerbation.

MATERIALS AND METHODS

The study was carried out on adult female guinea pigs sensitive to HSV-2; the morphogenetic folliculogenesis processes and reactive characteristics of the ovaries in these animals are similar to those in humans.

The animals were infected by intravaginal application of HSV-2 (strain MS) [6]. The animals were kept in boxes over 1 year (period of observation). Short-term spontaneous relapses of infectious processes manifested in vesicular rash and edema of genitals.

The animals were divided into 3 groups: 1) normal adult guinea pigs (control; n=10), 2) animals with HSV-2 infection in remission (n=12), and 3) animals with exacerbation of HSV-2 infection (n=14).

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Since all infected guinea pigs were in the stage of infectious process remission at the moment of the study, relapses were induced by 10-12-min UV exposure of animals [7].

The ovaries were fixed in 10% neutral formalin and routinely embedded in paraffin blocks. Serial sections (4 μ) were stained with hematoxylin and eosin. The sections were examined under an Axiostar plus microscope (Zeiss) and photographed.

The total content of follicles in the ovaries, percent of normal and atretic follicles by developmental stages were evaluated by the morphometrical method (labeling and quantitation of follicles in computer images of serial sections of the ovaries). The index of atresia was calculated as the ratio of the number of atretic forms to the total number of follicles in the ovary. Standard parameters served as the morphological criteria of normal and atretic follicles [1]. Quantitative analysis was based on classification of ovarian follicles: primordial, primary mono- and multi-lamellar, secondary (cavitary), and tertiary (preovulatory) follicles [5].

RESULTS

Morphologically intact (normal) and atretic follicles of all developmental stages were present in the ovaries of control animals (Fig. 1, *a*).

In groups 2 and 3, the total number of follicles was reduced in comparison with the control; no preovulatory forms were detected. In group 2 (remission of infection), atretic forms predominated in the total population of follicles, while in group 3 (exacerbation) virtually all follicles in the ovaries were in the state of atresia, and there were no normal follicles (Table 1).

Comparative analysis of atretic follicles of control and infected animals detected a number of differences. In group 2, the entire population of atretic primordial follicles had only a small portion of follicles with signs of physiological atresia, structurally identical to follicles in the control group. These were flat follicular cells with pyknotic nuclei, deformed oocytes with eosinophilic cytoplasm. The greater part of atretic primordial follicles differed from the control: their follicular cells had a clear swollen cytoplasm and "watch glass" nuclei, oocytes were vacuolated and had pyknotic nuclei (Fig. 1, *b*).

Atretic forms of primary multilayer follicles in the ovaries of group 2 females also differed from the control, but predominantly by the reaction of theca interna. In physiological atresia, thecocytes of this membrane proliferate, are hypertrophic, and replace the follicular epithelium, while in group 2 atretic follicles the thecal cells looked swollen with clear, virtually unstained cytoplasm and "watch glass" nuclei, and closely contacted with dilated thecal capillaries (Fig. 2, *a*). A characteristic sign distinguishing the primary atretic follicles in group 2 animals from the control was the absence of follicular cell mitoses already during the initial stages of atresia.

Atretic secondary (cavitary) follicles of group 2 animals were characterized by more pronounced (in comparison with the control) leukocytic infiltration of dying follicular epithelium, hypertrophic inner thecal membrane, formation of wide coil of collagen in the parafollicular zone (Fig. 2, *b*).

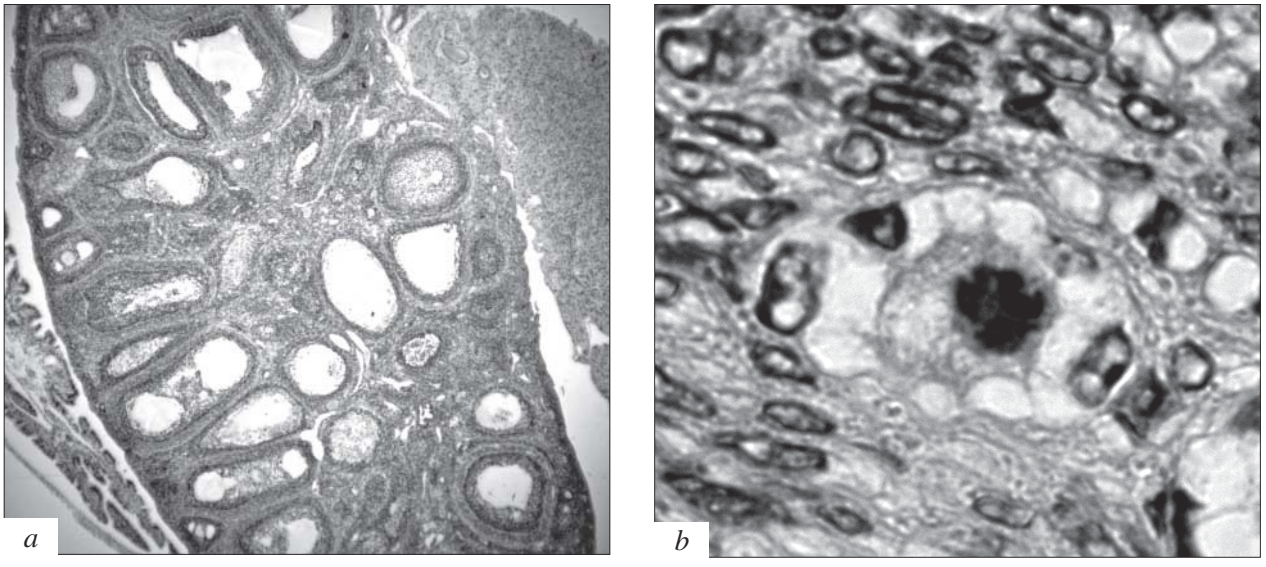

Fig. 1. Fragments of guinea pig ovaries. *a*) group 1. Common aspect. Hematoxylin and eosin staining, ×32. *b*) group 2. Atretic primordial follicle with hypertrophic and vacuolated follicular cells. Vacuolated cytoplasm in oocyte. Hematoxylin and eosin staining, ×1000.

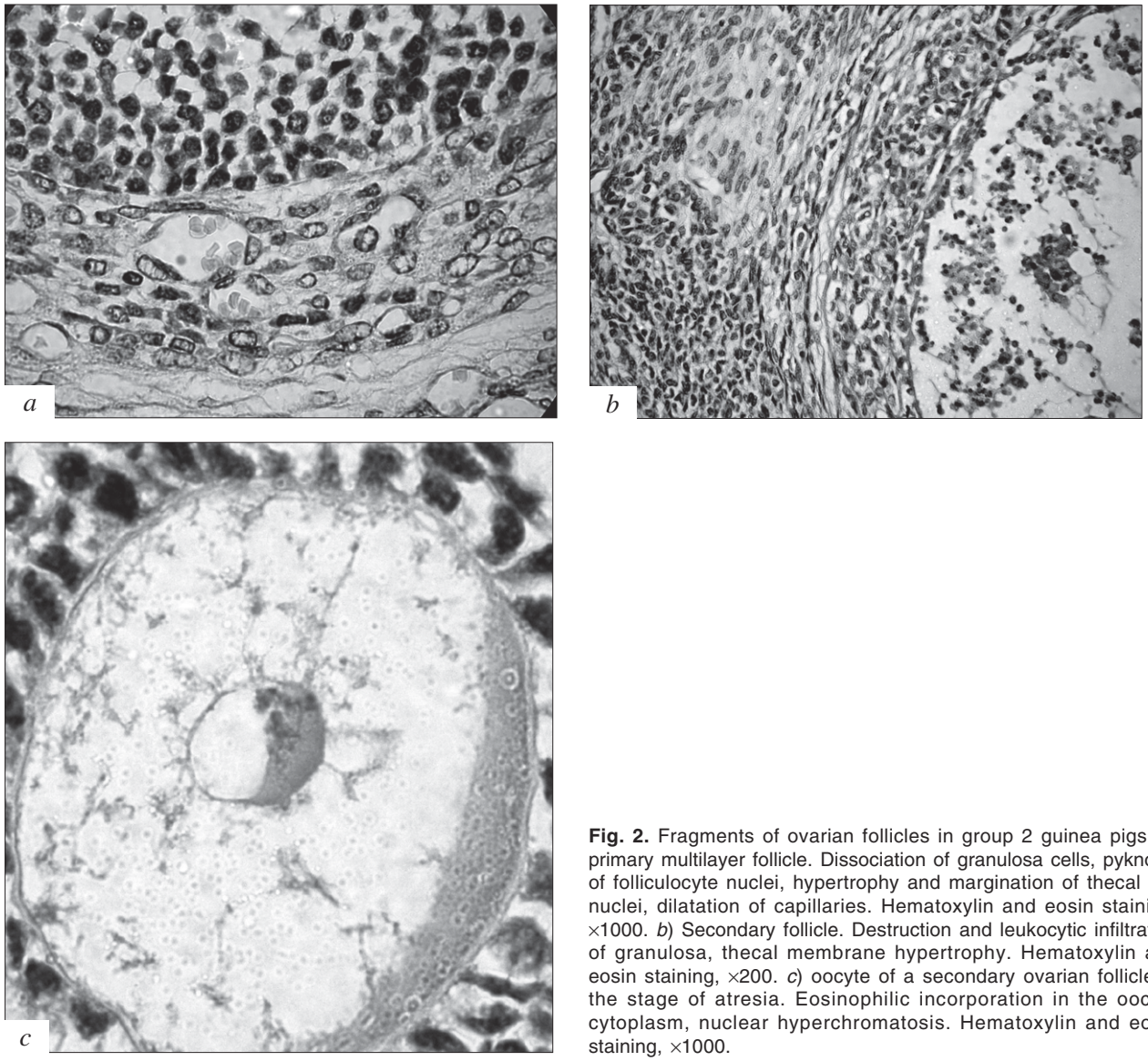

Fig. 2. Fragments of ovarian follicles in group 2 guinea pigs. *a*) primary multilayer follicle. Dissociation of granulosa cells, pyknosis of folliculocyte nuclei, hypertrophy and margination of thecal cell nuclei, dilatation of capillaries. Hematoxylin and eosin staining, ×1000. *b*) Secondary follicle. Destruction and leukocytic infiltration of granulosa, thecal membrane hypertrophy. Hematoxylin and eosin staining, ×200. *c*) oocyte of a secondary ovarian follicle at the stage of atresia. Eosinophilic incorporation in the oocyte cytoplasm, nuclear hyperchromatosis. Hematoxylin and eosin staining, ×1000.

TABLE 1. Percent Proportion of Follicles at Different Stages of Folliculogenesis and Atresia

Follicular types		Group 1 (control)	Group 2	Group 3
Primordial	normal	4.69	4.23	0
	atretic	43.47	45.42	48.59
Primary monolayer	normal	2.98	1.65	0
	atretic	5.18	6.77	11.11
Primary multilayer	normal	5.31	1.52	0
	atretic	3.42	3.5	5.97
Cavitary (secondary)	normal	21.58	5.0	0
	atretic	13.37	31.91	34.33
Index of atresia (proportion of the number of atretic follicles to total number of follicles)		0.65	0.86	0.99

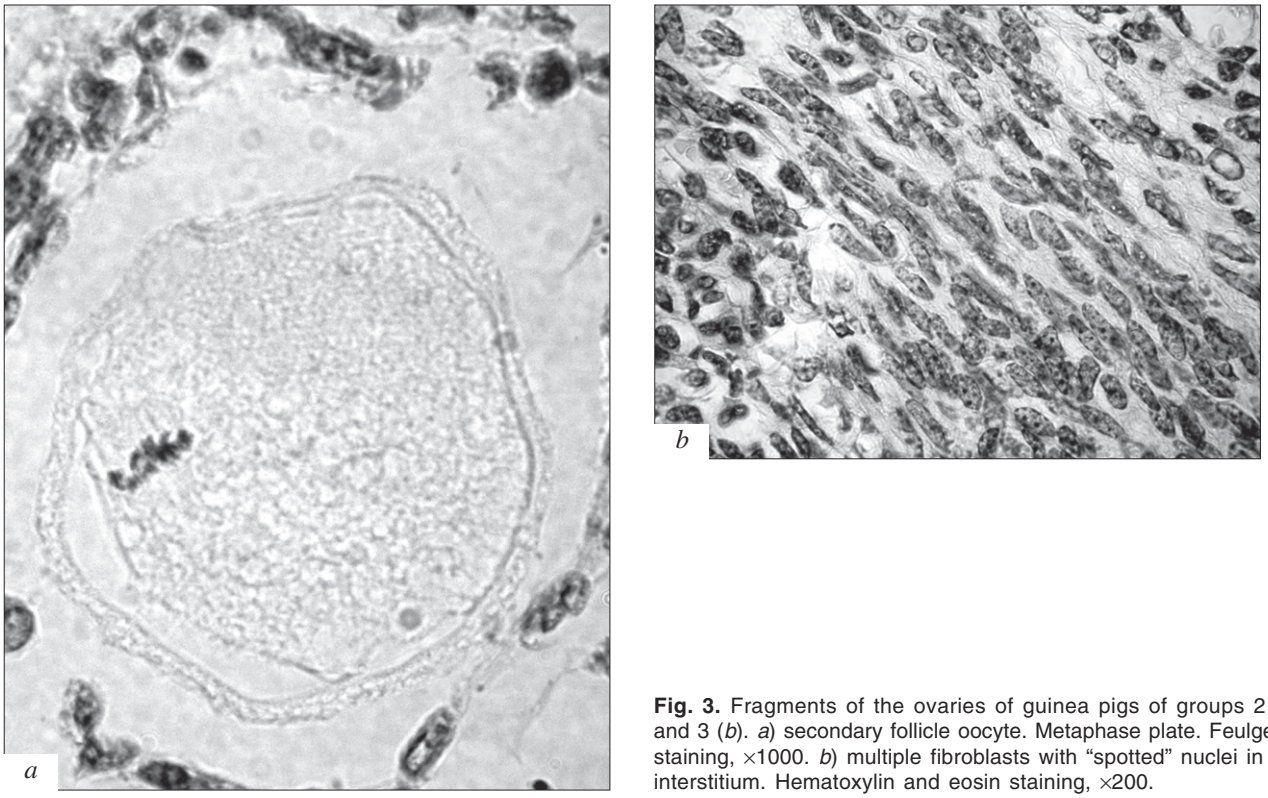

Fig. 3. Fragments of the ovaries of guinea pigs of groups 2 (*a*) and 3 (*b*). *a*) secondary follicle oocyte. Metaphase plate. Feulgen's staining, ×1000. *b*) multiple fibroblasts with "spotted" nuclei in the interstitium. Hematoxylin and eosin staining, ×200.

Oocyte nuclei were hyperchromatic, numerous finely grained eosinophilic incorporations, not characteristic of physiological atresia, were seen in the cytoplasm (Fig. 2, *c*).

Metaphase plates in oocytes of some atretic secondary follicles in group 2 animals (Fig. 3, *a*) indicated preterm resumption of meiosis, while normally meiotic division prophase 1 is blocked in oocytes during this stage of folliculogenesis.

No normal follicles were detected in the ovaries of group 3 animals; the entire population of follicles was presented by atretic forms with similar changes in the somatic and sex cells not typical for physiological atresia, characteristic of group 2.

In contrast to the control, ovarian stroma in groups 2 and 3 was formed by numerous oppositely directed cords of hypertrophic fibroblasts with nuclei containing numerous nucleoli (Fig. 3, *b*).

Hence, morphofunctional changes in guinea pig ovaries in experimental chronic herpesvirus infection consisted in significant reduction of the total number of follicles, activation of follicular atresia, morphogenetic changes in the stroma presented as fibroblast reaction and hypertrophy of the interstitial endocrine elements. The morphological characteristics of somatic cells and oocytes detected in atretic follicles of experimental animals were not characteristic of physiological atresia and presumably resulted from HSV-2 effects. This experimental model of genital herpes can be used for evaluation of the therapeutic effect of new drugs on the ovarian generative function.

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