Morphological Changes in the Ovaries during Modeling of Functional Cysts of Hormonal Genesis

S. V. Logvinov, O. A. Tikhonovskaya, I. A. Petrov, M. S. Petrova, Yu. V. Petrina, and A. O. Okorokov

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Morphological study of ovarian follicular cysts induced by chorionic gonadotropin (300 U) and insulin (Protafane HM; 2.5 U) in adult rats showed that superovulatory dose of chorionic gonadotropin and moderate hyperinsulinemia induced the development of ovarian cysts on days 3-5 in 100% cases. Dynamic study of ovarian morphology showed that changes were reversible and the cysts regressed within 60 days, which confirmed their functional nature.

Key Words: *morphology; ovaries; cysts; hyperinsulinemia; chorionic gonadotropin*

Follicular cysts (FC) are responsible for more than 50% tumor-like ovarian formations [2]. They can be caused by dysfunction of any component of the neuroendocrine system. One of the main causes is luteinizing hormone imbalance. Many morphogenetic aspects of the role of hyperinsulinemia and superovulatory secretion of chorionic gonadotropin (CG) in the formation of ovarian cysts remain little studied. There are numerous, but scattered data that FC can develop as a result of various influences [6]. On the other hand, just few authors took into account the role of insulin and insulin-like growth factors in the morphogenesis of ovarian cysts [3-5].

We created a model of functional ovarian cysts corresponding to those in females by pathogenetic and pathomorphological criteria.

MATERIALS AND METHODS

Experimental model of ovarian cyst formations was created in adult outbred female albino rats (n=54; 180-200 g). Colpocytological study was carried out in all animals in order to identify the phase of the estrous cycle. The rats were taken into experiment during the diestrus stage. Experimental animals (n=36) received daily (for 7 days) intramuscular injection of CG in a superovulatory dose of 300 U and 2.5 U Protafane HM (the dose inducing moderate hyperinsulinemia). Intact animals (n=18) served as controls. Treatment with CG and insulin for 7 days induced permanent estrus in all rats. Experimental animals were sacrificed under ether narcosis on days 3, 5, 10, 15, 30, and 60 after the end of treatment. The ovaries were directly fixed in Carnoy fixative and neutral formalin. Deparaffinized serial 5-6-μ sections were stained with hematoxylin and eosin. Follicular and lutein cysts, primordial and growing follicles, atretic follicles and bodies, and corpora lutea were counted in serial sections of the ovaries (150-200 μ intervals between sections). The specific volume of ovarian tissue components was evaluated by ocular measuring grid [1].

The results of morphological quantitative analysis and hormonal study were statistically processed using nonparametric Mann—Whitney test.

RESULTS

Histological study showed the formation of FC by day 3 after the end of treatment in 100% rats. In

Department of Histology, Embryology, and Cytology and Department of Obstetrics and Gynecology, Siberian State Medical University, Tomsk

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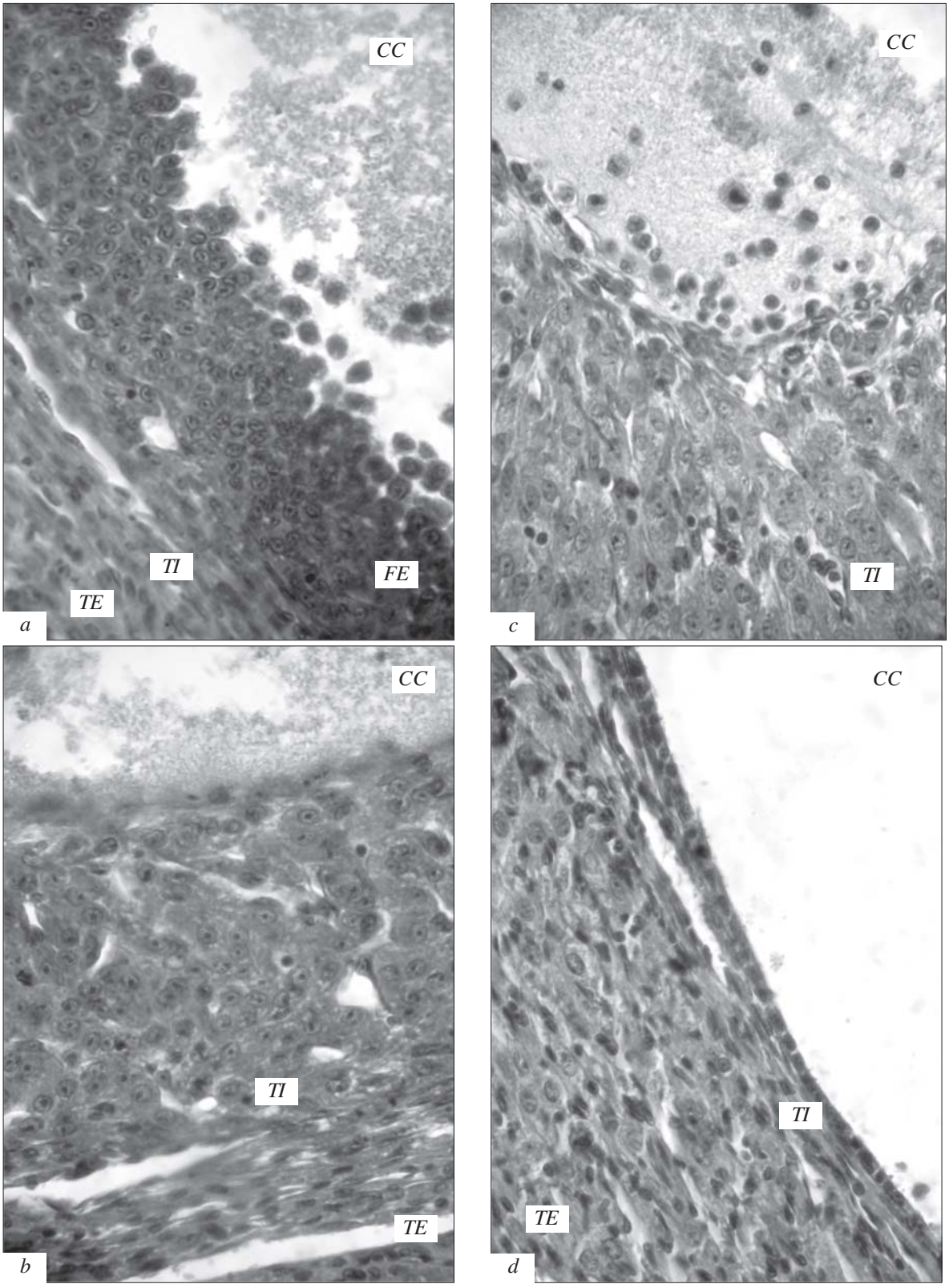

Fig. 1. Morphogenesis of ovarian cysts on day 3 after injections of CG and Protafane HM. *a*) FC (hormonally active); *b*) devastated cyst lined with single layer of flat cells (hormonally inert); *c*) partially luteinized FC; *d*) completely luteinized FC (lutein cyst). Hematoxylin and eosin staining, ×600. *CC*: cyst cavity; *FE*: follicular epithelium; *TI*: theca interna; *TE*: theca externa.

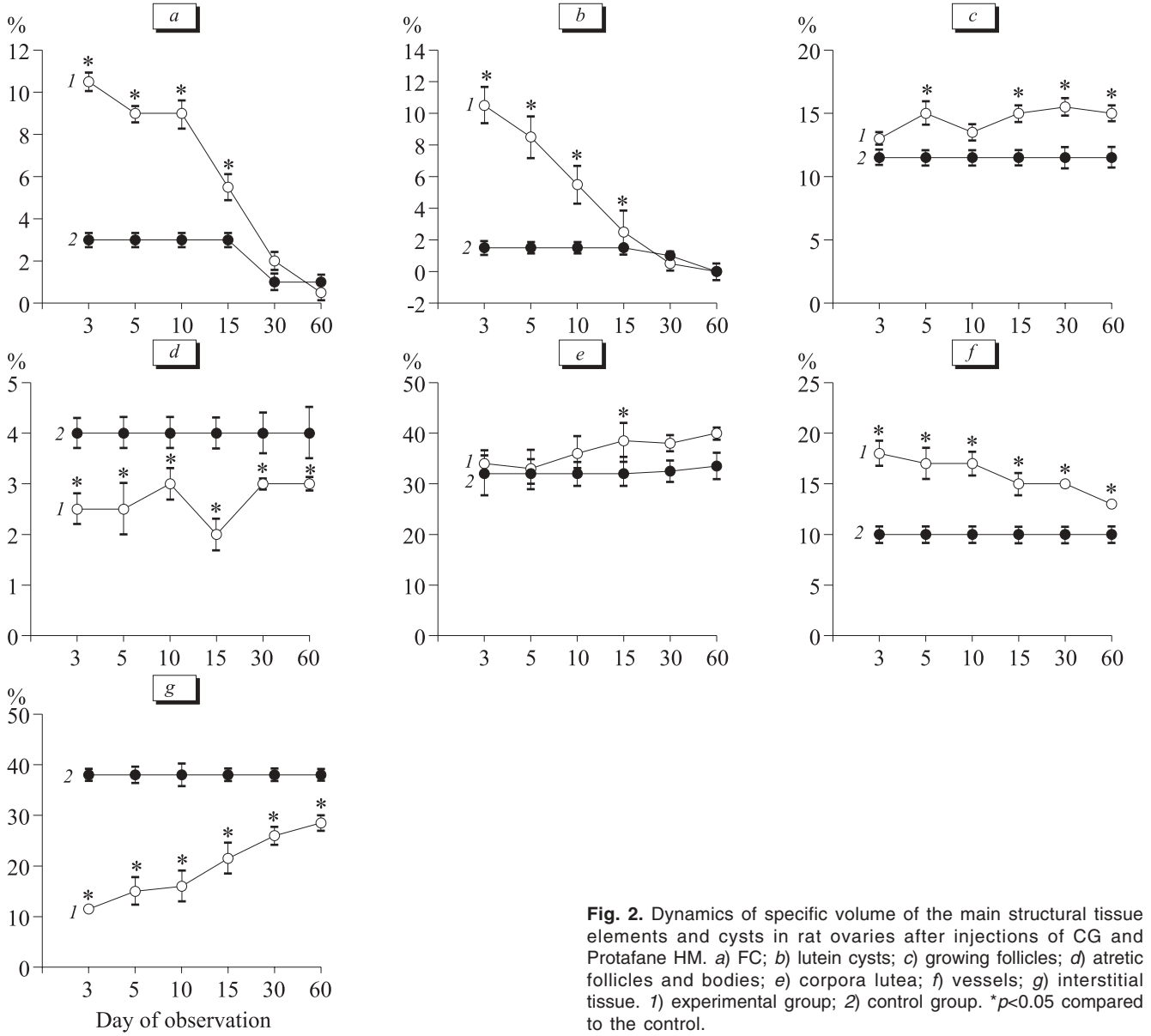

Fig. 2. Dynamics of specific volume of the main structural tissue elements and cysts in rat ovaries after injections of CG and Protafane HM. *a*) FC; *b*) lutein cysts; *c*) growing follicles; *d*) atretic follicles and bodies; *e*) corpora lutea; *f*) vessels; *g*) interstitial tissue. *1*) experimental group; *2*) control group. **p*<0.05 compared to the control.

most cases, several cysts were simultaneously seen in rat ovaries. These cysts were at different stages of their development: typical FC with multilayer granulosa sheath (Fig. 1, *a*), cysts undergoing luteinization, lined with a single layer of flat cells, and presumably characterized by high steroid activity (Fig. 1, *b*), completely luteinized cysts (Fig. 1, *c*), degenerative, and devastated cysts (Fig. 1, *d*) presumably devoid of steroid activity with the cavity lined by one layer of flat cells with markedly hypertrophic theca and mononuclears displaced into the cavity. This morphological picture could indirectly indicate that the morphogenesis of FC included several stages. The cyst initially exhibited steroid activity, which gradually decreased. Other FC developed later and replaced the lost function of older cysts by their own activity. The number of cysts with pronounced granulosa negligibly decreased and the number of devastated cysts increased by days 30-60 in the experimental group. The functional type of ovarian cysts was confirmed by regression of the above morphological changes by day 60 of the experiment.

According to the results of morphological quantitative analysis of the dynamics of the relations between the main structural tissue elements of the ovaries, the maximum specific volume of FC and lutein cysts was observed on day 3 of the experiment and was 10.5±0.4 and 10.5±1.2% *vs.* 3.0±0.3 and 1.5±0.2% in the control, respectively ($p<0.05$; Fig. 2). By day 15, the volume of cysts decreased, but remained above the control. The specific vol-

ume of FC and lutein cysts continued shrinking from day 30 until day 60.

The volume content of growing follicles increased at all stages of the experiment (to 15.0±0.6% *vs.* 11.5±0.8% in the control; $p<0.05$), while specific volume of atretic follicles and bodies remained low in comparison with that in the control.

The specific volume of ovarian vessels drastically increased starting from day 3 of the experiment in comparison with the control (18.0±0.3 *vs.* 10.0±0.7% in the control; $p<0.05$), which was presumably caused by hemodynamic disorders in the ovarian microcirculatory system, manifesting in vasodilatation, stasis, and hyperemia. Presumably, for this reason the specific volume of interstitial tissue decreased (11.5±0.4% *vs.* 38.0±0.7% in the control; $p<0.05$). By day 60, the vascular reaction somewhat decreased, but the trend to an increase in the vascular bed volume persisted (13.0±0.1% *vs.* 10.0±0.7% in the control; $p<0.05$); the volume of interstitial tissue also increased (to 28.5±1.3% *vs.* 38.0±0.8% in the control; $p<0.05$).

The specific volume of the corpora lutea was significantly higher than in the control only on day 15 of the experiment (38.5±3.5% *vs.* 32.0±3.5%; $p<0.05$), while their number in an ovarian section was greater during the entire period of observation. The decrease in the specific volume of corpora lutea seemed to be also due to increased vascular bed volume.

Hence, moderate hyperinsulinemia and superovulatory doses of CG caused 100% development of multiple ovarian FC in experimental animals, which can serve as a model of the formation of ovarian functional cysts. Dynamic study of ovarian morphology showed that the described phenomena were reversible: the cysts regressed within 60 days after injections of CG and insulin, which confirmed their functional type. Morphologically the simulated cysts corresponded to cysts in women.

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