A. B. Zyuzyun. Cytomorphological research of rabbit oocytes in the process of in vitro embryogenesis

Introduction. Using genetic potential of rabbit ovaries and studying patterns of meiotic maturation of gametes in female *in vitro* is a basis for success in cloning and the creation of transgenic animals, so there is a need for in-depth study of cytomorphological characteristics of oocytes during *in vitro* embryogenesis.

The aim of the research is cytomorphological study of oocytes during embryogenesis, derived from matured rabbits' ovaries and before the sexual cycle.

Materials and methods of research. The ovaries of the rabbits (n = 8) aged 4 months and rabbits aged 11 months, coming into heat (n = 10) were used in the study. All the ovaries derived from females, were at follicular growth phase. Rabbits' oocyte-cumulus complexes were cultured *in vitro* during 24 hours in plastic Petri dishes (25 - 30 OCC per ml) in the medium for maturing – 199 with Earle's solution (Sigma, M 5017), supplemented by 20% heat-inactivated (56°C, 30 min.) homemade estrous cow serum, 0.068 mg/ml kanamycin sulfate, 0.11 mg/ml sodium pyruvate and 0.1 mg/ml glutamine. Granulosa cells derived from the antral follicles without atresia evidence were necessarily added in the culturing medium in amount of 3–5x10⁶ per ml. Received *in vitro* the ova were fertilized by freshly derived rabbit's epididymal spermatozoa.

Results. As a result of extracting oocytes from all the ovaries (n = 18), 245 OCC were received, including 115 OCC from eight rabbits' ovaries before the sexual cycle and 130 OCC from ten ovaries of mature rabbits. Analysis of cytomorphological studies found more (P < 0.05) oocyte-cumulus complexes can be received from rabbits' ovaries during puberty being suited for cultivation than from mature rabbits' ovaries.

After *in vitro* culturing 85,5 % of OCC (47 of 55) derived from rabbits' ovaries during puberty and 75.6% of OCC (62 of 82) – from mature rabbits' ovaries reached MII meiosis. It was revealed the level of *in vitro* maturation of oocytes was 10 % higher in the group derived from rabbits' ovaries at puberty, compared with the group derived from mature rabbits' ovaries. Ripened outside a body the oocytes were fertilized *in vitro* by freshly derived rabbit's epididymal spermatozoa. The embryos developed in the both groups, but with a significant difference in the level of division. Cytomorphological research found that the level of 2-4-cell embryos formation in the group of oocytes derived from rabbits at puberty was 68,1% and in group of gametes from mature rabbits – only 46.8 % (P < 0.05). 22.2 % of embryos on average developed to the morula stage *in vitro*. In terms of embryo development to early morula stage significant difference between the groups wasn't found. The significant difference between the study groups in the number of zygotes have not passed division block (P < 0.05) was noted. In the group of oocytes derived from mature rabbits' ovaries the zygotes which have not divided were 23.8 % more.

Conclusions. It was found that more oocyte-cumulus complexes (P < 0.05) were received from rabbits' ovaries during puberty, being suited for *in vitro* cultivation, than from mature rabbits' ovaries. And oocytes with degeneration signs, being unsuited for *in vitro* cultivation, were received more (P < 0.05) from mature

rabbits' ovaries. The level of maturation also was 10 % higher in the group of OCC derived from rabbits' ovaries at puberty.

So, for biotechnology research as oocyte donors more effective is use of rabbits during puberty, which have not yet begun sexual cycle, because significantly more (P < 0.05) fully-fledged oocytes cumulus complexes, being suited to culture outside a body, can be derived from their ovaries which will provide greater percentage of preimplantation embryos.

Keywords: rabbit oocyte-cumulus complexes (OCC), epididymal spermatozoa, in vitro maturation and fertilization, rabbit embryos