

Analysis of oocyte-cumulus complexes of goats and parameters of their maturation in conditions *in vitro*

Purpose. Analysis of populations of oocyte-cumulus complexes of goats and determination of optimal time parameters of their maturation under *in vitro* conditions. **Methods.** Genetic (cytogenetic analysis of chromosomal changes during maturation of oocytes *in vitro*), biometric. **Results** It was established that by cytomorphological features of oocyte-cumulus complexes of goats, suitable for further *in vitro* cultivation, ovaries at the stage of follicular growth were obtained by 15.9% more than in the luteal stage. **Conclusions** To obtain oocyte-cumulus complexes of goats it is expedient to use ovaries at the stage of follicular growth. Optimal time of cultivation of oocytes of goats *in vitro* for reaching stage II meioze metaphase - 27 hours.

Key words: oocyte-cumulus complexes, goats, in vitro, morphological analysis, cytogenetic analysis.

Formulation of the problem. Recent advances in reproductive biotechnology are improved methods for obtaining embryos outside the body and attempts to transplant stem cells [12]. The use of reproductive biotechnology in cucumbers will help to use genetically valuable but biologically weak sperm to produce a large number of embryos from one genetically valuable animal [1]. The stages of maturation of oocytes of goats and the production of embryos *in vitro* are very similar to those used for cattle and sheep, but certain features have been identified. The main stages of receiving embryos of goats outside the body: maturation of oocyte-cumulus complexes (OCs), fertilization of oocyte matured to metaphase II (M II) *in vitro* by freshly frozen or frozen-defatted sperm and cultivation of obtained embryos to preimplantation stages that can be transplanted Recipient or cryopreservation for further use [3 - 5]. The development of embryos *in vitro* depends on the processes occurring during the nuclear and cytoplasmic maturation of the oocytes [6, 11, 13]. At present, the level of maturation of oocytes of goats beyond the body is much lower than that obtained *in vivo* due to the quality of oocytes selected for cultivation [4]. Despite the number of studies conducted, the methods of selection and cultivation of OCS *in-vitro* goats need to be improved [9]. The purpose of the research is to analyze the populations of the OCZ of goats derived from the ovaries, and to determine the optimal time parameters of their maturation *in vitro*. **Materials and methods of research.** The research was conducted in the Biotechnology Laboratory of the Institute of Breeding and Genetics of Animals named after MV. Nail Stud

Fig. 1. Goat's ovaries in the phase of follicular growth

Fig. 2. Goat's ovaries on the luteal phase (the yellow bodies indicated by the arrows)

For research, ovaries of goats were selected at the stage of follicular growth and on the luteal phase [2]. Getting OCC preantralnyh of ovarian follicles follicles carried dissection blade safety razor in a petri dish with the environment Dyulbekko with the addition of 0.075 mg / ml kanamycin sulfate. Cultivation selected OCC goats *in vitro* carried out in plastic petri dishes in a medium maturing - 199 on the solution Earl (Sigm ^, M 5017), which complemented 20% inactivated estrusnoyi serum cows (homemade), 0,068 mg / ml kanamycin sulfate 0 , 11 mg / ml sodium pyruvate and 0.1 mg / ml glutamine. Cells of granulose - 3 - 5106 per ml were added to the culture medium. To study the chromatin from oocytes, dry-air preparations were prepared using the modified Tarkovsky method [10]. Coloring of the preparations was carried out with 2% solution of dye Gimza. To evaluate the morphological state of oocytes and cytogenetic drug analysis, a light microscope MBS-9, Jenaval was used. Statistical data processing was performed using the Student criterion. **Research results.** To make a comprehensive analysis of populations OCC removed from the ovaries goats at different stages of the estrous cycle, selected ovarian follicular growth phase and the luteal phase. Removed ovaries,

which were at the stage of follicular growth, had no signs of ovulation and corpora lutea and ovarian luteal phase had formed the corpus luteum (Fig. 1, 2). With studied ovarian (n = 8) received 166 OCC, with a 4-stage ovarian follicular growth OCC received 110, and from 4 x ovarian luteal phase - 56 OCC, ie by 32.6% less. Oocytes population, depending on the cumulus and ooplazmy, were divided into 4 groups: the group - OCC with cumulus dense, transparent shell intact and homogeneous nevakulizovanoyu ooplazmoyu correct round shape; li - OCC with loose cumulus and homogeneous ooplasm; lii - partially deprived of cumulus cells, but with a homogeneous ooplasm without signs of atresia; Group IV - atretic OCs (deposed or with a small number of cumulus cells, ooplasm with signs of degeneration).

According to the results of the morphological evaluation of OCK, it has been found that 15.9% more oocytes that are suitable for cultivation of oocytes (i - ii groups) from the ovaries at the stage of follicular growth were extracted from ovaries in the luteal phase. In addition, in the population of OCs extracted from the ovaries in the luteal phase, much more (P <0.05) degenerated oocytes was obtained (Table 1). The state of chromatin on the manufactured cytogenetic preparations was analyzed in the various stages of the esthetic cycle of the OCC. The results of the studies indicate that most of the OCS of goats removed from the ovaries in the follicular and luteal phases were at the stage of diploenia. In this case, the most gametes (49%) withdrawn from the ovaries in the phase of follicular growth, and 35.7% of the oocytes from the ovaries in the luteal phase were at the stage of diffuse diplotination (Table 2). A greater number of gametes (33.9%) (P <0.05) with signs of chromatin degeneration was found in the population of oocytes withdrawn from the ovaries on the lutein phase. Sources of literature report that the number of oocytes of goats matured to the metaphase and after 24-27 hours of cultivation is significantly greater than after 21 hours. There is also information that the level of fertilization of oocytes is significantly higher after cultivation for 24-27 hours than after cultivation of 21 or 30 hours [7, 8]. Therefore, we investigated 3 time parameters of cultivation of in vitro oocytes of goats - 24, 27 and 30 hours.

To cultivate ip vitro, 170 OCPs of excellent and good quality, coated with several layers of cumulus cells were selected (Fig. 3). The criterion for morphological evaluation of oocyte maturation was the presence of the first polar body (Fig. 4). According to the results of an analysis of cytogenetic oocyte preparations after their 24-hour cultivation, it was found that only 32.2% of oocytes initiated meiosis and these cells were at the stage of metaphase I and metaphase and meiosis. The remaining oocytes of goats (57.6%) remained at the stage of diploenia. Consequently, the 24-hour cultivation of immature oocytes of goats provides only 23.7% of maturation in the conditions of yip. After 27 hours of oocyte culturing, the metaphase II meiosis stage reached 52.7% oocytes, did not restore meiotic transformations, and remained at the stage of diffuse diffuse 16.4, fibrillar - 14.6% oocytes (Table 3). The prolongation of the cultivation time to 30 hours reduced the amount of gametes that were in the diffuse dipole stage by 11.1%, although this difference is unlikely and tends to increase the number of oocytes with degenerate chromatin by 10%.

1. Morphological analysis of oocyte-cumulus complexes extracted from the ovary of goats in different phases of the estral cycle (in groups)

The phase of the estral cycle Total number of OCS, n OCCs, suitable for further development outside the body, n (%) OCC, not suitable for further development Out of the body, n (%) and ii ii Follicular 110 46a (41.8 ± 4.7) 29b (26.4 ± 4.2) 19th (17.3 ± 3.6) 16d (14.5 ± 3, 3) Luteinova 56 18a (32,1 ± 6,2) 15b (26,8 ± 5,9) 6e (10,7 ± 4,1) 17e (30,4 ± 6,1) Notes: d: e - P <0.05, Student's criterion.

2. Cytogenetic analysis of oocyte-cumulus complexes removed from the ovaries goats at different stages of the estrous cycle, n (%) phase of the estrous cycle Total OCC, n number of oocytes at the stage dyploteny, n (%) chromatin degeneration, n (%) diffuse fibrillary visible bivalent metaphase II Follicular 54a 110 (49,0 ± 4,7) 28b (25,5 ± 4,1) sixth (5,5 ± 2,1) 4d (3,6 ± 1,7) 18s (16, 4 ± 3,5) 56 luteal 20a (35,7 ± 6,4) 13b (23,2 ± 5,6) second (3,6 ± 2,4) 2d (3,6 ± 2,4) 19f (33,9 ± 6,3) Note: e: f - - P <0.05, Student's criterion.

3. Determining the optimal time cultivating oocyte-cumulus complexes goats in conditions in vitro cultivation OCC, number of oocytes h, n number of oocytes at stage number of oocytes with chromatin dehenerovanym, n (%) dyploteny, n (%) and metaphase, n (%)) metaphase II, n (%) diffuse fibrillary visible bivalent 11a 24 59 (18,6 ± 5,0) 16s (27,1 ± 5,7) seventh (11,9 ± 4,2) syi co ^ ± 00 th tb 149 (23,7 ± 5,5) 6 (10,2 ± 3,9) 27 55 9ab (16,4 ± 4,9) 8ed (14,6 ± 4,7) second (3,6 ± 2 5) S ± cq co & CN 29h (52,7 ± 6,7) 5 (9,1 ± 3,8) 30 56 3b (5,3 ± 3,0) 7d (12,5 ± 4,4) 3rd (5,4 ± 3,0) 1f (1,8 ± 1,7), 6) 6, ±, April 5 (5 November 31 (19,6 ± 5,3) Notes: a: b; C: d - P <0.05; g: h - P <0.001, Student's criterion.

Conclusions

Based on the results of morphological evaluation and cytogenetic analysis of the OCK populations, it has been found that oocytes that are suitable for the cultivation of oocytes (I-III groups) in the follicular growth stage have been extracted more than from the lutein phase ovaries. In addition, in the population of OCs extracted from the ovaries on the luteal phase, much more ($P < 0.05$) degenerated oocytes was obtained. Therefore, to obtain OCK goats it is expedient to use ovaries at the stage of follicular growth. Cultivation of goat oocytes for 27 hours in vitro allowed to increase the level of ripeness to 52.7% of oocytes ($P < 0.001$) and reduce the percentage of gametes with degenerated chromatin to 9.1%. Thus, the optimal time of cultivation of oocytes of goats in vitro for reaching the stage of metaphase II meiosis is 27 hours.

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