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CYTOLOGICAL FEATURES OF PIG OOCYTES IN THE PROCESS OF SPONTANEOUS MATURATION

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Aim. To determine the cytological changes, related to the germinal vesicle (GV) of porcine oocyte during the follicular maturation. **Methods.** The ovaries were classified according to the estrous cycle phase, judging by the status of yellow bodies and follicles. The oocytes were isolated from each animal using dominating and atresiated follicles separately. The oocytes were used to prepare whole mounts by a specially designed express-method, which is thermal fixation (about 95 °C) and their further transfer into the medium for clarification. The whole procedure of preparing the mount from one oocyte or their group lasted for about 10 min. The microscopic studies were conducted using the phase contrast. A total of 831 oocytes were studied. **Results.** It was established that sow oocytes had eccentric location of GV which may be found in oocytes from early antral follicles. This condition is kept until the beginning of meiosis restoration and GV destruction. The nucleolus is also located eccentrically. Another relevant cytological change was gradual condensation of chromatin around the nucleolus, starting from separate clots on its surface and further formation of circular structures. **Conclusions.** Contrary to laboratory rodents, GV in porcine oocytes has eccentric localization, which is set even in early antral follicles and remains until the beginning of meiosis restoration. The process of chromatin transformation was found to be its gradual condensation around the nucleolus which may be defined as a nucleolus complex.

Keywords: estrous cycle, germinal vesicle, oocyte, sow, chromatin, nucleolus.

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INTRODUCTION

The matter of obtaining the robust embryos from oocytes via ripening and further *in vitro* insemination is still an urgent issue for the researchers of reproductive technologies in animal breeding [1, 2]. There has been considerable progress, in particular, in the application of sow oocytes when blastocysts and progeny were obtained from them [3, 4]. However, no acceptable technology for this complicated process has been suggested yet, and the efficiency of obtaining embryos is still low [5]. Its every stage requires improvement and corresponding actual data about the processes, taking place *in situ*. In particular, one of the distinguishing factors of successful maturation of oocytes *in vitro* is the selection of promising oocyte-cumulus complexes (OCC) from the ovaries. There are several known suggestions regarding the morphological and cytological indices for the selection of robust OCC for cultivation [6, 7]. Their distinguishing characteristic is the condition of the cumulus mass of the oocyte. At the same time, the cytological status of the oocyte has not been studied sufficiently yet. On the one hand, this is conditioned by the fact that the cumulus mass masks the oocyte during the microscopic study, and on the other – the ooplasma of porcine oocyte has the structure, which does not allow observing the status of its GV. Therefore, the data regarding the cytological structure of porcine oocytes were obtained mostly due to the investigation of isolated mounts. In particular, these are electronic microscopic studies [8, 9], the study of histological sections [10], dry air mounts for the study of chromatin status [11, 12] as well as whole mounts of oocytes [13] which are the most informative and accessible for application. The use of improved methods of investigation allowed determining the chromatin status

in porcine oocytes during the spontaneous (natural) and artificial (in conditions beyond the organism) maturation of a follicle, where two main types regarding its condensation around the nucleolus were determined. These are non-surrounded nucleolus (NSN) when there is no condensed chromatin and surrounded nucleolus (SN) with a formed ring or "horse shoe" around it [14]. Our aim was to study the cytological changes, related to GV of a porcine oocyte during its spontaneous follicular maturation using the original method of isolating whole mounts.

MATERIALS AND METHODS

The ovaries were selected at a local meat processing factory and transported in the isotonic solution to the laboratory. The status of ovaries was estimated by the development of yellow bodies and follicles, referring them to a specific phase of estrous cycle [15]. For instance, the ovaries in the diestrus phase were characterized by yellow bodies in the hiatus of their functional development and had the diameter of 8–10 mm, while the follicles did not exceed 5.5 mm. The sow ovaries in the proestrus phase were determined when they had the features of the beginning of regression of yellow bodies (weakening of color intensity), the reduction in their size down to 5–8 mm with follicles reaching up to 6–8 mm. The oocyte-cumulus complexes (OCC), isolated from follicles, did not have any mucinization. The estrus was assumed when the ovaries had well-expressed regressive changes in yellow bodies which decreased in their size down to 4–7 mm and changed their color to whitish-yellow. The follicles grew to maximal size of 7–10 mm. The cumulus cells were mucinized and formed a mucous mass with intercellular matrix.

The oocytes in OCC form were isolated separately from each pair of ovaries by dissecting the follicles with a razor blade in two stages. At first OCC were isolated from the pool of 12–15 largest dominating follicles, then the ones from the rest. OCC of the first selection were characterized by the largest cumulus mass. Depending on the phase of estrous cycle, they had from 3 to 8 layers of cumulus cells. Immediately after isolation from each group, whole mounts were prepared from each group of the estimated OCC. This involved a specially designed express method which envisaged the thermal fixation (about 95 °C), removal of cells of the cumulus mass and further transfer of the oocyte into the clarification environment. The whole procedure of preparing the mount from one oocyte or their group lasted for about 10 min. The prepared mount ensured the investigation of ooplasma structure, the status of the nucleus, nucleolus, and chromatin of the oocyte. The microscopic studies were conducted using the phase contrast. The status of ooplasma and nucleoplasma, the presence, location and form of GV, the status of oolemma, the structure of nucleolus and perinucleolar formations (nucleolus complex) were estimated in the preparations. A total of 831 oocytes were studied.

RESULTS AND DISCUSSION

The prevailing majority of oocytes from all the investigated morpho-functional groups are characterized with the presence of GV with the nucleolus. Here the nucleoplasma has a finer grained structure than ooplasma. The absence of GV was noted in oocytes with highly mucinized cumulus, which is considered to be the consequence of meiosis restoration. Sometimes a polar body could be found in such oocytes (Fig. 1). In some cases, the presence of GV was not found in degenerating oocytes. However, this should not be related to meiosis restoration. This was the place for deep destructive changes, confirmed by the corresponding status of ooplasma.

It was determined that GV was located eccentrically in all the investigated oocytes. This was true for oocytes from both healthy and atresiated follicles as well as the ones, which belong both to the category of NSN and SN by the degree of chromatin condensation (Fig. 2, 3). This fact proves that the eccentric localization of GV is determined in oocytes even in early antral follicles and stays stable further on. The localization of GV in porcine oocytes was practically not studied yet, which is largely conditioned by the non-transparency of their ooplasma, due to which it is impossible to determine the location of the nucleus without special preparation of the cytological mount. However, the results of previous studies, aimed at investigating the transformation of chromatin in GV during folliculogenesis, confirm its eccentric localization [16, 17]. Such localization of GV is also inherent for other mammals, which were under study, except for rats and mice [18].

Similar to porcine oocytes, where GV is eccentrically located, according to these studies, the nucleolus also takes an exclusively eccentric location in GV (Fig. 2, 3). It was determined in human oocytes that the location of GV is related to the location of nucleolus and the capability of oocytes to complete their maturation. Thus, the central location of GV correlates with the central location of the nucleolus, and the peripheral location of GV – with the peripheral nucleolus. Prior to the formation of the first polar body, human oocytes

with the central location of GV matured better than the ones with peripheral location [19].

The location of GV in mouse oocytes has been studied in the finest detail. Thus, the location of GV is related to their capability of completing meiotic maturation: the closer GV is to the center of oocyte, the higher is its capability of restoring meiosis [20]. The authors assumed that the central location of GV may be a morphological marker to forecast their capability of maturing. A similar conclusion was made by another group of researchers [21], however, to foresee the success of further maturation it is suggested to combine the estimates by GV localization and the status of chromatin organization. Another study [22] established that the central location of GV is the most frequent in mouse oocytes. However, in addition to the central location, the peripheral and interim ones are suggested for consideration as well, assuming this variant to be more informative. Besides, the authors relate the location of GV to the degree of condensation of chromatin and its capability of migrating.

It is assumed that the location of GV is a key factor for asymmetric division of the oocyte. It is especially relevant in the meiotic process, where a small polar body is formed along with a much larger egg. However, the information about this process in mammalian oocytes is still scarce [23]. It should be noted that the division of oocyte should take place in the cortical zone, due to which the central location of GV in mouse oocytes results in the migration of the spindle of the division further into the closest cortical zone of the oocyte [24].

Taking the known results into consideration, one may assume that the eccentric location of GV in the sow is achieved the soonest compared to all the investigated mammals and can already been confirmed in oocytes from early antral follicles. This is a considerable difference of a sow from all the other investigated mammals.

Another relevant specificity of porcine oocytes is the status of chromatin condensation in GV during the follicle maturation. This study revealed that condensed chromatin is absent around the nucleolus of oocytes in early antral follicles (smaller than 2 mm), it appears in nuclei from larger follicles in the form of a ring, also known as karyosphere. This formation is viewed as a complex nuclear structure, composed of chromosomes, united into a knot or a general chromatin mass, located around the nucleolus [25]. In porcine oocytes, this structure is described in the shape of a crown or a horse-shoe around the nucleolus, which is kept until the restoration of meiosis [26]. The study of pre-antral fol-

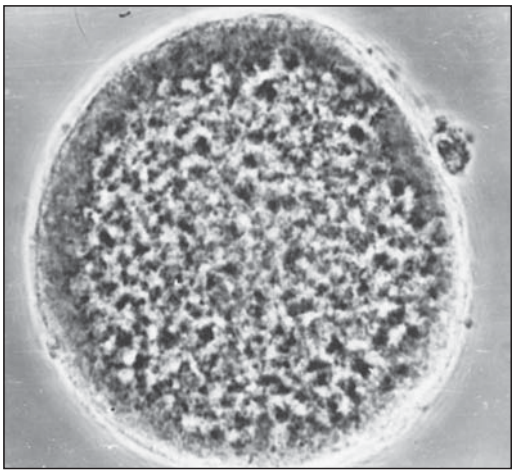

Fig. 1. The general overview of a fixed oocyte with the first polar body

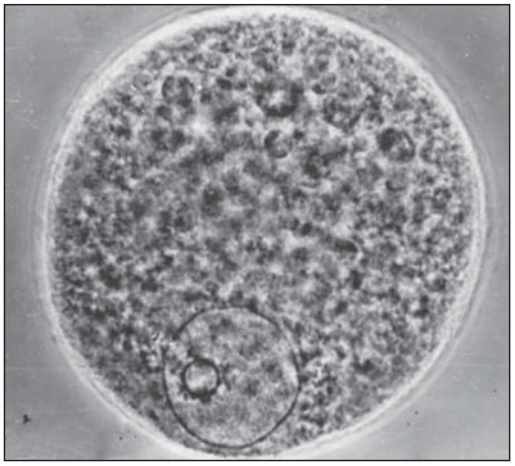

Fig. 2. The general overview of the fixed oocyte with the nucleus and nucleolus, surrounded by condensed chromatin (SN)

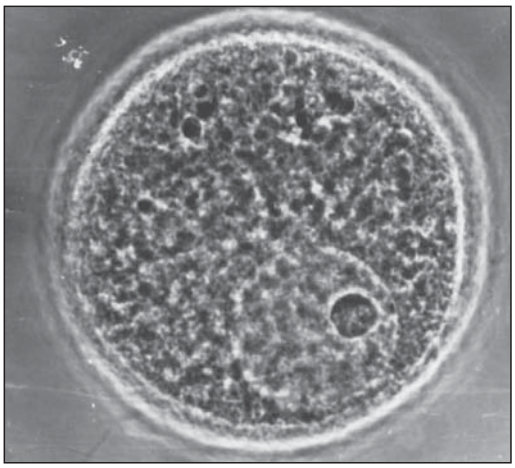

Fig. 3. The general overview of the fixed oocyte with the nucleus and nucleolus, not surrounded by condensed chromatin (NSN)

licles [27] did not reveal condensed chromatin around the nucleolus in their oocytes, while oocytes from medium and large antral follicles had such condensed chromatin.

In recent years there has been a notion formed about two types of nucleoli, including the porcine ones, which were related to chromatin condensation. At the initial stages of folliculogenesis in GV the diffuse status of chromatin prevails and the nucleolus does not have its condensed forms (NSN). While the follicle is developing, this chromatin appears around the nucleolus (SN) [14, 28]. These authors established that the diffuse state of chromatin in oocytes from follicles < 1.9 mm corresponds to NSN of the state of the nucleolus, known for mouse and many other mammals. In its turn, SN is remarkable for all the developing oocytes and precedes the restoration of meiosis.

The method of preparing mounts, employed in this study, allows visualizing both NSN and SN in the oocytes without using any special dyes. As it is believed that chromatin is condensed in other zones of the nucleus as well, the part, connected to the nucleolus, should be regarded as a nucleolus complex. The shape of this formation demonstrates some diversity in its dependence on the physiological state of the follicle, from which the oocyte originates. The conclusion lies in the possibility of estimating the state of the development for the process of forming condensed chromatin by the part, forming a structure, shared with the nucleolus. It is suggested to isolate a separate cytological structure – a nucleolus complex, which consists of a nucleolus and the associated condensed chromatin mass, which may be useful from the standpoint of estimating the status of oocyte maturation.

CONCLUSIONS

Contrary to laboratory rodents, GV in porcine oocytes has eccentric localization, which is set in oocytes even in the early antral follicles. This condition is kept until the beginning of meiosis restoration and GV destruction. The cortical location is noted both in NSN and SN oocytes as well as in the ones, originating from atresiated follicles. Among the investigated species of mammals, the sow has the earliest formation of the required location of the nucleus which is a key factor for asymmetric division of the oocytes.

The process of chromatin transformation was found to be its gradual condensation around the nucleolus and it may be defined as a nucleolus complex. As for the oocytes, originating from early antral follicles, the nucleolus is in NSN state. The increase in the follicle size occurs with simultaneous gradual condensation of chromatin on the surface of the nucleolus, which results in the formation of karyosphere or SN. The method of preparing whole mounts, used in these studies, ensures the possibility of investigating the status of the nucleolus and the associated chromatin without using any special dyes. The degree of chromatin condensation may be determined by the status of the nucleolus complex, which is a dynamic cytological structure, composed of the nucleolus and the surrounding condensed chromatin.

Цитологічні особливості ооцитів свині в процесі спонтанного дозрівання

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Мета. Завданням дослідження було виявлення цитологічних змін, пов'язаних із зародковим пухирцем (GV) ооцита свині, впродовж фолікулярного дозрівання. **Методи.** Яєчники класифікували відповідно до фази естрального циклу за станом жовтих тіл та фолікулів. Ооцити виділяли від кожної тварини окремо з домінуючих та атрезуючих фолікулів. З ооцитів готували тотальні препарати спеціально розробленим експрес-методом, що полягає у термічній фіксації (близько 95 °С) та наступному перенесенні їх у просвітлююче середовище. Вся процедура підготовки препарату з одного або групи ооцитів тривала близько 10 хв. Мікроскопічні дослідження виконували з використанням фазового контрасту. Всього було досліджено 831 ооцит. **Результати.** Встановлено, що у свині має місце ексцентричне розташування GV, яке може бути виявлене вже в ооцитах із ранніх антральних фолікулів. Такий стан зберігається аж до початку відновлення мейозу та руйнування GV. Ядерце так само розміщується ексцентрично. Іншою важливою цитологічною зміною була поступова конденсація хроматину навколо ядерця, починаючи від окремих згустків на його поверхні та наступного утворення кільцевих структур. **Висновки.** В ооцитах свині, на відміну від лабораторних гризунів, GV має ексцентричну локалізацію, яка встановлюється вже у ранніх антральних фолікулах і зберігається аж до початку відновлення мейозу. Виявлено існування процесу трансформації хроматину, що полягає у його поступовій конденсації навколо ядерця та може бути визначений як ядерцевий комплекс.

Ключові слова: естральний цикл зародковий пухирець, ооцит, свиня, хроматин, ядерце.

Цитологические особенности ооцитов свиньи в процессе спонтанного созревания

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Цель. Заданием исследования было выявление цитологических изменений, связанных с зародышевым пузырьком (GV) ооцита свиньи, в течение фоликулярного созревания. **Методы.** Яичники классифицировали в соответствии с фазой эстрального цикла по состоянию желтых тел и фолликулов. Ооциты выделяли от каждого животного отдельно из доминирующих и атрезирующих фолликулов. Из ооцитов готовили тотальные препараты специально разработанным экспресс-методом, который заключается в термической фиксации (около 95 °С) и последующем их перенесении в просветляющую среду. Вся процедура подготовки препарата из одного или группы ооцитов длилась около 10 мин. Микроскопические исследования проводили с использованием фазового контраста. Всего было исследовано 831 ооцит. **Результаты.** Установлено, что у свиньи имеет место эксцентрическое размещение GV, которое может быть выявлено уже в ооцитах из ранних антральних фолликулов. Такое состояние сохраняется вплоть до начала возобновления мейоза и разрушения GV. Ядрышко также размещается эксцентрично. Другим важним цитологическим изменением была постепенная конденсация хроматина вокруг ядрышка, начиная от отдельных сгустков на его поверхности до последующего образования кольцевых структур. **Выводы.** В ооцитах свиньи, в отличие от лабораторных грызунов, GV имеет эксцентричную локализацию, которая устанавливается уже в ранних антральных фолликулах и сохраняется до начала возобновления мейоза. Установлен процесс трансформации хроматина, заключающийся в его постепенной конденсации вокруг ядрышка и может быть определен как ядрышковый комплекс.

Ключевые слова: зародышевый пузырек, эстральний цикл, ооцит, свинья, хроматин, ядрышко

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