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Molecular mechanisms of oogenesis

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ABSTRACT

This literature review is devoted to the molecular mechanisms of oogenesis and depletion of the ovarian reserve. One of the factors in this process is constantly changing environment of the ovaries, both during intrauterine development and the postnatal period. Numerous mechanisms and factors affecting the internal environment of the female gonad are described, such as stem cell factor (SCF), which regulates migration of primordial germ cells and survival of early oocytes, insulin-like growth factor I (IGF-I), and leukocyte migration inhibitory factor (LMIF). The capabilities of the endocrine system, namely sex steroids, which can both replenish the number of germ cells and deplete the ovarian reserve through the expression of apoptotic markers, were shown. Apoptosis causes degeneration of most of the germ cells formed during oogenesis. The molecular mechanisms and factors involved in this process are numerous.

Pathways mediated by mitochondria of germ cells and external pathways mediated by receptors of the cell surface were described. A mediator between two apoptotic pathways was established – the Bid protein (BH3-interacting domain death agonist), the activation of which triggers the apoptosis mechanism of the intrafollicular microenvironment. Some other factors were identified that mediate programmed germ cell death and result in diminished ovarian reserve: eukaryotic elongation factor 2 kinase (eEF-2 K), *PUMA* and *NOXA* genes, the absence of growth factors and members of tumor necrosis factor (TNF) family. Changes in the epigenetic modification of chromatin in the follicular and germ cells, oxidative stress, decreased DNA repair, and the involvement of the genes *BRAC1, RAD51, ERCC2,* and *H2AX* associated with this process can also affect reproductive health and the ovarian reserve. A significant role of mitochondrial dysfunction of granulosa cells in depletion of the ovarian reserve is of great interest, which leads to impaired oocyte competence, deteriorates the gamete quality, and depletes the ovarian reserve. Therefore, oogenesis depends on a huge number of factors and the internal environment of the ovaries, the knowledge of which can maintain the stability of the reproductive function and preserve the quality of the ovarian reserve.

Key words: oogenesis, molecular genetic mechanisms, folliculogenesis, apoptosis, ovarian reserve.

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Молекулярные механизмы оогенеза

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РЕЗЮМЕ

Обзор литературы посвящен молекулярным механизмам оогенеза и истощения овариального резерва. Одним из аспектов данного процесса является постоянно изменяющаяся среда яичников, как во время внутриутробной закладки, так и постнатальном периоде. Описаны многочисленные механизмы и факторы, влияющие на внутреннюю среду женской гонады, такие как SCF, регулирующий миграцию первичных половых клеток и выживание ранних ооцитов; инсулиноподобный фактор роста I и фактор ингибирования лейкоцитов. Показана возможность эндокринной системы, а именно половых стероидов, которые способны как пополнять количество половых клеток, так и истощать овариальный запас через экспрессию апоптозных маркеров. Апоптоз вызывает дегенерацию большей части образующихся в процессе оогенеза половых клеток. Молекулярные механизмы, факторы, участвующие в данном процессе, многочисленны.

Описаны собственные, опосредованные митохондриями половых клеток и внешние, опосредованные рецепторами клеточной поверхности пути. Установлен посредник между двумя апоптозными путями – белок Bid, активация которого запускает механизм клеточной смерти внутрифолликулярного микроокружения. Определены и некоторые другие факторы, опосредующие запрограммированную гибель половых клеток и, как следствие, приводящие к сокращению овариального резерва: фактор элонгации киназа-2, гены *PUMA* и *NOXA*, отсутствие факторов роста и членов факторов некроза опухоли. Изменения в эпигенетической модификации хроматина в клетках гранулезы и половых клетках, окислительный стресс, снижение репаративной способности ДНК и связанное с этим процессом участие генов репарации *BRAC1, RAD51, ERCC2* и *H2AX* также могут повлиять на репродуктивное здоровье и фолликулярный запас. Особо следует отметить значительную роль в истощении запаса половых клеток митохондриальной дисфункции клеток гранулезы, что приводит к нарушению компетентности ооцитов, ухудшает качество гамет и истощает овариальный резерв. Следовательно, оогенез зависит от огромного количества факторов и внутренней среды яичников, владение которыми способно сохранить стабильность репродуктивной функции и качество овариального резерва.

Ключевые слова: оогенез, молекулярно-генетические механизмы, фолликулогенез, апоптоз, овариальный резерв.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

The relationship between the size of the ovarian reserve and a woman's reproductive life span highlights the importance of understanding the regulatory factors and processes that determine its formation [1–4]. Studies conducted in mice describe in detail (some at the molecular level) the processes involved in determining the number of oogonia and oocytes, while our knowledge about these processes in the human body is not sufficient [3, 5].

The degree of change in the number of germ cells at every stage leading to the formation of the ovarian reserve is particularly worth noting. However, we have almost no understanding of the causes of the dynamics in folliculogenesis and germ cell death, which may be related to the nature or timing of triggers for each of the stages of oogenesis. We do not

understand why so many oocytes are formed and then lost at the pre-reproductive stage of the ovarian reserve. For example, in women, only 1 in 1,000 primordial follicles at birth will mature before ovulation and produce estradiol and progesterone necessary for fertility during the reproductive period of life [6].

It seems that mammalian fetal oocytes face a number of challenges to survive throughout all the stages of the oogenesis, especially in prophase I of meiosis up to the diplonema stage and the initial follicle assembly [7]. Depending on the period of development and experimental conditions, these oocytes can undergo various forms of programmed cell death. We assume that they require constant support of growth factors to carry out the activities necessary to overcome apoptotic death during prophase I. Before the formation of primordial follicles, a decrease in the amount of nutrients or growth factors can activate protective autophagy, but if fasting is prolonged, it can end in death.

In fact, elucidating the relationship between signaling growth factors (mainly the caspase cascade) and apoptotic and autophagic proteins that probably co-exist in fetal oocytes may be essential for understanding the causes of death of these cells. However, recent progress in molecular markers for the prophase of meiosis I, as well as for oocytes and stem cells has greatly helped in the identification and classification of developing gametes [1, 2, 7]. Such progress in apoptotic markers and many other pathways related to ovarian cellular functions contributes to our better understanding of oogenesis. However, it is important to understand how individual signals of local cell death accumulate, leading to changes in reproductive function at the level of the entire body.

MECHANISMS AFFECTING OOCYTE SURVIVAL: THE CHANGING OVARIAN ENVIRONMENT

It is still unclear to what extent the balance of individual oocyte decisions to continue development or initiate cell death may relate to the local effects of the developing ovarian somatic cell cluster in oocyte survival (for example, endocrine environment, intercellular signaling, extracellular matrix) or to the inherent properties of oocytes in meiotic transformations (for example, errors in synapsis that can delay meiotic progression or cause arrest).

Paracrine factors of oocyte survival. A number of paracrine factors identified in the human fetal ovary may affect oocyte survival at one stage or another [8–10]. For some paracrine factors, progress has been made in identifying the primordial cell type and receptor locations. However, the local environment of the ovaries is complex and constantly changes both during intrauterine development and in the future.

There are a number of so-called survival factors without which germ line cells die, for example, the stem cell factor (SCF), also known as the KIT ligand, which is essential during migration of primordial germ cells and for survival of early oocytes [11]. The KIT ligand has been noted by numerous studies as a critical regulator of primary follicle activation. By binding to its receptor, it sends signals along several pathways, including activation of the oocyte phosphotidylinositol-3-kinase (PI3K), which are particularly important for ovarian development, restoring intercompartment communication and reducing the rate of follicular atresia. SCF, along with insulin-like growth factor I (IGF-I) and leukocyte migration inhibitory factor (LMIF), support the survival of germ line cells after migration. They are overlaid with pro- and antiapoptotic mechanisms and other cell death pathways that function at certain stages [8]. For example, in mice, the absence of signaling in a complex of factors activates the Fas death pathway in pre-follicular oocytes [12], and tumor necrosis factor-α promotes apoptosis during follicle formation [13, 14].

Endocrine factors of oocyte survival. Within egg nests, inter-oocyte communication is mediated through cytoplasmic bridges, but after the collapse of this formation, interfollicular communication can continue, for example, through molecules produced by granulosa cells [14]. The developing ovary is also influenced by the embryonic endocrine system, and transcripts of some steroidogenic enzymes are present in the ovary for at least 15 weeks of gestation, while the ability to metabolize androgens into estrogens is only present for about 12 weeks of gestation. Estrogens and progesterone inhibit follicular assembly in rats, possibly by inhibiting apoptosis in oocytes [15, 16].

However, progesterone may be at least partially endocrine rather than local, since the removal of ovaries from the environment *in vivo* marked an accelerated transition from primordial to primary follicles in the absence of progesterone [14, 17]. Possible extragonadal sources of such steroids were identified in human embryonic tissues. Experiments in which ovaries of a specific genotype are transplanted in mice with severe combined immunodeficiency can be used to demonstrate the need for local effect of the gene.

Therefore, the local presence of the *Fas* gene in the ovary is necessary for normal elimination of oocytes in transplanted ovaries [9, 15].

Intraoocytic factors and intercellular interactions. Against the background of this complex local environment, internal factors of the oocyte can also influence the prospects of their survival. Some researchers noted an increase in the frequency of abnormal synapsis in genetically abnormal fetuses, as expected, and also associated some chromosomal abnormalities with increased apoptosis. However, when studying individual mouse oocytes in the prophase of meiosis I, only a slight relationship was found between apoptotic molecular markers and normal or abnormal SCF appearance in mice [7, 18, 19]. In contrast to the relative absence of an association between apoptotic markers and meiotic abnormalities in individual oocytes, significant differences were observed in the behavior of ovarian tissue samples *in vitro* in accordance with gestational age [20]. Consequently, this may affect the environment that surrounds oocytes entering meiosis at different stages of pregnancy.

It was found that cultures of ovarian fragments from fetuses at 14 weeks of gestation were prone to expansion *in vitro*, the cells moved from the original fragment and eventually covered most of the membrane with clusters and aggregates. Alkaline phosphatase staining showed that germ cells were mainly concentrated in the original tissue fragment and in clusters that were formed as a result of reproduction. The remarkable ability of oocytes to migrate during intrauterine development even during prophase I of meiosis was noted by previous authors [18].

For example, X. Wu et al. (2017) used organ culture techniques applied to adult tissues. Survival and growth of follicles *in vitro* from the tissue were observed at 16–22 and 22–23 weeks of gestation, respectively, and confirmed meiotic initiation and progression [19]. Optimization of culture methods for fetal ovaries will be a valuable tool for studying hormonal and paracrine effects on the key aspects of human ovarian development. The mechanism of movement of germ line cells is currently being studied. This ability was significantly improved by including growth factors in the culture medium (SCF 10 ng / ml and IGF-I 15 ng / ml).

In contrast, the ovarian tissue increased significantly *in vitro* at the 15th week of gestation, and the stimulating effect of growth factors was no longer significant. It was also noted that later gestational ages (up to 23 weeks) cultured under similar conditions tend to round off *in vitro* and form dense surface epithelium [21].

It is well known that mouse primordial germ cells can be successfully cultured and their number increases in cultures supplemented with growth factors, including SCF [22]. It is also known that c-kit is present on human oogonia and oocytes from 14–21 weeks of gestation and that the distribution of c-kit (SCF) in the human germ line varies depending on the status [23]. For example, S. Gkountela et al. (2013), using immunohistochemistry, found that primordial germ cells and oogonia are c-kit-positive, while free oocytes or those enclosed in primordial follicles are stained poorly or do not express these factors at all. C-kit is again found on the surface of oocytes in growing follicles [23, 24]. We know that progenitor cells remain in the fetal ovaries well after 15 weeks, so perhaps they multiply less or undergo apoptosis at later stages.

Meiosis as a survival factor. The authors also proposed to evaluate the progression of meiotic transformations of oocytes under cultured conditions using growth factors, such as SCF. The number of oocytes decreased significantly during the first week of cultivation, probably due to a drastic change in the environment [24]. After that, oogenesis was restored with an increase in the number of oocytes at the stages of leptonema and zygonema, progressing during the next week of cultivation. Recovery was more pronounced at 14 weeks than at 15 weeks, and it was evident with or without the addition of the growth factor until the second week *in vitro* [16, 21]. After that, cultures with growth factors were more likely to maintain recovery. It is not yet clear whether growth factors support progenitor cells, oocytes directly, or have an indirect effect through the somatic environment.

Therefore, in addition to expression of numerous genes and formation of growth factors described in previous works [2, 3], oogenesis also depends on the constantly changing environment of the ovaries, namely, endocrine and paracrine factors, inter-oocyte communication, meiotic transformations, and possible abnormalities of germ cell division. Further research will require the use of specific markers for differentiating oocytes and progenitor cells at different stages and using tissue samples from a wider range of gestational age.

DEPLETION OF THE OVARIAN RESERVE OF GERM CELLS

Apoptosis causes elimination of more than 99% of germ cells from the ovaries through follicular atresia

[2, 25, 26]. Less than 1% of germ line cells, following oocyte cultivation, further undergo apoptosis during the last phases of oogenesis and deplete the ovarian reserve in most mammalian species, including humans. The maximum number of germ cells in mice was determined at the time of entry of primary oocytes into the meiosis prophase (Figure). After that, up to two-thirds of the germ cells die and by the time of birth, the ovarian reserve is established, which remains for the rest of life [3, 27, 28]. The peak number of gametes in the human ovary occurs by 20 weeks of pregnancy, after which a drastic decrease in their number takes place, similar to that observed in mice. The degree of germ cell loss during this time (about 20 weeks of gestation) ultimately affects the size of the ovarian reserve [1, 2, 26, 29]. The molecular mechanisms via which oocytes are eliminated and the factors involved in this process remain largely unknown.

According to various authors, the death of mammalian ovarian somatic cells can occur in various ways: apoptosis, necrosis, autophagy, or necroptosis [29–31]. These processes differ death mode, as well as in morphological, biochemical, and molecular characteristics. However, there are currently no published data on which of these pathways is primary and responsible for germ cell death before the formation of the ovarian reserve [32, 33]. Some scientists report a decrease in the number of primordial follicles in prepubertal mice, not associated with the pronounced expression of classical markers of apoptosis and cleaving caspase-3, which results in authors' suggesting an alternative mechanism of oocyte death [34–36]. Consequently, multiple perinatal mechanisms affect the primary follicular reserve.

There are several factors that induce apoptosis directly or indirectly in oocytes at various stages of the cell cycle and meiosis. Premature removal of surrounding granulosa cells from immature oocytes, decreased levels of adenosine 3',5'-cyclic monophosphate and guanosine 3',5'-cyclic monophosphate, increased levels of calcium and oxidants, sustained decreased levels of maturation-promoting factors, depletion of survival factors, nutrients, and cell cycle proteins, decreased meiotic competence, and increased levels of proapoptotic and apoptotic factors lead to oocyte apoptosis [27–30].

Both internal (mediated by mitochondria) and external (mediated by cell surface receptors) pathways are involved in programmed germ cell death. An intermediary between two apoptosis pathways was found – the Bid protein, which is present in an inactive form in the cytosol. In response to the stimulus of the external apoptotic pathway, the N-terminal part of the protein is cleaved off to form the active form tBid. The activated protein moves to the mitochondria and, interacting with the proapoptotic proteins Bak and Bax, permeabilizes the mitochondria with the release of apoptogenic factors, such as cytochrome C [37, 38]. One of the eight helices (H3) of the Bid protein contains the BH3 domain, which activates the mechanism of cell death in the intrafollicular microenvironment. Oocyte apoptosis leads to depletion of the ovarian reserve, directly affecting the reproductive outcome of various mammals, including humans [2, 14, 28].

The role of programmed cell death has been well studied in the ovaries during the transition from mitosis to meiosis and degeneration of follicular clusters in mice [16, 30], when the number of germ cells decreases dramatically (Figure). Apoptotic death at this stage was also demonstrated in the human ovary [8, 26]. Apoptosis is probably of great biological significance for elimination of defective or "low-quality" oocytes with damaged nuclear or mitochondrial DNA [27, 37, 39]. However, there is no direct evidence that the quality of germ cells is maintained by apoptosis during oogenesis. It was suggested that eukaryotic elongation factor 2 kinase (eEF-2 K) is involved in this process by inhibiting antiapoptotic proteins in response to oxidative stress, which makes germ cells more susceptible to apoptosis and elimination [40, 41].

There is evidence that certain proteins mediate apoptosis in somatic cells and affect the number of ovarian germ cells [3, 26, 42]. M. Myers et al. (2014) reported that in mice that are genetically deficient in the *PUMA* gene, increased expression of apoptotic factors and reduction of germ cells entering meiosis by half are observed; therefore, the size of the ovarian reserve is significantly reduced [14, 43].

This effect cannot be associated with altered proliferative activity of germ cells. Data indicate that the *PUMA* gene affects only oogonia granulosa cells before the formation of egg nests, but not during the subsequent decrease in the number of germ cells during degeneration of the latter [44]. Conversely, the antiapoptotic Bcl-2-like protein, MCL-1, is expressed in oocytes relatively late, just before the formation of primordial follicles, and, therefore, may indirectly be involved in preserving the ovarian reserve during oogenesis and at the end of pregnancy [44]. Inactivation of antiapoptotic Bcl-x led to increased apoptosis in granulosa cells of embryonic follicles [36, 39, 40].

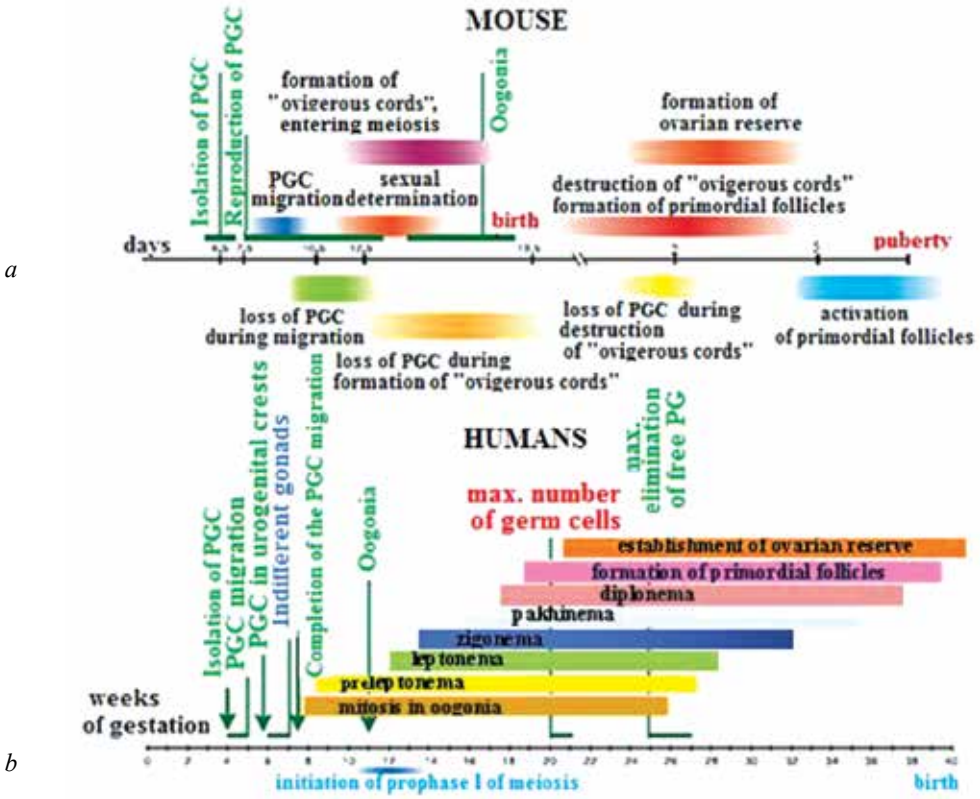

Figure. Comparative characteristics of the ovarian reserve in mice (*a*) and humans (*b*), the number of primordial germ cells (PGC) in different gestation periods

Apoptosis requires activation of either an internal or an external pathway and physiological or stress-related triggers responsible for the activation of these pathways, which have not yet been completely elucidated in granulosa cells. In somatic cells [30, 40] and postpartum mouse oocytes [43, 45], failure to repair DNA damage was shown to cause apoptotic death via *PUMA* and *NOXA*. Other factors responsible for apoptosis in somatic cells include the absence of growth factors (internal pathway) and the absence of tumor necrosis factors (TNF) (external pathway). TNF/TNFR1 and FasL/Fas are expressed in the neonatal ovaries of rodents [3, 13, 46]. In addition, TNF contributes to oocyte death *in vitro*, and deletion of TNFa or Fas in mice increases the initial number of follicles at birth [9, 47]. These data indicate a crucial role of apoptosis for death receptor signaling in female germ cells, particularly during the period of egg nest breakdown and primordial follicle formation.

We know very little about how epigenetic modifications of chromatin in granulosa cells and germ cells can affect reproductive health and follicular supply. Epigenetic modification of chromatin occurs mainly due to DNA methylation, modification of histones, or non-coding RNA, but it is unknown to what extent these processes affect the final number of oocytes in the reserve [34, 36, 48]. Oxidative stress causes oocyte apoptosis with activation of the Fas/FasL system, and oocyte competence correlates more closely with histone modification than with chromatin configuration [36, 40].

The age-related decline in reproductive function in women is not poorly understood, and apoptosis is considered in this process as one of the reasons for the decrease in the primary follicular reserve. One of the papers described a decrease in the DNA repair capacity in age-related rats and, as a result, demonstrated a fall in the mRNA level of the *BRCA1* and *H2AX* repair genes [40, 49]. This study identified 13 differentially expressed proteins involved in a wide range of biological functions, including apoptosis, DNA repair, and the immune system. The differentially expressed FIGNL1 proteins responsible for DNA repair and BOK, a apoptotic protein found in primary follicles, were described for the first time and are associated, according to the authors, with some common features of ovarian aging, loss of follicular reserve, and genome integrity [14, 49].

Another similar study measured the mRNA levels of DNA repair genes in aging animals compared to young ones. The results showed a significant decrease in the mRNA levels of the *BRAC1, RAD51, ERCC2*, and *H2AX* genes for DNA repair and the levels of BRAC1 and H2AX phosphoproteins in the primordial follicles of elderly rats [49]. Therefore, the impairment of DNA repair is confirmed as a mechanism of oocyte aging.

More and more studies are devoted to finding new methods that identify the size of the ovarian reserve. Of course, ovarian biopsy and histological examination provide the most accurate representation of the follicular reserve compared to ultrasound or other indirect laboratory tests. Currently, more data appear on other, non-invasive, but reliable methods for diagnosing the ovarian reserve. In one of these studies, it was determined that mitochondrial biogenesis in granulosa cells may be associated with impaired oocyte competence in patients with reduced ovarian reserve [38, 50].

Mitochondria, which contribute to the quality of oocytes, may be involved in the pathogenesis of follicular depletion. The study of granulosa cells offers a non-invasive approach to assessment of the quality of oocytes and the metabolic processes affecting it. If mitochondrial dysfunction is involved in depletion of the ovarian reserve, it is likely to affect the functioning of cumulus cells. The content of mitochondria in oocytes and cumulus cells was evaluated by quantitative determination of mitochondrial DNA by PCR and expression of 13 genes involved in mitochondrial functions, such as apoptosis and antioxidant protection [38, 40, 50].

Therefore, we can state that follicular cells can regulate mitochondrial biogenesis, creating an adequate pool of mitochondria in oocytes for further development. Changes in this process in patients with diminished ovarian reserve may explain the deterioration of the quality of germ cells. Consequently, some characteristics of the mitochondria in cumulus cells can serve as indicators of oocyte competence, and the quality of germ cells can be improved by products that enhance mitochondrial biogenenesis.

CONCLUSION

Even after decades of study, oogenesis is still not known completely. It is obvious that the internal environment of a pregnant woman at key stages of fetal ovarian development can directly affect both fertility of her future daughter (by controlling the size of the ovarian reserve) and the quality of her oocytes (by influencing the degree of selection and apoptosis). Oogenesis is an integral process of ovarian reserve formation, and the preservation and depletion of the latter depends on a huge number of factors of intraovarian and extragonadal origin, proapoptotic and apoptotic agents, mitochondrial dysfunction, expression of certain genes at all stages of oogenesis and follicle formation, epigenetic modification of chromatin, the ability to repair DNA, and many other, still unknown, markers.

The study of follicular dynamics is gaining momentum due to the use of modern methods that allow to determine the factors of germ cell survival and ovarian reserve formation, as well as to search for possible regulators in preventing pathological germ cell death.

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