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Chapter

Female Germline Stem Cells: A Source for Applications in Reproductive and Regenerative Medicine

Hong-Thuy Bui, Nhat-Thinh Nguyen, Truc Phuong Lam Do, Anh My Le Ba and Nguyen Van Thuan

Abstract

One of the most significant findings in stem cell biology is the establishment of female germline stem cells (FGSCs) in the early 21st century. Besides the massive contribution of FGSCs to support ovarian function and fertility of females, the ability to create transgenic animals from FGSCs have high efficiency. Whether FGSCs can differentiate into mature oocytes for fertilization and complete embryonic development is a significant question for scientists. FGSCs were shown to produce oocytes, and the fertilized oocytes could generate offspring in mice and rats. This discovery has opened a new direction in human FGSCs research. Recently, cryopreservation of ovarian cortical tissue was already developed for women with cancer. Thus, isolation and expansion of FGSCs from this tissue before or after cryopreservation may be helpful for clinical fertility therapies. Scientists have suggested that the ability to produce transgenic animals using FGSCs would be a great tool for biological reproduction. Research on FGSCs opened a new direction in reproductive biotechnology to treat infertility and produce biological drugs supported in pre-menopausal syndrome in women. The applicability of FGSCs is enormous in the basic science of stem cell models for studying the development and maturation of oocytes, especially applications in treating human disease.

Keywords: ovarian stem cell, primordial germ cell, female germline stem cell, oocyte-like cell, regenerative medicine

1. Introduction

It is widely known that mammals begin their lives with a fixed number of oocyte-containing follicles, which do not increase but only degenerate after birth. This explains why fertility decreases with age, and the phenomenon of menopause in women is an indication that reserve resources of oocytes have been depleted over age. This differs from the male that can produce sperm throughout their life due to the presence of spermatogonia stem cells (SSCs), which could allow them to

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constantly proliferate and differentiate to maintain their persistent spermatogenesis, allowing male mammals to have a long-lasting reproductive age [1]. Since the 1950s, the dogma in reproductive biology has been widely accepted that primordial germ cells (PGCs)-derived oogonia in mammals cease proliferation and differentiate into primary oocytes shortly after birth, which will arrest in prophase of meiosis I until fertilization triggers the completion of meiosis [2]. In other words, shortly after birth, mammalian ovaries can differentiate to produce new oocytes to compensate for the consumption of ovulation. This explanation was well accepted due to the shorter gestational age of females compared to that of males. This theory of a fixed ovarian reserve had been the central principle in the field of reproduction.

The evolutionary and molecular processes of female reproductive aging have been highly debated. In the early 21st century, scientists at Harvard Medical University ignited the debate about the unexpected ability of mouse ovaries to regenerate immature oocytes after destruction [3]. Then, the report raised numerous questions by showing that these proliferative ovarian cells, termed female germline stem cells (FGSCs), could produce immature oocytes. Transplantation of these FGSCs into the ovaries of adult mice, was able to differentiate them into mature eggs that are able to ovulate, fertilize, and produce viable offspring [4]. This study opens a new direction in the study of stem cells in human ovaries. If we succeed in establishing the human FGSCs (hFGSCs), they will play a very significant role in reproductive medicine and the treatment of menopause symptoms in women. This would not only recover fertility in infertile women but also delay early menopause in women and treat pre-menopausal syndrome for women without hormone replacement therapy. Notably, the preservation of hFGSC can restore fertility and endocrine function for patients after cancer treatment. This is a huge challenge for scientists "Do hFGSCs exist in humans as they do in mice or not?". Recent studies from researchers supporting the existence of oogenesis in postnatal mammalian ovaries raised some questions, and opened a new avenue for the investigation of stem cells in human ovarian tissue. Stem cells are thought to have numerous uses in cell therapy. Chemotherapy, radiation, genetic induction, or hormonal stress can all result in ovarian failure [5]. Additionally, premature ovarian failure (POF), which affects 1% of young women, is a common cause of ovarian dysfunction before the age of 40 [6]. Furthermore, young women are also rendered sterile by some diseases causing oocyte loss, such as polycystic ovary syndrome [7]. However, very little progress has been made in solving this problem. With assisted reproductive technologies, the findings that FGSCs play a role in fertility provide the future application for clinical therapies [8]. Cryopreservation of ovarian cortical tissue has already been developed for female patients with cancer. Isolation and expansion of FGSCs from this tissue before or after cryopreservation may be helpful for new fertility applications [9]. Some progress has been made in addressing this issue through assisted reproductive technologies. It has been discovered that FGSCs play a role in fertility, offering the potential for future clinical applications. However, the question arises whether the egg cells are produced from hFGSCs or not. If hFGSCs exist in ovaries, why do women still have the phenomenon of menopause? The remaining challenge is to clearly elucidate the origin, roles, and capabilities of these cells, and to be able to use them for therapeutic applications. To this end, studies in mammalian models other than the mouse need to be done because several mechanisms of biological processes for oocytes in mice are different from those in humans. This review will present recent studies on the existence of germline stem cells (GSCs) in the mammalian ovary and summarize the current understanding of ovarian germline stem cells (OSCs) and FGSCs (**Figure 1**).

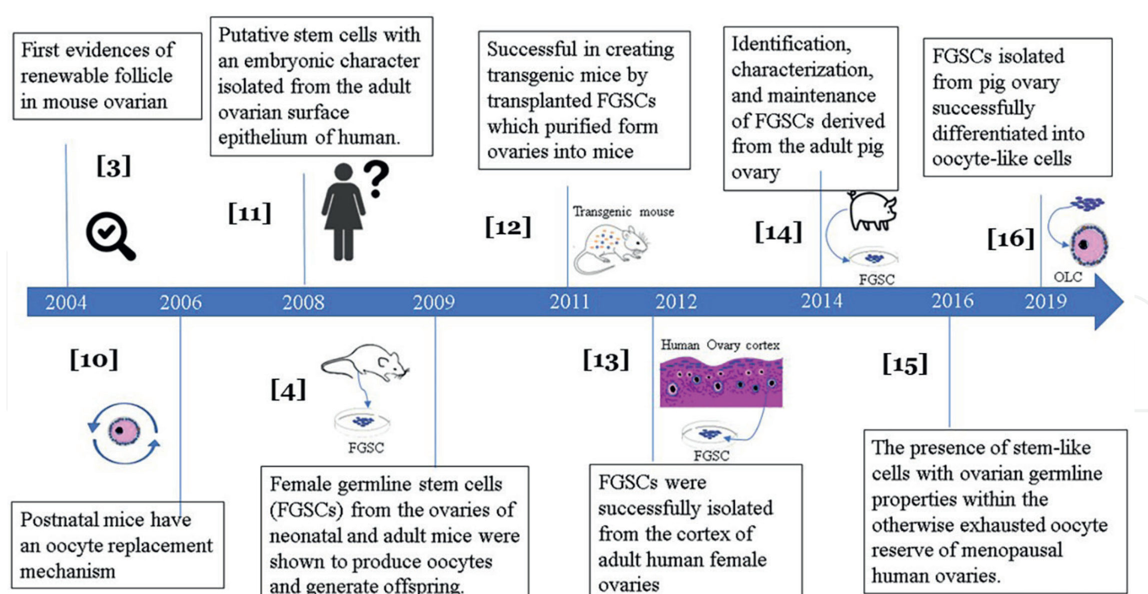

Figure 1.
The timeline of major discoveries in the history of female germline stem cells research.

2. The existence of germline stem cells in adult ovary

For several decades, it has been believed that females are born with a limited pool of oocytes and lose their capacity for oocyte renewal under perturbed conditions. The presence of mammalian FGSCshas been a highly debated area of reproductive technology since 2004, the Harvard University group suggested a source of cells for oogenesis during the reproductive period [3]. In this study, they discovered that the atresia follicle formation rate in mice was lower than the consumption rate of non-atretic follicles. Hence, they believed that there is a renewal of follicles in mice. Moreover, immunostaining showed the presence of proliferating germ cells by expression of germ cell markers. In their final set of experiments, wild-type mouse ovaries were grafted to transgenic GFP-positive mouse ovarian bursa to provide additional evidence for ongoing folliculogenesis in postnatal life. They found that GFP was positive after 3–4 weeks in the grafted ovarian tissue, leading to the conclusion that oogenesis continues in the postnatal ovary. This has sparked much debate about whether germline stem cells do exist in the postnatal ovary. In addition, it was reported that both immature and follicles were detected after transplanting the bone marrow-derived cells from the adult mice into the ovary of infertility mice [10]. Both reports opened many arguments on whether FGSCs existed in the postnatal ovary. Kerr's study in 2006, in which the number of ovarian follicles in mice were counted at different ages, revealed that the average number of follicles did not significantly decrease between 7 and 100 days after birth. Since postnatal mice mature sexually in around 8 weeks (56 days) and ovulation consumes a portion of the follicular pool, it is suggested that postnatal mice have an oocyte replacement mechanism [11]. The culture of cells attained from scrapings of the human ovarian surface epithelium (OSE) resulted in the formation of large oocyte-like cells (OLCs) expressing zona pellucida proteins [12], leading the authors to suggest that putative germ cells within the OSE of the postnatal ovary differentiate from mesenchymal progenitors in the ovarian tunica albuginea. In line with this possibility, small round (2–4 μm diameter) c-kit/stage-specific embryonic antigen (SSEA)-positive cells were isolated from human OSE cells. These cells expressed early PGC markers, including

OCT4 (POU5F1), NANOG, and SOX2 [13]. In 2009, the first successful result in isolating and purifying the FGSCs from the ovary of neonatal and adult mice was reported, by using a magnetic bead sorting technique against Vasa protein, a germline-specific marker [4]. In order to dissipate the doubts about the existence of putative FGSCs in postnatal mammalian ovaries, this group utilized another germline cell-specific protein, Fragilis, to isolate and purify putative FGSCs in postnatal mice. They successfully purified the cells using magnetic sorting techniques, which showed the same characteristics as FGSCs isolated by Vasa protein. Then, FGSCs were transplanted with different genes and subsequently implanted into the ovaries of infertile female mice to create transgenic animals after mating with normal male mice [14]. This research provided significant evidence to support the existence of germline stem cells in postnatal female mammals and opened a new direction in the study of stem cells in the human ovary. Suppose the FGSCs can be successfully isolated from humans, the benefit of this type of cells will play a key role in reproductive studying, medicine, and treatment for menopausal syndrome in women, and other relevant clinical applications. Infertile women can have the ability to give birth again. Besides, further investigation on FGSCs can open a new method for delaying early menopause in women and treating pre-menopause syndrome for women without using hormone replacement therapy.

In 2012, remarkably, FGSCs were able to be isolated from the cortex of adult human ovaries and differentiated into oocyte-like structure cells *in vitro*. Moreover, xenotransplantation of hFGSCs modified to express GFP into immunodeficient female mice resulted in the development of follicles harboring GFP-positive oocytes in the human ovarian cortex after 1–2 weeks [15]. Further, these isolated FGSCs formed into follicles containing oocytes when transplanted into the immunodeficiency mice and could be expanded for months and spontaneously generate 35–50 μm oocytes [16]. This discovery has opened a new direction in research on human FGSCs. Therefore, FGSCs can have an important role in the treatment of diseases caused by infertility females or in extending the period of menopause, as well as the application of stem cell therapy.

We have successfully established pig FGSC from ovarian tissue *in vitro* culture, and porcine PGCs-like Putative Stem Cells (PSCs) continue to maintain their germ stem cell identity *in vitro* and can differentiate into OLCs under appropriate culture conditions. Moreover, experimental evidence showed that PGCs-like PSCs are probably generated from Vasa-positive stem cells *in vitro*. Finally, we demonstrated the critical role of ovarian cell-derived regulatory factors and the proximal stem cell niche in the establishment of porcine PSCs [17]. In addition, many studies provided evidence available to support the existence and potential of putative germline stem cells in the adult mammalian ovary, such as bovine, monkey, and human [18–21]. A finding demonstrated the presence of stem-like cells with ovarian germline properties within the otherwise exhausted oocyte reserve of menopausal human ovaries. Using immunomagnetic enrichment based on membrane DDX4 expression followed by single-cell sorting under a dielectric field, large culture-derived OLCs expressing markers of mature and haploid oocytes were obtained from fertile women as well as menopausal women [22].

Besides, other groups failed to observe replenishment of the follicle pool by donor bone marrow-derived cells [23] or after chemical depletion [24]. They also failed to observe the generation of new follicles even after depletion with busulphan toxin, thus putting into question a need to explain the regeneration of follicle numbers, a finding further supported by mathematical modeling [25]. Lei and Spradling traced the numbers of follicles over time using tamoxifen-induced random labeling of cells. They argued that the follicle pool is highly stable with a half-life of 10–11 months, which would make the follicle pool at birth large enough to support ~500 ovulations required during

the life of a mouse [26]. In a study to confirm the existence of FGSCs in postnatal mouse ovaries, transplantation of premeiotic female PGCs and companion pre-follicular cells into the ovaries of adult mice has been shown to be capable of supporting the formation of new follicles. However, the transplanted PGCs could only form follicles with their pre-follicular cells and vice versa [27]. Although the authors concluded that neo-oogenesis does not normally occur in the ovaries of adult mice, the results nonetheless provide an answer to the important question of whether adult ovaries can support neo-oogenesis from transplanted PGCs. Taken together, we suggest that germline stem cells themselves may not persist in postnatal and adult mammalian ovaries but that progenitor cells/small PSCs in the ovary may instead differentiate into germline stem cells under appropriate conditions [17]. Therefore, although experimental evidence supports the existence of cells with germline progenitor/stem cell characteristics in ovaries of various species, including humans, the existence of GSCs in postnatal ovaries remains ambiguous.

3. Location of female germline stem cells (FGSCs) in the ovary

In female mammalian species, during the embryonic stage, a subset of blastula cells can form PGCs by germ cell determination under some signal induction. Millions of germ cells are formed during embryogenesis. However, most of the germ cells degenerate after embryonic development. Most oogonia die in this period, while the remaining enter the first meiotic division. These latter cells, called primary oocytes, proceed to the first meiotic prophase after replicating their genomes. These primary oocytes are then arrested at this stage of development until the first menstrual cycle. Only a few numbers of oocytes periodically resume meiosis after puberty. Millions of germ cells are produced during embryonic development, but only hundreds of oocytes mature during a female's lifetime [28]. In a menstrual cycle, when the primary oocyte enters metaphase I, its nucleus (germinal vesicle) breaks down, and the metaphase spindle migrates to the periphery of the cells. In telophase, the chromosomes are evenly divided, but one of the two daughter cells retains almost all of its cytoplasmic components, while the other cell has almost no cytoplasm. The smaller and larger cells are called the first polar body and the secondary oocyte, respectively. Moreover, the same phenomenon takes place during the second division of meiosis. Nearly all the cytoplasm is retained by the mature egg (the ovum), and a second polar body receives little more than a haploid nucleus. Thus, the purpose of oogenic meiosis is to conserve the volume of cytoplasm in a single oocyte. Ovulation begins shortly thereafter, in which the follicle ruptures and the secondary oocyte is released into the uterine tube, yet the second meiotic division has not occurred yet. Meiosis of a secondary oocyte is completed only if a sperm succeeds in penetrating its barriers. Meiosis II then resumes, producing one haploid ovum that, at the instant of fertilization by a (haploid) sperm, becomes the first diploid cell of the new offspring (a zygote) [29, 30].

The general idea in reproductive biology is that FGSCsdifferentiate into primordial oocytes through fetal development and that oogenesis begins with a pool of primordial follicles, which is the case in the majority of animals. Several studies have reported the presence of FGSCs in the ovary. Parte and colleagues in 2011 reported the discovery of very small pluripotent stem-like cells deposited in the OSE of adult rabbits, sheep, monkeys, and menopausal humans [18]. Two different populations of putative stem cells (PSCs) of varying sizes were found in scraped OSE. While the larger 4–7 μm cells with cytoplasmic localization of Oct-4 and little expression of SSEA-4 were likely the tissue-committed progenitor stem cells, the smaller

1–3 μm very small embryonic-like PSCs were pluripotent in nature. To demonstrate characteristics of these cells derived from OSE, the PSCs underwent spontaneous differentiation, c-Kit, DAZL, GDF-9, VASA, and ZP4 germ cell markers were used to immunolocalize in oocyte-like structures. Mammalian ovaries include a unique population of extremely small embryonic-like PSCs and tissue-committed progenitor stem cells that have the ability to develop into oocyte-like structures *in vitro*, contradicting the conventional belief that OSE is a bipotent source of oocytes and granulosa cells.

In 2014, we indicated that the ovary contains a considerable number of undifferentiated cells with stem cell characteristics. These might remain in the adult ovary and cannot proliferate normally, but they can undergo proliferation and differentiate into OLCs under appropriate conditions. PSCs were found to comprise a heterogeneous population based on c-kit expression, cell size, and expressed stem and germ cell markers. Analysis of PSCs molecular progression during establishment showed that these cells undergo cytoplasmic-to-nuclear translocation of Oct4 in a manner reminiscent of gonadal PGCs. Flow cytometry analysis revealed abundant PSCs proliferation after isolation and culture for 1 week. Of these, 4.65% of the cells were positive for the germ cell marker Vasa, and some were also positive for additional germ and stem cell markers, such as Fragilis, Thy-1, SSEA4, and c-kit. At this time, two populations of PSCs were observed: one with a cell diameter of 5–7 μm and one with a cell diameter of 10–12 μm. The cells became identical in size after 2 weeks in culture, at 10–12 μm, with an increasing percentage of cells positive for germ and stem cell markers [17]. About 2.8% of all mouse testicular cells were c-kit positive [31] and had the capacity to become multipotent germline stem cells, whereas c-kit-negative cells go on to become SSCs [32]. We similarly observed two distinct subsets of cells (c-kit positive versus c-kit negative) within the PSCs population. This finding was strengthened by immunofluorescence analysis showing that, after 1 month in culture, most of the PSCs expressed high levels of the reprogramming factor Oct4. In contrast, only 22% of the PSCs expressed high levels of c-kit [17].

4. FGSCs aging and stem cell niche in the ovary

While much evidence support the existence of OSCs, it raises the question that ovarian have follicle reserve. Why do they not appear to contribute to postnatal follicle formation, and why does the phenomenon of menopause occur only in females? The researchers believe that the FGSCs aging directly determines ovarian aging.

The stem cell niche is the key to elucidating the entire mechanism of stem cell senescence; Schofield proposed the hypothesis in 1978 that the components surrounding stem cells act as a microenvironment that promotes their growth and protects them from external damage [33]. Other than FGSCs, stem cell niche can be found in almost any stem cell, such as intestinal, myocardial, neural, and hematopoietic stem cells. Stem cell niche can support the growth of stem cell, and disturbance of these niches can cause stem cell damage and eventually leads to certain diseases. Ovarian stem cell niche aid FGSCs to continually proliferate to differentiate into postnatal follicles and oocytes, by regulating to divide into new stem cells and differentiate into germ cells. FGSCs niche is extensively studied in Drosophila Melanogaster. Although the structure and the function of FGSCs niche in mammals have not been fully understood, the component of the niche is believed to be similar to that of drosophila, thus predicting that it may at least be composed of follicular membrane-stromal cells, granulosa cells, extracellular matrix, blood vessels, immune system-related cells, and cytokines. It was suggested

that damage to the stem cell niche is a major cause of the ovarian recession and is more closely related to aging in the ovary than the stem cells themselves. Thus, factors that damage the stem cell niche may have a critical impact on ovarian regression.

Factors that lead to stem cell decay are nutritional and energy deficiencies in the stem cell niche. Insufficiency of energy is caused by mitochondrial depletion associated with aging women, thus only supplies limited amounts of ATP. As a result, the risk of birth defects and infertility is increased due to the reduced energy of FGSCs provided by stem cell niche. The immune system has a critical role in the maintenance of OSC niche to support FGSCs. The perivascular compartment of the stem cell niche forms a bridge to connect the niche and both cellular and humoral aspects of immunity. Cellular immunity that provides support in stem cell niche are monocytes, macrophages, and T cells. Cytokines and immunoglobulins are the humoral aspects of immunity that help stem cell niche maintenance. These are essential for the derivation of OSCs into new germline cells. Weakened immunity due to aging is causing difficulty in the maintenance of the stem cell niche and resulting in ovarian recession.

Dysfunctional gonads can be regenerated by transplanting niche cells of germline stem cells (mostly Sertoli cells or mesenchymal cells). Thus, it could be helpful as first-line therapy to permanently restore gonadal function in POF and cancer patients. Thus, it is clinically more important to reestablish the niche of the FGCSs than to inhibit the aging of the FGCSs themselves in order to delay ovarian aging. The study of the FGSC niche is relatively new, and there are still several issues that we still need to know about. To be able to use for the application of the FGSC niche in clinical practice, these should be addressed and clarified. Understanding the mechanism and application of stem cell niche in FGCS can be crucial in regenerative medicine. Although the result of transplanting FGSCs into infertile female ovaries remains controversial, this has the potential to regenerate.

Researchers have put out solutions to answer this question. One of the most widely accepted theories is that stem cell functions would decline with age, which would result in a loss of renewal capacity [34]. They explained that the aging of FGSC is related to the aging of the stem cell niche. The niche is a specialized microenvironment that gives stem cells specialized cues in the form of adhesion molecules, differentiation and self-renewal-regulating signals, spatial organization, and metabolic support to stem cells. As a result, the niche is crucial for controlling stem cells' fundamental processes and protecting them from cell damage and toxins. Changes in the niche may result in to decline in stem cell function. To demonstrate this hypothesis, Bukovsky observed that the niche of FGSCs formed during early embryonic development consists of nonspecific ovarian monocyte-derived cells (MDCs), T cells, and vascular endothelial cells, In contrast to the nests of adult ovarian germinal stem cells, which are made up of primary CD14 + MDCs, activated HLA-DR + MDCs, and T cells [12]. Furthermore, when the ovarian tissues of older mice were transplanted into young mice, the young mice's ovarian tissues were found to have fewer follicles and no mature follicles [35].

Another hypothesis proposed by researchers suggests that ovarian function may decline by systemic aging-related signals despite the presence of oogonial stem cells [36]. For example, progressive loss of ovarian estrogen (E2) production drives reproductive aging and menopause [37]. To demonstrate the hypothesis, they have shown that mouse OSCs express E2 receptor-α (Erα) by RT-PCR and western blot analysis. To test for potential interactions of E2-activated Erα with meiotic regulatory pathways in OSCs, chromatin immunoprecipitation (ChIP)-PCR assays were applied to assess the Stra8 promoter. Results showed that Erα occupied a consensus ER response element (ERE) in the Stra8 promoter. Moreover, E2 treatment increased the number of GFP-positive cells. Thus, OSCs have differentiated in response to E2. In reverse,

Era-deficient shows a loss of Stra8 expression and oocyte numbers. This study will provide more information on how changes in ovarian estradiol production with aging in women are related to age-related ovarian dysfunction and reproductive aging.

Moreover, oxidative stress also has an essential role in the aging of FGSCs. ROS is a chemically reactive oxygen atom or group of atoms produced during cellular metabolism. The development of ovarian granulosa cells was inhibited by ROS, which also damaged mitochondria and lowered the production of the anti-oxidative enzyme. Additionally, it might diminish ovarian function and trigger an inflammatory response, impairing fertility [38]. Resveratrol (RES) is a naturally occurring substance with many pharmacological roles, including antioxidant, anti-inflammatory, immune-regulating, cell-protective, anti-tumor, and anti-apoptotic effects. RES therapy can be employed to enhance ovarian follicle function by lowering TNF-levels, which was validated by lowering LH levels and the ratio of LH/FSH, two markers of ovarian function. The scientists discovered that RES significantly increased body weight, ovarian index, follicle quantity, and decreased follicular atresia in POF mice.

5. Isolation, maintenance, and characterization of FGSCs

After debating the existence of germline stem cells in ovaries, proponents of their existence continue to question what types of FGSCs exist in ovaries and their characteristics. FGSCs' sizes vary considerably, ranging from 2 to 8 μm. Tilly's group focused on bigger-sized (5–8 μm) OSCs [16], whereas smaller (2–4 μm) pluripotent stem cells, very small embryonic-like stem cells (VSELs) were found in ovary surface epithelium (OSE) [39]. However, OSCs of both sizes express germ-line markers and differentiate into OLCs. Thus, they concluded that there are two different populations of stem cells, the small-sized, pluripotent VSELs, and the bigger OSCs. VSELs are pluripotent stem cells produced from epiblast that are identical to PGCs and persist in small numbers in adult gonads [39]. In contrast, OSCs are tissue-specific progenitors that are bigger and have different gene expressions from pluripotent VSELs. It has been reported that VSELs are the most primitive population of quiescent SCs found in adult tissues compared to OSCs, which quickly divide and produce germ cell nests before differentiating into oocytes. FSH receptor (FSHR) expression was observed on both very small embryonic-like stem cells (VSELs) and ovarian stem cells (OSCs) by immune-localization and immunophenotyping studies. FSH treatment increases germ cell clusters and stimulates stem cells to undergo proliferation and clonal expansion to form germ cell nests. This was further confirmed by the differential expression of OCT-4 in VSELs and NUMB in OSCs. Immunohistochemical expression of OCT-4, proliferation, and FSHR were noted on stem cells located in the OSE of ovarian sections of sheep. Therefore, the establishment of FGSCs is significant for many applications.

Using adult porcine ovaries to isolate, identify, and characterize FGSCs to elucidate their origin and then examine the capability of these cells to proliferate, grow, and differentiate. These cells were heterogeneous, depending on both c-kit expression and cell size, and also expressed stem cell and germline markers. Importantly, we clearly demonstrated that cells with characteristics of early PGCs are present in the adult porcine ovaries. Once FGSCs were established, they could be expanded *in vitro* for months without the loss of identifying markers and proliferative potential. Under appropriate conditions, FGSCs can be differentiated into OLCs. These have the potential to make new oocytes, support ovarian function and fertility, and may support therapeutic

Figure 2.
The female germline stem cells (FGSCs) were successfully isolated from porcine ovarian tissue by enzymatic digestion and different adhesive selection, then cultured in vitro, in DMEM/F-12 and N21 free-serum supplemented. Two populations of FGSCs, one with a cell diameter of 5-7μm and the other with a cell diameter of 10-12μm, were observed with spherical morphology and expressed specific germline characteristics (Vasa, Stella, Oct4, c-kit). The cells became identical at 10-12 μm after ten days in culture, Oct4 expression was reduced in the cytoplasm and augmented in the nucleus, forming groups of cells clustered around theca cell colonies. The large clumps of ovarian cells will be removed during cell passages by the filter of 40μm, and FGSCs were maintained for one month. After one month, following the gradual death of the ovarian cells, the FGSCs were transferred onto mitomycin C-treated MEF feeder layers for long-term culture with the addition of stem cell factor.

strategies in humans [17]. The methods used to isolate, maintain, and characterize FGSCs from each of the research teams studied to date are summarized in **Figure 2**.

The formation of germ cell nests has provided evidence in support of neo-oogenesis from the stem cells [40]. VSELs may endure radiotherapy and chemotherapy and preserve lifelong homeostasis. Due to the loss of function brought on by disturbing ecology, its impairment results in host aging, and the existence of overlapping pluripotency markers raises the possibility that it may also be linked to epithelial ovarian cancer. Many methods for isolation and culture of FGSC have been studied in recent years. Briefly, FGSCs can be isolated by enzymatic digestions (collagenase and trypsin) and purified with either MVH/Fragilis-magnetic bead sorting or/with Flow Cytometer-based SSEA-4, FRAGILIS, DDX4 (VASA) or long-term passage methods to eliminate ovarian somatic cells. Isolated FGSCs are able to be cultured in both absence or presence of feeder cell layers, MEF, STO, or granulose cells. The gelatin-coated surface is typically used in the culture of non-sorting cells. The concentration of FBS varies from 5 to 15% and is used in maintenance and differentiation medium. Besides that, growth factors GDNF, EGF, and bFGF are important for the expansion of FGSCs; LIF is used for FGSCs maintenance, while bone morphogenetic protein and retinoic acid can significantly induce germ cell differentiation [41, 42]. In addition, SCF was also suggested to increase the number of Fragilis- and MVH-positive cells and enhance colony formation efficiency [17, 43]. Other supplements such as antibiotic and antimycotic, pyruvate, non-essential amino acids, antioxidants, insulin, transferrin, and selenium are added to increase the viability and proliferation of ovarian cells. Cultured cells normally express

Species	Isolation	Condition	Result	References
Mouse	MACS- based MVH	MEM α, 10% FBS, bFGF, EGF, GDNF, LIF Co-culture: STO	FGSCs/ Offspring.	[4]
	FACS-based DDX4	MEM α, 10% FBS, N2 supplement, bFGF, EGF, GDNF, LIF Co-culture: MEF	Oogonial stem cells	[15]
	FACS- and MACS-based DDX4	MEM α, 10% FBS, N2 supplement, bFGF, EGF, GDNF, LIF Co-culture: MEF	Oogonial stem cells	[16]
	Preplate culture	DMEM, 15% FBS, LIF, Co-culture: MEF	Oogonial stem cells	[45]
	FACS-based DDX4	MEM α, 10% FBS, bFGF, EGF, GDNF, LIF Co-culture: STO	FGSCs/ Offspring	[14]
	MACS-based Fragilis and MVH	MEM α, 10% FBS, LIF, SCF Co-culture: MEF	Putative stem cells	[43]
	MACS- based MVH	MEM α, 15% FBS, N2 supplement, LIF, GDNF, bFGF Co-culture: STO	FGSCs	[46]
	Whole ovarian cells culture	DMEM/F12, 5% FBS, LIF	Ovarian stem cells	[47]
	Repetitive different adhesion selection	MEM α, 10% FBS, N2 supplement, LIF, GDNF, bFGF, EGF	FGSCs	[37]
Rat	MACS-based Fragilis	MEM α, 10% FBS, bFGF, LIF, EGF, GDNF Co-culture: STO	FGSCs/Transgenic offspring	[48]
Sheep	OSE cells scraping	DMEM/F12, 10% FBS, FSH	Ovarian stem cells	[40]
Porcine	OSE cells scraping	DMEM/F12, 20% knock-out serum, LIF, EGF	FGSCs	[49]
	Differential adhesion selection	DMEM/F12, B27, bFGF, EGF, GDNF, LIF, SCF Co-culture: MEF	FGSCs	[17]
	Differential adhesive selection	DMEM/F12, N21 supplement, bFGF, EGF, GDNF, LIF	FGSCs	[50]
Bovine	Ovarian tissue culture	MEM α, bFGF, FSH	Ovarian stem cells	[20]
Monkey	Colony pickup	DMEM/F12, 10% FBS, LIF Co-culture: MEF	FGSCs	[19]
Human	FACS-based DDX4	MEM α, 10% FBS, N2 supplement, bFGF, EGF, GDNF, LIF	Oogonial stem cells	[15]
	FACS- and MACS-based DDX4	MEM α, 10% FBS, N2 supplement, bFGF, EGF, GDNF, LIF	Oogonial stem cells	[16]

bFGF: basic fibroblast growth factor; EGF: epidermal growth factor; FBS: fetal bovine serum; FGSCs: female germline stem cells; GDNF: glial cell line-derived neurotrophic factor; LIF: leukemia inhibitory factor; MEF: mouse embryonic fibroblast; OSE: Ovarian surface epithelium; SCF: stem cell factor; STO: SIM embryonic fibroblasts.

Table 1.
Summary of isolation and culture of FGSCs in mammalian.

general pluripotent, germ cell, and oocyte markers. While Oct4 is always strongly expressed in cultured OSCs, others, such as SOX2 and Nanog, are still in conflict. VASA, PRDM1, FRAGILIS, and DAZL are considered germline-associated genes. Oocyte-specific markers, including ZP1–3, GDF9, NOBOX, and SCP3, are rarely detected or weakly expressed [15, 44]. A summary of these methods is shown in **Table 1**.

6. FGSCs share characteristics with epiblast-derived PGCs

We have successfully established FGSCs from porcine ovaries and demonstrated that these FGSCs derived from PGCs have been retained and become inactivated. These PGCs were thought to exist only during the fetal period, and all transformed into oocytes before the individual was born [17]. In addition, a study has also shown similar patterns of gene expression profiles between FGSCs and PGCs [51].

In the current model, PGCs are derived from a small number of epiblast cells and are identified before differentiation into different germ layers begins. PGCs then undergo a complex migration process, passing through the abdominal cavity, along the developing hindgut, and finally into the dorsal mesentery, where gonads develop. Despite the apparent importance of these early developmental processes for future fertility, little is understood. It was suggested that PGCs temporarily reside in an "Allantoic Core Domain" (ACD), which they propose has similar functions to the Spemann organizer, consisting of a stem cell pool that extends the body axis in a posterior direction—contributing not only to the germ cell lineage but also the three germ layers. This creates a solid interface between the future umbilical cord and the developing embryo [52]. The stem cells in the ACD express Oct4, Blimp1, Stella, and Fragilis—markers thought to be specific for PGCs but appear to contribute also to other tissues [53]. These observations and the fact that hematopoietic stem cells also migrate from the proximal epiblast to the embryonic aorta-gonad-mesonephros during the same development period. Implies that it is theoretically possible that there may be "intermixing" or that there is a common precursor pool for PGCs and a subpopulation of bone marrow stem cells. Cell lineage tree analysis based on somatic mutations accumulated in microsatellites has shown that oocytes form a completely distinct cluster from other cell populations, suggesting no mixing of germline progenitor pools with other cell types but the bone marrow stem cells [54]. It is conceivable that very rare subpopulations of these cells would be missed in this type of analysis. Their result also shows that aging and unilateral ovariectomy increase the number of mitotic divisions of oocytes. This may be explained by the recruitment of oocytes in the order in which they first differentiate during development. However, it is also consistent with the idea that oocytes are continuously produced from circulating stem cells. Many researchers have suggested that if GSCs exist, they are most likely derived from normal developmental precursors of oocytes, that is, PGCs or oogonia (which have not yet differentiated into oocytes and can undergo mitosis) [55, 56]. The close relationship of PGCs to pluripotent cells is evidenced by the fact that they can be returned to a pluripotent phenotype called embryonic germ cells *in vitro* without genetic manipulation after isolation from the embryo [57, 58].

In 2014, we investigated the developmental origin of porcine PSCs. In normal development, c-kit, SSEA1, and SSEA4 are expressed by the majority of pre-gonadal PGCs and are progressively downregulated when PGCs enter meiosis in the embryonic ovary [59]. In contrast, Vasa protein is detectable only when PGCs enter the gonadal ridges and remains elevated in human fetal and postnatal oocytes [60]. VASA (DDX4)-negative VSEL stem cells (2–4 μm) isolated from the human OSE express genes typical

of ESCs, such as NANOG and SOX2, thereby indicating their undifferentiated status. After culture for 3 weeks under differentiation conditions, VASA-negative cells are transformed into OLCs expressing VASA and ZP2, a marker for oocytes. In the present study, small Vasa-positive porcine PSCs (5–7 μm in diameter) began to reduce their expression of Nanog, Sox2, and Rex1 after 1 week in culture, indicating their transformation to a differentiating status. Previous investigations showed that Vasa-positive VSEL stem cells isolated from adult organs express several characteristic markers of early PGCs, including fetal-type alkaline phosphatase, Oct4, SSEA-1, CXCR4, Stella, Fragilis, Nobox, and Hdac6. Since the porcine PSCs described herein similarly express a number of typical, early PGC markers, these findings might indicate a close association of PSCs with Vasa-positive VSELs and epiblast-derived PGCs [17].

7. Self-renewal capacity of FGSC

The studies have focused on the development of FGSCs into oocytes both *in vivo* and *in vitro*. Transplantation of the GFP-FGSCs back into ovaries leads to the generation of fertilization-competent eggs that produce embryos and offspring [4]. Furthermore, GFP_FGSCs have generated GFP-positive OLCs enclosed in host somatic cells, as characterized by morphology and expression of oocyte-specific markers after injection into adult human ovarian cortical tissue and transplantation into an immune-deficient mouse [61].

Maintaining and extending FGSCs *in vitro* is a crucial step to obtaining fully active germ cells. The effects of various supplements on FGSCs proliferation have been evaluated to optimize conditions for *in vitro* culture of FGSCs. Ovarian tissue plays an essential role in maintaining the properties of FGSCs *in vitro* culture [62]. Follicle-stimulating hormone (FSH) and basic fibroblast growth factor (bFGF) were considered to induce the proliferation of FGSCs and retain their potential for spontaneous differentiation into oocyte-like structures in extended cultures [39]. Preliminary studies by our group have successfully established porcine FGSCs and maintained them for more than 6 months without loss of proliferative potential. Expression of identified germline markers was also maintained. The estimated cell doubling time was 48–72 hours. Subsequently, long-term culture increased the number of differentiated cells among FGSCs, but many FGSCs that were positive for both BrdU and Oct4 or Vasa retained high proliferative potential [17].

GSK3 inhibitors are involved in this process by promoting the proliferation of FGSCs by activating both β-CATENIN and E-CADHERIN [63]. FGSCs that exhibit pluripotency are highly capable of self-renewal, which involves a number of genes and signaling pathways. CADHERIN-22 (CDH22), a member of the cadherin superfamily, functions in FGSC maintenance and self-renewal through its interaction with JAK–STAT and β-CATENIN. The knockdown of CDH22 strongly affected FGSC proliferation by its inhibition and triggering apoptosis, decreased phosphorylation levels of p-JAK2 and p-STAT3, and led to the downregulation of β-catenin [64, 65]. CDH22 also interacts with PI3K to phosphorylate AKT3 and increase the expression of N-myc and cyclin family of FGSCs to promote self-renewal [65].

A study in 2012 evaluated the effects of leukemia inhibitory factor (LIF) and other growth factors, epidermal growth factor (EGF), beg, and glia-derived neurotrophic factor (GDNF), on the proliferation and colony formation of FGSCs. Results showed that these growth factors promote FGSCs proliferation through activation and upregulation of β-CATENIN and E-CADHERIN, and LIF has a significant positive effect on cell colony number [63]. Activation of the GDNF signaling pathway is mediated

by GFRα1 (GDNF receptor), which is related to circGFRα1 by leading to the expression of GFRα1 [66]. In addition, YAP1, an effector of the Hippo signaling pathway, regulates FGSCs proliferation and differentiation *in vitro* and ovarian function [67]. The Hedgehog (Hh) signaling pathway plays a vital role in the fertility of FGSCs. Blocking the Hh pathway by GANT61 depletes ovarian germ cells and FGSCs [68].

Furthermore, gene expression analysis inferred increased expression of proliferation-related genes c-Myc and Cyclin A in the OSE and cortical cells, while expression of the differentiation marker Zp3 was significantly decreased. Rapamycin inhibits the activation of primordial follicles, promotes FGSCs proliferation, and inhibits their differentiation, thus providing a new prospect for delaying ovarian senescence. Furthermore, the novel administration of Daidzein to FGSCs promoted the survival and proliferation of FGSCs by activating the Akt signaling pathway through Type C lectin domain family 11 member a, which functions as a growth factor [66].

8. Differentiation of FGSCs

Stem cell differentiation is critically dependent on the ability to differentiate stem cells into a specific cell type with a highly efficient and scalable system. Recent evidence has demonstrated that the differentiation signals are strongly modified by adhesive and mechanical factors. Furthermore, an environment that mimics the microenvironment in tissues is desired to stimulate stem cell potential and differentiation. Mimicking the cellular microenvironment *in vitro* is increasingly influential in guiding stem cell proliferation and differentiation [69]. Studies claim that cells from both menopausal and non-menopausal women may produce *in vitro* oocyte-like cells (OLCs-large spherical cells). This suggests that neo-oogenesis may take place during ovarian senescence. Tilly and colleagues discovered that OSCs developed *in vitro* to produce OLCs with gradual expansion up to 30–50 μm in diameter. These cells have expressed terminal markers such as zona pellucida (ZP) glycoproteins, GDF–9, NOBOX, YBX2, and SYCP3. In addition, they have examined that OLCs have the haploid karyotype [15]. OLCs generated by regulatory factors or spontaneous development lose expression of developmental pluripotency-associated genes, resulting in strong expression of oocyte makers and maintenance of germline markers. However, although many groups have been studied for the establishment of FGSCs, the differentiation potential of FGSCs in mammalian ovaries remains a controversial issue among germline biologists and stem cell researchers. To date, no mammals other than mice and rats have successfully produced offspring from FGSCs [14, 48]. Whether FGSCs can undergo growth, maturation, and fertilization to become functional oocytes is one of the crucial questions for us.

While most researchers have focused on the role of media (cytokines and growth factors) in regulating FGSCs differentiation into OLCs, the ideal culture system condition has yet to be established. We have investigated the effect of culture medium on FGSCs and further studied the effect of different culture systems (gelatin-coated dish, MEF feeder layer, co-culture with granulosa cells, and co-culture with MEF cells) on isolated FGSCs differentiation into OLCs. Co-culture of stem cells with a somatic cell population has been investigated as an alternative growth factor to induce stem cell differentiation [70]. This system provides growth factors and overcomes the requirement for exogenous growth factors to promote stem cell differentiation [71]. Somatic cells have been demonstrated to support oocyte development through cell-to-cell communication. This plays a vital role in oocyte growth and functioning via the transport of metabolites [72, 73]. Granulosa cells are one of the most important cell types which support oocyte

development in the follicle. It was also proved that granulosa cells enhance oocyte development competence for *in vitro* culture [50, 74]. Another important cell type, MEF feeder cells, can produce and secrete growth factors and cytokines to provide an environment for stem cell migration, differentiation, and proliferation [75]. Hence, co-culture systems between FGSCs and somatic cells should be studied to improve the quality of OLCs.

2D culture system (monolayer) has been used as a conventional method for stem cell culture and differentiation. Recently, a new advanced 3D low attachment system has been developed. In this system, the primary cells exhibit a higher level of a specific function for a more extended period *in vitro* in 3D culture compared to monolayer culture. Furthermore, the 3D low attachment culture enhances the differentiation and stabilizes the functions of stem cells. [76]. During oocyte culture and development, it has been proved that improvement in ovarian culture systems increased the survival and growth of preantral follicles after the long-term culture period [77]. The majority of work on *in vitro* oocyte culture was undertaken using a conventional 2D culture system [72]. In recent years, further technical advancement has emerged form of 3D culture, which improved oocyte quality and development competence [78].

Our group also has successfully isolated FGSCs from adult pig ovaries and differentiated them into OLCs [50]. FGSCs were passed to the differentiation medium. Firstly, the old culture medium was gently removed from the tissue culture plate. Cells were then washed three times with PBS solution. After removing the PBS solution, 0.25% Trypsin-EDTA was added to the dish for 3–5 minutes. The dish was shaken slightly to separate FGSCs from the bottom of the surface. 10% FBS was added to stop trypsin

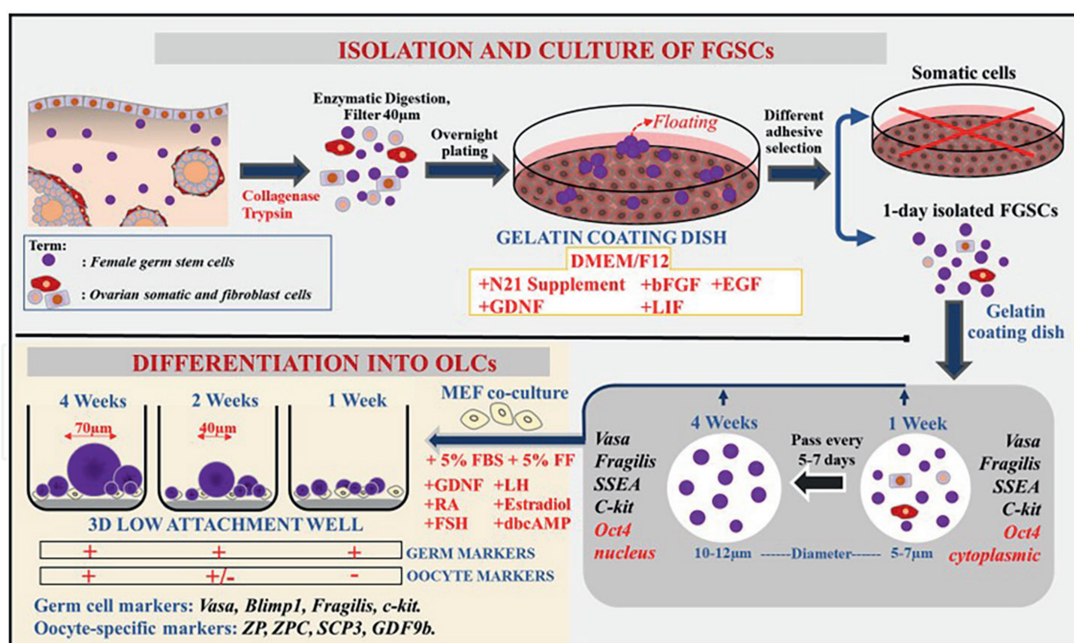

Figure 3.

The female germline stem cells (FGSCs) were isolated from porcine ovarian tissue and cultured in vitro, in DMEM/F-12, and N21 free-serum supplemented. These cells possessed spherical morphology and expressed specific germline characteristics (Vasa, Stella, Oct4, c-kit). For in vitro differentiation induction, using FGSCs 1-week after isolation, where ovarian somatic cells remained in the culture, or using 1-month cultured cells with less or no ovarian somatic cells. Co-culturing the isolated FGSCs with MEF cells under three-dimensional (3D) cell cultures supplemented with follicle fluid. After 1-month in differentiation culture, OLCs could reach about 70 μm in diameter, with a large number of surrounding somatic cells. OLCs expressed germ cell-specific markers (Vasa, Blimp1, Fragilis, C-kit) and oocyte-specific markers (ZP, ZPC, SCP3, GDF9b), contained large nuclei, about 25-30μm, with filamentous chromatin, similar to the oocyte.

action. The cell suspension was then divided into new tubes and centrifuged at 1000 rpm for 5 minutes twice using DMEM solution (Sigma). The supernatant was taken out, and the pellet was resuspended with a differentiation medium. This study evaluated four differentiation conditions to differentiate FGSCs into OLCs *in vitro* (**Figure 3**).

9. Application of FGSCs

Preservation of fertility is one of the most important qualities of life issues fertility preservation (FP) for young women with threatening premature ovarian insufficiency, especially for young cancer survivors who have not completed their family upon a cancer diagnosis. Among the currently available options for FP, especially in young patients, is cryopreservation of ovarian cortical tissue containing primordial follicles followed by autotransplantation [79]. Although ovarian tissue cryopreservation (OTC) currently represents an experimental approach, it offers not only FP but a restoration of endocrine function for women with cancer prior to undergoing gonadotoxic treatments. Re-implantation of OTC is currently the only option to use stored tissue, but in many cases, this procedure carries the potential risk of reintroducing the malignancy. However, in cancer patients, especially women with leukemia, re-transplantation is not an option due to the presence of malignant cells in the ovaries [80, 81].

FGSCs have the great clinical potential to be one of the options for the treatment in regenerative medicine for restoring declined female reproductive function caused by ovarian aging and perimenopausal-related diseases and preservation of fertility for patients with post-gonadotoxic therapy for ovarian cancer. In the former case, activation of FGSCs may restore ovarian function through their self-renewal and ability for committed differentiation into oocytes. The ovary is one of the most important female organs and reflects physiological signs of aging. Delay of ovarian age may avoid the negative consequences of menopause on one's health and its climacteric symptoms. It can also be applied to the fertility of women with POF to prolong their lives. Regarding the latter, FGSCs can be isolated and preserved for future use by cryopreservation after the biopsy of the ovarian cortex from the patient. Then, it might be able to use for *in vitro* fertilization by starting *in vitro* maturation to mature oocytes or injected back into the patient's ovaries to undergo neo-folliculogenesis. These will offer several advantages. First, the collection of ovarian cortex samples does not create the need to delay life-saving treatment, contrary to ovarian superovulation regimens. Additionally, more new follicles and oocytes may be obtained from FGSCs than from cryopreserved tissue or ovarian stimulation. However, the actual clinical application has not yet been achieved, due to technical and regulatory issues. Thus, research on FGSCs for therapeutic application has become an important topic.

Nowadays, OTCs and re-transplantation is a viable methods to preserve fertility in cancer patients. Therefore, most research focuses on technical aspects of OTCs, including follicle survival from freezing/thawing and fragment size, and duration of ovarian function after re-transplantation [82–84]. Study on *in vitro* culture for human OTCs is limited by the extreme difficulty of this technique and the unavailability of human tissue. Despite the undeniable advancements strengthening the protocols currently used for OTCs in domestic animals and endangered species has been achieved, this technique is still considered to be experimental for livestock such as swine [85], caprine [86], ovine [87], bovine [88], equine [89]. Using OTCs to establish FGSCs and differentiate into functional oocytes will answer the question about the possibility of creating gametes cells from adult mammalian ovaries. Gamete cells are ready for

insemination with sperm to form embryos. This study will contribute significantly to the study of biological processes in human eggs in infertility treatment.

Moreover, FGSCs have great applicability in the basic science of stem cell models to study oocyte development and maturation, especially for treating human disease. Besides, FGSC is also very important in the production of transgenic animals. Transgenic animals are the animals with modified genome. A foreign gene is inserted into the genome of the animal to alter its DNA. This method is done to improve the genetic traits of the target animal. Until now, people have created many products of this type, and many products are suitable for use as food or medicine. Zhang and colleagues established FGSCs in mice and transplanted various genes [14]. FGSCs were transferred into the ovaries of infertile female mice. The result was to create transgenic mice after coordination with normal male mice. Scientists have suggested that the ability to produce transgenic animals in this method would be a great tool for biological reproduction in the future. Producing transgenic livestock can significantly improve human health, enhance nutrition, protect the environment, increase animal welfare, and decrease livestock disease. Especially, the creation of transgenic animals with biotechnology-based pharmaceuticals to produce precious protein for human or animal organ replacements, such as miniature pigs, due to the size of their organs being similar to humans.

In order to control these factors, a suitable culture system must be designed and optimized. From the basic research on the ability to generate eggs from FGSCs, applying this method to produce transgenic animals would be carried out efficiently. A study showed that FGSCs were established in mice and transplanted different genes into them. Then these FGSCs were transferred into the mouse ovaries of infertile females. The result was to create transgenic mice after coordination with normal male mice [14]. Recently, scientists have proven that FGSCs were a useful tool for the genetic manipulation of animals by creating transgenic rats [48]. Moreover, a study reported the success to restore ovarian function that suffers from cancer chemotherapy treatment and eventually produces offspring for the first time by transplanting the FGSCs [90]. Therefore, FGSCs played an essential role in the treatment of diseases caused by infertility females or in extending the period of menopause, as well as the application of stem cell therapy. In addition, FGSCs may play a significant role in treating diseases caused by infertile females or in extending the period of menopause, as well as the application of stem cell therapy.

10. Summary

FGSCs have a vital role in the treatment of diseases caused by infertility of females or in extending the period of menopause, as well as the application of stem cell therapy. Research on FGSCs opened up a new direction in reproductive biotechnology to treat infertility and produce biological drugs supported in pre-menopausal syndrome in women.

Our results support the theory that the ovary contains a small number of undifferentiated cells with stem cell characteristics. They may remain in the postnatal and adult ovary, but they are generally unable to proliferate due to inhibitory factors in the ovary. Under appropriate conditions, however, they can proliferate, differentiate into OLCs, and self-renewal of FGSCs. The presence of such FGSCs in mammalian ovaries and the depletion of ovarian reserve as the female reproductive system ages leads to the hypothesis that such "neo-oogenesis" was present in ancestors and is still present in insects, some fish, and mollusks. Nevertheless, it has been lost in terrestrial vertebrates during evolution. FGSCs are usually unable to proliferate in the ovary

due to the presence of inhibitory factors unless placed under appropriate conditions. Although we have successfully established pFGSCs and differentiated them into OLCs, it is still inconclusive whether FGSCs become functional oocytes through their growth, maturation, fertilization, and embryonic development in large animals.

In summary, FGSCs appear to exist in ovaries and have been independently isolated by different research groups and from various species (e.g., humans, pigs, mice, rats, etc.). Furthermore, these cells can be manipulated *in vitro* and transplanted to produce offspring. However, only mice and rats have successfully produced offspring from FGSCs. Although the biological significance of these cells remains controversial, their identification and isolation are expected to provide a valuable model for understanding germ cell development and represent a significant step forward in the future for reproductive biotechnology and infertility treatment. Thus, research on the isolation and culture of FGSCs from ovarian tissue before or after cryopreservation may be helpful in the treatment of fertility in women.

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References

[1] Brinster RL. Male germline stem cells: From mice to men. Science. 2007;**316**:404-405. DOI: 10.1126/science.1137741

[2] Zuckermann S. The numbers of oocytes in the mature ovary. Recent Progress in Hormone Research. 1951:**6**;63-108

[3] Johnson J, Canning J, Kaneko T, Pru JK, Tilly JL. Germline stem cells and follicular renewal in the postnatal mammalian ovary. Nature. 2004;**428**:145-150. DOI: 10.1038/nature02316

[4] Zou K, Yuan Z, Yang Z, Luo H, Sun K, Zhou L, et al. Production of offspring from a germline stem cell line derived from neonatal ovaries. Nature Cell Biology. 2009;**11**:631-636. DOI: 10.1038/ncb1869

[5] Wang X-F, Zhang L, Wu Q-H, Min J-X, Ma N, Luo L-C. Biological mechanisms of premature ovarian failure caused by psychological stress based on support vector regression. International Journal of Clinical and Experimental Medicine. 2015;**8**:21393-21399

[6] Hernández-Angeles C, Castelo-Branco C. Early menopause: A hazard to a woman's health. The Indian Journal of Medical Research. 2016;**143**:420. DOI: 10.4103/0971-5916.184283

[7] Dumesic DA, Oberfield SE, Stener-Victorin E, Marshall JC, Laven JS, Legro RS. Scientific statement on the diagnostic criteria, epidemiology, pathophysiology, and molecular genetics of polycystic ovary syndrome. Endocrine Reviews. 2015;**36**:487-525. DOI: 10.1210/er.2015-1018

[8] Oktay K, Buyuk E, Rodriguez-Wallberg KA, Sahin G. In vitro maturation improves oocyte or embryo cryopreservation outcome in breast cancer patients undergoing ovarian stimulation for fertility preservation. Reproductive Biomedicine Online. 2010;**20**:634-638. DOI: 10.1016/j.rbmo.2010.01.012

[9] Silber S. Ovarian tissue cryopreservation and transplantation: Scientific implications. Journal of Assisted Reproduction and Genetics. 2016;**33**:1595-1603. DOI: 10.1007/s10815-016-0814-1

[10] Johnson J, Bagley J, Skaznik-Wikiel M, Lee H-J, Adams GB, Niikura Y, et al. Oocyte generation in adult mammalian ovaries by putative germ cells in bone marrow and peripheral blood. Cell. 2005;**122**:303-315. DOI: 10.1016/j.cell.2005.06.031

[11] Kerr JB, Duckett R, Myers M, Britt KL, Mladenovska T, Findlay JK. Quantification of healthy follicles in the neonatal and adult mouse ovary: Evidence for maintenance of primordial follicle supply. Reproduction. 2006;**132**:95-109. DOI: 10.1530/rep.1.01128

[12] Bukovsky A, Svetlikova M, Caudle MR. Oogenesis in cultures derived from adult human ovaries. Reproductive Biology and Endocrinology. 2005;**3**:17. DOI: 10.1186/1477-7827-3-17

[13] Virant-Klun I, Zech N, Rožman P, Vogler A, Cvjetičanin B, Klemenc P, et al. Putative stem cells with an embryonic character isolated from the ovarian surface epithelium of women with no naturally present follicles and oocytes. Differentiation. 2008;**76**:843-856. DOI: 10.1111/j.1432-0436.2008.00268.x

[14] Zhang Y, Yang Z, Yang Y, Wang S, Shi L, Xie W, et al. Production

of transgenic mice by random recombination of targeted genes in female germline stem cells. Journal of Molecular Cell Biology. 2011;**3**:132-141. DOI: 10.1093/jmcb/mjq043

[15] White YAR, Woods DC, Takai Y, Ishihara O, Seki H, Tilly JL. Oocyte formation by mitotically active germ cells purified from ovaries of reproductive-age women. Nature Medicine. 2012;**18**:413-421. DOI: 10.1038/nm.2669

[16] Woods DC, Tilly JL. Isolation, characterization and propagation of mitotically active germ cells from adult mouse and human ovaries. Nature Protocols. 2013;**8**:966-988. DOI: 10.1038/nprot.2013.047

[17] Bui H-T, Van Thuan N, Kwon D-N, Choi Y-J, Kang M-H, Han J-W, et al. Identification and characterization of putative stem cells in the adult pig ovary. Development. 2014;**141**:2235-2244. DOI: 10.1242/dev.104554

[18] Parte S, Bhartiya D, Telang J, Daithankar V, Salvi V, Zaveri K, et al. Detection, characterization, and spontaneous differentiation In vitro of very small embryonic-like putative stem cells in adult mammalian ovary. Stem Cells and Development. 2011;**20**:1451-1464. DOI: 10.1089/scd.2010.0461

[19] Fereydouni B, Salinas-Riester G, Heistermann M, Dressel R, Lewerich L, Drummer C, et al. Long-term oocyte-like cell development in cultures derived from neonatal marmoset monkey ovary. Stem Cells International. 2016;**2016**:1-17. DOI: 10.1155/2016/2480298

[20] de Souza G, Costa J, da Cunha E, Passos J, Ribeiro R, Saraiva M, et al. Bovine ovarian stem cells differentiate into germ cells and oocyte-like structures after culture *in vitro*. Reproduction in Domestic Animals. 2017;**52**:243-250. DOI: 10.1111/rda.12886

[21] Clarkson YL, McLaughlin M, Waterfall M, Dunlop CE, Skehel PA, Anderson RA, et al. Initial characterisation of adult human ovarian cell populations isolated by DDX4 expression and aldehyde dehydrogenase activity. Scientific Reports. 2018;**8**:6953. DOI: 10.1038/s41598-018-25116-1

[22] Silvestris E, Cafforio P, D'Oronzo S, Felici C, Silvestris F, Loverro G. In vitro differentiation of human oocyte-like cells from oogonial stem cells: Single-cell isolation and molecular characterization. Human Reproduction. 2018;**33**:464-473. DOI: 10.1093/humrep/dex377

[23] Eggan K, Jurga S, Gosden R, Min IM, Wagers AJ. Ovulated oocytes in adult mice derive from non-circulating germ cells. Nature. 2006;**441**:1109-1114. DOI: 10.1038/nature04929

[24] Kujjo LL, Chang EA, Pereira RJG, Dhar S, Marrero-Rosado B, Sengupta S, et al. Chemotherapy-induced late transgenerational effects in mice. PLoS One. 2011;**6**:e17877. DOI: 10.1371/journal.pone.0017877

[25] Bristol-Gould SK, Kreeger PK, Selkirk CG, Kilen SM, Mayo KE, Shea LD, et al. Fate of the initial follicle pool: Empirical and mathematical evidence supporting its sufficiency for adult fertility. Developmental Biology. 2006;**298**:149-154. DOI: 10.1016/j.ydbio.2006.06.023

[26] Lei L, Spradling AC. Female mice lack adult germ-line stem cells but sustain oogenesis using stable primordial follicles. Proceedings of the National Academy of Sciences of the United States of America. 2013;**110**:8585-8590. DOI: 10.1073/pnas.1306189110

[27] Zhang H, Zheng W, Shen Y, Adhikari D, Ueno H, Liu K. Experimental evidence showing that no mitotically

active female germline progenitors exist in postnatal mouse ovaries. Proceedings of the National Academy of Sciences of the United States of America. 2012;**109**:12580-12585. DOI: 10.1073/pnas.1206600109

[28] Inoue N, Matsuda F, Goto Y, Manabe N. Role of cell-death ligand-receptor system of granulosa cells in selective follicular atresia in porcine ovary. The Journal of Reproduction and Development. 2011;**57**:169-175. DOI: 10.1262/jrd.10-198E

[29] Baker TG. Comparative aspects of the effects of radiation during oogenesis. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 1971;**11**:9-22. DOI: 10.1016/0027-5107(71)90028-5

[30] Beaumont HM. Radiosensitivity of Oogonia and oocytes in the Foetal rat. International Journal of Radiation Biology and Related Studies in Physics, Chemistry and Medicine. 1961;**3**:59-72. DOI: 10.1080/09553006114550071

[31] Kanatsu-Shinohara M, Inoue K, Lee J, Yoshimoto M, Ogonuki N, Miki H, et al. Generation of pluripotent stem cells from neonatal mouse testis. Cell. 2004;**119**:1001-1012. DOI: 10.1016/j.cell.2004.11.011

[32] Izadyar F, Pau F, Marh J, Slepko N, Wang T, Gonzalez R, et al. Generation of multipotent cell lines from a distinct population of male germ line stem cells. Reproduction. 2008;**135**:771-784. DOI: 10.1530/REP-07-0479

[33] Schofield R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood Cells. 1978;**4**:7-25

[34] Ermolaeva M, Neri F, Ori A, Rudolph KL. Cellular and epigenetic drivers of stem cell ageing. Nature Reviews. Molecular Cell Biology. 2018;**19**:594-610. DOI: 10.1038/s41580-018-0020-3

[35] Massasa E, Costa XS, Taylor HS. Failure of the stem cell niche rather than loss of oocyte stem cells in the aging ovary. Aging. 2010;**2**:1-2. DOI: 10.18632/aging.100119

[36] Niikura Y, Niikura T, Tilly JL. Aged mouse ovaries possess rare premeiotic germ cells that can generate oocytes following transplantation into a young host environment. Aging. 2009;**1**:971-978. DOI: 10.18632/aging.100105

[37] Satirapod C, Wang N, Mac Donald JA, Sun M, Woods DC, Tilly JL. Estrogen regulation of germline stem cell differentiation as a mechanism contributing to female reproductive aging. Aging. 2020;**12**:7313-7333. DOI: 10.18632/aging.103080

[38] Wu J, Tu D, Yuan L-Y, Yuan H, Wen L-X. T-2 toxin exposure induces apoptosis in rat ovarian granulosa cells through oxidative stress. Environmental Toxicology and Pharmacology. 2013;**36**:493-500. DOI: 10.1016/j.etap.2013.03.017

[39] Bhartiya D. Ovarian stem cells are always accompanied by very small embryonic-like stem cells in adult mammalian ovary. Journal of Ovarian Research. 2015;**8**:70. DOI: 10.1186/s13048-015-0200-0

[40] Patel H, Bhartiya D, Parte S. Further characterization of adult sheep ovarian stem cells and their involvement in neo-oogenesis and follicle assembly. Journal of Ovarian Research. 2018;**11**:3. DOI: 10.1186/s13048-017-0377-5

[41] Miyauchi H, Ohta H, Nagaoka S, Nakaki F, Sasaki K, Hayashi K, et al.

Bone morphogenetic protein and retinoic acid synergistically specify female germ-cell fate in mice. The EMBO Journal. 2017;**36**:3100-3119. DOI: 10.15252/embj.201796875

[42] Teletin M, Vernet N, Ghyselinck NB, Mark M. Roles of retinoic acid in germ cell differentiation. In: Current Topics in Developmental Biology. 2017;**125**:191-225. DOI: 10.1016/bs.ctdb.2016.11.013

[43] Yazdekhasti H, Hosseini MA, Rajabi Z, Parvari S, Salehnia M, Koruji M, et al. Improved isolation, proliferation, and differentiation capacity of mouse ovarian putative stem cells. Cellular Reprogramming. 2017;**19**:132-144. DOI: 10.1089/cell.2016.0054

[44] Hainaut M, Clarke HJ. Germ cells of the mammalian female: A limited or renewable resource? Biology of Reproduction. 2021;**105**:774-788. DOI: 10.1093/biolre/ioab115

[45] Paravati S, Rosani A, Warrington SJ. Physiology, Catecholamines. Treasure Island (FL): Stat Pearls Publishing; 2021

[46] Liu J, Shang D, Xiao Y, Zhong P, Cheng H, Zhou R. Isolation and characterization of string-forming female germline stem cells from ovaries of neonatal mice. Journal of Biological Chemistry. 2017;**292**:16003-16013. DOI: 10.1074/jbc.M117.799403

[47] Esmaeilian Y, Atalay A, Erdemli E. Putative germline and pluripotent stem cells in adult mouse ovary and their *in vitro* differentiation potential into oocyte-like and somatic cells. Zygote. 2017;**25**:358-375. DOI: 10.1017/S0967199417000235

[48] Zhou L, Wang L, Kang JX, Xie W, Li X, Wu C, et al. Production of fat-1 transgenic rats using a post-natal female germline stem cell line. MHR: Basic Science of Reproductive Medicine. 2014;**20**:271-281. DOI: 10.1093/molehr/gat081

[49] Bai Y, Yu M, Hu Y, Qiu P, Liu W, Zheng W, et al. Location and characterization of female germline stem cells (FGSCs) in juvenile porcine ovary. Cell Proliferation. 2013;**46**(5):516-528. DOI: 10.1111/cpr.12058

[50] Nguyen H-H, Nhu BLQ, Uyen NNP, Nguyen V-T, Bui H-T. Isolation of female germline stem cells from porcine ovarian tissue and differentiation into oocyte-like cells. The Journal of Reproduction and Development. 2019;**65**:423-432. DOI: 10.1262/jrd.2019-050

[51] Xie W, Wang H, Wu J. Similar morphological and molecular signatures shared by female and male germline stem cells. Scientific Reports. 2015;**4**:5580. DOI: 10.1038/srep05580

[52] Mikedis MM, Downs KM. Mouse primordial germ cells. International Review of Cell and Molecular Biology. 2014;**309**:1-57. DOI: 10.1016/B978-0-12-800255-1.00001-6

[53] Mikedis MM, Downs KM. STELLA-positive subregions of the primitive streak contribute to posterior tissues of the mouse gastrula. Developmental Biology. 2012;**363**:201-218. DOI: 10.1016/j.ydbio.2011.10.003

[54] Reizel Y, Itzkovitz S, Adar R, Elbaz J, Jinich A, Chapal-Ilani N, et al. Cell lineage analysis of the mammalian female germline. PLoS Genetics. 2012;**8**:e1002477. DOI: 10.1371/journal.pgen.1002477

[55] Abban G, Johnson J. Stem cell support of oogenesis in the human. Human Reproduction. 2009;**24**:2974-2978. DOI: 10.1093/humrep/dep281

[56] De Felici M. Germ stem cells in the mammalian adult ovary: Considerations

by a fan of the primordial germ cells. Molecular Human Reproduction. 2010;**16**:632-636. DOI: 10.1093/molehr/gaq006

[57] Shamblott MJ, Axelman J, Wang S, Bugg EM, Littlefield JW, Donovan PJ, et al. Derivation of pluripotent stem cells from cultured human primordial germ cells. Proceedings of the National Academy of Sciences USA. 1998;**95**:13726-13731. DOI: 10.1073/pnas.95.23.13726

[58] Turnpenny L, Brickwood S, Spalluto CM, Piper K, Cameron IT, Wilson DI, et al. Derivation of human embryonic germ cells: An alternative source of pluripotent stem cells. Stem Cells. 2003;**21**:598-609. DOI: 10.1634/stemcells.21-5-598

[59] Kerr CL, Hill CM, Blumenthal PD, Gearhart JD. Expression of pluripotent stem cell markers in the human fetal ovary. Human Reproduction. 2008;**23**:589-599. DOI: 10.1093/humrep/dem411

[60] Castrillon DH, Quade BJ, Wang TY, Quigley C, Crum CP. The human VASA gene is specifically expressed in the germ cell lineage. Proceedings of the National Academy of Sciences USA. 2000;**97**:9585-9590. DOI: 10.1073/pnas.160274797

[61] Ding X, Liu G, Xu B, Wu C, Hui N, Ni X, et al. Human GV oocytes generated by mitotically active germ cells obtained from follicular aspirates. Scientific Reports. 2016;**6**:28218. DOI: 10.1038/srep28218

[62] Parte S, Bhartiya D, Manjramkar DD, Chauhan A, Joshi A. Stimulation of ovarian stem cells by follicle stimulating hormone and basic fibroblast growth factor during cortical tissue culture. Journal of Ovarian Research. 2013;**6**:20. DOI: 10.1186/1757-2215-6-20

[63] Hu Y, Bai Y, Chu Z, Wang J, Wang L, Yu M, et al. GSK3 inhibitor-BIO regulates proliferation of female germline stem cells from the postnatal mouse ovary. Cell Proliferation. 2012;**45**:287-298. DOI: 10.1111/j.1365-2184.2012.00821.x

[64] Zhang X, Yang Y, Xia Q, Song H, Wei R, Wang J, et al. Cadherin 22 participates in the self-renewal of mouse female germ line stem cells via interaction with JAK2 and β-catenin. Cellular and Molecular Life Sciences. 2018;**75**:1241-1253. DOI: 10.1007/s00018-017-2689-4

[65] Zhang X, Wei R, Sun Y, Xia Q, Xie W, Song H, et al. AKT3 is a pivotal molecule of Cadherin-22 and GDNF family receptor-α1 signal pathways regulating self-renewal in female germline stem cells. Stem Cells. 2019;**37**:1095-1107. DOI: 10.1002/stem.3030

[66] Li X, Tian G, Wu J. Novel circ GFRα1 promotes self-renewal of female germline stem cells mediated by m6A writer METTL14. Frontiers in Cell and Development Biology. 2021;**9**:640402. DOI: 10.3389/fcell.2021.640402

[67] Ye H, Li X, Zheng T, Hu C, Pan Z, Huang J, et al. The hippo signaling pathway regulates ovarian function via the proliferation of ovarian germline stem cells. Cellular Physiology and Biochemistry. 2017;**41**:1051-1062. DOI: 10.1159/000464113

[68] Jiang Y, Zhu D, Liu W, Qin Q, Fang Z, Pan Z. Hedgehog pathway inhibition causes primary follicle atresia and decreases female germline stem cell proliferation capacity or stemness. Stem Cell Research & Therapy. 2019;**10**:198. DOI: 10.1186/s13287-019-1299-5

[69] Eyckmans J, Lin GL, Chen CS. Adhesive and mechanical regulation of

mesenchymal stem cell differentiation in human bone marrow and periosteum-derived progenitor cells. Biology Open. 2012;**1**:1058-1068. DOI: 10.1242/bio.20122162

[70] Hendriks J, Riesle J, van Blitterswijk CA. Co-culture in cartilage tissue engineering. Journal of Tissue Engineering and Regenerative Medicine. 2007;**1**:170-178. DOI: 10.1002/term.19

[71] Hwang NS, Varghese S, Elisseeff J. Controlled differentiation of stem cells. Advanced Drug Delivery Reviews. 2008;**60**:199-214. DOI: 10.1016/j.addr.2007.08.036

[72] Eppig JJ, Wigglesworth K, Pendola FL. The mammalian oocyte orchestrates the rate of ovarian follicular development. Proceedings of the National Academy of Sciences USA. 2002;**99**:2890-2894. DOI: 10.1073/pnas.052658699

[73] Kidder G, Mhawi A. Gap junctions and ovarian folliculogenesis. Reproduction. 2002;**123**:613-620. DOI: 10.1530/rep.0.1230613

[74] Zarate-Garcia L, Lane SIR, Merriman JA, Jones KT. FACS-sorted putative oogonial stem cells from the ovary are neither DDX4-positive nor germ cells. Scientific Reports. 2016;**6**:27991. DOI: 10.1038/srep27991

[75] Brizzi MF, Tarone G, Defilippi P. Extracellular matrix, integrins, and growth factors as tailors of the stem cell niche. Current Opinion in Cell Biology. 2012;**24**:645-651. DOI: 10.1016/j.ceb.2012.07.001

[76] Abu-Absi SF, Hansen LK, Hu W-S. Three-dimensional co-culture of hepatocytes and stellate cells. Cytotechnology. 2004;**45**:125-140. DOI: 10.1007/s10616-004-7996-6

[77] Gupta P, Nandi S. Isolation and culture of Preantral follicles for retrieving oocytes for the embryo production: Present status in domestic animals: Isolation and culture of Preantral follicles. Reproduction in Domestic Animals. 2012;**47**:513-519. DOI: 10.1111/j.1439-0531.2011.01904.x

[78] Belli M, Vigone G, Merico V, Redi CA, Zuccotti M, Garagna S. Towards a 3D culture of mouse ovarian follicles. The International Journal of Developmental Biology. 2012;**56**:931-937. DOI: 10.1387/ijdb.120175mz

[79] Kim S-Y, Kim SK, Lee JR, Woodruff TK. Toward precision medicine for preserving fertility in cancer patients: Existing and emerging fertility preservation options for women. Journal of Gynecologic Oncology. 2016;**27**:e22. DOI: 10.3802/jgo.2016.27.e22

[80] Rosendahl M, Greve T, Andersen CY. The safety of transplanting cryopreserved ovarian tissue in cancer patients: A review of the literature. Journal of Assisted Reproduction and Genetics. 2013;**30**:11-24. DOI: 10.1007/s10815-012-9912-x

[81] Medrano JV, Andrés MDM, García S, Herraiz S, Vilanova-Pérez T, Goossens E, et al. Basic and clinical approaches for fertility preservation and restoration in cancer patients. Trends in Biotechnology. 2018;**36**:199-215. DOI: 10.1016/j.tibtech.2017.10.010

[82] Donnez J, Martinez-Madrid B, Jadoul P, Van Langendonckt A, Demylle D, Dolmans M-M. Ovarian tissue cryopreservation and transplantation: A review. Human Reproduction Update. 2006;**12**:519-535. DOI: 10.1093/humupd/dml032

[83] Yding Andersen C, Mamsen LS, Kristensen SG. Fertility preservation:

Freezing of ovarian tissue and clinical opportunities. Reproduction. 2019;**158**:F27-F34. DOI: 10.1530/REP-18-0635

[84] Hoekman EJ, Louwe LA, Rooijers M, Westerlaken LAJ, Klijn NF, Pilgram GSK, et al. Ovarian tissue cryopreservation: Low usage rates and high live-birth rate after transplantation. Acta Obstetricia et Gynecologica Scandinavica. 2020;**99**:213-221. DOI: 10.1111/aogs.13735

[85] Borges EN, Silva RC, Futino DO, Rocha-Junior CMC, Amorim CA, Báo SN, et al. Cryopreservation of swine ovarian tissue: Effect of different cryoprotectants on the structural preservation of preantral follicle oocytes. Cryobiology. 2009;**59**:195-200. DOI: 10.1016/j.cryobiol.2009.07.003

[86] Luz VB, Santos RR, Pinto LC, Soares AAX, Celestino JJH, Mafezoli J, et al. Dimethyl sulfoxide perfusion in caprine ovarian tissue and its relationship with follicular viability after cryopreservation. Fertility and Sterility. 2009;**91**:1513-1515. DOI: 10.1016/j.fertnstert.2008.07.1778

[87] Oskam IC, Asadi BA, Santos RR. Histologic and ultrastructural features of cryopreserved ovine ovarian tissue: Deleterious effect of 1, 2-propanediol applying different thawing protocols. Fertility and Sterility. 2010;**93**:2764-2766. DOI: 10.1016/j.fertnstert.2010.02.003

[88] Kagawa N, Silber S, Kuwayama M. Successful vitrification of bovine and human ovarian tissue. Reproductive Biomedicine Online. 2009;**18**:568-577. DOI: 10.1016/S1472-6483(10)60136-8

[89] Gastal GDA, Aguiar FLN, Alves BG, Alves KA, de Tarso SGS, Ishak GM, et al. Equine ovarian tissue viability after cryopreservation and in vitro culture. Theriogenology. 2017;**97**:139-147. DOI: 10.1016/j.theriogenology.2017.04.029

[90] Xiong J, Lu Z, Wu M, Zhang J, Cheng J, Luo A, et al. Intraovarian transplantation of female germline stem cells rescue ovarian function in chemotherapy-injured ovaries. PLoS One. 2015;**10**:e0139824. DOI: 10.1371/journal.pone.0139824