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Review

The role of germ cell loss during primordial follicle assembly: a review of current advances

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Abstract

In most female mammals, early germline development begins with the appearance of primordial germ cells (PGCs), and develops to form mature oocytes following several vital processes. It remains well accepted that significant germ cell apoptosis and oocyte loss takes place around the time of birth. The transition of the ovarian environment from fetal to neonatal, coincides with the loss of germ cells and the timing of follicle formation. All told it is common to lose approximately two thirds of germ cells during this transition period. The current consensus is that germ cell loss can be attributed, at least in part, to programmed cell death (PCD). Recently, autophagy has been implicated as playing a part in germ cell loss during the time of parturition. In this review, we discuss the major opinions and mechanisms of mammalian ovarian PCD during the process of germ cell loss. We also pay close attention to the function of autophagy in germ cell loss, and speculate that autophagy may also serve as a critical and necessary process during the establishment of primordial follicle pool.

Key words: Germ cell loss; Germ cell cyst; Apoptosis; Autophagy; Primordial follicle assembly

Introduction

In most mammals, it has been demonstrated that approximately 50 % - 70 % of oocytes do not survive to reach maturity [1-5]. The transition of the ovarian environment from fetal to neonatal involves a change from nutritive ingestion via the maternal-fetal blood interface to lactation, and appears to be a critical factor influencing this process. Many organs, including the ovary, have to adapt to post-parturition starvation by non-apoptotic or apoptotic types of PCD [6], a crucial process that is necessary for physiological development. PCD can be classified into three categories: Type I involves apoptotic cell death, which morphologically features cell shrinkage and nuclear fragmentation, type II which morphologically features the formation of autophagic vacuoles, and type III involving necrotic cell death, which morphologically features plasma membrane breakdown [7].

Analysis of the mouse ovary confirms the decrease in the whole number of around birth, with about 40 % - 50 % reduction of follicles from 19.5 days post coitum (dpc) to 2 days post partum (dpp) [8]. Apoptosis is believed to be the major mechanism of germ cell loss in newborn ovaries [9-12]. The expression and balance of the apoptotic-related genes Bcl-2 and Bax are believed to regulate the apoptosis of germ cells [13-15]. In addition, increased lysosome amplification and the expression of LAMP1, suggests a probable role for autophagy in this process [8]. The process of germ cell death occurring during the shift between germ cyst breakdown and establishment of primordial follicle remains poorly understood. Furthermore, the mechanism by which germ cells are lost during the perinatal period remains largely undefined. Complex mechanisms and pathways are believed to function together in fetal and neonatal

germ cell loss, and further work is needed to acquire a better understanding of this process.

Autophagy (type II PCD) is an evolutionarily conserved cellular process through which a cell degrades their own proteins, aggregates, or organelles and is seen in many species from yeast to mammals [16, 17]. Autophagy is an important process that is necessary for normal development, remodeling of tissues or organs, and cell survival. Recent reports provide evidence that autophagy is a lysosome mediated degradation pathway [18-23]. In this review, we discuss the major opinions and mechanisms of mammalian ovarian PCD. We also pay close attention to the function of autophagy in germ cell loss, and propose that autophagy may also act as a critical and necessary process during establishment of the primordial follicle pool.

Germ cell loss around birth

In the female early germ cell development includes the formation of PGCs and their colonization in the gonadal ridges. Following colonization, the PGCs form oogonia and arrest at meiotic prophase I (MPI), and the germ cell cysts undergo breakdown during the establishment of primordial follicle. In the mouse PGCs, the precursors of both oocyte and sperm, begin to appear at about 7.25 dpc [24]. Once PGCs are specified, they start to migrate from outside of the embryo at around 8.5 dpc and arrive at the undifferentiated genital ridge at about 10.5-11.5 dpc [25]. The PGCs colonize and proliferate in the fetal gonadal tissues and then initiate the developmental programs of spermatogenesis or oogenesis. In the female oogonia, the mitotically active germ cells, expand their numbers within the developing fetal ovary. Once the female germ cells enter into meiotic prophase they stop mitotic proliferation and form germ cell cysts [26-28]. Around the time of birth the germ cell cysts undergo a process of degradation to form primordial follicles, during which some oocytes are enclosed by ovarian somatic cells but the majority die during the process of germ cell cyst breakdown.

Exact germ cell numbers prior to degradation has proven extremely difficult to determine. For example, when the gonad germ cells of 13.5 dpc female mice were specifically labeled with a VASA antibody to recognize the total number of germ cells, it was determined that one gonad contained approximately 6,000 germ cells [4]; Conversely, when germ cells were identified by their high content of cytoplasmic alkaline phosphatase, they found one gonad contained 11000 germ cells at 13.5 dpc [29, 30]. Morphological studies in mice have shown that cell death mainly occurs in oocytes at MPI from 16.5 dpc to the early perinatal stage, generally in the first couple days following birth [31]. It was reported that the germ cell number decreases from about 12,000 at 13.5 dpc to 3,500 - 4,000 three days following birth [4]. In the human fetus 7 million female germ cells develop initially in the gonadal ridges at about the 20th week of gestation, however at birth only 1 - 2 million viable oocytes remain (Fig. 1) [32]. The function and mechanism underlying germ cell loss remains poorly understood and requires further studies.

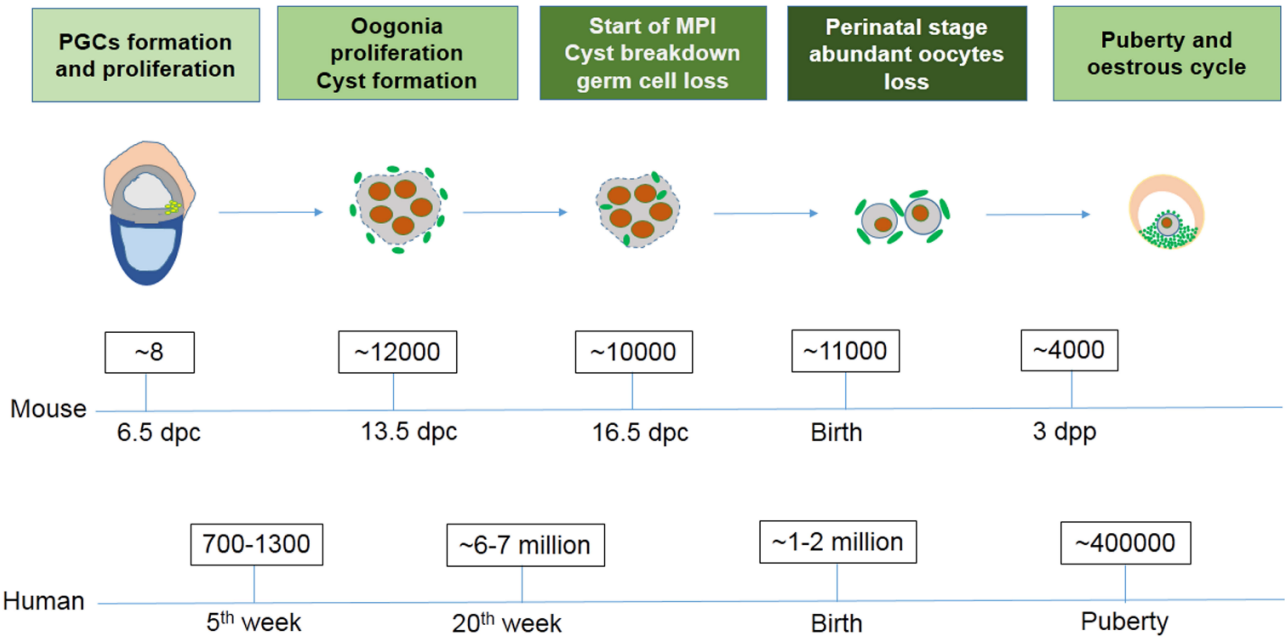

Figure 1. The number of germ cells in mouse and human ovaries at different stages.

Hypotheses of germ cell loss

With the dramatic loss of germ cells taking place, prior to and during follicular assembly, the final number of follicles is not determined by the number of germ cells entering meiosis but the number of survived oocytes [1, 5]. The mechanism or cause of this oocyte loss remains largely unknown. There is no evidence that only one factor or pathway can explain the entire germ cell loss in the fetal ovary. It is well accepted that PCD can be triggered by all kinds of intra and extracellular stresses including DNA damage, hypoxia, or growth factor withdrawal. Currently three hypotheses have been proposed to explain germ cell loss during this process.

The first hypothesis involves the limited amounts of trophic factors. Similar to other cell types the development and survival of germ cells depends on growth factors, and in most cases, an indigent supply of trophic factors would determine whether an oocyte enters apoptosis [8]. Supporting this hypothesis PGCs have been reported to suffer apoptosis with a lack of certain trophic factors, such as leukemia inhibitory factor (LIF) [34]. It has also been shown that Kit and its ligand stem cell factor (SCF) are essential for the survival of germ cells *in vivo* [35-38]. The access to trophic factors is altered with the transition of nutritive ingestion, from blood circulation to lactation, which is sufficient to influence the survival of germ cells. The second hypothesis involves a selection mechanism to guarantee the quality of oocytes by removing germ cells with genetic or meiotic pairing errors [28]. Finally, the third hypothesis suggests a more altruistic purpose for germ cells undergoing PCD. It proposes that the dying oocyte may act as a nurse cell supplying nutrition and energy to developing surviving oocytes [4]. Some evidence suggests that oocytes die in order to donate their cellular components and metabolites to surviving oocytes within a given cyst. However, none of these hypotheses can sufficiently explain the oocyte loss seen in fetal ovaries on their own. It is likely a combination of all these hypotheses playing a role in the dramatic loss of germ cells seen during ovarian development. Currently, the pathways as well as the individual signaling molecules involved in oocyte elimination remain largely unknown. Currently studies utilizing genetic mutations or the overexpression of various molecules, involved in the apoptosis pathway, have generated controversial results.

Multiple mechanisms of germ cell loss during primordial follicle assembly

Understanding the mechanisms involved in oocyte loss are particularly important in attempting to increase the oocyte pool and thereby, reproductive lifespan of an organism. Apoptosis has been implicated as playing a major role in germ cell death observed in neonatal ovaries. However, increased LAMP1 expression has been documented suggesting a probable role of autophagy in this process. During the establishment of primordial follicles, it is likely germ cells may require the cooperation of multiple mechanisms to ensure the survival of chosen oocytes.

Apoptosis and its function during germ cell loss

Generally, early germ cell loss occurs at two critical time points during development: (1) during meiotic prophase I, germ cell cyst breakdown in the prenatal and newborn ovaries, as well as the establishment of primordial follicles; (2) follicular atresia taking place during the ovulatory cycle [39].

Apoptosis was found to play a role at both time points [40]. Apoptosis, one type of PCD, is involved in the normal development process of many tissues. It has the morphologically features of nuclear fragmentation followed cell breakdown into a number of apoptotic bodies, and it is believed to function in the control of cell numbers in most organs [40-42]. Many studies have demonstrated that apoptosis functions a lot throughout the process of oocytes loss seen in the fetal mammalian ovaries.

During mammalian oogenesis oocyte loss occurs particularly at the pachytene stage of meiosis, with several reports suggesting apoptosis is playing a major role [3, 10]. In mice a number of 13.5 dpc female germ cells was observed to live with reduced DNA content, and the number of them increased in 15.5 to 17.5 dpc fetal ovaries [43, 44]. Others have demonstrated that DNA fragmentation was also detected by TUNEL stain in fetal ovaries and ranged from about 0.5 to 5 % [45-46]. In addition to the fetal ovaries it was also shown that apoptosis occurs in germ cells predominantly of newborn mouse ovaries from 1 to 3 days following birth [4]. Some reports have revealed the involvement of the three apoptosis-associated genes *Bcl2*, *Bax*, and *Caspase2* in impacting germ cell numbers in the fetal ovaries [13-15]. Other studies have also demonstrated that mammalian BCL-2 and BAX proteins regulate germ cell loss in fetal ovaries [47-49]. Albamonte *et al*. detected the expression of VASA, BAX, and BCL-2 in human fetal ovaries and revealed that high expression of both BAX and BCL-2 was found within female germ cells from week 12, while BCL-2 was undetectable from week 17. This suggests that the maximum number of apoptotic germ cells coincides with the expression of the BCL-2 protein, and the

balance between apoptosis-inhibition BCL-2 and apoptosis-induction BAX determines germ cell survival or death [50]. Therefore, it appears that apoptosis plays a significant role during primordial follicular assembly, though it remains unknown how it is regulated.

Germ cell cyst breakdown and germ cell loss

In mice, the establishment of primordial follicle pool and germ cell cyst breakdown occur in concert, suggesting a link between them. Throughout the transition from oogonia to oocyte both apoptosis and autophagy can be detected (Fig. 2). However, the significance of the role they play in germ cell cyst breakdown and follicular assembly remain poorly defined. In mice, PGCs start to express germ cell-specific genes including *Mvh* at about 10.5 - 11.5 dpc, and at about 12.5 - 13.5 dpc some PGCs, now referred as oogonia, enter into the first meiotic division prophase and develop into clusters called germ cell cysts [51, 52].

Because of incomplete cytokinesis germ cells within cysts divide synchronously but remain connected with each other by intercellular bridges during oogonial division. It was reported that in *Drosophila*, one oogonia would undergo mitotic divisions and form a 16 cell cyst, but in the end only one of them will develop into an oocyte while the rest act as nurse cells to supply nutrients for the chosen one [53]. This process is not as well understood in mice and remains poorly defined. Lei *et al*. developed a single-germ-cell tracking method allowing the lineage of marked germ cells to be detected during development and found that following labeling at 10.5 dpc, all the germ cell clones (100 %) contained labeled cells in one, two, four or eight cell containing cysts. At 12.5 dpc most of them contained four, eight and 16 cells, suggesting continued cyst production [54]. The germ cells within the cysts were shown to be connected via intercellular bridges. Using electron microscopy, Pepling *et al*. observed many examples of intercellular bridges in 11.5 dpc to 17.5 dpc ovaries. These bridges are similar in appearance and size to those described in previous studies of mammalian germ cells [55]. The Balbiani body is reported to be a distinctive organelle aggregate containing mitochondria, ER, and granulofibrillar material (GFM) that is observed in early oocytes of different species [56-58]. The mouse Balbiani body forms prior to the primordial follicles formation and is only short-lived within young oocytes. It is demonstrated that in mice early germ cells build the Balbiani body to form oocytes receiving energy and nutrients from the cyst-sister cells, whereas the cyst-sister germ cells die, thereby acting as nurse-like cells [59].

In mammals, during the formation of primordial follicles, oocytes are enclosed and separated by invading granulosa cells to form independent primordial follicles. It has been proposed that there are ovarian regional differences during the establishment of primordial follicles. In mice, it was reported that this process of breakdown begins at 17.5 dpc mainly in the medullary part of the ovaries [2, 9].

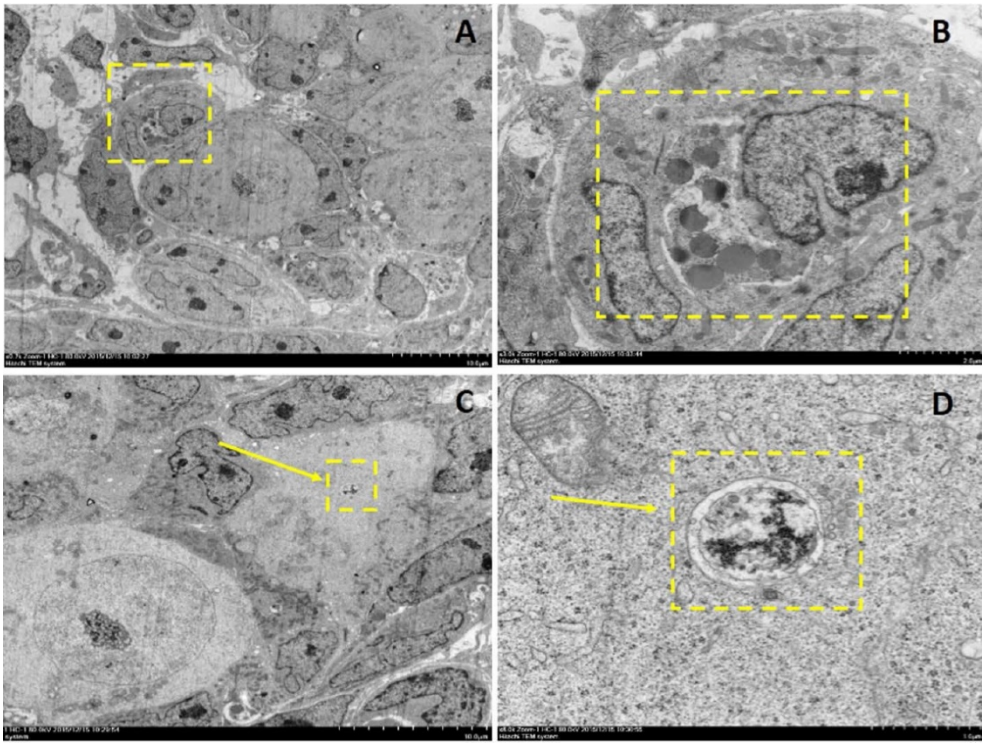

Figure 2. Electron microscopy depictions of apoptosis and autophagy in 2dpp mouse germ cell cysts. A depicts a 2-cell germ cyst within which one is undergoing apoptosis. B depicts the magnified section denoted by the dashed yellow box in A. C and D depict an autophagosome observed in a germ cell within a cyst.

Growth factors and signaling molecules of germ cell loss during primordial follicle assembly

PGCs were reported to enter apoptosis with the lack of trophic factors including LIF and SCF [34, 35]. Several growth factors have been reported to be important for follicle formation.

Wang *et al*. cultured fetal mouse ovaries for 9 days *in vitro* to match the time when distinct primordial germ cells could be detected and they found that adding SCF significantly stimulated the primordial follicle formation. In contrast to SCF, growth differentiation factor-9 (GDF-9) showed dose-dependent stimulation following the formation of the primordial follicles. Adding GDF-9, at a low-dose, stimulated the development of primary follicles leading to a slight decrease in primordial follicle number, but a high dose of GDF-9 surprisingly increased the percentage of both primordial and primary follicles [61]. As mentioned, KIT signaling has also been reported to play a role during several phases of ovarian development. In Reynaud's study heterozygous mice carrying the mutation $Kit^{Wv\text{-}lacZ}$ had decreased KIT and SCF and were used to explore the function of KIT signaling. Compared with the control group they found folliculogenesis of the heterozygous $Kit^{Wv\text{-}lacZ/+}$ mice was disturbed including oocyte growth, and the proliferation of granulosa cells [62].

There is growing evidence that the transforming growth factor (TGF) superfamily plays an important role during primordial follicular assembly [63]. To study the role of multiple TGF-β family members in the formation of primordial follicles bone morphogenetic protein 15 (BMP15) or GDF9 knockout mice were used. GDF-9 mutant mice showed infertile due to a failure in primary follicle formation. While BMP15 null females are subfertile with minimal dysfunction in their ovaries. They also found that BMP15 and GDF9 double homozygous females display oocyte loss at the primary follicle stage, resembling the GDF-9 mutants [64]. When mouse ovaries were exposed to Activin A (ActA), another member of TGF-β family, they showed an increase in the primordial follicle numbers [65]. Liang *et al*. cultured fetal mice ovaries of 12.5 dpc adding ActA for 28 days *in vitro* to explore the effects of ActA during early development of oocytes. In accordance with the previous results they found that the ovaries treated with ActA showed a higher number of oocytes with better growth. Furthermore, when 12.5 dpc ovaries were cultured *in vitro* with the adding of ActA for 10 days, they showed less germ cell death and more primordial follicle survival compared to the control group [66].

To our knowledge, Notch signaling pathway is important for the formation of primordial follicles. Wang *et al*. isolated and cultured 2 dpp mouse ovaries *in vitro* by adding DAPT or L-685,458, to examine the role of Notch signaling during primordial follicle assembly, which are inhibitors of Notch pathway. They found that adding DAPT or L-685,458 significantly decreased the expression of Notch pathway components, including Notch1, Notch2, Jagged1 and Jagged2. Following a 5-day culture mouse ovaries treated with DAPT showed a decreased number of growing follicles compared with the control, however, the primordial follicle number was higher than in the control group. Similarly, treatment with L-685,458 displayed consistent results as with DAPT. Nevertheless, the total number of oocytes in ovaries that were cultured with inhibitors of Notch pathway, was significantly reduced compared with the control group. According to these results, the process of primordial follicle assembly was also effected by regulation of the Notch signaling pathway [67].

Besides endogenous growth factors and signaling pathways the external environment and oestrogen-like hormones can also affect the formation of primordial follicles. For example, Bisphenol A (BPA), a synthetic additive that is ubiquitous in our everyday environment, was noted to have effects on mammalian reproductive functions. Zhang *et al*. found that early development of oocyte was significantly depressed when the newborn mice ovaries were cultured *in vitro* by adding BPA and diethylhexyl phthalate (DEHP) [68]. Another study revealed that BPA influenced oocyte growth through the hypomethylation of imprinted genes including *Peg3* and *Igf2r*, as well as the enhanced estrogen receptor (ER) expression [69]. Although a limiting supply of trophic factors may be one of the causes of fetal germ cell loss, the underlying mechanisms and molecular pathways and the link to PCD remain unclear.

A potential mechanism: autophagy and its function during germ cell loss

Autophagy is a conserved mechanism for the bulk recycling of proteins and organelles. Although a number of studies have demonstrated that apoptosis plays a role in oocyte loss during follicular assembly other mechanisms have also been implicated. Lobascio *et al*. proposed a different mechanism of PCD operating during follicular assembly, namely autophagy [70]. The occurrence of LAMP1 expression during follicular assembly suggests a role for autophagy, which is controlled by insulin/target of rapamycin (TOR) signaling [71-73]. In *Drosophila* nutrient depletion induced autophagy and TOR signaling has been shown to influence the function and structure of the ovaries [74-77]. Furthermore, *Drosophila* ovaries develop abnormally when lacking autophagy-related gene (ATG) in the germline cells and follicle cells. This suggests a relationship between autophagy and germ cell development. It was reported that autophagy is also important for

spermatogenesis. Wang *et al*. reported that the knockout of autophagy-related gene 7 (*Atg7*) specifically in germ cells will result in infertility in female mice. Knock-out of *Atg7* failed to affect the early development of spermatogenesis but impeded the formation of the acrosomal vesicle. Further study found that *Atg7* depleted germ cells showing disruption in the autophagic flux, and resulted in an abnormality of conjugation between LC3 and Golgi apparatus-derived vesicles [78]. These findings uncovered a new role for ATG in germ cell development.

Similarly, some reports demonstrated that autophagy appeared to exist and play an important role in mammalian germ cell loss [18-23]. Studies looking at mouse fetal oocyte loss found that adding rapamycin, an inhibitor of mTOR signaling, increased the number of TUNEL positive germ cells presumably lowering the amount of autophagy (De Felici *et al*. 2008). Along with this the relatively less number of apoptotic cells seen in fetal ovaries suggests that additional mechanisms of PCD may be involved in germ cell loss [8].

Autophagy was found to play an important role in follicular atresia, particularly in primordial and primary follicles, in the spiny mouse [80]. In addition, ultrastructural observation of ovaries has demonstrated the existence of autophagosomes and lysosomes in the majority of species [81, 82]. In accordance with previous studies Rodrigues *et al*. explored the role of autophagy during the early germ cell loss through LAMP1 immunocytochemistry and special markers of lysosomes, investigating the lysosome amplification of oocytes around the time of birth. Firstly, oocytes enclosed within primordial follicles showed an apparent lysosomal accumulation when compared to those extruded or undergoing PCD. Secondly, prominent expression of *LC-3B* and *Beclin 1* were found in 19.5 dpc ovaries, 2 dpp and 6 dpp, whereas *Caspase-3* is present in fetal mice ovaries only [8]. These results support the role of lysosome mediated autophagy in germ cell loss.

Gawriluk *et al*. found that interdicting autophagy by knocking out of *Beclin1* or *Atg7*, resulted in an over loss of oocytes within the perinatal ovary. The number of germ cells in *Beclin1* null 1 dpp ovaries showed a 50 % - 60 % reduction compared to normal wildtype ovaries. Whereas *Atg7* null ovaries at this stage lack discernable germ cells. Their study suggested that autophagy acts as a cell survival mechanism during germ cell loss and oocyte survival in the mouse ovary [18].

To confirm the involvement of autophagy during rat oogenesis, Choi *et al*. investigated the expression of LC3 in immature rat ovaries to determine the site of autophagy in follicular cells. They found that primordial follicles showed weak LC3 expression. Conversely, primary and preantral follicles exhibited very intense immunoreactivity for *LC3*. In addition, granulosa cells of atretic follicles were identified that showed intense *Caspase-3* and *LC3* staining, indicating that autophagy has a good correlation with apoptosis. [22]. In another study a germ cell specific *Atg7* null-mutation was demonstrated to cause subfertility due to loss of follicles. When *Atg7* was knocked out in 17.5 dpc mouse ovaries, the expression of LC3 decreased significantly compared to wildtype ovaries. Similarly, *Atg7* Tnap-Cre ovaries showed a significant reduction of about 50 % in the number of oocytes and follicles when compared with the control ovaries. When *Atg7* Tnap-Cre neonatal mouse ovaries were cultured under a starvation condition *in vitro* the oocytes and follicles were reduced drastically compared with the control group [23]. All of these results suggest that autophagy protects germ cells from loss in the neonatal ovaries under starvation conditions. Further investigation revealed that autophagy inhibition will lead to oocyte death by apoptosis in cultured ovaries *in vitro*, suggesting a close connection between autophagy and apoptosis.

Conclusions

In most female mammals, it has been well accepted that significant germ cell loss occurs shortly after birth [1-5]. While it is generally accepted that germ cell and oocyte loss involves the concerted effort of several processes, the exact underlying mechanisms remain unknown. Apoptosis is an evolutionarily conserved genetically regulated cell removal system which is morphologically distinct from necrosis. A majority of studies have demonstrated that germ cell loss takes place through apoptosis in fetal and neonatal mammalian ovaries. Three apoptosis-associated genes *Bcl2*, *Bax*, and *Caspase-2*, and the balance between BAX and BCL-2 proteins is believed to determine the death or survival of germ cells. TUNEL-positive oocytes observed in fetal mouse ovaries also suggest the role of apoptosis during germ cell loss. The surviving germ cells within cysts are reported to build a Balbiani body and receive nutrients and organelles from cyst-sister cells, whereas the sister germ cells die acting as nurse cells [83, 84]. Growth factors are important and necessary for germ cell development and survival, and a limited supply or change in levels might influence the survival of oocytes. Signaling pathways, aspects of the external environment, and oestrogen-like hormones may also affect the formation of primordial follicles. Autophagy is also reported to play a part in the

process of germ cell and oocyte loss. The observation of autophagosomes and lysosomes in many species ovaries provides support for this assertion. Furthermore, *Atg7* and *Beclin1* knockout studies display the crucial role of autophagy during germ cell loss during the transition from the fetal to newborn stages.

In summary, it is clear a level of complexity involving multiple mechanisms is involved in the establishment of primordial follicles (Fig. 3). The relative contributions of growth factors, apoptosis, autophagy, along with other mechanisms may vary at distinct stages during ovarian development. Future studies directed at improving the reproductive lifespan need to take these diverse mechanisms into account and understand how they work together during every stage over the development of most mammalian ovaries.

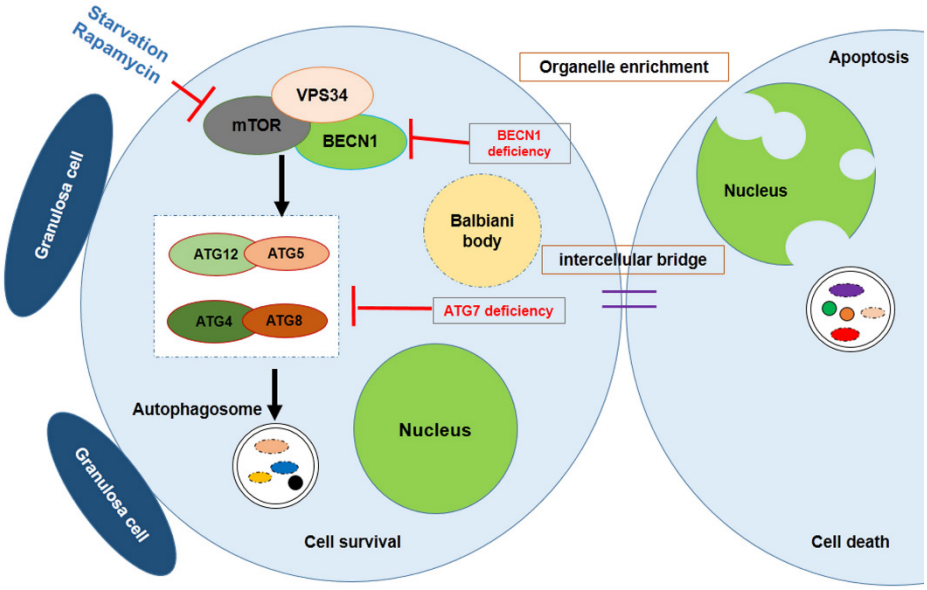

Figure 3. Survival of the chosen oocyte, showing that the mouse germ cells receive organelles from neighboring cyst cells and build a Balbiani body to become oocytes. Autophagy is regulated by mTOR signaling; Atg7 or BECN1 deficiency will interdict the formation of the autophagosome, causing germ cell loss.

Table 1. Major studies of germ cell loss in the mammalian ovary

Researcher	Species	Mechanism involved	The experimental introduction	Year
De Pol et al.	Human	Apoptosis	Numerical decrease of human female germ cells during the fetal period is due to apoptosis	1997
De Felici et al.	Mouse	Bcl-2 and Bax regulation of apoptosis under a partial control by SCF	Elimination of excrescent oocytes might be an apoptotic phenomenon controlled by SCF availability	1999
Pepling et al.	Mouse	Germ cyst breakdown and apoptosis	Germ cell cysts undergo programmed breakdown to form primordial follicles	2001
McClellan et al.	Mouse	GCNA-1	Quantify continuous oocyte loss throughout MPI using GCNA-1	2003
Jefferson et al.	Mouse	Germ cyst breakdown and the formation of MOFs	Genistein treatment inhibits germ cell breakdown and increases oocyte survival	2005
Pepling et al.	Mouse	Balbiani body	oocytes contain a Balbiani body	2006
Michelle et al.	Mouse	BAX-independen apoptosis	While progressing through meiotic prophase, oocytes are eliminated by a BAX-independent mechanism	2006
Bristol-Gould et al.	Mouse	ActA	ActA increased the number of postnatal mouse primordial follicles	2006
Chen et al	Mouse	Germ cyst breakdown and the formation of MOFs	Estradiol, progesterone and genistein inhibit germ cyst breakdown	2007
Ghafari et al.	Mouse	Apoptosis	Apoptosis occur throughout MPI in fetal and postnatal oocytes, with greatest incidence at diplotene stage	2007
Lobascio et al.	Mouse	Germ cell apoptosis	Fetal oocytes undergo degeneration mostly by apoptosis	2007
Albamonte et al.	Mouse	BCL-2/BAX expression balance and apoptosis	apoptotic germ cells was coincident with the lack of detectable BCL-2 protein	2008
Wen et al.	Mouse	The expression of PAR6	PAR6, a potential marker for the germ cells celected to form Primordial Follicles	2009
Rodrigues et al.	Mouse	Germ cell apoptosis and autophagy	Multiple perinatal mechanisms establish the primordial follicle reserve in mice	2009
Lei et al.	Mouse	Cyst fragmentation and germ cell apoptosis	PGCs initially develop into cysts that undergo fragmentation into smaller cysts prior to meiotic entry	2013
Song et al.	Mouse	Autophagy	Atg7 knockout resulted in germ cell over-loss at the neonatal transition period	2015
Lei et al.	Mouse	Cytoplasmic and organelle transport	germ cells receive organelles from sister cyst cells to become oocytes, nurse-like germ cells die	2016

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Competing Interests

The authors have declared that no competing interest exists.

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