INTRODUCTION

The fertility and reproductive lifespan of female mammals are supported by ovarian function, which is responsible for hormone production and oocyte supply, and the associated functions of uterine and oviduct. Concerning the ovaries, a fixed stockpile of primordial follicles, which is termed the "ovarian reserve," determines the intrinsic fertility and reproductive lifespan of a female. Primordial follicles are formed in the fetal period in bovines, sheep, and humans, and from the fetal to neonatal period in pigs and mice. After several rebuttal studies over the past years, it is generally accepted that primordial follicles are not formed afterwards in vivo. In addition, studies using genetically edited experimental animals have shown that the failure of primordial follicle formation results in a premature ovarian insufficiency. Therefore, technological advances aimed at improving the size of the primordial follicle pool, and maintaining them would extend their reproductive lifespan. This would contribute to global food security while also reducing the environmental impact of industrial animal production as well as livestock products, and would also permit the preservation of endangered species.

1 | INTRODUCTION

The impact of oocyte death on mouse primordial follicle formation and ovarian reserve

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Abstract

Background: Ovaries, the source of oocytes, maintain the numbers of primordial follicles, develop oocytes for fertilization and embryonic development. Although it is well known that about two-thirds of oocytes are lost during the formation of primordial follicles through cyst fragmentation and the aggregation of oocytes within the cyst, the mechanism responsible for this remains unclear.

Methods: We provide an overview of cell death that is associated with the oocyte cyst breakdown and primordial follicle assembly along with our recent findings for mice that had been treated with a TNFα ligand inhibitor.

Main Findings: It is generally accepted that apoptosis is the major mechanism responsible for the depletion of germ cells. In fact, a gene deficiency or the overexpression of apoptosis regulators can have a great effect on follicle numbers and/or fertility. Apoptosis, however, may not be the only cause of the large-scale oocyte attrition during oocyte cyst breakdown, and other mechanisms, such as aggregation, may also be involved in this process.

Conclusion: The continued study of oocyte death during primordial follicle formation could lead to the development of novel strategies for manipulating the primordial follicle pool, leading to improved fertility by enhancing the ovarian reserve.

KEYWORDS
apoptosis, cell death, fertility, ovarian reserve, primordial follicle formation
In animals that produce primordial follicles before birth, an approach to elucidating the mechanism is not simple. Studies on the formation of human primordial follicles are also hampered by limited access to tissue samples. Therefore, research that is intended to identify the factors and pathways involved in that mechanism has largely used mice as model animals. We recently reported on the ovarian phenotype in autophagy inducers-administrated neonatal mice and cystine-glutamate transporter (xCT)-knockout mice. In these animals, the numbers of stored primordial follicles are upregulated.8,9 This review focuses on recent developments in our understanding of cell death, which affects the formation of primordial follicles and their maintenance in mice.

2 | THE PROCESS OF PRIMORDIAL FOLLICLE FORMATION IN THE MOUSE

The germline originates from primordial germ cells (PGCs) differentiated from the extraembryonic germ layer region of the prolapsed blastocyst. In mice, approximately 45 PGCs are observed near the base of the incipient allantois at embryonic day 7.5 (E7.5).10 At E8-E9, PGCs begin to move to the genital ridge, then move to the cranial region via the posterior intestine, and finally dorsally through the dorsal intestinal membrane. By E10.5, they enter the genital ridge. They subsequently proliferate around 500 PGCs while undergoing sexual differentiation to adopt an ovarian fate, and primordial germ cells become oogonia.11,12 The major events leading up to the formation of primordial follicles in mice are shown in Figure 1. Clusters of up to 30 proliferating oogonia are observed to be connected by intercellular bridges due to incomplete cytokinesis and are known as germ cell cysts. In the process of cyst formation, the cysts can fragment into smaller cysts and become reassociated with unrelated cysts with the formation of nests where some germ cells are still connected by intercellular bridges with others are associated by aggregation.13 At E13.5, the oogonium transitions from mitosis to meiosis to become an oocyte. Oocytes stop for a long period of time at the diplotene stage of prophase I meiosis.14 By E14.5, the nests undergo cystic division, and cell-to-cell communication is maintained at the intercellular bridge with the formation of an “oocyte cyst” which is surrounded by follicular epithelial cells (pre-granulosa cells). With the formation of cysts, the number of oogonia reaches about 15,000 around E15, which is the maximum for life. From E17.5 to 5 days after birth (PD5), “primordial follicles,” a unit of dormant oocytes and surrounding flat granulosa cells, are formed through oocyte cyst breakdown. During this process, two-thirds of the oocyte pool is lost due to cell death.15 Cyst breakdown, the predominant form of “cyst fragmentation,” is a process in which larger oocyte cysts become smaller cysts. This process involves “aggregation” of the cytoplasm into the dominant oocytes within cysts, and the sister germ cells die acting as nurse cells. On the other hand, the number of germ cells are decreased significantly during this cyst breakdown process, and about 2500–6000 primordial follicles typically observed in the ovary at PD5.15,16

From around PD2, “the first wave of folliculogenesis” occurs in which some primordial follicles on the ovarian medulla region are recruited to primary follicles.17 They serve as sources of the steroid hormones that are required for the establishment and sexual maturation of the hypothalamic–pituitary–ovary (H-P-O) axis and become atretic during follicle development.18

It is generally thought that primordial follicles are not formed afterwards. Therefore, recruitment as first-wave follicle development, periodic follicle development and atresia after sexual maturity, along with the depletion of follicle stock due to ovulation affects the reproductive function and reproductive life of an individual.

3 | INVOLVEMENT OF OOCYTE DEATH DURING PRIMORDIAL FOLLICLE FORMATION

3.1 | Types of cell death in the perinatal ovary

During the breakdown of oocyte cysts and primordial follicle assembly, some oocytes in each cyst die by programmed cell death with only one-third of the total surviving. A detailed study of
programmed oocyte death would be important for developing a better understanding of the pathogenic mechanism of premature ovarian insufficiency or even female infertility.\textsuperscript{19,20} However, the exact mechanisms underlying this process have not yet been fully elucidated.

Several possible mechanisms, including apoptosis,\textsuperscript{15,21} autophagy,\textsuperscript{22,23} and the direct extrusion from the ovarian surface (ovarian shedding),\textsuperscript{19} have been proposed for explaining oocyte loss during primordial follicle formation. Furthermore, in a recent study by Niu et al.,\textsuperscript{24} the presence of acidified nurse oocytes labeled by lysotracker has also been reported. They observed that the addition of an inhibitor of vacuolar ATPase-dependent acidification, Bafilomycin (BafA1), to in vitro culture system of fetal ovaries strongly inhibited oocyte development.\textsuperscript{19} More recently, it has been reported that the addition of Bafilomycin to in vitro culture system of fetal ovaries strongly inhibited oocyte development.\textsuperscript{19} However, it has been reported that the addition of an inhibitor of vacuolar ATPase-dependent acidification, Bafilomycin (BafA1), to in vitro culture system of fetal ovaries strongly inhibited oocyte development.\textsuperscript{19}

3.2 | Apoptosis regulators involved in primordial follicle formation and ovarian reserve

This section focuses on apoptosis during oocyte cyst breakdown and the assembly of primordial follicles. Examples of oocyte death due to granulosa cell death have been reported,\textsuperscript{25} but for each factor, it has not been clearly demonstrated that the death of the oocyte is caused by the death of the oocyte itself or by the death of somatic cells such as granulosa cells. Therefore, this aspect is not covered here. Understanding the mechanisms involved in oocyte loss during primordial follicle formation are particularly important in attempting to increase the primordial follicle pool and thereby, the reproductive lifespan of an organism. It is generally accepted that apoptosis is the major mechanism responsible for the depletion of germ cells. In support of these conclusions, studies have demonstrated that mutations in regulatory genes associated with apoptosis lead to the dysregulation of follicular endowment, and an excess of or a decreased number of primordial follicles in neonatal ovaries (shown in Table 1).

Programmed cell death occurs through two distinct mechanisms, namely, the extrinsic and intrinsic pathways.

Extrinsically, death receptors can initiate the programmed death of cells. FAS (also called CD95 or APO-1), a cell surface receptor, is a member of the death receptor family. In Fas-deficient mice, higher numbers of germ cells were found in perinatal ovaries than in the same-aged wild-type mice.\textsuperscript{26} This can be attributed to a lower rate of germ cell/oocyte death induced by FAS.\textsuperscript{27} Additionally, in Kit/Fas double knockout mice, the loss of the Fas partially rescues the loss of germ cells seen in the case of Kit knockout.\textsuperscript{26} These results indicate that FAS is one of the regulators of oocyte death in fetal and early postnatal ovaries.

Intrinsically, apoptosis is triggered by diverse sources of cellular stress, such as cytokine deprivation, DNA damage or oncogene activation.\textsuperscript{28} The intrinsic apoptosis pathway is regulated by the activities of pro- and anti-apoptotic members of the B-cell lymphoma/leukemia (BCL2) protein family.\textsuperscript{29,30} The BCL2 family is comprised of multiple pro- and anti-apoptotic members that function together to control Caspase-dependent cell death.\textsuperscript{29,31,32} The pro-apoptotic members of the family can be divided into two groups: multidomain pro-apoptotic BAX-like molecules and the BCL2 homology 3 (BH3)-only proteins. The deletion of various members of the BCL2 family has been shown to produce similar alterations in primordial follicle numbers in neonates.

Greenfeld et al.\textsuperscript{33} reported that the deletion of Bax results in increased oocyte numbers in embryonic ovaries and increased follicle numbers in neonatal ovaries when compared with wild-type ovaries. In contrast to their hypothesis that BAX promotes oocyte death, and therefore that a Bax deficiency would result in a reduced level of oocyte apoptosis, the authors demonstrated that the proportion of perinatal oocytes that are undergoing apoptosis is higher than that for wild-type littermates.\textsuperscript{33} Instead, they reported that the regulatory activity of BAX in follicular endowment likely occurs during PGC migration, prior to PGC colonization of the gonad. On the other hand, another study using Bax-deficient mice reported that the normal numbers of primordial follicles were observed in the initial ovarian reserve at PD4, but three-fold more primordial follicles compared to their wild-type sisters were observed at PD42 because of reduced primordial and primary follicle atresia.\textsuperscript{34} As a result, Bax-deficient mice aged 20–22 months, well past typical murine reproductive senescence, still possess follicles and fertilizable oocytes indicating that the reproductive lifespan could be a dramatically extended.\textsuperscript{34} Moreover, a lack of BCL2 modifying factor (BMF), a pro-apoptotic BH3-only member of the BCL2 family, increases the numbers of germ cells in E15.5 and PD1 compared to wild-type mice of the same age.\textsuperscript{35} Furthermore, a deficiency of BMF in female mice was associated with a decrease in apoptosis at E15.5 and E17.5. However, the numbers of germ cells in ovaries from Bmf-deficient females after PD1 were similar to the numbers observed in wild-type females at PD3, PD5, and PD10.\textsuperscript{35} In addition, a study by Liew et al.\textsuperscript{36} reported that Bmf-deficient females had significantly more primordial follicles at PD100, PD200, PD300, and PD400 than wild-type controls, while there was no difference in the number of primordial follicles at PD20. These animals produced litters more frequently over a 6-month period and, consequently, more offspring than wild-type females. The fertile lifespan of Bmf-deficient females was also significantly extended compared to wild-type females.\textsuperscript{35} In mice lacking the Puma gene (also referred to as Bbc3), a pro-apoptotic BH3-only member of the BCL2 family, the number of germ cells remained elevated compared to the numbers for wild-type female mice throughout their embryonic and early postnatal life, resulting in a 1.9-fold increase in the number of primordial follicles in the ovary on PD10.\textsuperscript{37} The elimination of Puma in mice also prevented some primordial follicles from undergoing γ-irradiation-induced oocyte death.\textsuperscript{38} No difference, however, was observed in the fertility of the Puma-deficient mice, such as litter size.\textsuperscript{38,39}
Anti-apoptotic BCL2 family members may also regulate oocyte apoptosis. In previous studies, the BCL2 was identified as a possible regulator of germ cell death in female mice. Both the overexpression and knockout of the BCL2 protein altered ovarian histology. The overexpression of Bcl2 in germ cells results in ovaries with more oocytes at PD8; however, by PD60, transgenic ovaries have the same number of oocytes as wild-type ovaries.40 Additionally, in a Bcl2 knockout model, PD42 murine ovaries were found to contain follicles devoid of oocytes and had fewer primordial follicles.41 However, Jones et al.,42 in a study of the effects of a global Bcl2-deficiency or overexpression during the in vivo assembly of mouse primordial follicles, reported that these alterations in gene expression had no effect, even though the endogenous expression of BCL2 was observed in oocytes at all perinatal time points that were profiled. Furthermore, the authors also tested the effect of MCL1 ablation in mouse ovary organ cultures. MCL1 is a widely recognized member of the pro-survival BCL2 family. The inhibition of MCL1 with an antibody to MCL1 in an organ culture-reduced oocyte survival and promoted the breakdown of oocyte cysts, and this effect was dose-dependent.42 This study was expanded upon by Omari et al.,43 who observed these same effects in vivo as a result of the germ cell-specific depletion of Mcl1.

On the other hand, Caspases also appear to be involved in oocyte apoptosis. Caspase-2-deficient mice also exhibited a significant increase in the number of primordial follicles at birth.44 The oocytes that normally undergo apoptosis in response to doxorubicin (a chemotherapeutic agent) were resistant in Caspase-2 knockout.44 In contrast, Caspase-3-deficient mice showed no evidence of alterations in oocyte numbers at birth, although the apoptosis of granulosa cells in growing follicles was impaired.45 Moreover, Caspase-9 is essential for the developmentally regulated death of oocytes in the early stages of the first meiotic prophase, and is possibly involved in the elimination of oocytes with meiotic crossover errors.46

### 3.3 The role of TNFα on primordial follicle formation and ovarian reserve and subsequent fertility

TNFα is a type of cytokine that is responsible for a diverse range of signaling events within cells, including apoptosis and necrosis. TNF exerts its function through binding to two different transmembrane receptors, TNFR1 (also known as p55, TNFRSF1A, CD120a) and

| Gene | Expressing cells in ovaries | Animal model | Animal phenotype | References |
|------|-----------------------------|--------------|------------------|------------|
| **Fas** | Not reported | Fas-deficiency | Increased primordial follicles in neonates. | [26] |
| **Tnfr1** | Oocyte | Tnfr1-deficiency | No observed effect on primordial follicle assembly. | [49] |
| **Tnfr2** | Oocyte | Tnfr2-deficiency | No observed effect on primordial follicle assembly. | [50] |
| **Bax** | Oocyte | Bax-deficiency | Increased oocyte numbers in embryonic ovaries and increased follicle numbers in neonatal ovaries. | [33] |
| **Bcl2** | Not reported | Bcl2-deficiency | Decreased abundance of oocytes and primordial follicles in postnatal ovary. | [41] |
| | Oocyte and somatic cells | Bcl2-deficiency | No observed effect on oocyte cyst breakdown and primordial follicle assembly or abundance. | [42] |
| | Not reported | Bcl2-overexpression | Increased primordial follicles in postnatal ovary. | [40] |
| | Oocyte and somatic cells | Bcl2-overexpression | No observed effect on oocyte cyst breakdown and primordial follicle assembly or abundance. | [42] |
| **Bmf** | Oocyte | Bmf-deficiency | Increased oocytes due to attenuated embryonic germ cell apoptosis until birth, but does not influence the number of primordial follicles initially established in ovarian reserve. | [35] |
| **Puma** | Somatic cell | Puma(Bbc3)-deficiency | Increased primordial follicle in fetal and newborn. | [37] |
| **Mcl1** | Oocyte and somatic cells | MCL1 antibody treatment in organ culture | Reduces oocyte survival and promotes cyst breakdown in a dose-dependent manner. | [42] |
| | Oocyte | Mcl1 conditional knockout | Reduced primordial follicle abundance in postnatal ovary. | [43] |
| **Caspase 2** | Not reported | Caspase 2-deficiency | Increased primordial follicles in neonates. | [44] |
| **Caspase 3** | Oocyte and somatic cells | Caspase 3-deficiency | No effect on oocyte abundance or follicle assembly. | [45] |
| **Caspase 9** | Oocyte and somatic cells | Caspase 9-deficiency | Increased primordial follicles in neonates. | [46] |

Note: Not reported—not mentioned in the cited paper.
TNFR2 (also known as p75, TNFRSF1B, CD120b). TNFR1 contains an intracellular death domain, which is required for the cell signaling associated with apoptotic processes. In contrast, TNFR2 can induce the gene transcription associated with cell survival, growth, and differentiation.\(^\text{47}\) Study by Roby et al.\(^\text{48}\) reported that Tnfr1-deficient mice exhibit early evidence of puberty and underwent normal estrous cycles but enter periods of acyclicity associated with aging earlier than wild-type mice. Furthermore, Tnfr1-deficient mice delivered significantly fewer litters as compared with C57BL6 and Tnfr2-deficient mice.\(^\text{48}\) On the other hand, Cui et al.\(^\text{49}\) reported that Tnfrα-deficient female mice had approximately a 1.8-fold larger primordial follicle pool than wild-type animals at PD4. The numbers of primordial follicles were also found to be higher at PD42 and PD90 than those for wild-type animals. Moreover, Tnfrα-deficient mice gave birth to 21% more pups than wild-type mice during a 12-month breeding period.\(^\text{49}\) Meanwhile, Greenfeld et al.\(^\text{50}\) reported that the primordial follicle numbers in the early neonatal period for Tnfr1 nor Tnfr2 knockout mice were affected. Moreover, Tnfr2 deletion led to an apparent acceleration in follicular growth in neonates, while the number of primordial follicles at PD80 was significantly greater in Tnfr2-deficient compared with wild-type ovaries.\(^\text{50}\) These conflicting results suggest that the functions of TNFα are quite complex. Despite some studies, the roles of TNFα in primordial follicle reserve and fertility remain poorly understood.

Here, we report on an evaluation of the effect of the administration of the TNFα inhibitor, Infliximab (IFX, a chimeric monoclonal antibody against TNFα) on follicle number. In animals that received IFX (2 μg/g body weight) by 54 hours (h) after birth, the number of primordial follicles were 1.6-fold higher compared with the control group at 60 h after birth (Figure 2B). However, there was no significant difference in the numbers of each follicle types in the IFX groups compared with control groups at 3–4 weeks of age (Figure 2C). In addition, no significant difference was observed in the delivery rate and litter size between control and IFX groups when mated with 3–4 months old C57BL/6j males for 2 weeks (Table 2). Interestingly, the abundance of Cleaved PARP, an apoptosis maker, tended to be higher in the IFX group than in control group at 60 h after birth (Figure 3A–C). The expression ratio of BAX/BCL2 proteins also tended to be higher in the IFX group at 60 h after birth (Figure 3D,E).

In a study by Greenfeld et al.,\(^\text{50}\) TNF, TNFR1, and TNFR2 were observed in the cytoplasm of oocytes in cysts, as well as in the cytoplasm of oocytes in primordial and growing follicles in neonatal ovaries. In a preliminary experiment, we also observed the strong localization of TNF and TNFR1 in oocytes at 60 h after birth. Some studies reported that TNFα induce a decrease in the number of oocytes in rodents.\(^\text{50,51}\) From the results of our research and Cui et al.,\(^\text{49}\) the TNFα ligand is a factor that controls primordial follicle formation, since the inhibition/deficient of TNFα results in an increase in the number of primordial follicles in mice. The mechanism by which TNFα acts, however, is unclear, as Tnfα1 or Tnfα2 gene deficient did not affect primordial follicle formation. In other cell types, transmembrane TNF (tmTNF), which is a precursor of soluble (active) TNF, also functions as a ligand, while it acts as a receptor that transmits outside-to-inside (reverse) signals.\(^\text{52}\) As far as we know, a study of the involvement of tmTNF in primordial follicle formation has not been reported, and further studies will clearly be needed to reveal details of the molecular mechanism by which TNFα acts.

In addition, a temporary increase in the number of primordial follicles was observed in the case of the IFX group, but in Tnfrα-deficient mice, the expanded primordial follicle pool has been shown to remain high after sexual maturation. These results suggest that TNFα also plays a key role in ovarian follicular development and maintenance.\(^\text{49}\)

### 3.4 The impact of apoptosis on oocyte death in cyst breakdown during primordial follicle formation

In the previous section, we alluded to the importance of apoptosis regulators on primordial follicle formation and ovarian reserve. However, the question arises as to whether this drastic loss of oocytes in cyst breakdown during primordial follicle formation is due to apoptosis. Our findings concerning TNFα ligand inhibitor-administered mice showed an increase in the number of primordial follicles but no difference in germ cell apoptosis markers in the neonatal period (Figure 3). The previously reported administration of the autophagy inducer, Tat-beclin1 D-11, to neonatal mice resulted in an increase in the number of primordial follicles, but no TUNEL-positive oocytes were detected regardless of the administration. There was also no significant difference in the expression of the apoptosis initiator Caspase-9.\(^\text{8}\) It should also be noted here that, while Bax-deficient mice exhibited a significant increase in the number of primordial follicles at PD4 and PD7, this increase was associated with a significant increase in the number of TUNEL-positive oocytes at PD1 and PD4.\(^\text{39}\) Therefore, if oocyte death were entirely due to apoptosis, it would be difficult to explain the increase in the number of primordial follicles in these mouse models. In fact, some studies have reported that TUNEL-positive oocytes are rarely observed in vivo during oocyte cyst breakdown.\(^\text{3,15,53}\) Given these facts, the frequency of production of apoptotic oocytes during primordial follicle formation may actually be low.\(^\text{15}\)

In this regard, recent studies have focused on the aggregation of nurse oocytes into dominant oocytes. Oocytes in cysts are divided into dominant oocytes with Balbiani bodies, and nurse oocytes (sister germ cells) without them. The Balbiani body (named after the nineteenth century Dutch microscopist) is a highly conserved oocyte-specific structure, and is characterized by a region in the oocyte with enriched organelles, including the ring-shaped Golgi apparatus, the endoplasmic reticulum, mitochondria, and germ plasm.\(^\text{54,55}\) Lei et al.\(^\text{56}\) reported that Balbiani body-positive oocytes differentiate into primary oocytes of primordial follicles without undergoing oocyte death. Balbiani bodies have been also reported in marsupials\(^\text{57}\) and many mammalian species including the goat,\(^\text{58}\) rat,\(^\text{56}\) hamster,\(^\text{59}\) and in humans.\(^\text{60,61}\) On the other hand, Dhandapani et al.\(^\text{62}\) developed live-imaging methods and showed that human and Xenopus oocytes contain a Balbiani body, but that mouse oocytes do not. The function of mammalian Balbiani
FIGURE 2  Effect of the administration of Infliximab (IFX), a TNF-α ligand inhibitor, on primordial follicle formation. (A) Timeline showing the injection of IFX. C57BL/6j female mice were intraperitoneally injected with 2 μg/g body weight IFX (A1097-200, Bio Vision) at 6, 30, and 54 h after birth. Histological follicle counting, immunofluorescence staining, Western blotting and evaluation of fertility performed after administration were conducted according to our protocol. The differences between the control and IFX groups were analyzed by means of a Student’s t-test. A p value of <0.05 was considered significant. (B) The number of primordial follicles per ovary at 60 h after birth. The number of primordial follicles in the IFX group were significantly greater than that in the control group (p < 0.01). (C) The number of primary follicles per ovary at 60 h after birth. Data are expressed as mean ± SD (n = 6–8 per group). The number of primary follicles in the IFX group tended to be higher than that in the control group. (D) The number of follicles at each developmental stage in the control and IFX groups at 3–4 weeks old. Data are expressed as mean ± SD (n = 5–6 per group). The numbers of each follicle types in the IFX groups at 3–4-week-old did not show significant difference as compared with control.

| Experimental groups | n   | Body weight (Mean ± SD) | Delivering rate (%) | No. of offspring (Mean ± SD) |
|---------------------|-----|--------------------------|---------------------|-----------------------------|
| 2-month             |     |                          |                     |                             |
| Cont.               | 12  | 20.3 ± 1.7               | 75.0 (9/12)         | 7.7 ± 1.8                   |
| IFX                 | 13  | 20.1 ± 1.8               | 76.9 (10/13)        | 7.8 ± 0.7                   |
| 6-month             |     |                          |                     |                             |
| Cont.               | 10  | 25.9 ± 3.2               | 70.0 (7/10)         | 8.3 ± 0.5                   |
| IFX                 | 11  | 25.8 ± 2.7               | 100 (11/11)         | 6.8 ± 2.7                   |
| Accumulated total   |     |                          |                     |                             |
| Cont.               | 10  | 27.2 (16/22)             | 11.3 ± 5.2          |                             |
| IFX                 | 11  | 87.5 (21/24)             | 12.5 ± 4.9          |                             |

TABLE 2  Fertility in 2- and 6-month-old mice from control and IFX groups
bodies is currently unclear and further research will be needed to address this issue.

4 | CONCLUSION

A pool of primordial follicles established at birth represents the total population of oocytes available for being utilized throughout the female reproductive lifespan in mice. This review summarizes previous studies showing that apoptosis regulators can contribute to the expansion of the ovarian reserve. On the other side, we suggest that apoptosis is only one of the mechanisms of oocyte death, and there are likely other mechanisms in the large-scale oocyte depletion that occurs during primordial follicle formation. Continued studies of oocyte death during the formation of primordial follicles should provide clearer explanations of the regulatory mechanisms that determine whether a primordial follicle will live or die. Not all results from mouse models can be extrapolated to industrial animals such as pigs, cows, and humans, but such studies can involve the exploration of new strategies for controlling oocyte mortality for the storage and protection of primordial follicle pool and for the improvement of female fertility.

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CONFLICT OF INTEREST

Ken Umeno, Ayana Sasaki and Naoko Kimura declare that they have no conflict of interest.

HUMAN RIGHTS STATEMENT AND INFORMED CONSENT

This review article does not include any study with human participants, and thus, it did not require approval from an ethics committee.

HUMAN/ANIMAL RIGHTS

This review article did not contain any human materials. Our studies in this review were performed according to the institutional guidelines for the use of experimental animals and were approved by the Ethics Committee of Yamagata University.

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