The association between the oocyte pool and aneuploidy: a comparative study of the reproductive potential of young and aged mice

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Abstract

Purpose The present study examined the effect of aging on female reproductive potential.

Methods Six-week-old and 9-month-old CD1 mice were referred to as the ‘young’ and ‘aged’ groups, respectively. Oocytes were collected after superovulation, and their viability were compared using parthenogenetic activation. The aneuploidy of the oocytes (MII) was assessed using chromosome spread, and the whole ovarian follicle number was counted using an unbiased stereological method. Serum hormone levels were measured using the radio-immunity method, and the expression of the Cohesin subunit genes in the oocytes (GV) were assessed using RT-PCR.

Results The mean number of recovered (25.8 vs. 16.2; $P<0.05$) and live oocytes (24.0 vs. 11.73; $P<0.01$) per head in the young-mice group (6-week-old) was significantly higher than that of the aged group (9-month-old). The aneuploidy rate of the ovulated oocytes in the aged group was significantly higher than that of the young group (36.8 % vs. 10 %; $P<0.01$), and the rate of blastocyst formation in the young group (85.23 %) was significantly higher than that of

the aged group (81.2 %; $P<0.05$). The number of primordial follicles (the oocyte pool) per ovary in the aged group was significantly decreased compared with the young group (330 ± 33.51 vs. 2079.6 ± 420.70 ; $P<0.01$), and the level of AMH in the aged group was significantly higher than that of the young group (4.66 ± 0.11 ng/ml vs. 4.07 ± 0.18 ng/ml; $P<0.01$).

Conclusions We propose that maternal aging significantly reduces the oocyte pool, superovulation efficiency and developmental potential and increases the oocyte aneuploidy rate.

Keywords Mouse · Oocyte · Meiosis · Aging · Aneuploidy · Cohesin

Introduction

At present, increasing numbers of women are acquiring advanced educations and participating in the labor force, which changes their reproductive behavior and delays childbearing. For example, in the Netherlands, the mean age at which women deliver their first child has risen from 24.6 years in 1970 to 29.1 years in 1999 [1]. A survey performed during the American census in 1995 demonstrated that women who had attempted but did not succeed in conceiving their first child within 1 year increased from 6 % for the 15 to 24 year-old age group to >30 % for the 35 to 44-year-old age group [2]. Hence, increasing numbers of “test tube babies” have been born since the first case of Louise Brown in 1978 [3]. This mode of conception does provide hope to many couples with infertility issues; however, some women are still unable to conceive after a certain age as a consequence of female reproductive aging [4]. Many reasons might lead to this problem, such as the endometrium, neuro-endocrine and ovarian et al. [1].

Compelling evidence has revealed that maternal ageing has detrimental effects on reproductive performance. Compared to

Capsule The association between the oocyte pool and aneuploidy.

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young mice, older counterparts show poor response to superovulation [5–7], increased aneuploidy rates [6–8] and increased FSH levels [9]. Recent data also demonstrated that oocytes from aged females contained higher levels of DNA damage [10]. These oocytes show decreased fertilization rates [11] due to delayed pronuclear formation [7] and abnormal calcium oscillations [12]. While it is clear that maternal ageing has multifaceted detrimental effects on oocyte development, the association between oocyte pool size and aneuploidy rates of ovulated oocytes has not been directly examined. It has been reported that oocyte quality decreases in parallel with the progressive loss of follicle number [13–15]. However, according to our knowledge, this is the first study that provides direct evidence for how ovarian follicle number is related to aneuploidy rates in young and aged mice by using whole ovarian follicle counting.

Chromosome aneuploidy is considered the major cause of the age-related decline in oocyte quality [1, 4]. Chromosome segregation errors (aneuploidy) during female meiosis lead to aneuploidy in the resulting egg and embryo, which makes these errors one of the leading genetic causes of spontaneous abortions and developmental disabilities in humans and mammals [8]. Chromosomal abnormalities in gametes that are predisposed to aneuploidy in the embryo can lead to implantation failure, congenital abnormalities, spontaneous abortion, mental retardation or genetic disease in the offspring [16]. It is known that aneuploidy of a meiotic origin increases dramatically with the age of the female, and current evidence suggests that most errors occur during meiosis I [8]. Several hypotheses regarding the cause of maternal age-related aneuploidy have been proposed, including recombination errors during early meiosis, a defective spindle assembly checkpoint in meiosis I and the deterioration of sister chromatid cohesion with age [17, 18].

Cohesion between sister chromatids is established during DNA replication by Cohesin, which is a highly conserved protein complex consisting of four main subunits: RAD21, SCC3, SMC1 and SMC3. Cleavage of Cohesin at anaphase releases sister chromatids and allows them to segregate [4]. Cohesion is established during premeiotic S phase during fetal development and must remain functional until meiosis resumes before ovulation in the adult, which can be up to 50 years later in humans [19]. In mice, cohesion can only be established during S phase before birth, and the cohesion protein does not exchange after birth once it is loaded [8]. It is known that defective cohesion is associated with age-dependent misaligned chromosomes in SAM (senescence-accelerated mice). Immunostaining REC8 in mouse oocytes illustrates that compared with oocytes from young SAM, oocytes from aged SAM display absent or disruptive REC8 staining between the arms and centromeres of chromatids [20].

For ethical considerations, human oocytes are restricted to studies on the effect of maternal age on aneuploidy. In animal models, the age effect is also present, and large numbers of oocytes are available for chromosomal, developmental and biological analysis. Therefore, such a model would provide insight into reproductive aging in women [7]. Although a mouse model has its limitations when studying human issues, it is a good model for studying age-related aneuploidy because advanced maternal age is associated with an increase in aneuploidy incidence in naturally aged mice [6, 7, 19], and mice have been used in many age-related aneuploidy studies [6, 7, 19–22].

Six-week-old and 9-month-old CD1 mice were used to determine whether a reduction in oocyte quality was highly associated with a decline in oocyte pool size during maternal ageing. Data from these experiments could provide direct evidence to support the “bottom of the barrel” hypothesis, which predicts an accelerated decline in the proportion of good quality oocytes from the oocyte pool due to increased aneuploidy rates [15]. During the first part of the study, the effect of aging on superovulation and developmental efficiency and aneuploidy rates were compared. In the second set of experiments, the ovarian reserve and three indicator hormones (AMH, FSH and inhibin B) in serum were calculated and measured. Finally, mRNA expression levels of four Cohesin complex subunits (Rec8: meiosis-specific paralogue of Rad21, SMC3, SMC1 β and Stag3) were compared to contribute to our understanding of molecular age-related aneuploidy through cohesion exhaustion because recent studies have suggested that deterioration of cohesion with increasing maternal age is a leading cause of age-related aneuploidy. The present findings in mice offer the possibility of studying the link between the ovarian reserve and oocyte aneuploidy in a more tractable system.

Materials and methods

All chemicals and media were purchased from Sigma Chemical Co. (St. Louis, MO, USA), unless otherwise indicated.

Ethics statement

All animal procedures were performed with the approval from the animal ethics committee of the China Agricultural University, P.R. China. All experimental protocols concerning the handling of mice were in accordance with the requirements of the Institutional Animal Care and Use Committee at China Agricultural University.

MII oocyte collection

Swiss CD1 female mice were housed under temperature-controlled (20 °C) and light-controlled conditions (lights on, 06:00–20:00) with free access to food and water. Female mice (6-week-old, young, and 9-month-old, aged) were superovulated using 10 IU of equine chorionic gonadotropin (eCG, Ningbo Hormone Product Co. Ningbo, Zhejiang Province, China) followed by IP injection with 10 IU of human chorionic gonadotropin (hCG, Ningbo Hormone Product Co. Ningbo, Zhejiang Province, China) 48 h later. Oocytes were retrieved 13 h after hCG administration with M2 medium and stripped of their cumulus cells by pipetting in M2 medium containing 300 IU/mL hyaluronidase. The numbers of ovulated and morphologically normal (live) oocytes were recorded to analyze the superovulation efficiency.

Oocyte activation and embryo culture

All treated oocytes were allowed to recover in a CO₂ incubator for 2 h before activation. The activation medium that was used was Ca²⁺ free human tubal fluid (HTF) supplemented with 10 mM SrCl₂. After being washed twice in M2 medium and once in activation medium, the oocytes were first incubated in activation medium for 2.5 h and then in regular HTF without SrCl₂ for 3.5 h at 37.5 °C in a humidified atmosphere with 5 % CO₂. The activation medium and HTF for the subsequent short culture were supplemented with 5 m g/mL cytochalasin D (CD). Six hours after the onset of activation, the oocytes were removed from the medium and cultured in regular HTF without CD for 4 days. Embryos at the two-cell and blastocyst stages were examined and recorded at 24 and 96 h after the start of culturing, respectively.

Follicle counting

The follicle number estimating method was performed according to a previous report [23]. Briefly, 10 ovaries from 5 young and 5 aged animals were collected in Bouin's fixative. After fixation for 4 to 6 h, the samples were dehydrated, embedded in paraffin and cut thoroughly into 8-μm-thick sections, followed by staining with Harris' hematoxylin and eosin (HE). Follicle counting was performed using an unbiased, stereological method and a physical detector without counting frames. Every 12th pair of adjacent sections was sampled, starting at a random section for the first 11. For each pair, the first section was the "look-up" section and the second was the reference. Only follicles with an oocyte nucleus in the reference section, but not the look-up section, were counted. The total number of follicles that was observed was then multiplied by 12, and the process was reversed, with the look-up section becoming the reference section and vice

versa. The follicles were classified according to the reported classification [24].

MII oocytes chromosome spread

Oocyte chromosome spreads were conducted according to a previous report [25] with modifications. Briefly, mouse oocytes were collected and cultured using standard protocols. The zona pellucid of the oocytes was removed prior to fixation by a brief exposure to 1 % pronase in culture medium. Zona-free oocytes were washed in medium and transferred to a Petri dish that was coated in 1 % agar to prevent attachment. For fixation, a clean microscope slide was dipped into a solution of 1 % paraformaldehyde in distilled H₂O (pH 9.2) containing 0.15 % Triton X-100 and 3 mM dithiothreitol. With a finely drawn pipette, up to 20 oocytes were carefully pipetted along the length of the slide. The oocytes burst within seconds of exposure to the fixative and slowly "melted" onto the slide. Optimal spreading of the chromosomes as achieved when the cells were expelled evenly across the slide, and the slide was allowed to dry slowly in a humid chamber for several hours before being washed in 0.1 % PBS and dried at room temperature. Chromosomes on slides were stained with DAPI, and spread chromosomes were counted using a microscope (Fig. 1).

Serum hormone measurements

Serum hormone levels were measured using the radioimmunity method (r-911 Automatic RIA Counter, University of Science and Technology of China Industrial Company, China) according to the manufacturer's instructions (AMH RIA KIT (HY-101340), INH B RIA KIT (HY-133), FSH RIA KIT (HY-10024), Beijing Sino-uk Institute of Biological Technology, China). The experiment was repeated thrice.

cDNA preparation and real-time PCR analysis

Total RNA was isolated from 200 GV stage oocytes of each groups and treated with DNase I to eliminate DNA contamination. RNA was then reverse transcribed using an Ambion Cells-to-cDNA II kit (Life Technologies, Inc., Grand Island, NY, USA) following the manufacturer's instructions. Real-time PCR analyses were performed as described previously [26] to quantify the steady-state mRNA levels of Rec8, SMC3, SMC1β, STAG3 and the housekeeping gene Rpl19 (endogenous control). The sequences for the Rec8, SMC3, SMC1β, STAG3 and Rpl19 primer pairs are listed in Table 1. Calculations of the relative fold changes of Rec8, SMC3, SMC1β and STAG3 were performed using the 2^{-ΔΔC_t} method, as described previously [27]. All experiments were repeated three times, and the values shown are the mean±SEM.

Fig. 1 Ploidy and aneuploidy of spread mouse chromosomes. Number of chromosomes, ploidy ($n=20$, *Middle*) and aneuploidy ($n=19$, *left*, $n=21$, *Right*), after chromosome spread

Statistical analyses

The percentage of aneuploidy oocytes (Table 2) were analyzed using a chi-square test. Superovulation efficiency, parthenogenetic development, the serum hormone concentration and the number of ovarian follicles (Tables 3, 4, 5 and 6) were analyzed using Student's *t*-test with the SPSS (Statistical Package for the Social Sciences) 1 2.0 software (SPSS, Inc., Chicago, IL, USA). $P<0.05$ was the criterion used for statistical significance.

Results

Oocyte numbers recovered from superovulated females

The young and aged animals were stimulated with hormones and superovulated oocytes at metaphase II (MII) were collected. The mean number of oocytes that were recovered from the young mice was significantly higher than that of the aged groups (25.8 vs. 16.2; $P<0.05$), the number of live oocytes that were collected from the young group was significantly higher than that of the aged groups (24.0 vs. 11.73; $P<0.01$), and not surprisingly the number of dead oocytes was significantly lower (1.93 vs. 4.4; $P<0.05$). These results (Table 3.) suggest that maternal age adversely affects the oocyte recovery rate after superovulation and the oocyte quality declines significantly in aged animals.

Increased aneuploidy in eggs from older mice

We found that the overall rate of aneuploidy in the young group was 10 % (16/160). However, this rate was significantly increased in the aged group (36.8 %, 32/87) ($P<0.01$). These results are similar to those of previous reports [6], which showed a rate of aneuploidy equal to 12.5 % for a 9-month-old group but a significantly increased to rate of 37.5 % for a 12-month-old group.

Parthenogenetic development of mouse oocytes

As shown in Table 4, the percentage of oocytes that developed into two-cell embryos was similar between the groups ($P>0.05$). However, the percentage of oocytes that developed into blastocysts in the young group (85.23 %) was significantly higher than that of the aged group (81.2 %; $P<0.05$).

Comparison of the serum hormone concentration

We examined three hormonal indicators of ovarian age: anti-Mullerian hormone (AMH), follicular-stimulating hormone (FSH) and inhibin B (Table 5). No significant differences were observed between the young and aged mice for FSH and INB levels. However, the level of AMH in the aged group was significantly higher than that of the young group ($P<0.01$).

Table 1 Primer sequences

Gene symbol	Forward	Reverse
<i>REC8</i>	TAGTGTGCTGGTAAGAGTGCAAC	TGTCTTCCACAAGGTACTGGC
<i>SMC3</i>	CGAAGTTACCGAGACCAAACA	TCACTGAGAACAACTGGATTGC
<i>SMC1β</i>	GAAGATGGCATACGAGCCTTAG	AGCAGTTTGTAACGATCCAGC
<i>STGE3</i>	TCCTCAGGCAGTGAGTCTTCC	GTTCCCTGTGAGTCTCTGTGCAT
<i>Rpl19</i>	TCAGGCTACAGAAGAGGCTTGC	ATCAGCCCATCCTTGATCAGC

Table 2 Increased aneuploidy in eggs from older mice

Group	No. of spread	Aneuploidy(%)
Young	160	16 (10) ^A
Aged	87	32 (36.8) ^B

Different superscripts (A, B) represent a significant difference ($P<0.01$)

Ovarian follicle pool comparison between young and aged mice

As shown in Table 6, the oocyte pool (primordial follicles) ($P<0.01$) for the young group was significantly higher than that of the aged group, as were the primary and secondary follicles ($P<0.05$). However, no significant differences were observed between the groups in terms of pre-antral, early antral and antral follicles. This result provides direct evidence that advanced maternal age and depletion of the follicle pool are closely associated. Similar amounts of antral follicles could guarantee that certain numbers of oocytes would ovulate in aged mice even with high risks of error in chromosome segregation (Table 2)

Real-time PCR analysis

The messenger RNA expression levels for the Cohesin subunit proteins (Rec8, SMC3, SMC1 β and STAG3) were compared between the GV-stage oocytes of the young and aged mice. The relative levels of SMC3, SMC1 β and STAG3 mRNA did not appear to differ between the groups. However, no Rec8 expression was detected in the young or aged groups (Fig. 2).

Discussion

In this study, a drop in the number of eggs that were collected from aged superovulated females was observed which is consistent with results from a previous studies in Kunming [7] or CD1 mice [6]. In Kunming mice, the mean number of ovulated MII oocytes decreased significantly with maternal age and that number declined from 41.7 ± 19.6 (6–8 week) to

Table 3 Effect of aging on superovulation efficiency

Group	No. of Animals	No. of oocytes		
		Ovulated	Live	Dead
Young	15	25.8 $\pm$ 3.31 ^a	24.00 $\pm$ 3.36 ^A	1.93 $\pm$ 0.40 ^a
Aged	15	16.2 $\pm$ 2.13 ^b	11.73 $\pm$ 1.42 ^B	4.4 $\pm$ 1.07 ^b

Values are the mean $\pm$ SD. Different superscripts (a, b) represent a significant difference ($P<0.05$). Different superscripts (A, B) represent a significant difference ($P<0.01$)

Table 4 Parthenogenetic development of the mouse oocytes

Group	No. of oocytes	No. of 2-cell embryos (%)	No. of blastocysts (%)
Young	149	140(94 $\pm$ 0.6)	127(85.23 $\pm$ 1.36) ^a
Aged	133	119(89.5 $\pm$ 1.12)	108(81.2 $\pm$ 1.55) ^b

Values are the mean $\pm$ SD. Different superscripts (a, b) represent a significant difference ($P<0.05$)

16.5 ± 7.9 (9 month) [7]. The mean number of eggs that were recovered from 3-month-old females in CD1 mice following superovulation peaked at 22.5 ± 3.8 eggs/oviduct and this number decreasing markedly between 6 and 9 months [6]. This phenomenon can be inferred from the results of the follicular counting experiment (Table 6) that was performed in this study. Cui et al. compared the histology of mouse ovaries from one microscopic field of a section of ovaries and found a reduced number of follicles of different stages [7]. The whole number of follicles was counted and analyzed in this study, and the number of primordial follicles in the aged mice was significantly decreased when compared with those of young mice. Although no significant differences were observed between the antral (pre-antral, early antral and antral) follicles, it could be inferred that a lower number of oocyte reserves restricts the number of eggs that are collected from aged, superovulated females.

In addition, a high rate of aneuploidy has been observed during similar experiments using aged mice [6, 28, 29]. Aneuploidy in these CD1 mice eggs was 3–4 % in mice aged 1 and 3 months, and this number rose to 12.5 % in 9-month-old mice and to 37.5 % in 12-month-old mice [6]. These results are consistent with previous reports of ageing mice having reduced numbers of ovarian follicles and ovulated MII oocytes as well as increased percentages of oocytes that display premature chromatid segregation [7]. However, in contrast with the rate of premature chromatid separation, the percentage of MII oocytes with 20 chromosomes was not different between groups due to the small number ($n=54, 35, 36$) of oocytes that were examined [7]. We observed a significant rise in aneuploidy rates, and aneuploidy in the young group was 10 % (16/160). However, this rate was significantly increased in the aged group (36.8 %, 32/87). It has been suggested that an association between lower egg numbers and aneuploidy does not necessarily indicate that maternal age-associated aneuploidy is caused by a smaller ovarian reserve [6]. However, as the ‘bottom of the barrel’ hypothesis mentions, an accelerated decline in the proportion of good-quality oocytes occurs, and those ovulated later in life may be less able to segregate their chromosomes normally [15]. In this study, the results indicate that along with a declined proportion of good quality oocytes in the CD1 mice, the aneuploidy of the ovulated oocytes increased.

Table 5 Serum hormone concentration comparison

Group	Number of samples	FSH (mIU/ml)	AMH (ng/ml)	INB (pg/ml)
Young	31	8.62±0.15	4.07±0.18 ^A	96.36±5.99
Aged	30	8.31±0.17	4.66±0.11 ^B	98.55±7.07

Values are the mean±SD. Different superscripts (A, B) represent a significant difference ($P<0.01$)

It is proposed that age-dependent changes in hormonal feedback mechanisms, especially in aged individuals who have a depleted pool of oocytes, may lead to a predisposition to aneuploidy in mammalian oocytes [7]. In this study, three hormones (AMH, FSH and inhibin B) were chosen as indicators of the oocyte pools. AMH, which is a product of primary, pre-antral and early antral follicles, is expressed by granulosa cells and is detectable in some primary and secondary follicles (2–6 granulosa cell layers) and in essentially all pre-antral and small (<6 mm) antral follicles [30, 31]. Other researchers have used the fractionator/optical disector technique to estimate the human numbers of primordial follicles (oocyte pools) and determined that the age-adjusted correlation between serum AMH and the oocyte pool is 0.48, which supports the view that AMH reflects the size of the oocyte pool [14]. It has been reported that in 4- to 18-month-old C57BL/6j mice, the serum AMH levels decline with increasing age and directly correlate with declining numbers of growing and primordial follicles [32]. However, in Kevenaar's study, no difference between the AMH levels of 4- and 8-month-old mice was observed. Despite species' differences, we believe that the low level of AMH in the 6-week-old CD1 mice in this study is possible. Moreover, studies using AMH-deficient mice have found that, in the absence of AMH, the rate of primordial follicles entering into the growing pool is enhanced. As a consequence, the primordial pool is exhausted at an earlier age in those AMH-deficient mice [33]. Suggesting that the inhibitory role of AMH in primordial follicle recruitment is further confirmed by in vitro culture experiment of mouse neonatal ovaries [34] and human ovarian cortical tissue biopsy strips. AMH, at a concentration of 100 ng/ml suppresses the initiation of primordial follicle growth in human ovaries in vitro [13]. Based

upon the above reports, we propose that in the aged group, high levels of AMH are likely maintained to inhibit the recruitment of primordial follicles to prolong the reproductive span. Inhibin B, which is produced by the granulosa cells of the antral follicles, reflects the quality and quantity of antral follicles and reflects the size of the underlying oocyte pool [35]. FSH is in a negative feed-back loop with the ovarian hormone inhibin B and provides an indirect measure of the characteristics of the underlying oocyte pool [35]. In this study, the FSH and inhibin B levels appeared unrelated to aging, which is consistent with the results from the follicle counting experiment (Table 6), that is, no significant difference in the number of antral follicles between the young and aged mice were observed.

The rate of blastocyst formation in the oocytes from the aged group still reached 81 %, although this number was significantly lower than that of the younger group (85 %). Thus, it is suggested that although the rate of oocyte recovery is low, many of the recovered oocytes that are recovered from the aged group still possess high developmental ability to form blastocysts after parthenogenetic activation. These results are consistent with previous results indicating that the fertilization rates of 6–8 week young (88.9 %), 9-month middle-aged (89.0 %) and 12-month aged (84.6 %) mice are similar and suggests that oocytes from aged mice are competent for fertilization and undergo normal cleavage despite the decrease in their quality [7]. One important reason for this is that usually only oocytes with high viabilities (as judged optically) are chosen to conduct further experimentation. Many theories concerning the reduced viability of aged oocytes, such as mitochondrial and epigenetic changes, exist. After natural mating, the pregnancy rate of young Kunming mice was higher than that of the older individuals [36, 37]. Studies have demonstrated the decreased expression of Dnmt1, Dnmt3a, Dnmt3b and Dnmt3L [37] and the significantly increased expression of AcH4K12 [36] in oocytes during the aging process in mice. Those age related DNA methylation and histone acetylation patterns as well as mitochondrial dysfunction change [38] and may lead to a reduction in oocyte developmental potential.

High fidelity chromosomal segregation is essential for maintaining genomic stability. Most autosomal aneuploidies and all autosomal monosomies are lethal, and only a small number of

Table 6 Proportion of ovarian follicles in the young and aged mice

Follicle type	Primordial	Transitional	Primary	Primary+	Secondary	Pre-antral	Early antral	Antral
Young	2079.6±420.70 ^B	302.4±52.87	434.4±46.54 ^b	75.6±16.50	208.8±14.00 ^b	19.2±8.78	62.4±13.76	27.6±6.18
Aged	330±33.51 ^A	190.8±36.96	254.4±42.54 ^a	102±17.29	140.4±24.49 ^a	24±4.24	66±13.42	32.4±6.18

Values are the mean±SD. Different superscripts (a, b) represent a significant difference ($P<0.05$). Different superscripts (A, B) represent a significant difference ($P<0.01$)

Fig. 2 Effect of aging on the gene expression of four Cohesin subunits. Effect of aging on the gene expression of four Cohesin subunits (Rec8, SMC3, SMC1 β and STAG3). The housekeeping gene Rpl19 was used as an endogenous control. Steady-state expression levels of SMC3, SMC1 β and STAG3 were detected in the young and aged group; however, no Rec8 expression was detected. The results represent the mean $\pm$ S.E.M from three experiments

trisomies are compatible with full-term development and often result in severe congenital abnormalities, such as Down syndrome. More than 70 years ago people reported the relationship between Down syndrome and advanced maternal age for the first time, and it was later found that trisomy 21 was the actual genetic cause [8]. It has been suggested that the increased incidence of trisomy 21 and spontaneous abortion in older women are a “tip of the iceberg” manifestation of the age-dependent loss of female fertility that is caused by increasing rates of aneuploidy in oocytes [1]. It has been reported that the incidence of trisomy in clinically recognized pregnancies remains low in woman in their 20s (approximately 2 %–3 %) but dramatically increases to approximately 35 % in woman in their 40s [4]. In humans, it is known that aneuploidy of a meiotic origin increases dramatically with female age, and several lines of evidence from mice are consistent with the cohesion hypothesis [20, 22, 39]. A leading hypothesis for explaining age-related aneuploidy is the deterioration of chromosome cohesion with increasing maternal age. In human eggs, the maternal-age-associated deterioration of chromosome cohesion is indicated by increased inter-kinetochore distances between sister chromatids [40], and the precocious loss of chromosome cohesion contributes to a high risk of meiotic errors [20, 41].

Protein complexes known as Cohesins maintain chromatid adhesion, facilitate the segregation of homologous chromosomes and maintain sister chromosomes pairing at the centromere until their separation at meiosis II during fertilization [41, 42]. It has been speculated that the deterioration of this protein complex over time might be relevant to the age-related rise in aneuploidy [42], and several recent publications support this idea. At least in mice, the loss of chromosome-associated cohesions in aging oocytes is associated with aneuploidy [19, 20]. It is reported that the expression of oocyte

genes that are related to cell cycle regulation, cytoskeleton structure, metabolic pathways, transcription control, and stress responses, were influenced by maternal age [43]. In this study, no difference in the relative expression levels of the SMC3, SMC1 β and STAG3 mRNAs between the groups was observed, and no REC8 mRNA expression was found (Supplementary Data Figure 1), most likely because little or no Cohesin turnover occurs for over 2 weeks during oocyte growth [44].

Several observations support the idea that the size of the oocyte pool influences the trisomy risk. After unilateral oophorectomies, mice display higher rates of aneuploidy compared to sham-operated mice [45]. It is also reported that a low follicular reserve has been associated with poor fertility in cattle [46]. Because the oocytes pool size cannot be directly measured in women, the size of the ovarian pool that contributes to the incidence of aneuploidy is an intrinsically difficult association to resolve. It is known that the average age at menopause among women with trisomic spontaneous abortions (SAs) is 1 year earlier compared to women with chromosomally normal pregnancies [47]. As we know, menopause occurs when the oocyte pool falls below a critical threshold, and this observation is consistent with the oocyte pool hypothesis. A third study indicated fewer retrieved oocytes, which is a possible indicator of a diminished ovarian reserve, among women with trisomic pregnancies [48]. The present findings in mice offer the possibility of studying the link between the ovarian reserve and aneuploidy in a more tractable system.

In conclusion, we propose that maternal aging significantly reduces the ovary reserve, superovulation efficiency and developmental potential, and increases the oocyte aneuploidy rate.

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