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—Original Article—

Meiotic arrest with roscovitine and sexual maturity improve competence of mouse oocytes by regulating expression of competence-related genes

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Abstract. We have studied the mechanisms by which meiotic arrest maintenance (MAM) with roscovitine, female sexual maturity, and the surrounded nucleoli (SN) chromatin configuration improve the competence of mouse oocytes by observing the expression of oocyte competence-related genes in non-surrounded nucleoli (NSN) and SN oocytes from prepubertal and adult mice following maturation with or without MAM. The results demonstrated that MAM with roscovitine significantly improved the developmental potential of adult SN and prepubertal NSN oocytes, but had no effect on that of prepubertal SN oocytes. Without MAM, while 40% of the 2-cell embryos derived from prepubertal SN oocytes developed into 4-cell embryos, none of the 2-cell embryos derived from prepubertal NSN oocytes did, and while 42% of the 4-cell embryos derived from adult SN oocytes developed into blastocysts, only 1% of the 4-cell embryos derived from prepubertal SN oocytes developed into blastocysts. Furthermore, MAM with roscovitine, SN configuration, and female sexual maturity significantly increased the mRNA levels of competence-beneficial genes and decreased those of competence-detrimental genes. In conclusion, our results suggest that MAM with roscovitine, SN chromatin configuration, and female sexual maturity improve oocyte competence by regulating the expression of competence-related genes, suggesting that *Oct4*, *Stella*, *Mater*, *Zar1*, *Mapk8*, and *Bcl2* are oocyte competence-beneficial genes, whereas *Foxj2*, *Ship1*, and *Bax* are competence-detrimental genes.

Key words: Chromatin configuration, Gene expression, Meiotic arrest, Oocyte competence, Roscovitine

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Many studies have demonstrated that the developmental competence of *in vitro* matured (IVM) oocytes is significantly lower than that of their *in vivo* matured counterparts. Further observations indicated that the low developmental competence of the IVM oocytes was due to a sudden premature nuclear maturation that occurred *in vitro* without adequate cytoplasmic maturation when oocytes were transferred from follicles into the culture medium [1, 2]. Thus, great efforts have been made to improve oocyte cytoplasmic maturation by meiotic arrest maintenance (MAM) *in vitro*. However, limited progress has been achieved in various species, mainly due to our insufficient knowledge of the ongoing cellular events during MAM. Furthermore, although Romero *et al*. [3] reported that cytoplasmic maturation was significantly improved following MAM of mouse oocytes using C-type natriuretic peptide, the mechanisms remain to be explored.

Fully grown mouse oocytes at the germinal vesicle (GV) stage show two distinct chromatin configurations: non-surrounded nucleolus (NSN) and surrounded nucleolus (SN) patterns [4, 5]. It is generally accepted that oocytes with an NSN configuration do not have full meiotic competence, and only oocytes with an SN configuration have the capacity to support blastocyst development [6]. However, the molecular mechanisms underlying this competence difference between NSN and SN oocytes remain largely unknown. Furthermore, the mechanisms underlying the lower oocyte developmental competence in prepubertal animals compared to that in adult animals [7–9] are largely unclear.

Recently, Chen *et al*. [10] observed that MAM with roscovitine improved embryo development to the 4-cell stage in NSN oocytes from prepubertal mice and increased rates of morulae in SN oocytes from adult mice, while having no effect on SN oocytes from prepubertal mice. Further observations indicated that MAM with roscovitine improved the competence of prepubertal NSN oocytes and adult SN oocytes by enhancing their global gene transcription. However, the genes which MAM with roscovitine had used to improve oocyte competence remain to be identified. Furthermore, according to Walker and Biase [11], although cattle oocytes can express over 15,000 protein-coding genes during their growth, less than 2% of these expressed genes have been linked to developmental competence.

The objective of the present study was to observe the expression of oocyte competence-related genes in NSN and SN oocytes from prepubertal and adult mice following maturation with or without MAM with roscovitine to reveal the mechanisms by which MAM, female sexual maturity and the SN chromatin configuration improve

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oocyte competence and to validate genes that contribute to oocyte developmental competence.

Materials and Methods

Our experimental procedures were carried out in accordance with the guidelines approved by the Animal Care and Use Committee of Shandong Agricultural University, P. R. China (Permit number: SDAUA-2014-011). Unless otherwise stated, all chemicals and reagents used in this study were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Animals and oocyte recovery

Mice of the Kunming strain, originally derived from ICR (CD-1) mice, were raised in a room under a 14-h light and 10-h dark photoperiod, with lights off at 2000 h. To collect ovaries, prepubertal female mice were sacrificed 18 to 19 days after birth without equine chorionic gonadotropin (eCG)-stimulation, whereas adult female mice were sacrificed 8 weeks after birth at 48 h following an intraperitoneal injection of 10 IU eCG (Ningbo Hormone Product Company Limited, Ningbo, China). The large follicles on the ovary were ruptured in M2 medium using a needle, and the cumulus-oocyte complexes (COCs) were recovered. Only COCs with oocytes larger than 70 μm in diameter and that showed a homogenous cytoplasm were selected for subsequent experiments.

Observation for GV chromatin configurations

Living oocytes were stained with Hoechst 33342 and observed under a fluorescence microscope. Briefly, cumulus cells were removed by pipetting COCs in M2 medium to prepare cumulus-denuded oocytes (DOs). Then, the DOs were stained for 10 min with 50 ng/ml Hoechst 33342 in M2 medium. The stained oocytes were then placed in M2 medium containing 200 μM 3-Isobutyl-1-methylxanthine (IBMX) and examined for chromatin configurations under a Leica DMIRB fluorescence microscope. Finally, DOs with known chromatin configurations were washed in M2 medium and MAM or maturation medium before culturing in MAM or maturation medium.

Preparation of cumulus cell monolayers

Approximately 25 COCs were collected from adult mice 48 h after eCG injection and were repeatedly pipetted into Dulbecco's modified Eagle's medium (DMEM) to obtain cumulus cells. The cumulus cells obtained were washed three times in DMEM and transferred into wells of a 96-well plate along with 150 μl DMEM. The cumulus cells were then cultured at 37°C in humidified air with 5% CO_2. When the cumulus cells grew to approximately 80% confluence, the spent medium in each well was replaced with 100 μl oocyte MAM or maturation medium and equilibrated for 3 h at 37°C in humidified air with 5% CO_2 before use for MAM or maturation culture of oocytes.

Oocyte MAM with roscovitine

The MAM culture was conducted as previously reported by Chen *et al*. [10]. Briefly, oocytes were cultured in microdrops of 100 μl (25–30 oocytes per drop) at 37°C in humidified air containing 5% CO_2. The medium used for MAM was 199–1 supplemented with different concentrations of roscovitine. To prepare the 199–1 medium, TCM-199 medium (Gibco, Grand Island, NY, USA) was supplemented with 10 IU/ml eCG, 0.23 mM sodium pyruvate, and 1 mg/ml bovine serum albumin (BSA, Gibco). Roscovitine (50 mM) was dissolved in dimethyl sulfoxide (DMSO, Gibco), and the stock solution was stored in aliquots at −20°C before use. While COCs from adult mice were cultured directly in MAM medium, DOs from prepubertal mice were cultured on cumulus cell monolayers in MAM medium supplemented with 200 μM cystine and 400 μM cysteamine.

Observation for GV breakdown (GVBD)

Immediately at the end of the MAM culture, oocytes were examined for GVBD using a phase-contrast microscope. While DOs from prepubertal mice were observed directly, COCs from adult mice were stripped of cumulus cells before observation. Oocytes with a clearly visible GV were considered GV intact, and the disappearance of the intact GV was indicative of the sample having undergone GVBD.

Maturation culture of oocytes

Oocyte maturation culture was conducted as previously reported [10]. Briefly, 25–30 oocytes were placed in 100 μl drops of 199–2 maturation medium and cultured at 37°C in humidified air with 5% CO_2. The 199–2 maturation medium was composed of TCM-199 supplemented with 10% (v/v) BSA, 1 μg/ml 17β-estradiol, 24.2 mg/ml sodium pyruvate, 0.05 IU/ml follicle stimulating hormone (FSH), 0.05 IU/ml luteinizing hormone (LH), and 10 ng/ml epidermal growth factor (EGF). While COCs from adult mice were cultured directly in 199–2, DOs from prepubertal mice were cultured on cumulus cell monolayers in 199–2 supplemented with 200 μM cystine and 400 μM cysteamine.

Oocyte activation and embryo culture

At the end of maturation culture for 24 h, while DOs from prepubertal mice were activated directly, COCs from adult mice were denuded of cumulus cells by pipetting in M2 medium with 0.1% hyaluronidase. After being washed twice in M2 and once in the activating medium, the DOs with PB1 were cultured in an activating medium for 6 h at 37°C in humidified air containing 5% CO_2. The activating medium consisted of a Ca^{2+}-free CZB medium, 10 mM $SrCl_2$, and 5 μg/ml cytochalasin B. At the end of the activation treatment, oocytes were observed under a phase-contrast microscope and were considered activated when each showed one or two pronuclei. Activated oocytes were cultured in regular CZB (25–30 oocytes per 100 μl drop) at 37°C in humidified air containing 5% CO_2. Glucose was added to CZB when embryos developed beyond the 3- or 4-cell stages. At 24, 48, 72, and 96 h of culture, embryos were examined to determine the number of 2-cell embryos, 4-cell embryos, morulae, and blastocysts, respectively. To determine the cell number per blastocyst, 20 blastocysts were randomly selected from each replicate and stained with 10 μg/ml Hoechst 33342 before cell counting.

One-step real-time-PCR

At the end of maturation culture for 24 h, while DOs from prepubertal mice were used directly, COCs from adult mice were denuded of cumulus cells by pipetting in M2 medium with 0.1% hyaluronidase. The DOs with PB1 were lysed to extract RNA using

a commercial cell lysis kit (CellAmp Direct Prep Kit for RT-PCR & Protein Analysis, TaKaRa, code no. 3733Q, Shiga, Japan). The lysate obtained was preserved at –80°C before use. Quantification of mRNA was conducted using a One-Step TB Green PrimeScript PLUS RT-PCR Kit (Perfect Real Time, TaKaRa, code no. RR096A). A 10 µl reaction volume was used for the amplification reaction (1 µl template, 2.2 µl RNase Free d H_2O, 5 µl 2 × One Step TB Green RT-PCR Buffer 4, 0.6 µl TaKaRa Ex Taq HS Mix, 0.2 µl PrimeScript PLUS RTase Mix, 0.2 µl ROX Reference Dye II, and 0.4 µl each of forward and reverse primers (10 µM)). The gene-specific primers used are listed in Table 1.

Data analysis

Each treatment was repeated at least three times. Percentage data were arcsine-transformed before analysis by ANOVA. Duncan's multiple comparison test was performed to identify differences during the ANOVA analysis. The software used was SPSS 25 (SPSS Inc., IBM Corp., Armonk, NY, USA). Data are expressed as mean ± SE, and statistical significance was set at $P < 0.05$.

Results

Classification of GV chromatin configurations in mouse oocytes

Freshly recovered oocytes at the GV stage were processed for chromatin configuration observation. In contrast to our previous study, which examined chromatin configuration in fixed oocytes [10], this study observed chromatin configuration in living oocytes after Hoechst 33342 staining. Four chromatin configurations were observed, including the NSN, partly NSN (pNSN), partly SN (pSN), and SN configurations (Figs. 1A, B, C, and D). In the NSN configuration, chromatin was mostly decondensed with sparse fine heterochromatin threads/granules that did not surround the nucleolus (Fig. 1A). In the pNSN pattern, some fine heterochromatin threads/granules began to close to the nucleolus (Fig. 1B). In both the SN and pSN oocytes, the nucleoli were enclosed by heterochromatin. However, while the chromatin in SN oocytes was the most condensed with little diffuse chromatin (Fig. 1D), the chromatin in pSN oocytes was less condensed, with some diffuse chromatin in the nucleoplasm (Fig. 1C). For convenience, NSN and pNSN were designated as NSN, whereas pSN and SN were classified as SN configurations in this study. Our calculation indicated that in adult mice, while 90.3 ± 1.2% oocytes showed an SN configuration, 9.7 ± 1.2% oocytes had an NSN configuration (Fig. 1E). In prepubertal mice, however, the number of oocytes with an NSN (49.5 ± 2.1%) and that of oocytes with an SN configuration (50.5 ± 2.1%) were almost equal.

Optimal concentrations of roscovitine for MAM of adult and prepubertal mouse oocytes

In our previous study [10], only one concentration of roscovitine (100 µM) was used for MAM in both prepubertal and adult mouse oocytes. Optimal concentrations of roscovitine for MAM in adult and prepubertal mouse oocytes were determined in this study. COCs from adult mice or DOs from prepubertal mice were cultured for 4 h in MAM medium containing various concentrations of roscovitine before examination for GVBD. The results showed that both 100 µM and 125 µM roscovitine could successfully prevent GVBD in both adult and prepubertal oocytes (Fig. 2A). Furthermore, while 25 µM and 50 µM roscovitine significantly reduced GVBD rates to approximately 10% in adult oocytes, these two concentrations decreased GVBD rates to 63% and 51% in prepubertal oocytes.

To optimize roscovitine concentrations during MAM to improve oocyte competence, developmental potential after maturation was observed following MAM of adult and prepubertal oocytes with various concentrations of roscovitine. For adult oocytes, the percentage of activated oocytes (94–96%), 2-cell embryos (83–88%), 4-cell embryos (77–80%), and blastocysts (60–62%) did not differ among different concentrations of roscovitine, and control oocytes matured without MAM. However, the percentages of morulae were significantly higher after MAM with 100 or 125 µM roscovitine than with 150 µM roscovitine and control oocytes (Fig. 2B). Furthermore, the cell number per blastocyst was significantly higher after MAM at 125 µM than at other concentrations of roscovitine. For prepubertal oocytes, the percentages of activated oocytes (79–83%), 2-cell embryos (63–68%), and blastocysts (0–7%) did not differ among different concentrations of roscovitine, and control oocytes matured without MAM. The percentages of 4-cell embryos, however, were significantly higher after MAM at 100 µM than at other concentrations of roscovitine or control oocytes (Fig. 2C). Thus, the optimal concentrations of roscovitine for MAM in adult and prepubertal mouse oocytes were 125 and 100 µM, respectively.

Table 1. Sequences of primers used for one-step RT-PCR in this study

Genes	Forward	Reverse
Oct4	TGTTCAGCCAGACCACCATCT	ACTCCACCTCACACGGTTCTCA
Stella	AGGGTCCGCACTTTGTTGTC	GGCTCACTGTCCCGTTCAAA
Mater	TCAAGAACTGGAACTAGTGGAC	GCTTGGTTGTTGTGATCATACA
Zar1	GACGCCTCGGTGCAGTGTTC	CACAGAAGGTCACGGACGAGAAC
Mapk8	AAACTGTTCCCCGATGTGCTT	CGTTGATGTATGGGTGCTGGA
Foxj2	TCAGCAAAGATGAGGCAGCG	ACCGATGCCAGCGTTCTTGTA
Shp1	GGGCTAGACTGTGACATTGATA	TTTCTTCTTGGTCGTTTCGATG
Bcl2	GAGCGTCAACAGGGAGATG	GGGCCATATAGTTCCACAAAGG
Bax	TGCAGAGGATGATTGCTGAC	GATCAGCTCGGGCACTTTAG

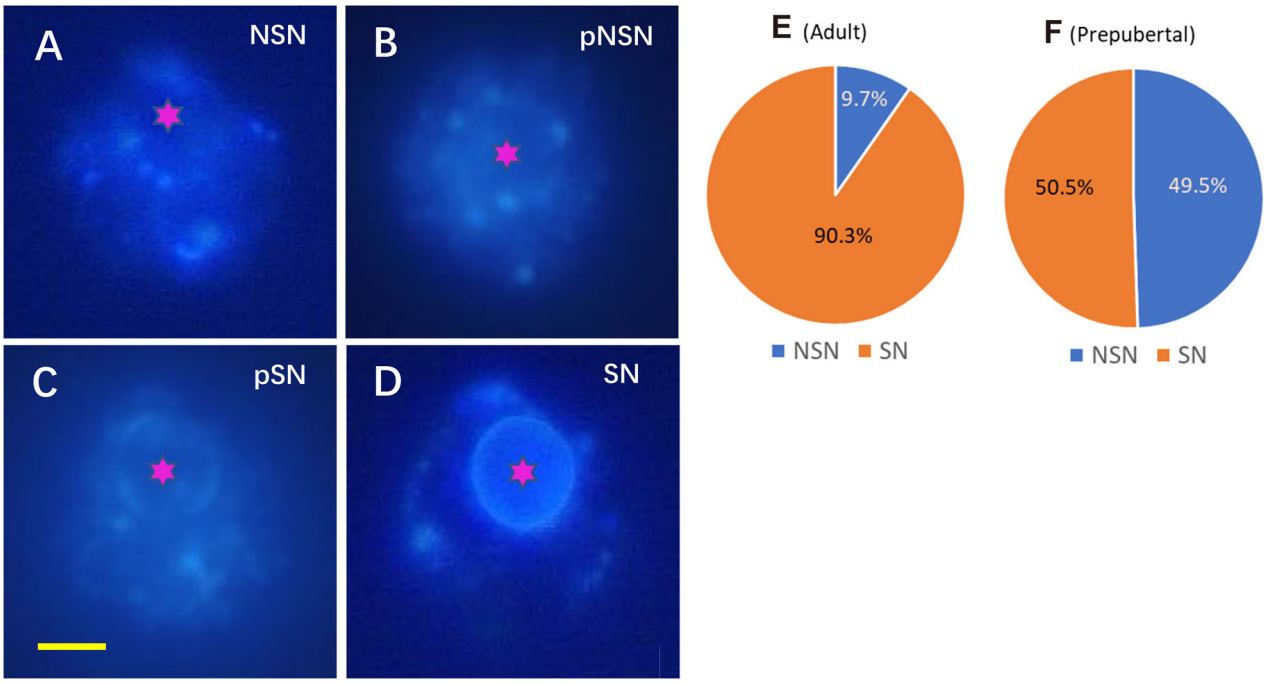

Fig. 1. Classification of GV chromatin configurations in mouse oocytes. Photographs A, B, C, and D show oocytes with NSN, partly NSN (pNSN), partly SN (pSN), and SN configurations, respectively. Bar is 25 μm and applies to all images. * indicates the location of the nucleolus. In this study, NSN and pNSN were designated as NSN, whereas pSN and SN were classified as SN configurations. Graphs E and F are pie charts showing percentages of NSN and SN oocytes in adult and prepubertal mice, respectively. Each treatment was repeated 4 times with each replicate including about 40–50 oocytes.

Effects of MAM with roscovitine on the developmental potential of adult and prepubertal mouse oocytes

Adult and prepubertal mouse oocytes were treated for 4 h with 125 and 100 μM roscovitine, respectively, before maturation and activation treatment for embryo development. While adult oocytes were treated immediately following recovery, prepubertal oocytes were classified into NSN and SN types before treatment. Freshly collected oocytes were also matured without MAM and activated as controls. For adult oocytes, while rates of mature and activated oocytes and 2-cell, 4-cell, and blastocyst embryos did not differ between MAM and control oocytes, the percentage of morulae and the cell number per blastocyst were significantly higher in MAM than in control oocytes (Fig. 3A). For prepubertal oocytes, although MAM had no effect on SN oocytes, it significantly increased the percentages of 4-cell and 2-cell embryos of NSN oocytes compared to those in control oocytes matured without MAM (Fig. 3B). Thus, the results suggest that MAM with roscovitine significantly improved the developmental potential of adult mouse oocytes and prepubertal NSN oocytes, while having no effect on that of prepubertal SN oocytes.

Effects of roscovitine on the expression of competence-related genes in adult and prepubertal mouse oocytes

One-step RT-PCR was performed to determine the mRNA levels of competence-related genes. The COCs from adult mice or the DOs of the NSN configuration from prepubertal mice were cultured for 4 h for MAM and then for 24 h for maturation. Control oocytes were matured for 24 h without MAM. At the end of the maturation culture, mature oocytes showing PB1 were examined for gene expression by one-step RT-PCR. In both adult oocytes (Fig. 4A) and prepubertal NSN oocytes (Fig. 4B), whereas the mRNA levels of the POU5F1-POU class 5 homeobox 1 (Oct4), Stella (also known as primordial germ cell 7, PGC7), maternal antigen that embryos require (Mater), zygote arrest 1 (Zar1), and Mapk8 (also known as JNK1) genes were upregulated, that of Forkhead Box J2 (Foxj2) and Src-homology protein tyrosine phosphatase-1 (Shp1) genes were significantly downregulated by MAM compared to that in control oocytes matured without MAM. The ratio of the anti-apoptotic Bcl2: proapoptotic Bax mRNA was also significantly higher in MAM oocytes than in control oocytes.

Comparison of competence-related gene expression between NSN and SN oocytes of prepubertal mice

Both the present results and previous studies indicate that SN mouse oocytes are more developmentally competent than NSN oocytes. Thus, we compared the expression of competence-related genes between these two groups of oocytes following maturation without MAM. The results showed that the mRNA levels of all the competence-beneficial genes, including *Oct4*, *Stella*, *Mater*, *Zar1*, and *Mapk8*, as well as the *Bcl2/Bax* ratio, were significantly higher in SN oocytes than in NSN oocytes (Fig. 4C). While the mRNA level of the competence-detrimental gene *Foxj2* was significantly lower, as expected, that of *Shp1* was unexpectedly higher in SN oocytes than in NSN oocytes. Overall, the results confirmed that compared to that of the NSN oocytes, the higher developmental

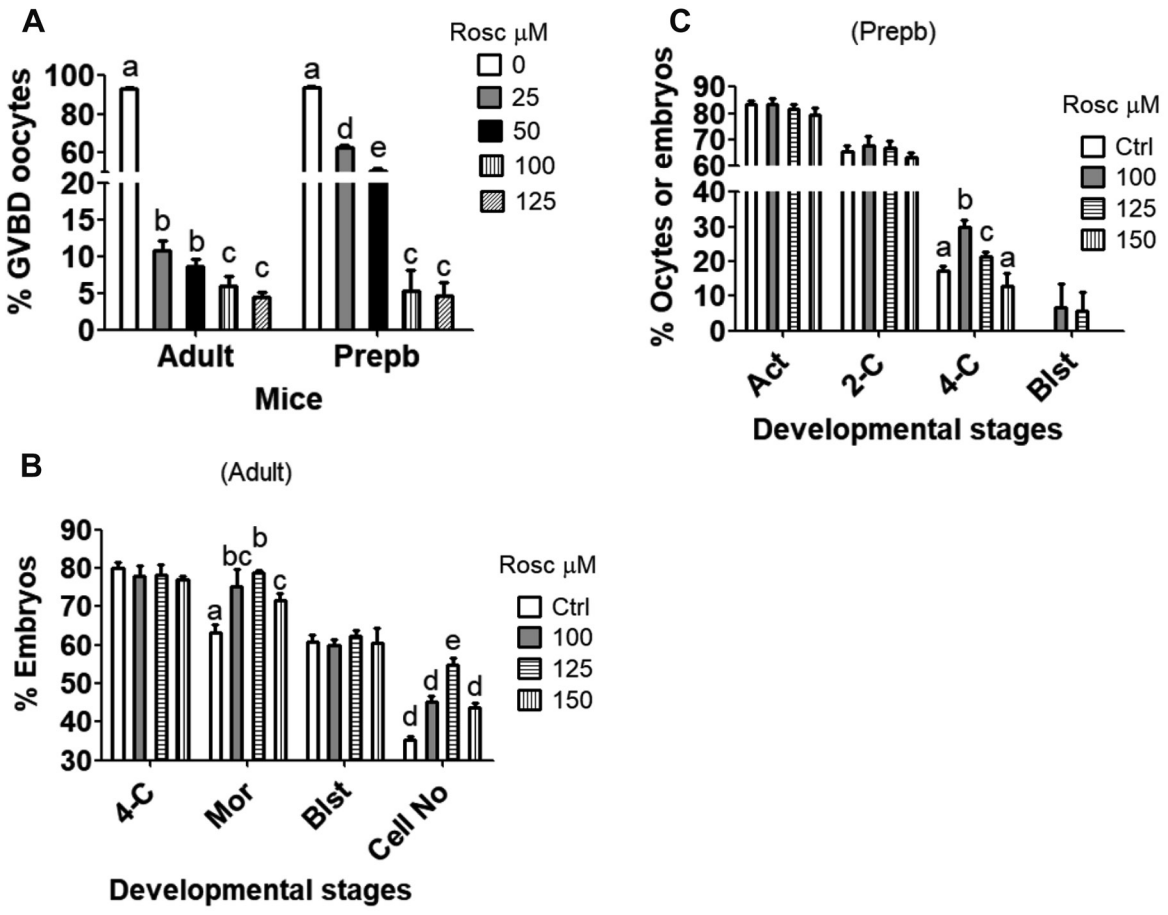

Fig. 2. Selection of optimal roscovitine concentrations for MAM of adult and prepubertal mouse oocytes. Graph A shows percentages of oocytes undergoing GVBD following a 4-h MAM of adult or prepubertal (Prepb) mouse oocytes with different concentrations of roscovitine (Rosc). Each treatment was repeated 5–6 times with each replicate including about 30–35 oocytes. Graphs B and C show embryo development in adult and prepubertal mouse oocytes, respectively, following MAM with different concentrations of roscovitine. Freshly collected oocytes were also matured and activated to serve as controls (Ctrl). Each treatment was repeated 3–4 times with each replicate containing 30–35 oocytes. Act: % Activated/MII oocytes; 2-C: % 2-cell embryos/activated oocytes; 4-C: % 4-cell embryos/2-C embryos; Mor: % Morulae/4-cell embryos; Blst: % Blastocysts/Morulae (for adult mouse oocytes) or /4-cell embryos (for prepubertal mouse oocytes); Cell No: Cell number per blastocyst. a–e: Values with a different letter above bars differ significantly ($P < 0.05$).

potential of the SN oocytes was associated with increased expression of competence-beneficial genes and decreased expression of competence-detrimental genes.

Comparison of competence-related gene expression between adult and prepubertal SN oocytes

To reveal the mechanisms by which female sexual maturity improves oocyte competence, we compared the expression of competence-related genes between adult and prepubertal SN oocytes. The results showed that while the mRNA levels of both *Foxj2* and *Shp1* were lower, the mRNA levels of all other competence-beneficial genes and the *Bcl2/Bax* ratio were significantly higher in adult SN oocytes than in prepubertal SN oocytes (Fig. 4 D), suggesting that female sexual maturity improves oocyte competence by enhancing the expression of competence-beneficial genes while downregulating the expression of competence-detrimental genes.

Discussion

By co-culturing mouse DOs with cumulus cell monolayers in medium supplemented with cystine and cysteamine, both the present study and our previous study [10] showed that over 85% of the NSN DOs from prepubertal mice completed nuclear maturation *in vitro* and developed to the MII stage after a short Hoechst staining and classification under UV light. This is a significant improvement over early studies, which observed that only about 15% of the NSN DOs isolated from adult mice matured *in vitro* under simple conditions following similar treatments of Hoechst staining and UV light classification [5, 12, 13]. In this study, while COCs from adult mice were cultured directly, DOs from prepubertal mice were co-cultured with cumulus cell monolayers. Because direct substance transfer through gap junctions could not occur in the coculture system, the developmental potential of prepubertal mouse oocytes might be

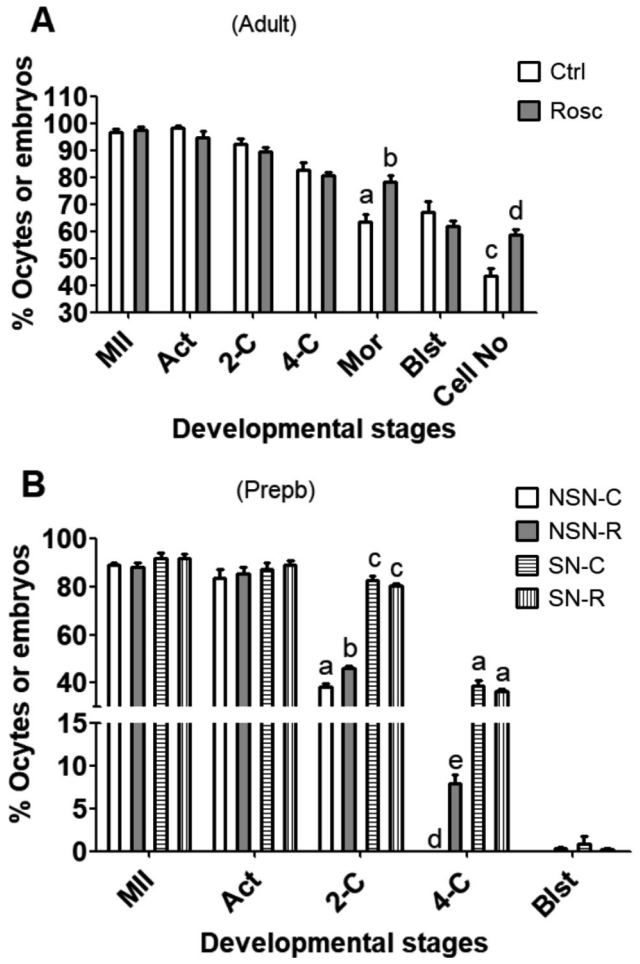

Fig. 3. Effects of MAM with roscovitine on the developmental potential of adult and prepubertal mouse oocytes. Graph A shows percentages of mature (MII) and activated (Act) oocytes, 2-cell (2-C) and 4-cell (4-C) embryos, morulae (Mor) and blastocysts (Blst), and cell number per blastocyst (Cell No) after MAM of oocytes from adult mice with 125 μM roscovitine (Rosc) compared to those in control (Ctrl) oocytes matured without MAM. Graph B shows percentages of MII and Act oocytes, 2-C, 4-C and Blst embryos after MAM with 100 μM roscovitine of NSN (NSN-R) and SN (SN-R) oocytes from prepubertal mice compared to those in control NSN-C or SN-C oocytes. Each treatment was repeated 3–4 times with each replicate including about 40 oocytes. Percentages of MII, Act, 2-C, and 4-C were calculated from cultured oocytes, MII oocytes, Act oocytes, and 2-C embryos, respectively. For adult mouse oocytes, % Mor and Blst were calculated from 4-C and Mor, respectively, and for prepubertal mouse oocytes, % Blst was calculated from 4-C embryos. a-e: Values with a different letter above bars differ significantly ($P < 0.05$).

further improved by direct culture of COCs.

In contrast to our previous study [10], which used 100 μM roscovitine, this study used 125 μM roscovitine for MAM of adult mouse oocytes, which significantly improved cell counts per blastocyst and morula rates. Furthermore, in this study, while 25 μM and 50 μM roscovitine significantly reduced GBVD rates to approximately 10% in adult oocytes, these two concentrations decreased GVBD rates to 63% and 51% in prepubertal oocytes. Han *et al*. [14] reported that GVBD inhibition required more roscovitine in prepubertal mouse oocytes than in adult oocytes because the prepubertal oocytes contained significantly higher MPF activities than did adult oocytes.

The current results suggest that MAM with roscovitine, SN chromatin configuration, and female sexual maturity might improve oocyte competence by regulating the expression of competence-related genes. Thus, MAM with roscovitine significantly improved the developmental potential of adult SN and prepubertal NSN oocytes, but had no effect on that of prepubertal SN oocytes. Without MAM, while 40% of the 2-cell embryos derived from prepubertal SN oocytes developed into 4-cell embryos, none of the 2-cell embryos derived from prepubertal NSN oocytes did. Whereas 42% of the 4-cell embryos derived from adult SN oocytes developed into blastocysts, only 1% of the 4-cell embryos derived from prepubertal SN oocytes developed into blastocysts. Furthermore, MAM with roscovitine, SN configuration, and female sexual maturity significantly increased the mRNA levels of competence-beneficial genes, including *Oct4*, *Stella*, *Mater*, *Zar1*, *Mapk8*, and *Bcl2*, and decreased those of competence-detrimental genes including *Foxj2*, *Ship1*, and *Bax*.

Among the competence-beneficial genes we observed in this study, the OCT4 protein is expressed only in SN oocytes and is never expressed in NSN oocytes during oocyte growth [14]. The absence of OCT4 at the very end of folliculogenesis might be detrimental for the acquisition of full developmental competence in oocytes [16]. Expression of Stella is significantly lower in fully grown NSN oocytes than in SN oocytes [17]. This close resemblance in expression between the Stella and Oct4 genes suggests a critical role for Oct4 in regulating Stella expression [16]. Most oocytes lacking Stella expression arrested development at the 2-cell stage following *in vitro* maturation and fertilization [18]. The Stella gene protects against demethylation of the maternal genome and some paternal imprinted genes at the early stages of embryo development [19]. Furthermore, Stella protected the unique oocyte epigenome by guarding against aberrant de novo DNA methylation, which is mediated by DNA methyltransferase (DNMT1) and DNA methylation regulator (UHRF1), and impairs zygotic genome activation in embryos [20].

The increased *in vitro* developmental competence of bovine oocytes was linked to their higher levels of *Mater* and *Oct4* transcripts immediately following oocyte retrieval 4 h after slaughtering [21]. Developmental arrest at the 2-cell stage of NSN mouse oocytes was associated with reduced expression of the *Mater* gene [22]. Less than 20% of the embryos derived from *Zar1* gene knockout (–/–) oocytes developed to the 2-cell stage, and none developed to the 4-cell stage [23]. Furthermore, inhibition of the c-Jun NH2-terminal kinase (JNK) pathway, such as MAPK8, with drug inhibitors or by RNAi resulted in the inhibition of cavity formation in mouse embryos during preimplantation development [24].

Among the competence-detrimental genes we observed in this study, the expression of the *Foxj2* gene is regulated by Oct4, and there was a 2.1-fold increase in Foxj2 transcripts in NSN mouse oocytes compared with SN oocytes following maturation [15]. Overexpression of *Foxj2* had a negative effect on both pre- and post-implantation embryo development [25]. Yin et al. [26] observed that mouse embryos overexpressing *Shp1* were mainly arrested at the 8-cell stage, and their further observation indicated that SHP1 blocked

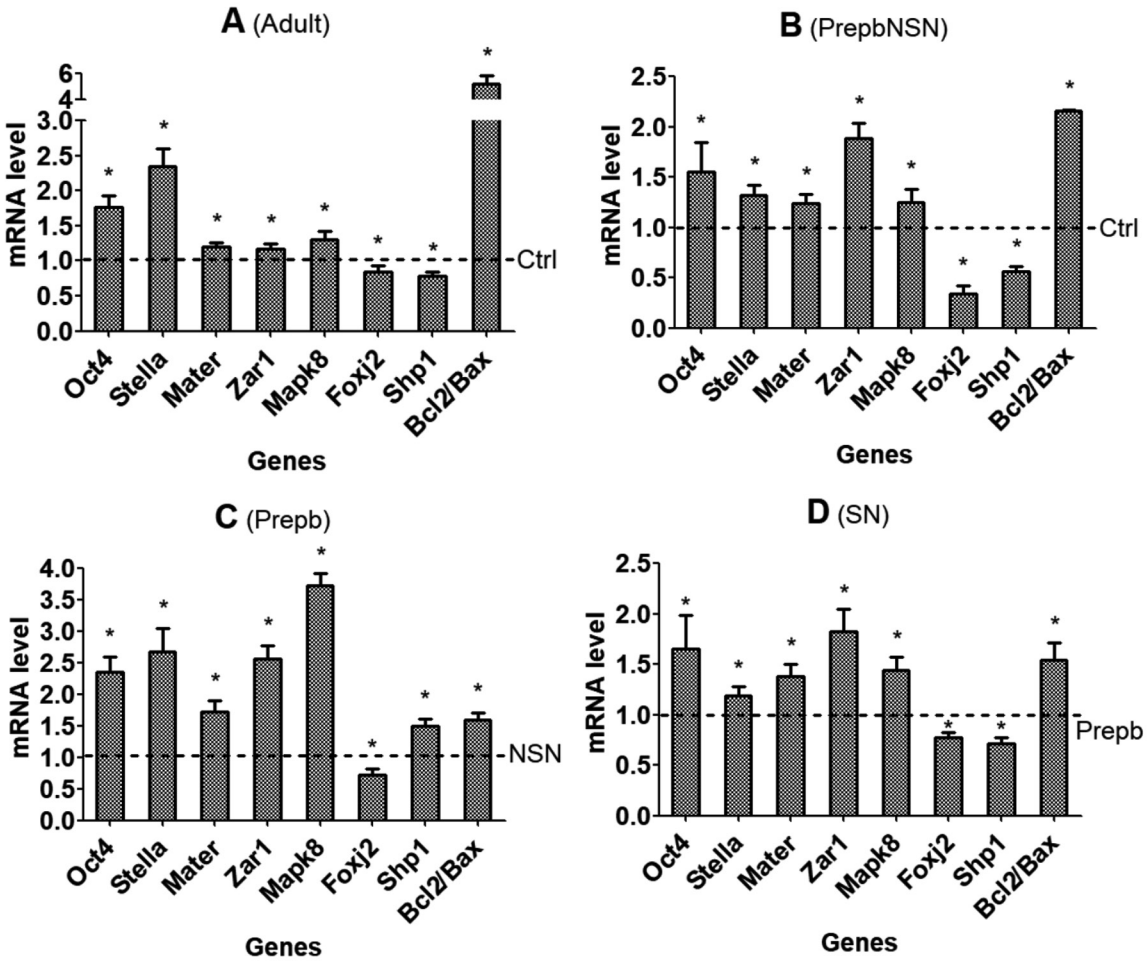

Fig. 4. Effects of MAM with roscovitine on expression of competence-related genes in adult and prepubertal mouse oocytes. Graph A compares relative mRNA levels of different genes between adult oocytes matured after MAM with roscovitine and control (Ctrl) oocytes matured without MAM. Graph B compares relative mRNA levels of different genes between prepubertal NSN (PrepbNSN) oocytes matured after MAM with roscovitine and control (Ctrl) oocytes matured without MAM. Values of the control oocytes were set to one, and those of roscovitine-treated oocytes were expressed relative to it. Graph C compares relative mRNA levels of different genes between prepubertal SN and NSN oocytes matured without MAM. Values of the NSN oocytes were set to one and those of SN oocytes were expressed relative to it. Graph D compares mRNA levels of different genes between adult and prepubertal SN oocytes. Each treatment was repeated at least 4 times with each replicate containing 7 oocytes. * indicates significant differences ($P < 0.05$) from control oocytes in Graphs A and B, from NSN oocytes in Graph C or from prepubertal oocytes in Graph D.

embryo development by downregulating Nanog via dephosphorylating STAT3. Furthermore, the anti-apoptotic BCL2 and proapoptotic BAX meet at the surface of mitochondria to compete for the regulation of cytochrome c release. If pro-apoptotic molecules prevail, a series of molecules, including cytochrome c, are released from the mitochondria to induce apoptosis [27]. Thus, it is the ratio of BCL2/BAX, but not their contents, which determines the susceptibility of a cell to apoptogenic stimuli.

Although our recent studies suggested that MAM with roscovitine enhanced global gene transcription by inhibiting cyclin-dependent kinase 5 (CDK5) and improved the competence of mouse [10] and pig oocytes [28], this study showed that the competence-detrimental genes were downregulated with the improvement of oocyte competence. Thus, MAM with roscovitine improved oocyte competence not only by enhancing but also by reducing gene transcription. In this study, the beneficial effect of MAM with roscovitine appeared from the morula stage in adult oocytes, while it appeared at the 4-cell stage in prepubertal oocytes. Furthermore, adult oocytes treated for 4 h with roscovitine during MAM showed a significant increase in morula rates and cell numbers 72 to 96 h later. Taken together, these results suggest that MAM with roscovitine improves oocyte competence through more complicated epigenetic mechanisms that are worth exploring in future studies.

In summary, we have studied the mechanisms by which MAM with roscovitine, female sexual maturity, and the SN chromatin configuration improve the competence of mouse oocytes by observing the expression of oocyte competence-related genes in NSN and SN oocytes from prepubertal and adult mice following maturation with or without MAM. The results suggested that MAM with roscovitine, SN chromatin configuration, and female sexual maturity improve

oocyte competence by regulating the expression of competence-related genes, suggesting *Oct4*, *Stella*, *Mater*, *Zar1*, *Mapk8*, and *Bcl2* as oocyte competence-beneficial genes, and *Foxj2*, *Ship1*, and *Bax* as oocyte competence-detrimental genes. The data are important for our understanding of not only the mechanisms by which MAM, SN chromatin configuration, and female sexual maturity improve oocyte competence, but also the general basic mechanisms for oocyte maturation.

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