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Strain specific spontaneous activation during mouse oocyte maturation

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Abstract

Objective—To examine whether spontaneous oocyte activation is determined by genetic differences and interacted with culture environment.

Design—Experimental Study.

Setting—Temple University School of Medicine.

Animals—C57BL/6, DBA/2, C3H/HeJ, and A/J strains, along with reciprocal F1 hybrid female mice (5–6 weeks).

Intervention(s)—Immature oocytes from different mouse strains were collected and cultured in different maturation conditions including different serum, serum replacement, bovine serum albumin (BSA) and follicle stimulation hormone (FSH).

Main Outcome Measure(s)—The emission of first polar body, pronucleus formation, meiotic arrest, spontaneous activation, and expression of maturation regulators.

Result(s)—Oocytes from C57BL/6 mice display a high rate of delayed first meiotic division and spontaneous activation after the first meiotic division with in vitro maturation (IVM), and the second meiosis with in vivo maturation (VVM) following superovulation. Spontaneous activation with IVM is sensitive to culture environment. Oocytes spontaneously activated during the first meiotic division with IVM have unusual paired tetrad chromosomes with slight connections at centromeres, whereas oocytes activated in vivo display haploidization from the second meiosis. Spontaneous activation is also seen in F1 hybrid oocytes, indicating a dominant trait from C57BL/6. Delayed meiosis was associated with reduced cyclin B and securin expression.

Conclusion(s)—Both mouse strain and culture environment have a significant effect on the incidence of meiotic defects and spontaneous activation. Reduced expression of meiotic regulators may underlie this effect.

Keywords

oocyte; meiosis; in vitro maturation; spontaneous activation; assisted reproduction

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Reprint requests: Keith E. Latham, Ph.D., 3400 North Broad Street, PAHB 561, Philadelphia, PA 19140 (klatham@temple.edu). Y.C has nothing to disclose. Z.Z has nothing to disclose. K.E.L has nothing to disclose.

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The correct control of oocyte maturation is a fundamental requirement for reproduction and for female health. Failure to regulate maturation correctly can lead to infertility, due to a failure to produce competent matured oocytes capable of fertilization. Moreover, spontaneous activation in vivo can create ovarian teratomas (1).

Mammalian oocyte maturation is complex, requiring meiosis resumption, spindle assembly, polar body extrusion, and meiotic arrest prior to insemination. Key molecules regulate these processes, such as cyclic adenosine monophosphate (cAMP), protein kinase type A (PKA), v-mos Maloney Murine Sarcoma Oncogene Homologue (MOS), mitogen activated protein kinases (MAPKs), and other cell cycle regulators such as cell division cycle 25 (CDC25) (2–5) as well as components of the centromere and spindle that drive chromosome pairing and segregation (6–9). Maturing mammalian eggs arrest at the metaphase of the second meiosis, awaiting either fertilization to induce endogenous calcium activation to become embryos, or degeneration.

Parthenogenesis, by which oocytes can initiate embryonic development without fertilization, is a common life pattern in non-mammalian species, but parthenogenetic development to term is prevented in mammals due to genomic imprinting. In mammals, parthenogenesis can be initiated artificially in vitro by activating agents such as ethanol, strontium and 6-dimethylaminopurine (6-DMAP) to elevate intracellular free calcium. Spontaneous activation in vivo is unusual in mammals, but occurs in a few species such as rat, hamster and human (10–13). Ovulated eggs from these species can initiate division once released from the oviduct ampulla. Extracellular calcium is required for spontaneous activation. Oocytes cultured in calcium free medium or medium containing the calcium antagonist decreases spontaneous activation (10–11). One mouse strain, LT/Sv, displays a high incidence of spontaneous activation during in vivo and in vitro maturation (14–16). Other strains such as BALB/cJ, SWR/J and C58/J rarely display spontaneous activation in vivo, suggesting genetic variation.

We report here that another strain, C57BL/6, which has been utilized extensively in laboratory and genetic studies, displays a higher incidence of spontaneous activation than other inbred strains. Germinal vesicle (GV) stage oocytes from C57BL/6 mice display a high rate of delayed first meiotic division and spontaneous activation after the first meiotic division with in vitro maturation. A significant fraction of superovulated C57BL/6 eggs matured in vivo also initiate spontaneous activation at the second meiotic metaphase. Oocytes from A/J, DBA/2 and C3H/HeJ females do not display the activation. Spontaneous activation is sensitive to culture environment. These observations confirm that spontaneous activation is subject to genetic control that varies with strain, and moreover reveal for the first time an important gene-environment interaction that regulates oocyte maturation, offering a novel platform for studying the regulation of oocyte maturation.

MATERIALS AND METHODS

Animals

Mice of the C57BL/6 strain were from Harlan Sprague-Dawley (Indianapolis, IN), DBA/2, A/J, C3H from The Jackson Laboratory (Bar Harbor, ME) and (B6D2)F1 hybrid mice were from the National Cancer Institute (Rockville, MD). All studies adhered to procedures consistent with the National Research Council Guide for the Care and Use of Laboratory Animals.

Retrieval and culture of oocytes

Fully-grown germinal vesicle stage (GV) oocytes were collected from ovarian follicles of 5–6 week old female mice 46–48 hours after injection of 5 IU equine chorionic gonadotropin

(eCG) (Sigma-Aldrich, St. Louis, MO). In order to prevent spontaneous germinal vesicle breakdown (GVBD) during isolation, cumulus-enclosed GV oocytes (CEO) from needle-punctured antral follicles were collected in HEPES buffered M2 medium with 0.2 mM 3-isobutyl-1-methylxanthine (IBMX) (Sigma-Aldrich, St. Louis, MO) and cultured in MEM α (Invitrogen Corporation, Carlsbad, CA cat# 12561-056) medium with 0.2mM IBMX containing either 20% fetal bovine serum (FBS, ATLAS Biologicals, Fort Collins, CO; Hyclone, Logan, UT; GIBCO/Life Technologies, Grand Island, NY), serum replacement supplement (GIBCO/Life Technologies, Grand Island, NY) or 5mg/mL BSA (Sigma-Aldrich, St. Louis, MO) for 1 hour to develop a perivitelline space. Cumulus cells were removed by pipetting. Denuded oocytes (DO) were completely rinsed to remove IBMX and cultured in MEM α medium containing 20% FBS, serum replacement supplement or 5mg/mL BSA for 14–16 hours for maturation. Follicle stimulating hormone (FSH, 7 IU/mL) (Sigma-Aldrich, St. Louis, MO) was added to maturation medium with BSA to prompt cumulus cell expansion. Matured eggs were identified after 16 hours by presence of the first polar body. The rate of spontaneous activation was determined thereafter on the basis of pronucleus formation.

In vivo superovulated eggs were collected from adult (7–8 weeks old) female mice after the injection of 5 IU eCG followed 48 h later by 5 IU human chorionic gonadotropin (hCG) (Sigma-Aldrich, St. Louis, MO) injection, and isolated at 14–16 hours post-hCG injection. The surrounding cumulus cells were removed by hyaluronidase treatment and the eggs were cultured in CZB medium to evaluate spontaneous activation and pronucleus formation by 8 hours after collection.

Immunofluorescence

Oocytes were fixed and processed for immunofluorescent detection of microtubules by confocal laser microscopy. Oocytes were fixed in 3.7% paraformaldehyde (Sigma-Aldrich, St. Louis, MO) in 0.1 mol/L PBS (pH7.4) for 40 minutes, then permeabilized in PBS buffer containing 0.25% Triton X-100 (Sigma-Aldrich, St. Louis, MO). After rinsing in PBS buffer three times, the oocytes were incubated with 1:50 FITC-labeled anti- β -tubulin (Sigma-Aldrich, St. Louis, MO) antibody for 1 hour. Oocytes were exposed to 10 μ g/mL propidium iodide (Sigma-Aldrich, St. Louis, MO) to stain DNA, and then examined by confocal laser microscopy.

Cytogenetic Analysis

Metaphase oocytes or zygotes mitotically arrested after 8 hours culture with 10 μ g/mL Nocodazole (Sigma-Aldrich, St. Louis, MO) were fixed for chromosome analysis as described (17) with some modification. Briefly, cells were removed from the zona pellucida by exposure to Tyrode Acid (pH 2.2) solution for 1 minute and then transferred to 1 mM KCl hypotonic solution for 2 minutes. Zona-free swollen cells were transferred to microscope slides which had been dipped in a solution containing 1% paraformaldehyde and 0.15% Triton X-100. After complete drying at room temperature, the spread chromosomes were stained by 10 μ g/mL propidium iodide for counting.

Western Blotting

Analysis of expression of maturation promoting factor (MPF), mitogen-activated protein (MAP) kinases (a.k.a. ERK) and securin (a.k.a., pituitary tumor transforming gene 1, PTTG1) proteins was performed by western blotting as described (18). Briefly, oocytes were lysed in Laemmli lysis buffer (BioRad Laboratories, Hercules, CA). Proteins were separated by 1-dimensional SDS-polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane (Bio-RAD Laboratories, Hercules, CA). Blots were blocked with 5% skim milk (BD, Franklin Lakes, NJ) in TBST (TBS containing 0.1% Tween-20), and then incubated

with 1:1000 mouse monoclonal antibody to cyclin B1 (Abcam Inc. Cambridge, MA), 1:250 mouse monoclonal antibody p-ERK antibody (Santa Cruz Biotechnology, Santa Cruz, CA), or 1:200 mouse monoclonal antibody to securin (Abcam Inc. Cambridge, MA) for 2 hours. The secondary antibody was horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG (Jackson ImmunoResearch Laboratories, Inc., PA). The membrane was washed in TBST and then processed using the ECL detection system (Amersham, GE Healthcare, Piscataway, NJ). For all of the treatments, 37 eggs were loaded into each lane.

Statistical Analysis

Chi-squared test was used to evaluate the significance of differences between groups. Samples sizes and values for the phenotypic categories were appropriate for this test.

RESULTS

Oocytes maturing in vivo or in vitro can progress through four different developmental patterns (Fig. 1). The typical pattern of maturation involves resumption of meiosis (GVBD) followed by completion of the first meiotic division (with first polar body extrusion) and then arrest at the second meiotic metaphase (MII) (Fig. 1 pattern A, Fig. 2 A–B). With the second pattern, oocytes complete the first meiotic division and then fail to arrest at the second metaphase, activating parthenogenetically instead (Fig. 1 pattern B, Fig. 2 E–F). If both polar bodies are extruded, this yields haploid parthenotes (Fig. 2 E–F). Alternatively, as the third pattern, oocytes exhibit delayed first polar body extrusion and then extrude a first polar body and activate directly without the second meiosis (Fig. 1 pattern C, Fig. 2 C–D, G–H) and display 20 replicated sister chromatid pairs (RSCPs) at first mitosis. The RSCPs have the appearance of meiotic bivalents, but they are not paired homologs (the first polar body was extruded), and instead arise when the sister chromatids fail to separate (nondisjunction) during the transition from MI to interphase, and then undergo replication during the first cell cycle (Fig. 2 H). In the fourth pattern oocytes remain arrested during the first meiotic division (MI) (Fig. 1 pattern D).

Spontaneous activation affected by different strains and culture conditions

To determine whether genetic differences exist between mouse inbred strains with respect to in vitro maturation outcomes, and whether genotype interacts with culture environment, we compared C57BL/6, DBA/2, C3H/HeJ, and A/J strains, along with reciprocal F1 hybrid strains, under different maturation conditions. These strains were chosen based on availability and extensive prior experience in their use. An analysis of genetic relatedness amongst strains indicates that C57BL/6 is quite distinct from the other strains, strains C3H/HeJ and DBA/2 share some ancestry and A/J is slightly more distant (1). We observed a significant effect of both mouse strain and culture environment on the incidence of meiotic defects and spontaneous activation (Table 1).

Using the ATLAS fetal bovine serum, approximately half (58%) of the oocytes from C57BL/6 females followed the normal meiosis pattern A when examined at 14–16 hours during IVM, whereas significantly more of the oocytes from DBA/2 and C3H/HeJ females matured normally (71% and 90%, respectively, $P < 0.001$, Table 1). The A/J strain displayed inefficient IVM, with only 19% of oocytes progressing to MII, and the remaining 81% arrested at MI (pattern D). Of the 42% of C57BL/6 oocytes that did not extrude the first polar body at 16 hours of IVM and had longer meiosis I metaphase, half (21% of total) extruded the first polar body late at 19–21 hours followed by meiosis pattern C during IVM. The remaining oocytes (21% of total) did not extrude the polar body and arrested at the MI stage (meiosis pattern D). DBA/2, C3H/HeJ and A/J oocytes did not display this late extrusion of the first polar body during MI (pattern C).

We examined further the C57BL/6 oocytes that delayed polar body extrusion and spontaneously activated afterwards to form pronuclei (meiosis pattern C, Fig. 2 G–H). Chromosome analysis at the first mitotic metaphase (arrested with Nocodazole treatment) revealed 20 RSCPs, (Fig. 2 G–H). This contrasts with activation after the second polar body extrusion, which yields 20 replicated chromosomes (haploidy) at the first mitosis (pattern B, Fig. 2 E–F). DBA/2, C3H/HeJ and A/J mice did not display spontaneous activation during IVM (Table 1).

Oocytes from reciprocal F1 hybrid females prepared by crossing C57BL/6 and DBA/2 individuals behaved like C57BL/6 oocytes, displaying a meiosis I delay defect and spontaneous activation (meiosis pattern C). The rate of spontaneous maturation (pattern C) in B6D2F1 and D2B6F1 oocytes was not significantly different from C57BL/6 oocytes, but higher than DBA/2 during IVM. This indicates genetic dominance for the predisposition to these meiotic abnormalities during IVM.

To test the effect of serum, FBS from different companies, serum replacement supplement, and the use of bovine serum albumin (BSA) instead of FBS were compared. Additionally, because FSH and cumulus cells are important for oocyte maturation, the effects of FSH and cumulus cells were also tested.

The source of serum and use of serum replacement had significant effects on the incidence of spontaneous activation (pattern C) in C57BL/6 oocytes during IVM. Compared with ATLAS serum, the rate of spontaneous activation was significantly reduced with Hyclone and GIBCO sera, with serum replacement supplement, and with substitution of BSA for serum (Table 1). Use of Hyclone and GIBCO sera resulted in a shift from pattern C to pattern D, and thus reduced spontaneous activation. Use of BSA and serum replacement led to a significant increase in the rate of normal meioses for C57BL/6 oocytes. There was no significant effect of serum replacement factor or substitution with BSA on the incidence of meiotic pattern D, leading to MI arrest. The C3H/HeJ and DBA/2 oocytes did not display spontaneous activation at the first meiotic division (pattern C) in these culture conditions, but displayed MI arrest (pattern D). The incidence of the abnormal pattern D was significantly reduced using BSA instead of serum for DBA/2 and increased for C3H oocytes. A reduced fraction of B6D2F1 oocytes also underwent spontaneous activation in BSA medium compared to medium with ATLAS serum. BSA medium rescued F1 oocytes from pattern C, shifting them to the normal pattern A (Table 1). These data thus reveal a genetic difference in response of oocytes of different mouse strains to variation in the IVM environment.

Addition of follicle stimulating hormone (FSH) into maturation medium prompted cumulus cell expansion from cumulus enclosed oocytes (CEO). Interestingly, spontaneous activation at the MI stage (pattern C) was not observed for the C57BL/6 and B6D2F1 strains following IVM of CEOs in the medium supplemented with FSH. Meiotic MI arrest (pattern D) with FSH was observed at roughly twice the rate compared with the other conditions for C57BL/6 oocytes, indicating an ability to prevent spontaneous activation. For F1 oocytes, the number of normal meioses was increased instead. For the C3H/HeJ strain, FSH treatment of CEOs yielded a large increase in the rate of MI arrest and overall lower rate of normal meiosis, as seen for the C57BL/6 strain. There was no difference between serum and FSH treatment of CEOs for the DBA/2 strain.

Strain-specific spontaneous activation displayed in oocytes matured in vivo

To determine whether strain-specific meiotic delay or spontaneous activation seen with IVM also occurs in vivo, we examined in vivo matured oocytes from C57BL/6, DBA/2, C3H/HeJ and B6D2F1 mice. In C57BL/6 mice, 28% of VVM eggs from oviduct ampula displayed

spontaneous activation at the MII stage (pattern B, Table 2 Fig. 2 E–F). Examining pronucleus stage parthenotes from C57BL/6 superovulated eggs revealed 20 chromosomes (haploidy) (Fig. 2 E–F). B6D2F1 strain oocytes also displayed spontaneous activation in superovulated in vivo matured oocytes at a rate of 22%, slightly lower ($P < 0.05$) than C57BL/6. All DBA/2 and C3H/HeJ oocytes obtained from in vivo maturation were arrested normally at the second meiosis and displayed no spontaneous division. Meiosis patterns C or D were not observed.

Expression analysis of maturation regulatory molecules

We examined the expression of three oocyte maturation associated molecules (maturation promoting factor (MPF), mitogen-activated protein (MAP) kinases, and securin) in oocytes with defective meiotic regulation (Fig. 2 I). We compared protein expression in oocytes with normal (14–16h) (meiosis pattern A) versus delayed (17–19h) polar body extrusion (meiosis pattern C). The expression of cyclin B (a regulatory subunit of MPF) and securin was decreased in oocytes with delayed polar body extrusion compared to the normal oocytes (meiosis pattern A). MAPK expression was not affected.

DISCUSSION

Meiosis is a complex process that is vital for fertility, reproduction, and species evolution. Dissecting the molecular controls of this process is likewise complex. This objective can be facilitated by the development and application of genetic tools for studying specific steps in the process. We show here that different mouse strains display genetic variation in patterns of meiosis both in vivo and in vitro. During in vitro maturation, oocytes from DBA/2 and C3H/HeJ mice display a high frequency of normal patterns of meiosis while oocytes from the A/J strain display a very poor efficiency of maturation. Most strikingly, oocytes from C57BL/6 females undergo a reduced rate of normal maturation in vitro and a high incidence of delayed polar body extrusion with spontaneous activation during the first meiotic division. This effect is sensitive to the culture environment, indicating a genetic difference in oocyte response to in vitro conditions. Studies of reciprocal F1 hybrids (B6D2F1 and D2B6F1) demonstrate that the delayed MI and spontaneous activation phenotype of C57BL/6 oocytes in vitro is a dominant trait. However, changes in culture medium can have contrasting effects on C57BL/6 and F1 oocytes, with a shift toward normal meiosis in F1 oocytes but a shift toward permanent arrest (pattern D) in C57BL/6. This suggests a possible hybrid vigor effect of F1 ooplasm in response to maturation conditions. In vivo maturation of C57BL/6 and B6D2F1 oocytes resulted in some degree of spontaneous activation at the MII stage. These results indicate that the C57BL/6 strain is genetically predisposed to spontaneous activation both in vitro and in vivo, but that strain-specific responses to the culture environment can elicit activation at an earlier stage of meiosis than when it occurs in vivo.

Supplementation of the culture medium with FSH with the presence of associated cumulus cells prevented spontaneous activation with C57BL/6 oocytes. However, meiotic delay still occurred, but these oocytes did not activate at the MI stage as in the other culture situations. Taken together with the occurrence of spontaneous activation in vivo, this result indicates that variation in the amount of FSH stimulation either in vivo or in vitro may determine whether spontaneous activation occurs, but that the follicular environment in vivo suppresses the meiotic delay that leads to activation at MI in vitro.

We observed abnormal chromosome segregation during C57BL/6 oocyte spontaneous activation, as well as delays in meiotic progression and prolonged first meiotic division. Two cell cycle regulators, cyclin B and securin, were altered in the aberrant oocytes. Securin binds and inactivates separase to maintain chromosome pairing and its deficiency leads to

mitotic errors. Chromosome analysis revealed a failure of centromeres division and chromatid separation with activation and transition from MI to interphase in activated C57BL/6 oocytes. Defects in chromosome segregation could arise from incorrect expression and regulation of securin and separase. Anaphase promoting complex/cyclosome (APC/C) mediates metaphase exit by promoting destruction of multiple targeted substrates including securin and cyclin B (19–23). The destruction of securin occurs concomitantly with degradation of cyclin B to allow the exit from metaphase and chromosome separation. The reduced expression of securin and cyclin B in C57BL/6 oocytes may thus contribute to spontaneous activation. The detailed temporal-spatial regulatory mechanisms responsible for the spontaneous activation remain to be determined.

Studies on the mechanism of parthenogenesis are clinically relevant. Recent studies reported that human unfertilized eggs undergo parthenogenetic activation in vivo and in vitro (12–13). This may contribute germ cell derived ovarian teratomas in humans. Early studies on the high frequency of ovarian teratomas in LT/Sv mice revealed spontaneous parthenogenetic oocytes within the ovarian follicle (14, 24), but teratomas are rare in C57BL/6 strain compared to LT/Sv mice. Mechanisms may exist to suppress activation within the follicle in C57BL/6 mice, and subsequent formation of ovarian tumors. The degree to which this genetic difference is affected by superovulation remains to be tested. An earlier study concluded that spontaneous activation was less common in oocytes from unprimed females (25). The data reported here provide a foundation for evaluating how genotype affects spontaneous activation in oocytes obtained with or without hormonal stimulation.

Our findings that C57BL/6 oocytes display a genetic predisposition to meiotic delay and spontaneous activation at the MI stage in vitro and spontaneous activation at the MII stage in vivo, along with a genetic difference in response to culture environment, provide a useful platform for genetic and molecular studies to identify novel regulators of mammalian meiosis. Moreover, the ability of FSH to suppress these aberrant processes in vitro provides a valuable means of studying signaling process and downstream molecular events that regulate chromosome behavior and meiosis. The availability of genetic tools incorporating the C57BL/6 strain, a wealth of genetic polymorphism, DNA sequence, and mapping data, and a wide range of genetically engineered mutations positions this as a new and valuable model for the field.

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FIGURE 1.

Schematic diagram of different patterns of meiosis observed in mouse in vivo and in vitro maturation. Pattern A: MII arrest; Pattern B: MII activation; Pattern C: MI activation; Pattern D: MI arrest

FIGURE 2.

A–D, Morphology of C57BL/6J oocytes undergoing normal or delayed in vitro maturation. A, Matured eggs were selected after emitting the polar body at 14~16 hours of in vitro maturation and continue to culture to 19 hours. B, Spindle structure after immunofluorescence staining. The spindle structure was stained by anti β -tubulin antibody in green and chromosomes were stained by propidium iodide in red. C, Eggs with delayed polar body extrusion at 17~19 hours of in vitro maturation. D, Anaphase spindle structure after immunofluorescence staining. E–H, Cytogenetic analysis to characterize the ploidy of C57BL/6J oocytes following activation at MII (E–F) or MI (G–H). E, MII activation, second polar body extruded and pronucleus formed. F, Twenty replicated chromosomes (haploidy)

at first mitosis with nocodazole treatment in embryo activated from MII stage. G, MI activation, pronucleus formation after extruding first polar body. H, Twenty RSCPs are seen at the first mitotic metaphase of nocodazole treated embryos activated after first meiotic division. The magnification shows the connection between replicated chromosomes. I, Western blotting showing the expression of cyclin B1, MAPK and securin in C57BL/6J mice immature oocyte in vitro maturation. Lane 1: Matured eggs without spontaneous activation collected at 19 hours. Lane 2: Oocytes with delayed first polar body extrusion at 17 hours and collected at 19 hours.

Occurrence of aberrant patterns of meiosis in oocytes of different mouse strains with different in vitro maturation conditions

Table 1

Conditions	Number of Oocytes	Pattern A (%)	Pattern B (%)	Pattern C (%)	Pattern D (%)
ATLAS	741	58 ^{a,e}	0	21 ^{a,e}	21 ^{a,e}
Hyclone	89	62 ^a	0	7 ^c	31 ^b
GIBCO	143	64 ^a	0	7 ^d	29 ^b
CS7BL/6	90	73 ^c	0	8 ^c	19 ^a
BSA	157	71 ^c	0	13 ^b	16 ^a
BSA+FSH+ Cumulus	125	54 ^a	0	0	46 ^d
ATLAS	1460	71 ^{a,h,i}	0	0	29 ^{a,e}
BSA	212	80 ^c	0	0	20 ^c
BSA+FSH+ Cumulus	87	71 ^a	0	0	29 ^a
ATLAS	115	90 ^{a,h,k}	0	0	10 ^{a,j}
BSA	86	77 ^c	0	0	23 ^b
BSA+FSH+ Cumulus	83	48 ^d	0	0	52 ^d
A/J	74	19 ^j	0	0	81 ^l
ATLAS	203	61 ^{a,e}	0	15 ^{a,e}	24 ^{a,e}
BSA	261	72 ^b	0	5 ^d	23 ^a
BSA+FSH+ Cumulus	102	78 ^c	0	0	22 ^a
D2B6F1	108	70 ^j	0	15 ^e	15 ^e

Denuded immature oocytes were collected and cultured in MEMa with different conditions.

Patterns are shown in Fig. 1.

^{a-d} Values with different letters are significantly different within pattern and strain as follows (a-b, $p < 0.05$; a-c, $b-c$ $p < 0.01$; a-d, b-d, c-d $p < 0.001$)

^{e-h}Values different between strains as follows (e-f, $p < 0.05$, e-g and i-j, $p < 0.01$, e-h, e-i, e-k, e-l, i-k, i-l, j-l, and k-l, $p < 0.001$)

Table 2

Percentage of spontaneous activation from different mouse strains for in vivo matured oocytes

	Number of eggs	Pattern A (%)	Pattern B (%)	Pattern C (%)	Pattern D (%)
C57BL/6	537	72 ^a	28 ^a	0	0
DBA/2	439	100	0	0	0
C3H	130	100	0	0	0
B6D2F1	445	78 ^b	22 ^b	0	0

Cumulus cells were removed from the MII stage oocytes after superovulation, and the oocytes cultured in CZB medium

Pattern A: MII arrest; Pattern B: MII activation; Pattern C: MI activation; Pattern D: MI arrest a–b, values significantly different within column P < 0.05.