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Comparison of parthenogenetic oocyte activation in different mouse strains on in vitro development rate and quality

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ABSTRACT:

The aim of our research is to investigate the effects of parthenogenetic activation on in vitro embryo development rates in different mouse strains. B6CBAF1, C57BL/6j, and B6D2F1 mouse strains were used in this study. Superovulated mice were sacrificed and oocytes were obtained 14 hours after the human chorionic gonadotrophin (hCG) injection and the parthenogenetic activation started 18 hours after hCG injection. The oocytes were activated for 6 hours in 10 mM $SrCl_2$ + 5 µg/mL^{-1} Cytohalasine B (CB) + 5 nM Trichostatin A (TSA) containing Ca^{2+} free Chatot Ziomek Brinster (CZB) activation medium. After this, further incubation was performed for two hours in an incubator at 37 °C and 5% CO_2 in embryo culturing medium + TSA. Finally, embryos were cultured for 120 hours. Parthenogenetic activation success of the B6D2F1 mouse strain was found to be higher than C57BL/6j and B6CBAF1 strains.

Farklı fare ırklarında parthenogenotik oosit aktivasyonun in vitro gelişim oran ve kalitesinin karşılaştırılması

ÖZET:

Çalışmamızın amacı, partenogenetik aktivasyonda farklı fare ırklarında in vitro embriyo gelişimi ve kalitesi üzerindeki etkilerinin araştırılmasıdır. Bu çalışmada, B6CBAF1, C57BL/6j, and B6D2F1 farelerin superovulasyon ile elde edilen oositleri kullanılmıştır. Superovule edilen fareler, insan koryonik gonadotropin (hCG) uygulamasından 14 saat sonra oositler elde edildi ve 18 saat sonra partenogenetik aktivasyona başlandı. Oositler, 10 mM $SrCl_2$ + 5 µg/mL^{-1} sitokalazin B (CB) + 5 nM trikostatin A (TSA) Ca^{2+} içermeyen Chatot Ziomek Brinster (CZB) medyumu içerisinde 6 saat bekletildi. Aktivasyon sonrası, embriyo kültür medyumu + TSA'da inkübatörde 37°C ve %5 CO_2 ortamında 2 saat bekletildi. Son olarak, tüm embriyolar 120 saat süre ile kültüre edildi. Bu çalışmadan elde edilen sonuçlar göre, B6D2F1 ırkının partenogenetik aktivasyon başarısı, C57BL/6j ve B6CBAF1 ırklarına göre daha yüksek bulundu.

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1. Introduction

Studies on reproductive biotechnology primarily focus on areas such as obtaining more embryos, cryopreservation, embryo culture, and developmental mechanisms. Parthenogenetic activation, which is used in reproductive biotechnology, provides in vitro embryo development without the presence of sperm (1,2).

Parthenogenetic activation is used in areas like fertilization modeling, somatic cell nuclear transfer (SCNT) as cloning, and research on embryonic stem cell. In cloning, the most important aspect of in vitro development is the activation of the oocyte after somatic cell transfer (3-6). Electrical and chemical methods are widely utilized in mouse embryos to achieve parthenogenetic activation. Among these, the $SrCl_2$ chemical activation method is the most commonly used protocol in mouse embryos (7-9).

Research of ESC purposes is most widely obtained from fertile embryos nowadays. Due to ethical restrictions especially on human embryo studies, parthenogenetic embryos are used as an alternative in stem cell researches. Parthenogenetic embryonic stem cells provide a suitable research model for regenerative medicine studies like cell therapy and tissue repair (10-12).

There are variations among different mouse strains in terms of in vitro development rates (13-14), embryonic stem cell obtaining rates (15), and also cloning success ratios. B6CBAF1 (C57BL/6j×CBA/J), C57BL/6j, and B6D2F1 (C57BL/6j×DBA/J) mouse strains are common used in reproductive biotechnology (ICSI, SCNT and transgenic model) (16-18). No research was found in literature about comparative results of in vitro developments among oocytes belonging to these strains mouse. In this study; therefore, in vitro culture development rates and development qualities of widely used mouse strains through parthenogenetic activation were evaluated.

2. Materials and methods

All animal applications and animal care and maintenance procedure were approved (approval number: 2014 - 05) by the Local Ethics Committee for Animal Experiments of Koç University. The animals were kept in the Koç University, Animal Research Facility (KUARF) of Centre for Translational Medicine (KUTTAM), and the animals were cared on cages (IVC) with HEPA-filtered individual ventilation, 12 hours light - 12 hours dark cycle. Commercial rodent food and water containers were provided ad libitum. In this work, we used 12 female mice. Four mice were used for each group.

Superovulation and oocyte collection:

6-8 weeks old female mice were used in this research. Female mice were chosen from unmated fertile adults. The animals were intraperitoneal injected 10 international unit (IU) pregnant mare serum gonadotropin hormone (SIGMA G4877 - PMSG) at 5:00 pm by intraperitoneal injection for superovulation. 48 hours after, 10 IU human chorionic gonadotropin (SIGMA C8554-hCG) were also applied intraperitoneally at 5:00 pm. Superovulated mice were then sacrificed and then a small incision was cut at ampulla regions of each oviduct in this medium with the help of a sterile toothed forceps. The oocytes were derived from rupturing oviduct ampulla and washed in Human Tubal Fluid + HEPES buffered (HTF, global total w / HEPES) medium + 80 IU/mL hyaluronidase (Sigma H - 3506) + 4 mg/mL Bovine Serum Albumin (BSA, Fraction V. Sigma A3311) and isolated oocyte washed three times in 500 µl HTF medium and selected only high-quality mouse oocytes. Then, oocytes were transferred into four well plates (18-21).

Parthenogenetic oocyte activation and embryo culture:

18 hours after hCG injection, oocytes were incubated for 6 hours in 10 mM $SrCl_2$ + 5 µg/mL $^{-1}$ CB + 5 nM TSA containing Ca^{2+} - free CZB medium. After this, further incubation was performed for two hours in humidified atmosphere of 5% CO_2 at 37°C in embryo culturing medium (LifeGlobal Media, LGGG - 020) + TSA. To assess the embryo development, all embryos were transferred into the embryo medium. Embryo culture drops (10 µl each) were

formed in a petri dish and drops were covered completely with mineral oil (LifeGlobal® Oils, LGOL - 500) to prevent contamination, evaporation, and preserve integrity. At least 2 hours prior, embryo culture media were incubated at 5% CO_2 and 37°C temperature and high humidified in an incubator for gassing. Oocytes were cultured at embryo culture medium (4 mg/mL BSA) for 120 hours until blastocyst stage (18-21).

Determination of cell numbers by differential staining:

Blastocysts were incubated in a solution of 100 µg/mL propidium iodide (for trofectorderm determination) + HTF medium + 1% Triton X 100 for 10-12 seconds and then transferred to 100 µg/mL 100% ethanol (EMPROVE) + 25 µg/mL Hoechst 33258 (H1398, Molecular Probes, Inc.) solution for overnight incubation at 4°C. Next day, blastocysts washed in a 5 µl glycerol droplet were formed on each glass slide for blastocyst stabilization. Blastocysts were transferred to the droplet and covered with a coverslip. These blastocysts preparations were investigated in an inverted microscope with red (propidium iodide) and blue (Hoechst)fluorescence attachment for the determination of trophectoderm (TE) and inner cell mass (ICM) numbers (20,23). For each replication, four blastocysts were used for each group.

Statistical analyses:

Experiments were performed in at least four replications. SPSS Statistics 22.0 program was used for statistical evaluation of the results. One Way ANOVA with Bonferroni post hoc test was used for comparison of the differences among groups.

3. Results

In vitro culture results

According to development evaluations done after in vitro culture, blastocyst development rates were 77.71%, 57.32% and 96.67% in B6CBAF1, C57BL/6j, B6D2F1 groups, respectively. The differences were found significantly important between B6D2F1 and C57BL/6j ($p < 0.05$), B6CBAF1 and B6D2F1 ($p < 0.05$) and B6CBAF1 and C57BL/6j ($p < 0.05$) mouse strains in terms of in vitro development rates (Table 1).

Table 1: In vitro development rates of parthenogenetically activated mouse oocytes (Mean Rate± Std. Error of Mean)

Tablo 1: *Partenogenetik olarak aktive edilmiş fare oositlerinin in vitro gelişim oranları (Aritmetik Ort. ± Std. sapma)*

Group	Number of Embryo (n)	Number of Blastocysts	In Vitro Development Rate (%)
B6CBAF1	88	69	77.71 ± 5.07^{a}
C57BL/6j	65	37	57.32 ± 9.37^{b}
B6D2F1	59	57	96.67 ± 4.08^{c}

Differences between the same columns with different symbols ($^{a, b, c}$) were found to be significant ($p < 0.05$).

Results of cell numbers determined by differential staining

According to differential staining results, mean of total cell numbers in B6CBAF1, C57BL/6j, B6D2F1 groups were determined as 54.8, 44 and 82, respectively. The mean cell number of trophectoderm of each group was calculated as 41, 33.33 and 62, respectively. The mean of ICM of each group were determined as 13.8, 10.67 and 20, respectively. The differences were found significantly important between B6D2F1 and C57BL/6j ($p < 0.05$), B6CBAF1, and B6D2F1 ($p < 0.05$) and, B6CBAF1 and C57BL/6j ($p < 0.05$) mouse strains in terms of total cell numbers, trophectoderm cell numbers and inner cell numbers (Table 2).

Table 2: Cell number of blastocysts according to differential fluorescence labeling (Mean± Std. Error of Mean)

Tablo 2: *Farklı floresan etiketlemeye göre blastosistlerin hücre sayısı (Aritmetik Ort. ± Std. sapma)*

Group	Mean Inner Cell Mass Number	Mean of Trophectoderm Cell Number	Total Cell Number Mean ± St. Dev.
B6CBAF1	13.8 ± 1.47^{a}	41 ± 1.1^{a}	54.8 ± 1.6^{a}
C57BL/6j	10.67 ± 0.94^{b}	33.33 ± 3.4^{b}	44 ± 3.27^{b}
B6D2F1	20 ± 2.16^{c}	62 ± 9.42^{c}	82 ± 11.43^{c}

Differences between the same columns with different symbols ($^{a, b, c}$) were found to be significant ($p < 0.05$).

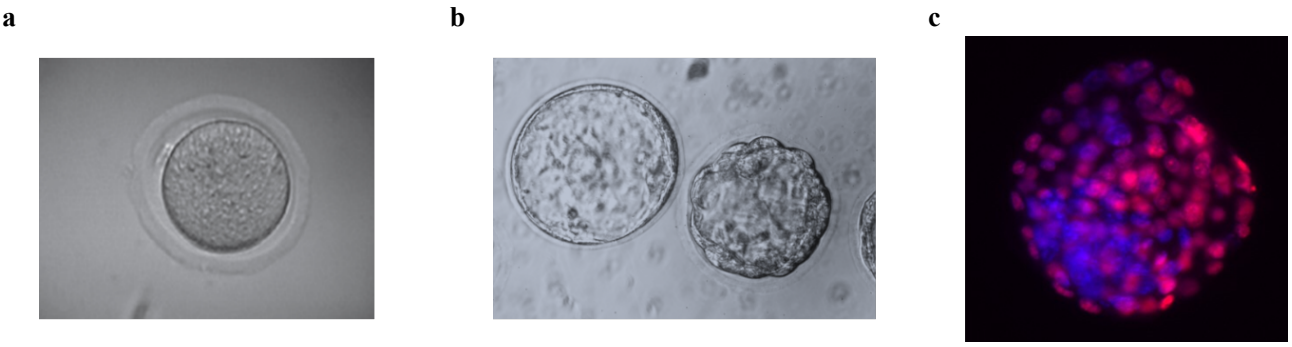

Figure 1: (a) oocyte for activation (20X), **(b)** developed blastocyst at 96 hours (20X), **(c)** differential stained of blastocyst (40X)

4. Discussion and Conclusion

Mice are appropriate in research of parthenogenetic development for improving of cloning technology. In mouse cloning studies, due to the genetic effects of different mouse strain, clone embryo development, as well as the information obtained in this study. However, the effects of different mouse strains are seen in the development effects of parthenogenetic mouse oocyte activations in this study results (24,25). In this study, we showed the importance of genetic influence on parthenogenetic development and embryo quality among different strains.

In their study to identify the optimum conditions for parthenogenetic activation through strontium chloride on mouse oocytes, Ma et al. (2005) achieved a 50.8% blastocyst development ratio 18 hours after hCG injection in Kunming-strain mouse oocytes at 2.5 hours of activation with 10 mM $SrCl_2$ + Ca^{2+}- free +CB 5 µg/mL (8). In the study presented as high as 90% blastocyst development rates were observed with 6 hours of activation in B6D2F1 then, we demonstrated animal strain importance for parthenogenetic activation result.

The activation period has been reported to increase the blastocysts development rate, especially 6 hours of activation in the B6DF1 mouse strain. In the present study, 6 hours of activation of B6DF1 strain were observed significantly higher in vitro embryonic development. Activated B6D2F1 strain mice oocytes for 3 hours at 10 mM

$SrCl_2$ + Ca^{2+}- free + Cytohalasine D 17 hours after hCG injection and observed 65% blastocyst development rate (23). Our study presented also used similar mouse strain mice but obtained blastocyst development rate. Thus, it is possible that our activation period was longer, and Cytohalasine B was used in activation medium instead of Cytohalasine D. The one of the study, 4 hours of activation with 10 mM $SrCl_2$ + Ca^{2+}- free + Cytohalasine D 2 µg/mL to B6D2F1 hybrid mice and obtained 89% blastocyst development rate and mean of total cell numbers, trophectoderm cell numbers, and inner cell mass cell numbers were defined as 75.61, 60.55 ve 15.06 in differential staining to evaluate embryo quality (7). The study we presented also utilized a similar mouse strain but, we used 6 hours of activation with 10 mM $SrCl_2$ + Ca^{2+}- free + Cytohalasine D 5 µg/mL to B6D2F1 hybrid mice and obtained %96 blastocyst development rate and mean of total cell numbers, trophectoderm cell numbers, and inner cell mass cell numbers were also found to 82, 62, 20 cell number. The other study, B6D2F1 mice oocytes were activated for 6 hours with $SrCl_2$ + Ca^{2+} - free + CB 5 µg/mL by Sung et al. (27) and blastocyst development rate was found to be 97.3% Similar B6D2F1 strain mice and same activation period were also used in our presented study and similar in vitro development rate was defined as 96.67%.

The chemical of activation method applied 6-DMAP (2 mmol/l) activation for 4 hours to oocytes of C57BL/6j strain mice obtained a blastocyst development rate of 20.73% (28). C57BL/6j strain mice were also used in our presented study but 6 hours $SrCl_2$ activation was and blastocyst development rate was found to be 57% in our study.Our results of C57BL6j suggest that 6 hours (prolonged activation) can have a positive effect on in vitro development rates, and the longer the duration of strontium treatment, the greater the calcium oscillations in mouse meiotic oocytes. Moreover, mouse oocyte activation was found to increase in aged oocytes more as the oocyte ages, and also as mitogen-activated protein kinase (MAPK) activity decreases (29).

Calcium channels are crucial for oocyte maturation and parthenogenetic activation (30-31). Different mouse strains have different properties in Ca^{2+} storage area and the post-fertilization (32,34). Our results demonstrated that calcium channels might enhance the parthenogenetic embryo development in certain mouse strains consistent with previous reports (30-32,34).

The conclusion of this study allowed the creation of an ideal protocol for chemical activation by comparing in vitro embryo development rates and cell numbers of parthenogenetic mouse oocytes of different strains. These findings are likely to have important applications in reproductive biotechnology. Further studies about molecular mechanisms of parthenogenetic development will be important in research areas such as cloning, ICSI, and stem cell studies.

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Conflict of interest

The author declared no conflict of interest.

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Ethical Approval

All animal applications and animal care and maintenance procedure were approved (Approval number: 2014 - 05) by the Local Ethics Committee for Animal Experiments of Koç University.

References

1. **Cuthbertson KS, Whittingham DG, Cobbold PH** (1981): *Free Ca2+ increases in exponential phases during mouse oocyte activation*. Nature, **294,** 754-757.
2. **Kline D** (1996): *Activation of the mouse egg*. Theriogenology, **45**, 81–90.
3. **Kishikawa H, Wakayama T, Yanagimachi R** (1999): *Comparison of oocyte-activating agents for mouse cloning*. Cloning, **1**, 153–159.
4. **Campbell KHS** (1999): *Nuclear equivalence, nuclear transfer, and the cell cycle*. Cloning, **1**, 3–15.
5. **De Sousa PA, Dobrinsky JR, Zhu J, Archibald AL, Ainslie A, Bosma W, Bowering J, Bracken J, Ferrier PM, Fletcher J, Gasparrini B, Harkness L, Johnston P, Ritchie M, Ritchie WA, Travers A, Albertini D, Dinnyes A, King TJ, Wilmut I** (2002): *Somatic cell nuclear transfer in the pig: control of pronuclear formation and integration with improved methods for activation and maintenance of pregnancy*. Biol Reprod, **66**, 642–650.
6. **Ma SF, Liu XY, Miao DQ, Han ZB, Zhang X, Miao YL, Yanagimachi R, Tan JH** (2005): *Parthenogenetic activation of mouse oocytes by strontium chloride: A search for the best conditions.* Theriogenology, **64**, 1142–1157.
7. **Versieren K, Heindryckx B, Lierman S, Gerris J, De Sutter P** (2010): *Developmental competence of parthenogenetic mouse and human embryos after chemical or electrical activation*. Reprod Biomed Online, **21**, 769-775.
8. **Han BS, Gao JL** (2013): *Effects of chemical combinations on the parthenogenetic activation of mouse oocytes*. Exp Ther Med, **5**, 1281-1288.
9. **Bai GY, Song SH, Wang ZD, Shan ZY, Sun RZ, Liu CJ, Wu YS, Li T, Lei L** (2016): *Embryos aggregation improves development and imprinting gene expression in mouse parthenogenesis*. Dev Growth Differ, **58**, 270-279.
10. **Fulka H, Hirose M, Inoue K, Ogonuki N, Wakisaka N, Matoba S, Ogura A, Mosko T, Kott T, Fulka J** (2011): *Production of mouse embryonic stem cell lines from maturing oocytes by direct conversion of meiosis into mitosis*. Stem Cells, **29**, 517-527.
11. **Didié M, Christalla P, Rubart M, Muppala V, Döker S, Unsöld B, El-Armouche A, Rau T, Eschenhagen T, Alexander P. Schwoerer AP, Ehmke H, Schumacher U, Fuchs S, Lange C, Becker A, Tao W, Scherschel JA, Soonpaa MH, Yang T, Lin Q, Zenke M, Han DW, Schöler HR, Rudolph C, Steinemann D, Schlegelberger B, Kattman S, Witty A, Keller G, Field LJ, Zimmermann WH** (2013): *Parthenogenetic stem cells for tissue-engineered heart repair*. J Clin Invest, **123**, 1285-1298.
12. **Daughtry B, Mitalipov S** (2014): *Concise review: parthenote stem cells for regenerative medicine: genetic, epigenetic, and developmental features*. Stem Cells Transl Med, **3**, 290-298.
13. **Dandekar PV, Glass RH** (1987): *Development of mouse embryos in vitro is affected by strain and culture medium*. Gamete Res, **17**, 279–285.
14. **Herrick JR, Paik T, Strauss KJ, Schoolcraft WB, Krisher RL** (2016): *Building a better mouse embryo assay: effects of mouse strain and in vitro maturation on sensitivity to contaminants of the culture environment*. J Assist Reprod Genet, **33**, 237-245.
15. **Czechanski A, Byers C, Greenstein I, Schrode N, Donahue LR, Hadjantonakis AK, Reinhold LG** (2014): *Derivation and characterization of mouse embryonic stem cells from permissive and nonpermissive strains*. Nat Protoc, **9**, 559-574.
16. **Ogura A, Inoue, K, & Wakayama T** (2013): *Recent advancements in cloning by somatic cell nuclear transfer*. Philos Trans R Soc Lond B Biol Sci, **368**, 20110329.
17. **Bagis H, Aktoprakligil D, Gunes C, Arat S, Akkoc T, Cetinkaya G, Kankavi O, Taskin AC, Arslan K,**

Dundar M, Tsoncheva VL, Ivanov IG (2011): *Expression of biologically active human interferon gamma in the milk of transgenic mice under the control of the murine whey acidic protein gene promoter*. Biochem Genet, **49**, 251-257.

18. **Mallol A, Santaló J, Ibáñez E** (2014): *Psammaplin a improves development and quality of somatic cell nuclear transfer mouse embryos.* Cellular Reprogram, **16**, 392-406.
19. **Taşkın AC, Akkoç T, Sağırkaya H, Bağış H, Arat S** (2016): *Comparison of the development of mouse embryos manipulated with different biopsy techniques*. Turk J Vet Anim Sci, **40**, 157-162.
20. **Taşkın AC, Kocabay A, Ebrahimi A, Karahüseyinoğlu S, Şahin GN, Ozcimen B, Ruacan A, Onder TT** (2019): *Leptin treatment of in vitro cultured embryos increases outgrowth rate of inner cell mass during embryonic stem cell derivation*. In Vitro Cell Dev Biol Anim, **55**, 473-481.
21. **Taşkın AC, Kocabay A** (2019): *Leptin supplementation in embryo culture medium increases in vivo implantation rates in mice*. Turk J Vet Anim Sci, **43**, 359-363.
22. **Mallol A, Santaló J, Ibáñez E** (2014): *Psammaplin a improves development and quality of somatic cell nuclear transfer mouse embryos*. Cellular Reprogram, **16**, 392-406.
23. **Mallol A, Santaló J, Ibáñez E** (2013) *Comparison of three differential mouse blastocyst staining methods*. Syst Biol Reprod Med, **59**, 117-122.
24. **Wakayama T, Perry AC, Zuccotti M, Johnson KR, Yanagimachi R** (1998): *Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei*. Nature, **394**, 369-374.
25. **Beck JA, Lloyd S, Hafezparast M, Lennon Pierce M, Eppig JT, Festing MF, Fisher EM** (2000*): Genealogies of mouse inbred strains*. Nat Genet, **24**, 23–25.
26. **Heytens E, Soleimani R, Lierman S, De Meester S, Gerris J, Dhont M, Van der Elst J, De Sutter P** (2008): *Effect of ionomycin on oocyte activation and embryo development in mouse*. Reprod Biomed Online, **17**, 764-771.
27. **Sung LY, Chang CC, Amano T, Lin CJ, Amano M, Treaster SB, Xu J, Chang WF, Nagy ZP, Yang X, Tian XC** (2010): *Efficient derivation of embryonic stem cells from nuclear transfer and parthenogenetic embryos derived from cryopreserved oocytes*. Cellular Reprogram, **12**, 203-211.
28. **Gao W, Yu X, Hao J, Wang L, Qi M, Han L, Lin C, Wang D** (2019): *Ascorbic acid improves parthenogenetic embryo development through TET proteins in mice*. Biosci Rep, **39**, 1-8.
29. **Alberio R, Zakhartchenko V, Motlik J, Wolf E** (2001): *Mammalianoocyte activation: lessons from the sperm and implications for nuclear transfer*. Int J Dev Biol, **45**, 797-809.
30. **Carvacho I, Lee HC, Fissore RA, Clapham DE** (2013): *TRPV3 channels mediate strontium-induced mouse-egg activation.* Cell Rep, **5**, 1375-1386.
31. **Miao YL, Stein P, Jefferson WN, Padilla-Banks E, Williams CJ** (2012): *Calcium influx-mediated signaling is required for complete mouse egg activation*. Proc Natl Acad Sci U S A, **109**, 4169-4174.
32. **Haverfield J, Nakagawa S, Love D, Tsichlaki E, Nomikos M, Lai FA, Swann K, FitzHarris G** (2016): *Ca 2+ dynamics in oocytes from naturally-aged mice*. Sci Rep, **6**, 19357.
33. **McDonough CE, Bernhardt ML, Williams CJ** (2020): *Mouse strain-dependent egg factors regulate calcium signals at fertilization*. Mol Reprod Dev, **87**, 284-292.
34. **Czajkowska K, Walewska A, Ishikawa T, Szczepańska K, Ajduk A** (2020): Age*-related alterations in fertilization-induced Ca2+ oscillations depend on the genetic background of mouse oocytes*. Biol Reprod, **103**, 986-999.