

Effects of Different Parthenogenetic Activation Periods on Mouse Embryo Development and Quality

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ABSTRACT

In the present study we investigate the effects of parthenogenetic activation on *in vitro* embryo development and quality in different activation periods. oocytes were obtained 14 hours after human chorionic gonadotropin (hCG) injection from superovulated B6D2F1 female mice then parthenogenetic activation started 18 hours after hCG injection. The oocytes were activated at different activation periods for 3, 4, 5 or 6 hours in 10 mM strontium chloride (SrCl₂) + 5 µg/mL⁻¹ Cytohalasine B (CB) + 5 nM Trichostatin A (TSA) containing a Ca²⁺ free Chatot Ziomek Brinster (CZB) activation medium, followed by further incubation for two hours at 37°C and 5% CO₂ in embryo culturing medium + TSA. The results in the present study suggested that the parthenogenetic activation of the 6 hour activation period was found to be higher than at 3, 4 and 5 hours.

Keywords: Mouse, Oocyte, Parthenogenetic, Activation period

Farklı Partenogenetik Aktivasyon Sürelerinin Fare Embriyo Gelişimi ve Kalitesi Üzerine Etkileri

ÖZ

Çalışmamızın amacı, partenogenetik aktivasyonda farklı aktivasyon sürelerinin *in vitro* embriyo gelişimi ve kalitesi üzerindeki etkilerinin araştırılmasıdır. Superovule B6D2F1 ırkı dişi farelere uygulanan insan koryonik gonadotropin (hCG) enjeksiyonundan 14 saat sonra oositler elde edildi ve 18 saat sonra partenogenetik aktivasyona başlandı. Oositler, 10 mM stronsiyum klorür (SrCl₂) + 5 µg/mL⁻¹ sitokalazın B (CB) + 5 nM trikostatın A (TSA) Ca²⁺ içermeyen Chatot Ziomek Brinster (CZB) medyumunu içerisinde 3, 4, 5 ve 6 saat bekletildi. Aktivasyon sonrası, embriyo kültür medyumunu + TSA'da inkübatörde 37°C ve %5 CO₂ ortamında 2 saat bekletildi. Son olarak, tüm embriyolar 120 saat süre ile kültüre edildi. Bu çalışmadan elde edilen sonuçlar göre, 6 saatlik partenogenetik aktivasyon başarısının, 3, 4 ve 5 saatlik sürelerle göre daha yüksek olduğu saptandı.

Anahtar Kelimeler: Fare, Oosit, Partenogenetik, Aktivasyon süresi

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INTRODUCTION

Studies of reproductive biotechnology tend to focus primarily on the obtaining of more embryos, increased storage capacity (cryopreservation), embryo culturing or developmental mechanisms. Parthenogenetic activation, which is used in reproductive biotechnology, allows for *in vitro* embryo development without presence of sperm (Cuthbertson et al. 1981, Kline 1996).

Parthenogenetic activation is used in such activities as fertilization modeling, somatic cell nuclear transfer (SCNT) (cloning) and stem cell research. In cloning, the most important stage in *in vitro* development is the activation of the oocyte following the somatic cell transfer (Kishikawa et al. 1999, Campbell 1999, De Sousa et al. 2002, Ma et al. 2005, Demir et al. 2014). Electrical and chemical methods are widely utilized in mouse oocytes to achieve parthenogenetic activation, with the SrCl₂ chemical activation approach being the most common (Versieren et al. 2010, Han and Gao 2013, Bai et al. 2016).

Due to ethical concerns, human embryo studies in embryonic stem cell research in particular make use of parthenogenetic embryos as an alternative. Parthenogenetic embryonic stem cells serve as a suitable research model for regenerative medicine studies into, for example, cell therapy and tissue repair (Fulka et al. 2011, Didić et al. 2013, Daughtry and Mitalipov 2014).

In vitro parthenogenic embryo development rates may differ, depending on the activation period (Ma et al. 2005, Versiersen et al. 2010, Han and Gao 2013, Heytens et al. 2008, Sung et al. 2010, Gao et al. 2019), and the cloning success ratios. A review of literature identified no studies comparing the effects of the most common activation periods on the rate and quality of parthenogenetic embryo development. To address this situation, the present study evaluates the *in vitro* development rates and development qualities of parthenogenetic activations at different activation periods.

MATERIALS and METHODS

All mice experiments and animal care protocols were approved by the Koç University Local Ethics Committee for Animal Experiments (approval number: 2014 - 05). The animals were kept in the Koç University Animal Research Facility of Centre for Translational Medicine (KUTTAM) in individually ventilated cages with individual HEPA-filtered ventilation and in a 12 hours light–12 hours dark cycle. The mice were fed a diet of commercial pellet food *ad libitum*, and automatic water containers were provided.

Superovulation and oocyte collection

B6D2F1 female mice aged 6–8 weeks were obtained for the study (n=12), selected from among unmated

fertile adults. The mice were given 10 IU pregnant mare serum gonadotropin hormone (SIGMA G4877-PMSG) at 5:00 pm via intraperitoneal injection for superovulation, and 48 hours later, 10 IU hCG (SIGMA C8554-hCG) was given intraperitoneally, again at 5:00 pm. The superovulated mice were then sacrificed and a small incision was made in the ampulla region of each oviduct with the help of sterile-toothed forceps. The oocytes with cumulus cells were collected via the rupture of the oviductal ampulla, and were washed with 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, SIGMA A-3311), and the isolated oocytes were washed three times in different 500 µl HTF media. The oocytes were again washed three times in different HTF media with HEPES, and selected high quality oocytes were kept in a four-well plate (Mallol et al. 2014, Taskin et al. 2019a).

Parthenogenetic oocyte activation and embryo culture

Eighteen hours after the hCG injection, the oocytes were incubated for 3, 4, 5 or 6 hours in a 10 strontium chloride (SrCl₂) + 5 µg/mL⁻¹ Cytohalasine B (CB) + 5 nM Trichostatin A (TSA) containing a Ca²⁺ free Chatot Ziomek Brinster (CZB) medium, and then incubated for 2 hours in an incubator at 37°C and 5% CO₂ in an embryo culturing medium (LifeGlobal Media, LGGG-020) + TSA. For the assessment of embryo development, all embryos were transferred into embryo culture drops (10 µl each) and covered completely with mineral oil (LifeGlobal® Oils, LGOL-500) to prevent contamination and evaporation, and to preserve integrity. The embryo culture media were incubated at 5% CO₂ and 37°C temperature and high humidity in an incubator for equilibration for at least 2 hours before embryo culturing. The oocytes were then cultured in an embryo culture medium supplemented with 4 mg/mL BSA (Fraction V. A3311) for 120 hours (Sung et al. 2010, Mallol et al. 2014).

Determination of cell numbers

Blastocysts were incubated in a solution of 100 µg/mL propidium iodide (PI) + HTF medium + 1% TritonX100 for 10–12 seconds, and then transferred to a 100 µg/mL 100% ethanol (EMPROVE) + 25 µg/mL Hoechst 33258 (H1398, Molecular Probes, Inc.) solution for overnight incubation at 4°C. On the following day, the blastocysts were transferred to the glycerol droplet after washing in 5 µl glycerol droplet on each glass slide, and covered with a coverslip for blastocyst stabilization. The blastocyst preparations were observed using an inverted microscope with a red and blue fluorescence attachment for the determination of trophectoderm (TE) and inner cell mass (ICM) cell numbers (Mallol et al. 2013, Taşkın et al. 2019b).

Statistical analyses

All experiments were replicated three times. The SPSS Statistics 22.0 program was used for the statistical evaluation of the results. A One-Way ANOVA with a Berferroni post hoc test was used to identify between-group differences.

RESULTS

Results of *In Vitro* Culture

The development evaluations carried out after the *in vitro* culture revealed blastocyst development rates of 66.09%, 70.00%, 73.87% and 87.73% in the 3, 4, 5 and 6 hour activation groups, respectively, and the

development rate of the 6 hour parthenogenetic activation was found to significantly higher than that of the 3, 4, 5 ($P < 0.05$) hour groups (Table 1).

Total Cell Numbers

Differential staining of blastocysts revealed the total cell numbers in the 3, 4, 5 and 6 hour groups to be 44.33 ± 4.19 , 37.33 ± 6.60 , 46 ± 1.63 and 51.33 ± 1.89 , respectively. The numbers of cell in the 6 hour group was found to be significantly greater than in the 3, 4 and 5 ($P < 0.05$) hour groups (Table 2).

Table 1: *In vitro* development rates.

Parthenogenetic Activation Period	Number of Oocytes (n)	Number of Blastocysts	<i>In Vitro</i> Development Rate (%) \pm St. dev
3 h	42	28	66.09 ± 6.13^b
4 h	40	28	70.00 ± 8.16^b
5 h	41	30	73.87 ± 17.53^b
6 h	39	34	87.73 ± 5.47^a

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

Table 2: Cell numbers of activated blastocysts according to differential fluorescence labeling.

Parthenogenetic Activation Period	Mean of Inner Cell Mass Number \pm St. Dev.	Mean Trophoctoderm Cell Number \pm St. Dev.	Mean of Total Cell Number \pm St. Dev.
3 h	34.67 ± 4.64	10.67 ± 0.94	44.33 ± 4.19^b
4 h	24 ± 5.89	12.67 ± 0.94	37.33 ± 6.60^b
5 h	38.33 ± 6.34	11 ± 0.82	46.00 ± 1.63^b
6 h	37 ± 2.16	14.33 ± 3.68	51.33 ± 1.89^a

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

DISCUSSION

Fertilization occurs *in vivo* when an egg is covered by spermatozoa, and when one spermatozoon manages to penetrate the egg through membrane fusion. Oocyte activation is a serial cell mechanism that occurs during fertilization. Parthenogenetic embryo development mimics this condition without spermatozoa, and has been used as a model in human embryonic stem cell research due to ethical concerns, especially in the cloning studies of many species over the last 20 years. A low embryonic development rate is a fundamental problem in cloning studies in particular. In their Honolulu method, Wakayama et al.

(1998) injected nuclei from cumulus cells into enucleated oocytes, thus producing the first cloned mouse, who they named "Cumulina". Cloning research now focuses on increasing the birth rates of cloned embryos transferred into surrogate mothers. The success rates from cloning are 0–20%, while the birth rates of cloned mice are 1–2%. The SrCl₂ chemical activation method is widely used in cloning. Studies have been performed on such SrCl₂ activation parameters as activation time, concentration rate and manipulation media, and have shown that embryo culturing success in particular varies according to the mouse oocyte activation period (Dandekar and Glass 1987, Heytens et al. 2008).

Ma et al. (2005) achieved 50.8% blastocyst development ratio 18 hours after hCG injection in Kunming-strain mouse oocytes at 2.5 hours of activation with 10 mM SrCl₂ + Ca²⁺- free + CB 5 µg/mL. Han et al. (2013), on the other hand, identified a 27.62% blastocyst development ratio in CD1-strain mouse oocytes at 30 minutes following activation with 10 mM SrCl₂ + Ca²⁺- free + CB 5 µg/mL, 18 hours after hCG injection. In the present study, blastocyst development rates as high as 80–90% were observed in the 5 and 6 hour activation groups. Our results suggest that prolonged activation (5 or 6 hours) 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 (Alberio et al. 2001).

Heytens et al. (2008) activated B6D2F1-strain mice oocytes for 3 hours at 10 mM SrCl₂ + Ca²⁺- free + Cytohalasine D, 17 hours after hCG injection, and observed a 65% blastocyst development rate. We recorded a similar result 3 hours following activation in B6D2F1-strain mice, although our activation period was shorter, and Cytohalasine B was used in the activation medium instead of Cytohalasine D.

B6D2F1 mice oocytes were activated for 6 hours with SrCl₂ + Ca²⁺-free + CB 5 µg/mL by Sung et al. (2010), and a blastocyst development rate of 97.3% was recorded. A similar B6D2F1-strain of mice and the same activation period were used in the present study, and the best activation results were recorded at 6 hours.

Gao et al. (2019) applied 4 hours of chemical activation to the oocytes of C57BL/6j-strain mice and obtained a blastocyst development rate of 84.61%. We also used a 4 hour activation period in the present study, but recorded a lower blastocyst development rate (70.00%). The different strains of mice used in the studies could be one of the reasons for the differences in activation results.

In conclusion, the present study has identified the ideal protocol for chemical activation through a comparison of the *in vitro* development rates and total cell numbers of parthenogenetic mouse blastocysts obtained after different activation periods. Further studies into the molecular mechanisms of parthenogenetic development will support such research areas as cloning, intracytoplasmic sperm injection and stem cell studies.

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REFERENCES

- Alberio R, Zakhartchenko V, Motlik J, Wolf E.** Mammalian oocyte activation: lessons from the sperm and implications for nuclear transfer. *Int J. Dev. Biol.* 2001;45(7): 797-809.
- Bai GY, Song SH, Wang ZD, Shan ZY, Sun RZ et al.** Embryos aggregation improves development and imprinting gene expression in mouse parthenogenesis. *Dev Growth Differ.* 2016;58 (3): 270-279.
- Campbell KHS.** Nuclear equivalence, nuclear transfer, and the cell cycle. *Cloning.* 1999; 1(1): 3–15.
- Cuthbertson KS, Whittingham DG, Cobbold PH.** Free Ca²⁺ increases in exponential phases during mouse oocyte activation. *Nature.* 1981; 294(5843): 754-757.
- Dandekar PV, Glass RH.** Development of mouse embryos *in vitro* is affected by strain and culture medium. *Gamete Res.* 1987;17(4): 279–285.
- Daughtry B, Mitalipov S.** Concise review: parthenote stem cells for regenerative medicine: genetic, epigenetic, and developmental features. *Stem. Cells. Transl. Med.* 2014;3(3): 290-298.
- De Sousa PA, Dobrinsky JR, Zhu J, Archibald AL, Ainslie A et al.** Somatic cell nuclear transfer in the pig: control of pronuclear formation and integration with improved methods for activation and maintenance of pregnancy. *Biol. Reprod.* 2002;66(3): 642–650.
- Demir K, Can A, Ertürk E, Özdemirci S, Karacam, H et al.** Effect of different activation techniques on immature and *in vitro* matured cat oocytes. *Kafkas Univ. Vet. Fak. Derg.* 2014;20: 565-570.
- Didié M, Christalla P, Rubart M, Muppala V, Döker S et al.** Parthenogenetic stem cells for tissue-engineered heart repair. *J. Clin. Invest.* 2013;123(3): 1285-1298.
- Fulka H, Hirose M, Inoue K, Ogonuki N, Wakisaka N et al.** Production of mouse embryonic stem cell lines from maturing oocytes by direct conversion of meiosis into mitosis. *Stem Cells.* 2011;29(3): 517-527.
- Gao W, Yu X, Hao J, Wang L, Qi M et al.** Ascorbic acid improves parthenogenetic embryo development through TET proteins in mice. *Biosci. Rep.* 2019;39 (1): 1-8.
- Han BS, Gao JL.** Effects of chemical combinations on the parthenogenetic activation of mouse oocytes. *Exp. Ther. Med.* 2013;5(5): 1281-1288.
- Heytens E, Soleimani R, Lierman S, De Meester S, Gerris J et al.** Effect of ionomycin on oocyte activation and embryo development in mouse. *Reprod. Biomed. Online.* 2008;17 (6): 764-771.
- Kishikawa H, Wakayama T, Yanagimachi R.** Comparison of oocyte-activating agents for mouse cloning. *Cloning.* 1999;1(3): 153–159.

- Kline D.** Activation of the mouse egg. *Theriogenology*. 1996; 45(1): 81–90.
- Ma SF, Liu XY, Miao DQ, Han ZB, Zhang X et al.** Parthenogenetic activation of mouse oocytes by strontium chloride: A search for the best conditions. *Theriogenology*. 2005;64(5): 1142–1157.
- Mallol A, Santaló J, Ibáñez E.** Comparison of three differential mouse blastocyst staining methods. *Syst. Biol. Reprod. Med.* 2013;59 (2): 117-122.
- Mallol A, Santaló J, Ibáñez E.** Psammaplin a improves development and quality of somatic cell nuclear transfer mouse embryos. *Cell Reprogram*. 2014;16 (5): 392-406.
- Sung LY, Chang CC, Amano T, Lin CJ, Amano M et al.** Efficient derivation of embryonic stem cells from nuclear transfer and parthenogenetic embryos derived from cryopreserved oocytes. *Cell Reprogram*. 2010;12 (2): 203-211.
- Taşkın AC, Kocabay A, Ebrahimi A, Karahüseyinoğlu S, Şahin GN et al.** 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.* 2019a;55 (7): 473-481.
- Taşkın AC, Kocabay A.** Leptin supplementation in embryo culture medium increases *in vivo* implantation rates in mice. *Turk J Vet Anim Sci.* 2019b;43 (3): 359-363.
- Versieren K, Heindryckx B, Lierman S, Gerris J, De Sutter P.** Developmental competence of parthenogenetic mouse and human embryos after chemical or electrical activation. *Reprod Biomed Online*. 2010;21(6): 769-775.
- Wakayama T, Perry AC, Zuccotti M, Johnson KR, Yanagimachi R.** Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. *Nature*. 1998;394 (6691): 369-374.