ABSTRACTS FOR POSTER PRESENTATION

Oocyte Activation

297 A METHOD TO DRIVE CALCIUM SIGNALLING DYNAMICS IN FERTILIZED MOUSE EGGS

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Following fertilization, eggs exhibit a series of repetitive increases in intracellular calcium that activate development. The developmental impact of the long-lasting series of Ca^{2+} signals is still a subject of controversy. Although several studies using parthenogenetically activated eggs suggest that Ca^{2+} dynamics affect post-implantation development, artificial stimulation of Ca^{2+} signaling after ICSI in bovine eggs shows that development still remains poor in comparison to fertilized eggs. Such divergence between parthenogenetic studies and those aimed at stimulating ICSI eggs makes it impossible to draw any conclusions regarding the function of Ca^{2+} signaling for two reasons. First, non-fertilized eggs do not release Ca^{2+} from intracellular stores and their development is compromised due to the absence of paternally-derived chromosomes. Second, because ICSI eggs are excitable, Ca^{2+} stimulation generates additional Ca^{2+} oscillations that might compromise their development. Moreover, in both cases, Ca^{2+} signaling is not physiological. To understand better the function of Ca^{2+} signaling at fertilization, we developed a new approach based on micro fluidic technology that makes it possible to drive Ca^{2+} signal dynamics of fertilized eggs with no apparent deleterious effects. This method relies on the fact that the properties of the IP3 receptor (IP3R) calcium channel are changed after fertilization, and IP3 and Ca^{2+} act as co-agonists to cause Ca^{2+}-induced Ca^{2+} release (CICR) from intracellular stores. Because Ca^{2+} has both an inhibiting and a stimulating function, we exploited these opposing properties. First, we inhibited Ca^{2+} release by external washing with Ca^{2+}-free medium; this extra cellular washing decreases cytosolic $[Ca^{2+}]I$, and facilitates dissociation of Ca^{2+} ions from the IP3R that in turn decreases the probability of IP3R channel opening. Second, once the IP3R is inhibited, a simple injection of Ca^{2+} ions by electropermeabilization triggers channel opening and induces Ca^{2+} release. Then, by just varying the time interval and the number of the electrical pulses, it is possible to drive the dynamics of the CICR process that initiates development. Intracellular Ca^{2+} imaging demonstrated that fertilized eggs subjected to 24 electrical pulses (1.45 kV cm^{-1}) every 8 min for 3 h in the microfluidic processor responded by exhibiting 24 induced-Ca^{2+} transients that are caused by calcium release from intracellular stores. All auto-regenerative responses between pulses were inhibited. Among 60 treated embryos transferred to pseudo-pregnant recipients, 40 (67%) developed to term, with birth of live offspring, thus demonstrating that this new methodology does not compromise development. Because the eggs are fertilized, it now becomes possible to study the function of Ca^{2+} signaling during egg activation and to evaluate its developmental impact, if any, in association with genomic approaches.

298 RED DEER (*CERVUS ELAPHUS*) PARTHENOGENETIC BLASTOCYSTS PRODUCED USING IONOMYCIN/6DMAP ACTIVATION

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Successful activation of red deer oocytes is a necessary prerequisite for the cloning of red deer individuals with desirable genetic characteristics. To investigate this, an established biphasic protocol used for oocyte activation in sheep was investigated for suitability. The method chosen was 5 μM Ionomycin for 5 min followed by 2 mM 6DMAP for 3 h (Loi P *et al*., 1998 Biol. Reprod. 58, 1177–1187). The medium used during activation and subsequent culture was Deer Synthetic Oviduct Fluid, which has been shown to support routine in vitro fertilization and blastocyst development (15%) of in vitro-matured red deer oocytes (DSOF, Berg D *et al*., 2003 Theriogenology 59, 189–205). Red deer abattoir-derived COCs were matured in vitro for 22 h before random allocation across 3 treatment groups comprising a standard IVF group, the activation group and a negative control

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group exposed to medium only. Activation treatment oocytes were stripped of cumulus by vortexing in 0.1% hyaluronidase before selecting for first polar body extrusion. First-step activation was performed in medium comprising HEPES-buffered IVF-DSOF containing 4 mM Ca^{2+}. Second-step activation used 3 mM Ca^{2+} early DSOF under 7% O_2, 5% CO_2, and 88% N_2 at 38.5°C. Standard IVF was conducted at 23 h post-IVM using 4 mM Ca^{2+} IVF-DSOF and 0.5×10^6 mL^{-1} final sperm concentration. Following activation and IVF, oocytes were washed 3 times in HEPES DSOF before culture for 7 days in sequential DSOF with late DSOF on Day 4 containing 1.5 mM Ca^{2+}. Cleavage was assessed 24 h after activation, and all blastocysts were fixed for cell counts. Four replicates of each treatment were performed. Cleavage and blastocyst rates were examined by chi-square analysis and cell numbers by ANOVA. First polar body extrusion rate was 84%. Cleavage was similar between the activation treatment and IVF ($P > 0.05$); but a significant difference was found in blastocyst development rates ($P < 0.05$) with the Ionomycin and 6DMAP protocol being superior to the IVF treatment. Exposure to high Ca^{2+} media alone resulted in only 5% of the negative control oocytes cleaving to 2 cells. Results show that Ionomycin and 6DMAP are effective in activating red deer oocytes and DSOF is a suitable medium to produce parthenogenetic blastocysts.

Activation protocol	No. oocytes	No. (%) 2-cell cleavage	No. (%) blastocycts from cleaved	Cell numbers (Mean ± SEM)
Ionomycin and 6DMAP	115	89 (77.4)	24 (27)*	93.8 ± 11.3
IVF	113	84 (74.3)	6 (7.1)	101.9 ± 23.1
Medium-negative control	121	6 (4.9)	0	NA

*Significant difference, $P < 0.05$.

299 OOCYTE ACTIVATION IS THE RESULT OF CALCIUM SIGNAL SUMMATION

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Egg activation in mammals is caused by a series of cytosolic Ca^{2+} oscillations that are essential for development. However, the specific functional significance of the number or the frequency of the Ca^{2+}signals is not clear. The objective of this study was to determine whether the efficiency of egg activation relies on the frequency or on the number of signals. Given the repetitive nature of the signal, it is nearly impossible to discriminate the specific effect of the frequency from the number because increasing the time interval between signals directly reduces their number in a given time unit and vice versa. To bypass such difficulty, we subjected non-fertilized mouse oocytes, which are not capable of releasing $Ca^{2+}$from intracellular stores, to a coherent series of four treatments. Repetitive intracellular Ca^{2+}signals were introduced into freshly ovulated mouse oocytes by electropermeabilisation of Ca^{2+} ions by means of a microfluidic processor . The first regime (T1) was a monotonic [Ca^{2+}]i increase lasting about 10 min and provoked by 4 consecutive electrical pulses (1.45 kV cm^{-1}) given at 2-min intervals. This large calcium signal gave a poor activation rate: 6% (4/67). The second regime (T2) was made up of six lower pulses (1.12 kV cm^{-1}) given at 30-min intervals. The rate of egg activation reached 38% (75/195). The third treatment (T3) consisted in subjecting oocytes consecutively to the T1 and the T2 treatments. In that case, the egg activation reached 88% (121/138). The remarkable increase in egg activation efficiency can be attributed to the increase in the total [Ca^{2+}]i load, which is the only change in T3. Thus, it appears that the large monotonic increase (T1) potentiates the response as revealed by the periodic stimulation delivered by T2. It therefore seems that the process downstream of the signal is incremental in nature, and entrance in interphase of the cell cycle is reached when the summation of individual signals reaches a sufficient level. We next evaluated whether the putative summation effect makes it possible to regularly get 100% of egg activation by increasing the cytosolic Ca^{2+}load. Oocytes were subjected to a last treatment (T4) that consisted of T1 and then 24 pulses (1.45 kV cm^{-1}) given every 8 min. In that case 100% of the oocytes were activated (80/80). Thus, it appears that all oocytes are subject to egg activation if they receive a sufficient amount of calcium signal. Moreover, the frequency does not seem to play a crucial role for entrance into interphase because reducing the time interval from 30 min (T3) to 8 min (T4) resulted in high activation rates in both cases (88% and 100%, respectively). The question remains as to whether the distribution of the Ca^{2+}signal throughout the process of egg activation might affect some epigenetic modifications that impact developmental processes.

300 MEIOTIC CELL CYCLE PROGRESSION AFTER PARTHENOGENETIC OOCYTE ACTIVATION: EFFECTS OF STRAIN AND ACTIVATION STIMULUS

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Parthenogenetic activation of mammalian oocytes can be induced by several stimuli in vitro. In the mouse, ethanol and Sr^{2+} are commonly used artificial activating agents. The aim of this study was to investigate both the effects of these two activating agents and the genetic background of the oocyte on meiotic cell cycle progression following activation. Metaphase-II (MII) oocytes were collected from superovulated (5 IU eCG/5 IU hCG) 8–10-week-old female mice of hybrid B6D2F1, inbred C57BL/6, outbred CF-1 and nude (NU/$^+$) strains at 16 h post-hCG. Oocytes were activated either by a 5-min exposure to 7% ethanol (E), followed by culture in KSOM, or by continuous culture in Ca^{2+}-free KSOM containing 10 mM $SrCl_2$ (S). Starting 5 min after the activation stimulus, oocytes were fixed at 5-min intervals, until 20 min post-activation (pa), and then at 30-min intervals, until 6 h pa. Fixed oocytes were processed for immunofluorescence analysis of chromatin (Hoechst), spindle (α/β-tubulin) and microfilaments (phalloidin) organization. The initial time course of activation (5–20 min pa), assessed by exit from MII arrest, was faster in E- than

in S-activated oocytes, except in the C57BL/6 strain in which anaphase (AII) onset after ethanol exposure was significantly delayed in comparison to the other three strains. Progression from AII into telophase (TII), at 50 min pa, was faster in CF-1 and C57BL/6 E-activated oocytes (78.3–87.1%) and in B6D2F1 and CF-1 S-activated oocytes (93.1–94.2%) when compared to the other strains (60–68.8% and 41–62.5%, respectively). NU/$^+$ S-activated oocytes showed the lowest activation rates (88.5% v. 100% at 6 h pa), and TII entry was also delayed both in comparison to the other strains (41% vs. 62.5–94.2% at 50 min pa) and in comparison to E-activated NU/$^+$ oocytes (68.8%). Rotation of the meiotic spindle began at 15 min pa in both E- and S-activated C57BL/6 oocytes, at 20 min pa in B6D2F1 and CF-1, and at 50 min pa in NU/$^+$ oocytes and, in general, spindle rotation was completed more rapidly following E activation. Second polar body (PB2) extrusion was also delayed in NU/$^+$ oocytes (starting at 1 h 20 min pa) when compared to all other strains (starting at 50 min pa) irrespective of the activating agent used. In E-activated oocytes, TII exit commenced earlier in C57BL/6 oocytes (1 h 20 min pa) than in the other three strains (1 h 50 min–2 h 20 min pa), but a significant proportion of CF-1 and NU/$^+$ oocytes (37–49% at 5 h 50 min pa) remained arrested at metaphase III. S-activation not only accelerated TII exit but also increased the rate of pronuclear formation in both CF-1 and NU/$^+$ oocytes to 100%. In conclusion, the results demonstrate that both the activating stimulus and the genetic background of the oocyte have significant effects on key events of meiotic cell cycle progression following activation. Supported by USDA NRI 2001-35205-09966 and Charles River Labs.

301 EFFECT OF THE MII-AGE AND ACTIVATION PROTOCOL ON THE PARTHENOGENETIC DEVELOPMENT OF PORCINE OOCYTES

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The completion of porcine oocyte nuclear maturation (MII) in vitro, characterized by the time of polar body extrusion, starts at about 32 h of maturation and lasts more than 12 h. This leads to the simultaneous presence in the population of matured oocytes with differing abilities to be activated. We investigated age-dependent changes in pig oocyte maturation, activation and development in SOFaa in response to electric impulse (EL) in the presence of cytochalasin B (CB) and EL in combination with cycloheximide and cytochalasin B (EL + CHX + CB). Oocytes were matured in TCM 199 with 10% FCS, cysteine, LH, FSH (Pergovet, Serono, Geneva, Switzerland) for 36 h and then decumulated. Matured oocytes were activated at 40 and 44 h by double pulse of 30 μs DC 1, 5 kV cm^{-1} and cultured in 5 μg mL^{-1} CB for 4 h or by EL followed by incubation in 10 μg mL^{-1} CHX + 5 μg mL^{-1} CB for 4 h. According to the MII-age before activation oocytes were divided into 2 age classes: 3–7 and 7–11 h after polar body extrusion. Embryos were cultured in SOFaa in 5% CO_2, 5% O_2 at 38.5°C. The rates of cleavage, blastocyst formation and cell number of BL on Day 7 (BLD7) were recorded. Our results showed that the average rate of maturation at 44 h was 72% ($n = 1377$). About 50% and 87% of oocytes, that eventually matured, extruded the polar body at 37 and 40 h, respectively. The average cell number of BLD7 developed in SOFaa was 80 ± 36 ($n = 52$) and was not affected by activation protocol. Seventy-nine and 27% of BL had more than 50 and 100 cells per BL, respectively. Porcine oocytes activated by EL acquired their developmental competence gradually, achieving the highest rates of cleavage and blastocyst formation 7 h after polar body extrusion. By contrast, oocytes activated by EL + CHX + CB showed their maximal developmental competence earlier (3–7 h group). In conclusion, we demonstrate that electric impulse in combination with CHX + CB treatment permits earlier efficient activation of porcine oocytes (3–7 h after polar body extrusion).

Activation	Age (h)	N	Cleavage (%)	BLD7 (%)
EL+CB	3–7	190	64 (33.7)[a]	16 (8.4)[c]
	7–11	139	123 (88.5)[b]	33 (23.7)[d]
EL+CHX+CB	3–7	155	141 (91)[b]	43 (27.7)[d]
	7–11	100	95 (95)[b]	34 (34)[d]

Chi-square test, $P < 0.05$. [a–d]Values in a column with the same letter do not differ significantly.

302 A MICROFLUIDIC DEVICE TO CONTROL Ca^{2+} SIGNALLING IN FERTILIZED AND NON FERTILIZED EGGS

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The process of egg activation has been the subject of much attention for many years. It has recently gained even more interest since nuclear transfer experiments have revealed the unique and extraordinary properties of the oocyte cytoplasm to cause massive reorganization of a somatic nucleus. The transduction mechanisms by which the incoming sperm triggers a series of intracellular Ca^{2+} oscillations is becoming clearer, but how activation impacts the process of remodelling is still unknown. One possibility to get a better view is to experimentally drive the dynamics of Ca^{2+} oscillations to monitor the changes in a sperm nucleus in maternal chromosomes or in a somatic nucleus during activation. For this purpose, we have used the modern technology of microfluidics in association with electropermeabilization of the cell membrane to drive the process of egg activation. We designed a microdevice that assures precise control of $[Ca^{2+}]I$ and makes it possible to slow down or accelerate the dynamics of Ca^{2+} signalling in fertilized and non-fertilized eggs during the process of activation. This device consists of a micro-chamber 400 μm wide, 0.5 mm high, and 25 mm long, making a volume of 5 μL. The lateral walls of the chamber are made of gold to subject the cells to an electric field pulse. The bottom surface

has a slot 20 μm wide used to hold the oocytes by suction. The space above is left open to allow access with a mouth-pipette to lodge a maximum of a hundred eggs. At any moment eggs can be put in place or removed. This chamber is embedded in a disposable cartridge made for us by Micronics (www.micronics.net). A series of computer-driven pumps and micro-valves embedded in the cartridge makes it possible to multiplex very rapid washing with four reagents. The total reagent content of the chamber can be replaced in less than 1 s while eggs are maintained aligned. This washing process makes it possible to control the electropermeabilization technique while embryos are cultured in vitro. To evaluate the survival to term of fertilized eggs cultured and stimulated in this microsystem, 60 fertilized embryos were subjected to 24 electrical pulses (1.45 kV cm^{-1}) every 8 min for 3 hours in the absence of Ca^{2+} ions during the electrical pulse. Imaging recording showed that the ongoing Ca^{2+} signal dynamic triggered by fertilization is not altered by the treatment. Among the 60 treated embryos transferred to pseudopregnant recipients, 45 (75%) were capable of giving birth to viable young. Since this new microtechnology appears to be biologically safe, it becomes possible to automatically drive a great variety of signals onto and into the cell during in-vitro culture, to better evaluate how oocytes integrate signals during the process of activation, and to give a biological response at later stages of development. Moreover, this new microtechnology will provide standardization of the biological response after fertilization, ICSI or nuclear transfer experiments.

303 PARTHENOGENETIC DEVELOPMENT OF PIG OOCYTES BY CHEMICAL ACTIVATION

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Efficient activation is essential for the success of animal cloning by nuclear transfer. The aim of this study was to investigate the effects of chemical activation agents on parthenogenetic development of pig oocytes matured in vitro. The medium used for oocyte maturation was TCM-199 supplemented with 26.19 mM sodium bicarbonate, 0.9 mM sodium pyruvate, 10 μg mL^{-1} insulin, 2 μg mL^{-1} vitamin B_{12}, 25 mM HEPES, 10 μg mL^{-1} bovine apotransferrin, 150 μM cysteamine, 10 IU mL^{-1} PMSG, 10 IU mL^{-1} hCG, 10 ng mL^{-1} EGF, 0.4% BSA, 75 μg mL^{-1} sodium penicillin G, 50 μg mL^{-1} streptomycin sulfate and 10% pFF. After about 22 h of maturation, oocytes were cultured without cysteamine and hormones for 22 h at 38.5°C, 5% CO_2 in air. Cumulus-free oocytes showing first polar body were selected for activation. Oocytes were activated as follows. First, all oocytes were activated with 25 mM HEPES buffered NCSU-23 medium containing 8% ethanol for 10 min. After that, in treatment 1, oocytes were incubated in the NCSU-23 medium supplemented with 7.5 μg mL^{-1} cytochalasin B for 3 h. In treatment 2, oocytes were incubated in the NCSU-23 medium supplemented with 10 μg mL^{-1} cycloheximide for 3 h. In treatment 3, oocytes were incubated in the NCSU-23 medium supplemented with 7.5 μg mL^{-1} cytochalasin B for 1.5 h, and then were incubated in the NCSU-23 medium supplemented with 10 μg mL^{-1} cycloheximide for 1.5 h. In treatment 4, oocytes were incubated in the NCSU-23 medium supplemented with 7.5 μg mL^{-1} cytochalasin B plus 10 μg mL^{-1} cycloheximide for 3 h. Following activation, oocytes were transferred into 500 μL NCSU-23 culture medium containing 0.4% BSA for further culture for 20 and 144 h. Activated oocytes were fixed and stained for evaluation of activation rate, cleaved oocytes, blastocyst formation rate and cell numbers per blastocyst. Data were analysed by ANOVA and Duncan's multiple range test using the SAS program. The rate of oocyte activation was higher in treatment 4 (62.1%) than in treatment 1, 2 and 3 (52.0, 49.6 and 58.0%, respectively). The percentage of cleaved oocytes was lower in treatment 1 and 2 (56.9 and 55.2%) than in treatment 3 and 4 (68.8 and 68.5%). The rate of blastocyst formation from the cleaved oocytes was higher in treatment 3 and 4 (19.8 and 22.0%) than in treatment 1 and 2 (12.1 and 11.7%). Mean cells per blastocyst were lowest in treatment 2 (21.2 ± 0.9) compared to treatment 1, 3 and 4 (27.3 ± 2.2, 30.4 ± 3.8 and 30.9 ± 3.4, respectively). In conclusion, cytochalasin B combined with cycloheximide was more efficient for parthenogenetic development of pig oocytes matured in vitro.

304 INFLUENCE OF ELECTRICAL STIMULUS ON ACTIVATION OF PIG OOCYTES MATURED IN VITRO

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Electrically induced activation of pig oocytes deserves particular attention for research on parthenogenesis. The aim of this study was to improve electrical activation of in vitro matured pig oocyte. The medium used for oocyte maturation was TCM-199 supplemented with 26.19 mM sodium bicarbonate, 0.9 mM sodium pyruvate, 10 μg mL^{-1} insulin, 2 μg mL^{-1} vitamin B_{12}, 25 mM HEPES, 10 μg mL^{-1} bovine apotransferrin, 150 μM cysteamine, 10 IU mL^{-1} PMSG, 10 IU mL^{-1} hCG, 10 ng mL^{-1} EGF, 0.4% BSA, 75 μg mL^{-1} sodium penicillin G, 50 μg mL^{-1} streptomycin sulfate and 10% pFF. After about 22 h of maturation, oocytes were cultured without cysteamine and hormones for 22 h at 38.5°C, 5% CO_2 in air. Cumulus-free oocytes involving first polar body were selected for activation. For electrical activation, oocytes were rinsed twice in 0.3 M mannitol solution supplemented with 0.1 mM $CaCl_2$, 0.2 mM $MgCl_2$, 0.5 mM HEPES and 0.01% BSA, and transferred to a chamber consisting of two electrodes 1 mm apart which were overlaid with the same activation solution. Experiment 1 was conducted to investigate the effect of electrical pulse on oocyte activation. Oocytes were activated with DC pulses of 1.0, 1.5, 2.0 and 2.5 kV cm^{-1} for 30, 60 and 90 μs, respectively. Experiment 2 was carried out to investigate the effect of electrical stimulus frequency on oocyte activation. Oocytes were activated one, two and three times, with a DC pulse of 1.0 kV cm^{-1} for 60 μs. After activation, oocytes were transferred into 500 μL NCSU-23 culture medium containing 0.4% BSA and cultured for 20 h. Activated oocytes were fixed for 48 h in 25% acetic acid (v:v) in ethanol at room temperature, and stained with 1% orcein (w:v) in 45% acetic acid (v:v) to examine pronucleus formation. Data were analyzed by ANOVA and Duncan's multiple range test using the SAS program. The rate of activation was highest in the DC pulse of 1.0 kV cm^{-1} for 60 μs (75.1%) compared with the other durations and strengths (62.5–63.1%). Activation rate by electrical stimulus frequency was highest (76.0%) when oocytes were activated by a one-time pulse. In conclusion, the results suggested that electrical stimulus with a single DC pulse of 1.0 kV cm^{-1} for 60 μs might be more efficient than other strengths and durations for activation of pig oocytes.

305 DEVELOPING AN ACTIVATION PROTOCOL FOR SOMATIC CELL NUCLEAR TRANSFER (SCNT) IN THE DOMESTIC CAT

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In order to establish an activation protocol for somatic cloning in the domestic cat, we evaluated the developmental competence of cat embryos derived from in-vitro matured ova after parthenogenetic activation treatment. The quality of parthenogenetic embryos was assessed by D3 cleavage rates, D8 rates of blastocyst formation and total nucleus numbers in expanded/hatching blastocysts. Parthenogenetic activation treatments were as follows; Treatment I: 3.0 kV cm^{-1} (25 μs, twice) in 0.3 M mannitol containing 0.1 mM $CaCl_2 \cdot 2H_2O$ and 0.1 mM $MgSO_4$, administered to matured cat oocytes and followed by 10 μg mL^{-1} cycloheximide +5 μg mL^{-1} cytochalasin B in TCM 199-Earle's salt supplemented with 0.3% BSA for 6–7 h. Treatment II: The first electric stimulation was performed as described for treatment I except that the activation medium consisted of 0.3 M mannitol containing Mg, but without Ca. Two hours later, pre-pulsed MII oocytes were electropulsed by applying 1.0 kV cm^{-1} (50 μs, twice, 5 s apart) in 0.3 M mannitol containing Ca and Mg for additional activation, followed by culture in 10 μg mL^{-1} cycloheximide +5μg mL^{-1} cytochalasin B treatment in TCM 199-Earle's salt supplemented with 0.3% BSA for 6–7 h. Immature cat oocytes were obtained from ovaries by mincing/dissection and matured in vitro for 26–30 h as previously described (Gomez *et al*., 2001, Therigenology, 55, 472). Only MII oocytes with a 1st polar body were utilized for the activation procedure after removal of cumulus cells with 0.1% hyaluronidase by gentle pipetting. A total of 1120 oocytes were collected and the overall maturation rate was 49.8% (551/1120). After parthenogenetic activation of the MII oocytes, the embryos were cultured in vitro as described previously (Pope *et al*., 2000, Theriogenology, 53, 163–174). The results are shown in Table 1. Treatment II resulted in significantly higher ($P < 0.01$) D3 cleavage rates; however, there were no significant differences in D8 blastocyst formation and total nucleus numbers. These data suggest that an additional electric activation (Treatment II) may increase the in vitro cleavage rates compared to using a fusion and electrical stimulation simultaneously (Treatment I). In addition, we demonstrated the developmental competence of domestic cat embryos derived from in vitro maturation, activation, and culture for development to the pre-implantation stage. By using these procedures for SCNT, several pregnancies were established and a healthy cloned kitten resulted in our laboratory (Shin *et al*., 2002, Nature, 415, 859). Therefore, this protocol can be useful, not only for prediction of the developmental competence of domestic cat oocytes matured in vitro, but also when used with SCNT to produce cloned cats.

Comparison of cleavage rates and developmental competence to blastocyst stage following parthenogenetic activation treatments in domestic cat oocytes matured in vitro

Treatment	# cultured	# cleaved D3 (%)	# BL at D8 (%)	# of nuclei
I	84	61 (72.6)[a]	19 (22.6)	154.9 ± 47.5
II	52	49 (94.2)[b]	13 (25.0)	185.2 ± 23.2

Values with different superscripts were significantly different (Chi-square test, $P < 0.01$)

306 SPONTANEOUS AND PARTHENOGENETIC ACTIVATION OF RAT OOCYTES

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Oocyte activation is one of the critical steps for the success in a mammalian cloning program. In rats, information regarding parthenogenetic activation of oocytes is limited and further studies are required to elucidate the phenomenon of spontaneous activation and to develop effective protocols of induced activation. The objectives of the study were (1) to characterize the kinetics of spontaneous activation and (2) to examine different activation regimens for rat oocytes. Oocytes were collected from 3–4-wk-old PMSG-primed Sprague Dawley rats at 14 h after hCG injection. HEPES-mR1ECM was used for oocyte collection and mR1ECM for in vitro culture (Miyoshi *et al.*, 1995 J. Reprod. Fert. 103, 27–32). Experiment 1: Cumulus-denuded oocytes were fixed and stained with bisbenzimide (Hoechst 33342) at 10, 40, 70, 100 and 130 min after recovery. Most oocytes (92.9%) were still arrested in the metaphase II stage at 10 min after recovery, but the proportion of activated oocytes increased in a time-dependent manner, i.e. at 40 min after recovery 50% were at anaphase. At 70 min, 32.4%, 40.5%, 8.1%; at 100 min, 4.8%, 57.1%, 23.8%; and at 130 min, 0%, 3.3%, 86.7% had progressed to anaphase II, telophase II and extruded a 2nd PB, respectively. Experiment 2: Oocytes were exposed to the following activation treatments: Two sets of electrical stimuli (ES) 1 h apart composed of 3 DC pulses, 1 s apart of 1.2 kV cm^{-1} field strength and 60 μs duration in 0.25 M mannitol solution (Roh *et al.*, 2003 Reprod. Fert. and Dev., 15, 135–140). After ES treatment, oocytes were exposed for 3 h to either cycloheximide (CHX, 10 μg mL^{-1}, Group 1), 6-dimethylaminopurine (DMAP, 2 mM, Group 2), or CHX (10 μg mL^{-1}) and DMAP (2 mM) (Group 3). Group 4 was exposed to Strontium (10 mM) for 1 h and then incubated with CHX (10 μg mL^{-1}) and DMAP (2 mM) for 3 h. Oocytes were washed thoroughly after treatment, transferred to 50-μL droplets of mR1ECM and cultured at 37°C in a humidified atmosphere of 5% O_2, 5% CO_2 and 90% N_2. Pronuclear formation and cleavage rates were significantly higher in Groups 2 and 3 than in Groups 1 and 4 (92.6%, 88.9% and 84.6%, 80.8% v. 49.1%, 45.6% and 62.8%, 55.8%, respectively). First, these results show that rat oocytes undergo spontaneous activation very rapidly after recovery from the oviducts. Second, activation protocols with two sets of triple DC electrical stimulation followed by exposure to DMAP or CHX/DMAP are effective means of activation. In conclusion, the above information will be useful in establishing effective protocols for cloning rats. (Financial support by NSERC and Canada Research Chairs.)