48 h and development to the blastocyst stage at 216 h after Ion treatment among groups. A total of 536 oocytes were used in 5 replicates. Parthenotes in Group 1 showed lower rates of cleavage and blastocyst development than those in other groups (20% and 1% *v*. 53–67% and 6–31%). Among the groups, parthenotes in Group 5 showed significantly ($P < 0.05$) higher blastocyst development. In Experiment 3, at 8 h after Ion treatment, oocytes from Groups 2, 3, and 5 were divided into two subgroups based on the presence or absence of the second PB, and assessed for cleavage rates and ploidy in 239 2-cell-stage parthenotes in 4 replicates, as described earlier by King *et al*. (1979 Vet. Sci. Commun. **3**, 51–56). The cleavage rates did not differ among activation treatments, or by the presence or absence of the second PB in any activation group. The haploid rate was significantly ($P < 0.05$) higher in Group 2 than in Groups 3 and 5 (38% *v*. 19% and 0%, respectively). The diploid rate was significantly ($P < 0.05$) higher in Group 5 than in Groups 2 and 3 (88% *v*. 69% and 45%, respectively). In Experiment 4, the diploid rate of Group 2 blastocyst-stage parthenotes was 100% (4/4), whereas the diploid rates of Groups 3 and 5 blastocyst-stage parthenotes were 50% (6/12) and 71% (17/24), respectively, but the rates did not differ among groups. These results indicate that oocyte activation by CHX/CCB for 5 h after Ion treatment could enhance parthenogenetic development in bovines with higher rates of diploidy by preventing the extrusion of the second PB.

233 STUDY OF CORTICAL GRANULE CONTENT IN *IN VITRO*-MATURED PORCINE OOCYTES BY MEANS OF PNA LECTIN PRECIPITATION

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Cortical granules (CG) are clue organelles in the oocyte since their content is released under oocyte activation (i.e. fertilization) modifying the zona pellucida and thus blocking polyspermy. Once released, CG are not renewed. Research on cortical reaction and putative CG enzymes has progressed slowly because mammalian eggs contain only picogram quantities of CG-derived proteins (Moller and Wassarman 1989 Dev. Biol. **132**, 103–112; Green 1997 Rev. Reprod. **2**, 147–156), so the protein(s) responsible for the physiological changes in ZP after cortical reaction are not well known. The objective of this project was to study porcine CG content in *in vitro*-matured porcine oocytes by means of lectin precipitation with peanut agglutinin (PNA), since this lectin binds to porcine CG (Yoshida *et al*. 1993 Mol. Reprod. Dev. **36**, 462–468). Immature porcine cumulus–oocytes complexes (COCs) from Landrace × Large White gilts were *in vitro*-matured for 44 h in NCSU-37 medium. After IVM period, COCs were stripped of cumulus cells, washed in PBS, and quickly washed through purified water. Then oocytes were lysed in a fresh water droplet by gentle pipetting using a narrow-bore glass pipette. Once lysed, zonae pellucidae were removed and oocyte cytoplasmic content (lysate) collected. Lysate from 1000 IVM-oocytes was incubated under continuous shaking (2 h, room temperature) with 100 μL PNA-agarose (Sigma, St. Louis, MO, USA) so that proteins bound to PNA could be precipitated by centrifugation. After lectin precipitation, proteins were detached from PNA-agarose beads by boiling in reducing sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) loading buffer (5 min, 100°C) (Laemmli 1970 Nature **227**, 689). Samples were then centrifuged (5 min, 7000*g*), the pellet containing PNA-agarose beads was discarded, and the supernatant containing the proteins was collected and further separated by SDS-PAGE. The silver staining of electrophoresis gels revealed eleven bands from 37 to 180 kDa, so a second gel was electrotransferred to a polyvinylidene fluoride (PVDF) membrane (100V, 1 h) and incubated with PNA-horseradish peroxidase (PNA-HRP, 10 μg mL^{-1}) for 1 h. Visualization was accomplished using the enhanced chemiluminiscence (ECL plus) method and Typhoon 9410 following the manufacturer's instructions (Amersham Biosciences, Freiburg, Germany); only four bands of approximately 57 kDa, 60 kDa, 70 kDa, and 80 kDa were observed. These bands will be cut and processed for proteomic analysis for further studies. Preliminary results show that porcine CG-derived proteins can be studied by PNA lectin precipitation. These results could be employed in the future to develop specific antibodies against porcine CG.

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234 PARTHENOGENETIC ACTIVATION OF DOMESTIC CAT OOCYTES USING STRONTIUM

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Cloning domestic cats is useful in comparative medicine programs as it may provide insight into unique disease mechanisms and facilitate investigation of new therapeutic options. It is also believed to be beneficial for the conservation of precious animal models. However, as in many species, low birth rates after nuclear transfer remain a formidable challenge. One potential reason for the low efficiency is poor embryo development following activation of the reconstructed oocytes. The number of methods available to induce a transient increase in the oocytes' cytosolic free calcium level to stimulate development is rather limited. Although strontium has been reported to successfully activate the developmental program of mature mouse and rat oocytes, it was without effect in all other species studied. Here we investigated the effect of strontium on mature cat oocytes. Oocytes collected from the cat ovaries were matured *in vitro* in Feline Optimized Culture Medium (FOCM) supplemented with 0.6 mM cysteine, 0.1 mM cysteamine, 1 IU mL^{-1} eCG, 2 IU mL^{-1} hCG, 25 ng mL^{-1} epidermal growth factor (EGF) for 24 h. For intracellular calcium measurements, mature oocytes were incubated in the presence of 2 μM fura-2 AM, a calcium indicator dye, and 0.02% pluronic F-127 for 40 min. Individual oocytes were transferred into calcium-free HEPES, and $SrCl_2$ was added to the medium at a final concentration of 20 mM. Changes in the intracellular free calcium levels were then monitored using an InCyt Im2^{TM} fluorescence imaging system (Intracellular Imaging, Cincinnati, OH, USA). Preimplantation embryonic development was also evaluated by incubating the oocytes with 20 mM $SrCl_2$ in calcium-free HEPES medium supplemented with 7.5 μg mL^{-1} cytochalasin B for 6 h. Control oocytes were activated by two 20-μs-long, 100 kV cm^{-1} direct current pulses and incubated in the presence of 7.5 μg mL^{-1} cytochalasin B