# ABSTRACTS

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on meiotic chromatin or chromosome and are essential for normal fertilization and embryo development. To determine chromatin modification during oocyte maturation, oocytes were collected at 0 h, 6 h, 9 h, 12 h and 24 h after maturation, respectively and the localization of remodeling factors and epigenetic markers was observed using immunofluoresence analysis. A variety of specific antibodies against Brg-1, BAF-170, Mi-2, hSNF2H, 5-MeC, AcH3K9, AcH4K5, mono-, di- and tri-methyl H3K9 were used in this study. All remodeling factors such as Brg-1, BAF-170, Mi-2 and hSNF2H were detected in germinal vesicle (GV) oocytes. Then, Brg-1 and BAF-170 disappeared gradually during oocyte maturation, whereas Mi-2 and hSNF2H vanished rapidly in the nucleus just before germinal vesicle breakdown (GVBD) stage. Epigenetic markers such as 5-MeC, AcH3K9, AcH4K5 and mono-, di-, and tri-methyl H3K9 were observed in GV and GVBD oocytes. Histone acetylation was disappeared from chromosome during meiotic progress. In contrast to histone acetylation, DNA methylation and histone methylation maintained throughout oocyte maturation. DNA methylation and tri-methyl H3K9 were enriched in the outside of chromosomes, whereas mono-and di-methylation of H3K9 were distributed in the whole chromosomes. This study demonstrated dramatic changes of some chromatin remodeling factors and epigenetic markers during oocyte maturation. Our findings suggest that dynamic changes of remodeling factors during oocyte meiosis may be a prerequisite process for the acquisition of developmental competence.

**56. Actin, Myosin, Calmodulin, Spectrin, and Gelsolin Are Components of Guinea Pig Sperm Acrosome.** Armando Zepeda-Bastida, Adela Mujica. National Polytechnic Institute, Mexico City. Mexico

The sperm acrosome in mammals is a highly specialized organelle that covers the apical part of the sperm nucleus and contains a large number of hydrolytic enzymes, which are required for fertilization. The acrosomal content is compartmentalized: morphological and biochemically. In the acrosome are consider several domains; these are the dorsal region (M1), an intermediate region (M2) and an electro-dense ventral region (M3). Similarly, the biochemical composition of acrosome is not homogeneous, the acrosome content is divided into both soluble and insoluble proteins, this is the acrosomal matrix. The purpose of this study was to identify some proteins associated with the acrosomal cytoskeleton of the guinea pig sperm. Results showed the presence of F-actin throughout the acrosome visualized by confocal microscopy optical sections; in the same way, we also observed the presence of spectrin, myosin, calmodulin and gelsolin throughout the acrosome. Likewise, the same five mentioned proteins were positively revealed in the Triton-X100 insoluble acrosomal protein fraction, by electron microscopy in immuno-gold stained samples. From results obtained we suggest that an F-actin cytoskeleton forms the acrosome; also, that myosin, spectrin, calmodulin and gelsolin are cytoskeleton associated proteins. The cytoskeleton might be involved in the morphological acrosome division; this is, in the three mentioned domains. In addition, to allow the gradual release of proteins required for the acrosome reaction. This study is supported by CONACYT Grant #59176 to AM.

**57. Ability of Freeze-Dried Bull Spermatozoa to Induce Calcium Oscillations and Meiosis Resumption.** Hany Abdalla, Masumi Hirabayashi, Shinichi Hochi. Shinshu University, Ueda, Japan; National Institute for Physiological Sciences, Okazaki, Japan

Freeze-drying is a candidate as alternative method for semen preservation. Rodent spermatozoa freeze-dried (FD) and stored for one year at a refrigerator temperature participated into full-term development. In contrast, information regarding ability of FD spermatozoa in large domestic species is limited. This study was designed to investigate the ability of FD bull spermatozoa to induce calcium oscillations in mouse oocytes and meiosis resumption in in-vitro-matured bovine oocytes after intracytoplasmic sperm injection (ICSI). Commercially available frozen semen from a Japanese Black bull was used. For freeze-drying, the post-thaw spermatozoa were suspended in 10 mM Tris-HCl, 50 mM EGTA and 50 mM NaCl (pH8.0) and frozen in $LN_2$. The sperm suspension was first dried for 14 h at 0.37 hPa and then for 3 h at 0.001 hPa in a programmable freeze-dryer (ALPHA 2-4; Christ, Harz, Germany). The spermatozoa were kept dried for one year in a desiccator at +25°C, a refrigerator at +4°C, or a $LN_2$ tank at –196°C. In the first experiment, rehydrated spermatozoa were subjected to sonication and sperm heads were microinseminated into B6D2F1 mouse oocytes loaded with 22 μM fluo-3/AM. The kinetics of intracellular calcium concentration was monitored for 1 h under a confocal laser-scanning microscope. Repetitive increases of intracellular calcium concentration occurred in the majority of injected oocytes, except that a few oocytes injected with FD spermatozoa stored at +4°C (11%, 4/38) and +25°C (8%, 3/39) exhibited a single increase or no response (non-oscillated). Proportion of oocytes oscillated with high frequency (≥10 spikes per hour) was higher in the non-dried control group (79%, 30/38) than those in the FD groups (58%, 22/38, 56%, 21/38, and 54%, 21/39 for storage at –196, +4, and +25°C, respectively). While all the other oscillated oocytes in the non-dried control group (21%) exhibited low frequency oscillations (2-9 spikes per hour), some of the oscillated oocytes in the FD groups (26, 8, and 10% for storage at –196, +4, and +25°C, respectively) exhibited an abnormal pattern of oscillations as a delayed first spike followed by repetitive, low amplitude spikes. In the second experiment, control and FD spermatozoa were microinseminated into in-vitro-matured and denuded bovine oocytes. The oocytes were fixed and stained 12 h after the ICSI. A lower proportion of bovine oocytes injected with +25°C-stored FD spermatozoa (47%, 17/36) resumed meiosis than those injected with control spermatozoa (75%, 47/63), –196°C-stored FD spermatozoa (71%, 29/41), and +4°C-stored FD spermatozoa (63%, 32/51). However, the proportion of ICSI oocytes that reached the pronuclear-stage (complete activation) was higher in the control group (65%) than in the FD groups (34, 27, and 14% for storage at –196, +4, and +25°C, respectively). Thus, the correlation between abilities of freeze-dried bull spermatozoa for inducing calcium oscillations and meiosis resumption was evaluated by combining interspecies assay with homogenous microinsemination. Ability of spermatozoa to induce frequent intracellular calcium spikes in mouse oocytes may be required for triggering meiosis resumption and leading to pronucleus formation in bovine oocytes.

**58.** NOT PRESENTED.

**59. Successful Microinsemination of Rabbit Oocytes with Round Spermatids.** Masumi Hirabayashi, Megumi Kato, Kaori Watanabe, Kensaku Kitada, Masao Hirao, Naoko Ohnami, Shinichi Hochi. National Institute for Physiological Sciences, Okazaki, Japan; Kitayama Labes Co. Ltd., Ina, Japan; Shinshu University, Ueda, Japan

Although the first report for rabbit offspring by round spermatid injection (ROSI) has already been published in 1994, the production of rabbit ROSI offspring is considered difficult due to the high incidence of chromosomal aberrations. The ROSI oocytes need to be activated artificially because sperm-borne oocyte-activating factor, SOAF, is present in elongating spermatids and spermatozoa but not round spermatids, and metaphase-promoting factor, MPF, in the oocytes causes premature chromosome condensation in injected spermatid nuclei. The present study was designed to investigate the effect of oocyte activation regimens on developmental ability of rabbit ROSI oocytes. Round spermatid cells were isolated from seminiferous tubules of Japanese White rabbits (Kbs:JW) with proven fertility and resuspended in GL-PBS (PBS supplemented with 5.6 mM glucose, 5.4 mM sodium lactate and 0.01% PVP). Oocytes were recovered from oviductal ampullae of Kbs:JW females superovulated with 3 AU FSH and 75 IU hCG, and were freed from cumulus cell layers. An individual round spermatid was introduced into the denuded oocyte using a piezo-driven micromanipulator. For activation regimens, oocytes were subjected to 5-min treatment with 5-10 μM ionomycin in RPMI1640/DMEM (RD) medium 50 min before ROSI (Group-1; n=108) or 10 min after ROSI (Group-2; n=87), 50 min before and 10 min after ROSI (Group-3; n=82), or 50 min before and 10 min after ROSI followed by incubation for 1 h in the RD medium and subsequent 1 h in the RD medium supplemented with 5 mg/ml cycloheximide and 2 mM 6-dimethylaminopurine (Group-4; n=131). All activation treatments were performed at 38.5°C. Oocytes microinjected with ejaculated spermatozoa (intracytoplasmic sperm injection; ICSI) were served as controls (Group-5; n=163). The ROSI/ICSI oocytes were cultured in TCM199+20% fetal bovine serum for 22-24 h at 38.5°C in 5% $CO_2$ in air. Oocytes cleaving to the 2-8 cell stage were selected for oviductal transfer to recipient rabbits. The activation regimens influenced cleavage rate of microinseminated oocytes (39, 29, 51, 68 and 60% in the Group-1, -2, -3, -4 and -5, respectively). The cleavage rate of ROSI oocytes in Group-4 (68%) was not significantly different from that of ICSI oocytes (60%), but higher than those of ROSI oocytes in the Group-1 (39%) and Group-2 (29%). However, proportions of advanced 4-8 cell-stage embryos among the cleaved ROSI embryos (45, 36, 43 and 52% in the Group-1, -2, -3 and -4, respectively) were significantly lower than that of ICSI embryos (67% in the Group-5). Caesarean section of recipient rabbits 4 weeks after the embryo transfer showed the presence of implantation signs in the Group-3 (2%) and Group-4 (9%), and full-term development only in the Group-4 (7%). The control ICSI (Group-5) resulted in implantation signs at 4% and full-term development at 8%. All the full-term offspring derived from the ROSI (n=6) / ICSI (n=8) appeared to be normal. In conclusion, rabbit ROSI oocytes were capable of developing into full-term when the oocytes were activated with a combined treatment of ionomycin and cycloheximide +6-dimethylaminopurine.

**60. Molecular Cloning and Expression of Dead-End Homologue in Chicken Germ Cells.** Shinya Aramaki, Takako Kato, Tomoki Soh, Nobuhiko Yamauchi, Yukio Kato, Masa-aki Hattori. Kyushu University, Fukuoka, Japan; Meiji University, Kawasaki, Japan

Chicken primordial germ cells (PGCs) dynamically migrate towards the prospective gonadal area through the germinal crescent region and bloodstream at early embryonic stages from stage X (Eyal-Giladi and