252 EFFECTS OF *IN VITRO* MATURATION AND AGE ON OOCYTE METAPHASE SPINDLE INTEGRITY AND CHROMOSOME SEGREGATION IN THE RHESUS MONKEY

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Oocyte IVM offers tremendous potential for the treatment of infertility by reducing the costs, risks, and side effects associated with ovarian stimulation. Although normal offspring have been produced using IVM/IVF technology in humans and monkeys, IVM is far from being a reliable technique for the production of developmentally competent oocytes in primates. This study examined oocyte karyotype and spindle integrity in rhesus monkey oocytes, and attempted to correlate anomalies with IVM and age of oocyte donors. Each of 14 females (ages 6 to 22 years) underwent both regimen A (FSH + hCG) and regimen B (FSH only) stimulation cycles at least once over the study period to facilitate collection of both mature and immature oocytes. Immature oocytes from regimens A and B underwent IVM to produce metaphase II oocytes. Metaphase II oocytes from each collection cycle cohort were fixed for either karyotype or metaphase spindle analysis. Statistical analysis of aneuploidy rates and spindle aberrations was performed using 2 × 2 G tests. Karyotype analysis revealed a significantly lower rate of aneuploidy in *in vivo*-matured (IVO) oocytes (6.0%) compared with IVM oocytes from regimen B (19.8%). Factoring age into these analyses revealed a significant difference in aneuploidy rate between IVO oocytes from young females (4.7%) and regimen B IVM oocytes from old females (25.0%; $P < 0.05$). Confocal analysis demonstrated a significant increase in metaphase spindle anomalies in IVM oocytes compared with IVO oocytes, which could be attributed to a significant increase in the rate of abnormal chromosome alignment on the metaphase spindle in IVM oocytes originating from either stimulation regimen: 7.0% in IVO oocytes *v.* 27.9% (regimen A) and 32.6% (regimen B) in IVM oocytes ($P < 0.05$). When donor female age was considered, regimen B IVM oocytes from old females displayed abnormal chromosome alignment on the spindle equator at a significantly higher rate than oocytes of any other maturation condition or age group ($P < 0.05$). In addition, IVO oocytes from young females showed this anomaly at a significantly lower rate than regimen B IVM oocytes from the same young females. We conclude that IVM can induce meiotic anomalies in the macaque oocyte, especially those obtained from older females. The results from this study provide possible explanations for the reported reduction in developmental competence of IVM *v.* IVO primate oocytes. This study provides information on the nuclear aspects of IVM, namely, the effect of maturation conditions and age on incidences of abnormal chromosome segregation during meiosis I and abnormal chromosome congression during meiosis II, data that may be helpful for determining optimal IVM culture conditions.

253 DEVELOPMENTAL COMPETENCE OF BOVINE OOCYTES MATURED IN SERUM-FREE MEDIUM UNDER LOW OXYGEN TENSION

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In vitro maturation (IVM) is a critical step in *in vitro* bovine embryo production. A number of factors can influence the IVM environment, such as media composition and protein supplementation. Serum and higher O_2 tension have been shown to reduce embryo quality; however, little is known about the effects of serum and O_2 tension during IVM on embryo quality and development. This study aimed to evaluate the effect of serum and O_2 tension on IVM of bovine oocytes. Immature oocytes obtained from slaughterhouse ovaries were randomly distributed in 4 groups of IVM: G1 ($n = 253$), 0.1% polyvinyl alcohol (PVA) in air; G2 ($n = 248$), 10% inactivated estrous cow serum (ECS) in air; G3 ($n = 270$), 0.1% PVA under 5% O_2; and G4 ($n = 236$), 10% ECS under 5% O_2. *In vitro* maturation was performed with TCM-199 culture medium supplemented with 20 μg mL^{-1} FSH, under 5% CO_2 at 38.5°C for 24 h. After maturation, oocytes were *in vitro* fertilized with 2.0×10^6 sperm mL^{-1} in Fert TALP medium, supplemented with heparin, for 20 h. Presumptive zygotes were denuded by vortexing and cultured in CR2aa medium with 2.5% fetal calf serum under 5% CO_2 and 5% O_2 at 38.5°C. Cleavage rate was evaluated 72 h postfertilization, and blastocyst rate and total cell number were evaluated 8 days postfertilization. Morphological classification of embryos was performed at Day 8 according to the International Embryo Transfer Society manual (1998). Cleavage, blastocyst, and grade 1 embryo rates were analyzed by chi-square, and total cell number was analyzed by ANOVA, with means compared by LSD. Results are presented as mean ± SEM. There was no difference ($P > 0.05$) in cleavage rates among G1, G2, and G4 (61.6, 65.3, and 57.6%, respectively), but cleavage rate was lower ($P < 0.05$) in G3 (52.5%). Blastocyst rates among G1, G3, and G4 (15.8, 17.7, and 20.3%, respectively) were similar ($P > 0.05$). However, blastocyst rate in G2 (25.0%) was higher ($P < 0.05$) than in G1 and G3, but was similar to G4 ($P > 0.05$). Total cell number was similar ($P > 0.05$) among G2 (194.1 ± 13.1), G3 (173.3 ± 9.0), and G4 (163.8 ± 8.7), but was lower ($P < 0.05$) in G1 (124.5 ± 11.4). The grade 1 embryo rate was lower ($P < 0.05$) in G1 (70.3%) than in G2 (89.5%), but was similar ($P > 0.05$) to G3 (77.0%) and G4 (83.9%). The results suggest that IVM with PVA in TCM-199 medium under 5% O_2 can be performed without reducing embryo development and quality, when compared with ECS. On the other hand, oocyte developmental competence seems to be affected when IVM is performed with PVA under air conditions.

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