THE INFLUENCE OF SPERM-OOCYTE INCUBATION TIME AND BREED OF BULL ON IN VITRO EMBRYO DEVELOPMENT IN CATTLE

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It has been reported that the length of sperm-oocyte-incubation time using Holstein sperm did not affect to the embryo development (Rehman, et al. Theriogenology 41:1447-1452, 1994). Therefore, the present study was designed as 5X4 factorial to investigate the effect of sperm-oocyte-incubation time (5h, 10h, 15h and 20h) and breed of bull (tetraparental Chimera, Japanese Black, Japanese Brown, Limousin and Holstein) on the fertilization, cleavage and blastocyst rates in an in vitro bovine embryo.

Cumulus oocyte complexes (COC) were aspirated from Holstein ovaries collected from a slaughterhouse and cultured in TCM-199 supplemented with 0.01 mg/ml. FSH and 5% superovulated cow serum (SCS). Frozen thawed semen was used for IVF. The fertilization medium (BO) medium contained 5mM caffeine and 20 mg/ml heparin. Fertilized oocytes were then cultured in TCM-199 supplemented with 5 mg/ml insulin and 5% SCS for further development. In experiment I acrosome reaction test was assessed at 1h after pre-incubation, aliquot of sperm suspension were fixed in 10% formaldehyde solution and staining with Napthol yellow S plus Aniline blue. In experiment II fertilization rate was assessed at 15h after fertilization, fertilized oocytes were fixed for 72h in Carnoy's solution and stained with 1% aceto-orcein. In experiment III, cleavage and blastocyst rates were assessed on Day 2 and Day 7, Day 8 and Day 9 after insemination. The proportion of acrosome reacted spermatozoa was higher ($P<0.01$) in Chimera than in Holstein (H) and Japanese Brown (JBr), but did not differ from Japanese Black (JB) and Limousin (L) sperm (79.0%, 71.2%, 72.5%, 57.0% and 57.8% for CH, JB, L, H and JBr sperm, respectively). Fertilization rate observed at 20h sperm-oocyte incubation time of IVM oocytes inseminated with Chimera (O-CH), Japanese Black (O-JB) and Limousin (O-L) did not differ from 5h (97.5% vs 81.5%, 83.8% vs 80.0% and 65.4% vs 69.4% for O-CH, O-JB, and O-L, respectively). However, the fertilization rate observed at 20h sperm-oocyte incubation time of IVM oocytes inseminated with Japanese Brown (O-JBr) and Holstein (O-H) sperm were higher ($P<0.01$) than 5h (71.4% vs 50.0% and 85.7% vs 18.2% for O-JBr and O-H sperm, respectively). At 5h of sperm-oocyte-incubation time, the cleavage rate of IVM oocytes inseminated Chimera sperm did not differ with Japanese Black, however there were higher ($P<0.01$) than IVM oocytes inseminated with Limousin, Japanese Brown and Holstein sperm (75.7%, 71.9%, 58.1%, 45.9% and 16.1% for O-CH, O-JB, O-L, O-JBr and O-H, respectively). The blastocyst rate observed at 5h of sperm-oocyte-incubation time of IVM oocytes inseminated with Chimera, Japanese Black and Limousin sperm were higher ($P<0.01$) than 20h (38.1% vs 20.0%, 39.0% vs 10.3% and 24.1% vs 8.0% for O-CH, O-JB and O-L, respectively). The proportion of blastocyst formation on Day 7 of IVM oocytes inseminated with Chimera, Japanese Black and Limousin sperm observed at 5h of sperm-oocyte-incubation time were higher ($P<0.01$) than 10h (34.8% vs 4.6%, 36.2% vs 5.9% and 34.6% vs 6.5%, for O-CH, O-JB and O-L, respectively). However, the proportion of blastocyst formation on Day 7 of IVM oocytes inseminated with Japanese Brown and Holstein sperm was not affected by sperm-oocyte-incubation time. In the present study indicate that (1) the optimal time of sperm-oocytes incubation for Chimera, Japanese Black and Limousin sperm is 5h, and (2) incubation longer than 15h resulted in a reduced blastocyst formation. However, the optimal time of sperm-oocytes incubation for Japanese Brown and Holstein is 15h. These results verify that the optimal of sperm-oocyte-incubation time in vitro is different from breed to breed.