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In-Vitro Fertilization and Culture of Pig Oocytes Matured *In-Vitro* by Liquid Boar Sperm Stored at 4℃

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This study was carried out to investigate the effects of liquid boar sperm stored at 4℃ on sperm motility, normal acrosome, and *in-vitro* fertilization and culture of pig oocytes matured in-vitro. The sperm-rich fraction (30~60 ml) of ejaculate was collected into an insulated vacuum bottle. Semen was slowly cooled to room temperature (20~23℃) by 2 h after collection. Semen was transferred into 15 ml tubes, centrifuged at room temperature for 10 min at 800×g, and the supernatant solution was poured off. The concentrated sperm was resuspended with 5 ml of lactose, egg yolk and N-acetyl-D-glucosamine (LEN) diluent to provide 1.0×10^9 sperm/ml at room temperature. The resuspended semen was cooled in a refrigerator to 4℃ and preserved for 5 days to examine sperm motility and normal acrosome. The medium used for oocyte maturation was modified tissue culture medium (TCM) 199. After about 22 h of culture, oocytes were cultured without cysteamine and hormones for 22 h at 38.5℃, 5% CO_2 in air. Oocytes were inseminated with liquid boar sperm stored at 4℃ for 2 days after collection. Oocytes were coincubated for 6 h in 500 ㎕ mTBM fertilization media with 0.2, 1, 5 and 10×10^6/ml sperm concentration, respectively. At 6 h after IVF, oocytes were transferred into 500 ㎕ Hepes-buffered NCSU-23 culture medium for further culture of 6, 48 and 144 h. There were significant differences in sperm motility and normal acrosome among preservation days and incubation times, respectively. The rates of sperm penetration and polyspermy were higher in 5 and 10×10^6 sperm/ml than in 0.2 and 1×10^6 sperm/ml. Male pronuclear formation was lower in 0.2×10^6 sperm/ml than in 1, 5 and 10×10^6 sperm/ml. Mean numbers of sperm in penetrated oocyte were highest in 10×10^6 sperm/ml compared with other sperm concentrations. The rate of blastocysts from the cleaved oocytes (2~4 cell stage) was highest in 1×10^6 sperm/ml compared with other sperm concentrations. In conclusion, we found out that liquid boar sperm stored at 4℃ could be used for *in-vitro* fertilization of pig oocytes matured *in-vitro*. Also, we recommend 1×10^6/ml sperm concentration for *in-vitro* fertilization of pig oocytes.

Key words) ***In-Vitro fertilization, Pig oocyte, Liquid boar sperm***