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Study on the Convenient Freezing Method in Boar Semen

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The purpose of this study was to establish the convenient freezing method for more cheap and simple. Semen quality was evaluated the motility, viability, abnormality, acrosome intactness and membrane integrity. And there were also examined the developmental rates of IVM/IVF embryos using frozen-thawed boar semen in each treatment group.

(1) Boar semen of all experimental groups were frozen until 5℃ for 3 hours using cell freezer and making the straws, and then were frozen by lowing the straws in different steps into styrofoam box above the LN_2. In different freezing methods, sperm motility, viability and abnormality were not differ in 1-step(-102℃ for 10 min) and 3-step(-47℃ for 4 min, -81℃ for 4 min and -137℃ for 2 min). In different pre-freezing temperature and holding times, sperm viability in 1-step(74.0%) was significantly higher than that of any other groups(58.0%, 48.3%, 21.2%, 33.3% and 38.3%, $P<0.05$). In thawing temperature, sperm viability was significantly higher in 37℃ group(60.5%) than in 52℃ group(30.7%). Using the Coomassie Brillient Blue(CBB), Hoechst 33258/Propidium Iodide(H258/PI) staining and Hypoosmotic swelling Test(HOST), the acrosome intactness, sperm survival and membrane integrity were not differ in 1 and 3-step. Employing the Chlortetracycline(CTC)/Hoechst 33258(H258), capacitated and acrosome intact sperm(B-type) and acrosome reaction sperm(AR-type) were 32.5%, 38.8% in 1-step and 39.7%, 25.3% in 3-step, respectively.

In the developmental rate of IVM/IVF embryos using frozen-thawed boar semen, the development rate of morula plus blastocysts were 27.5% in 1-step and 14.7% in 3-step, respectively. The developmental rate beyond morula stage in 1-step was significantly higher than that of 3-step($P<0.05$).

These results indicated that pre-freezing temperature, holding time above the LN_2 and thawing methods affects the semen characteristic, and also the frozen-thawed semen produced by 1 and 3-step affects the developmental rate of IVM/IVF embryos.

Key words: ***Freezing-thawed boar semen, Acrosome intactness, membrane integrity, HOST, IVM/IVF embryos***