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Antioxidant Effect of α-Tocopherol Supplementation on Boar Semen Cryopreservation

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Boar semen is extremely vulnerable to cold shock and sensitive to peroxidative damage due to the high content of unsaturated fatty acids in the phospholipids of the plasma membrane and the relative low antioxidant capacity of seminal plasma. Consequently, this study was conducted to evaluate whether supplementation of α-tocopherol in the semen extender would protect the spermatozoa from oxidative damage during cryopreservation of boar semen. Semen was collected from 4 boars (Duroc, 1~2 years) using gloved-hand technique, filtered through sterile cotton gauze, mixed (1:1) with Beltsville Thawing Solution (BTS) and transferred to the laboratory. Pooled semen was held for 2 h at room temperature, and subsequently centrifuged at 350 ×g for 15 min. After washing twice in BTS, the sperm pellet was diluted (1:1) in lactose-egg yolk (LEY) buffer. Initial cooling was performed from 25℃ to 4℃ during 3 h, and the same volume of lactose-egg yolk-containing 6% glycerol, 2% orvus es paste (LEYG) buffer supplemented with different concentration of α-tocopherol (0, 100, 200, 400, 600 and 800 μM) was slowly added. Semen samples were cryopreserved in 0.5 mL French medium straws using Kryo 360 (Planer series 300, UK), and then transferred into LN_2. Samples were thawed at 50℃ for 12 sec and evaluated for sperm motility using sperm analyser (Leica 500 DM), acrosome integrity by reaction with FITC-conjugated *pisum sativum agglutinin*, apoptosis and necrosis using FACS. Motility of frozen-thawed semen in all treatments was significantly ($p<0.05$) lower than that of fresh semen. However, sperm motility in 600 μM/mL α-tocopherol sup-

plemented sample was significantly ($p<0.05$) higher than in other concentrations. Acrosome damage was observed in all the frozen-thawed semen groups. Most of the fresh semen revealed acrosome intact, but approximately 30% frozen-thawed semen showed partially injured acrosome. In frozen-thawed semen a high incidence of early necrosis and apoptosis was observed. Frozen-thawed semen with 600 μM/ mL α-tocopherol exhibited similar characteristics to fresh semen. Thus, we conclude that supplementation of 600 μM/mL α-tocopherol in the semen extender improved the quality of frozen-thawed boar semen.

Key words) *Semen cryopreservation, α-tocopherol, Sperm motility, Acrosome integrity, Apoptosis, boar*

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