5°C/2 min or 37°C/40 s with cooling rates, the semen was diluted with the same volume of cryo-media in the first thawing temperature and refrozen. Sperm motilities were determined and compared between animals and groups after rethawing. The mean sperm concentration and motility was 45×10^6 mL^{-1} (range 2.3 to 89×10^6 mL^{-1}) and 40% (range 13 to 55%). Mean values of motility and viability of sperm that underwent second preservation were significantly higher in 5°C than in 37°C ($P < 0.01$). However, the activity of viable sperm thawed at 5°C was significantly decreased before refreezing. It is estimated that refreezing of frozen semen from rare Korean native cattle is possible with resistant properties of survived spermatozoa. The higher motility and viability of refrozen semen could be obtained with 5°C thawing procedure for reuse of frozen semen.

36 EFFECT OF cAMP MODULATORS DURING OOCYTE *IN VITRO* MATURATION ON GAP JUNCTIONAL ACTIVITY OF VITRIFIED BOVINE OOCYTES

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Oocyte cryopreservation is a strategic tool for *in vitro* embryo production, but low rates of cryosurvival are reported for bovine oocytes. Simulated physiological oocyte maturation system (Albuz *et al.* 2010 Hum. Reprod. **25**, 12) uses cAMP modulators to increase oocyte competence by the extension of meiosis block and gap junctional communications activity. The aim of this study was to investigate the effect of simulated physiological oocyte maturation system on gap junctional activity of vitrified bovine oocytes. Oocytes from slaughterhouse ovaries were divided into 4 groups: C (control: fresh immature oocytes); V (vitrified immature oocytes); PM-V (vitrified oocytes after a 2-h pre-*in vitro* maturation phase – in the presence of AMPc modulators, 100 μM Forskolin, and 500 μM IBMX); and PM (fresh immature oocytes subjected to pre-*in vitro* maturation). Viable oocytes ($n = 404$ obtained from 4 replicates) were stained with Calcein-AM using the protocol of Thomas *et al.* (2004 Biol. Reprod. **71**(4), 1142–1149) in order to measure gap junctions activity. Images were captured in fluorescence microscope, and fluorescence intensity was analysed with ImageJ software. Mean fluorescence intensity of each group was normalized to control group to obtain relative intensity value. Means were compared by Kruskal-Wallis test and Dunn post-test. A second analysis was performed considering the percentage of each staining pattern (low, middle, and high intensity) for each group. Results were analysed using Fisher exact test. All statistical analysis were performed in GraphPad Instat program with 5% significance level. Results demonstrated that all treatments induced an increase ($P < 0.05$) in fluorescence intensity (V: 1.76 ± 1.13; PM-V: 1.58 ± 0.98; PM: 1.38 ± 0.94) compared with control (C: 1.00 ± 0.48). Regarding the staining patterns analyses, immature vitrified oocytes (V group) differed from control group in middle and low patterns (G1, calibrator – high: 11.2%^{ab}, middle: 43.8%^{a}, low: 44.9%^{a}; G2 – high: 8.2%^{ab}, middle: 63.9%^{b}, low: 27.9%^{b}; G3 – high: 16.3%^{a}, middle: 42.3%^{a}, low: 41.3%^{a}; G4 – high: 6.7%^{b}, middle: 53.9%^{ab}, low: 39.3^{a}). In conclusion, unexpectedly, vitrification also increased gap junctional activity, as was found for pre-*in vitro* maturation group. However, staining pattern analysis results showed only vitrified group was different from control, suggesting vitrified and pre-*in vitro* maturation groups could have gap activity affected by different ways.

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37 DOUBLE FREEZING AND THAWING OF NGUNI BULL SEMEN

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Indigenous bulls semen are important for conservation programs. The objectives of this study were to evaluate the effects of repeated freezing and thawing on sperm motility characteristics. Semen was collected from 4 Nguni bulls by means of electro ejaculator and stored in a thermo flask (37°C). Sperm total motility, progressive and nonprogressive motility, and velocity were assessed using computer-aided sperm analysis before and after freezing. Semen was then diluted with egg yolk citrate extender (fraction A), then followed by 12% of glycerol + egg yolk citrate extender (fraction B, Seshoka *et al.* 2012). Diluted semen samples were equilibrated for 4 h at 5°C. After the equilibration period, samples were loaded into 0.25-mL straws and transferred into a controlled rate programmable freezer. After the target temperature of −130°C was reached, semen straws were stored in a LN tank (−196°C). After 3 months of storage, straws were thawed at 15°C (first and second freezing and thawing followed the same process) for 5 min and further evaluated post-thawed at 0 and 15 min during incubation at 15°C. Treatment means were separated using Fisher's protected *t*-test least. No significant differences were recorded between the raw semen total sperm motility percentage (93.2%) and first frozen-thawed at 0 min (82.6%), with the total sperm motility rate recovery of 88.6%. In addition, there was a marked decline recorded in sperm total motility during the first frozen-thawed at 15 min (77.6%), second frozen-thawed at 0 min (31.3%), and second frozen-thawed at 15 min (30.1%; $P < 0.05$). The sperm curvilinear velocity and average path velocity was reduced following first frozen-thawed ($P < 0.05$) but remained constant and stable between the treatment groups ($P > 0.05$). In conclusion, the freezing-thawing process did not reduce the Nguni bull total sperm motility during the first freezing and thawing process, compared with raw semen. However, a drastic decline was recorded during the second freezing-thawing processes, compared with raw semen.

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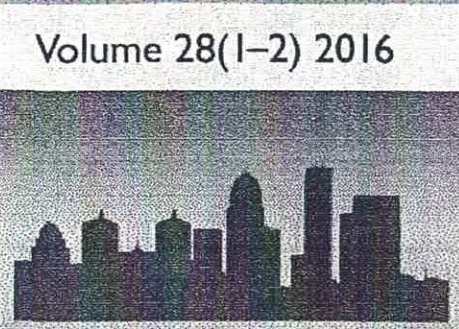

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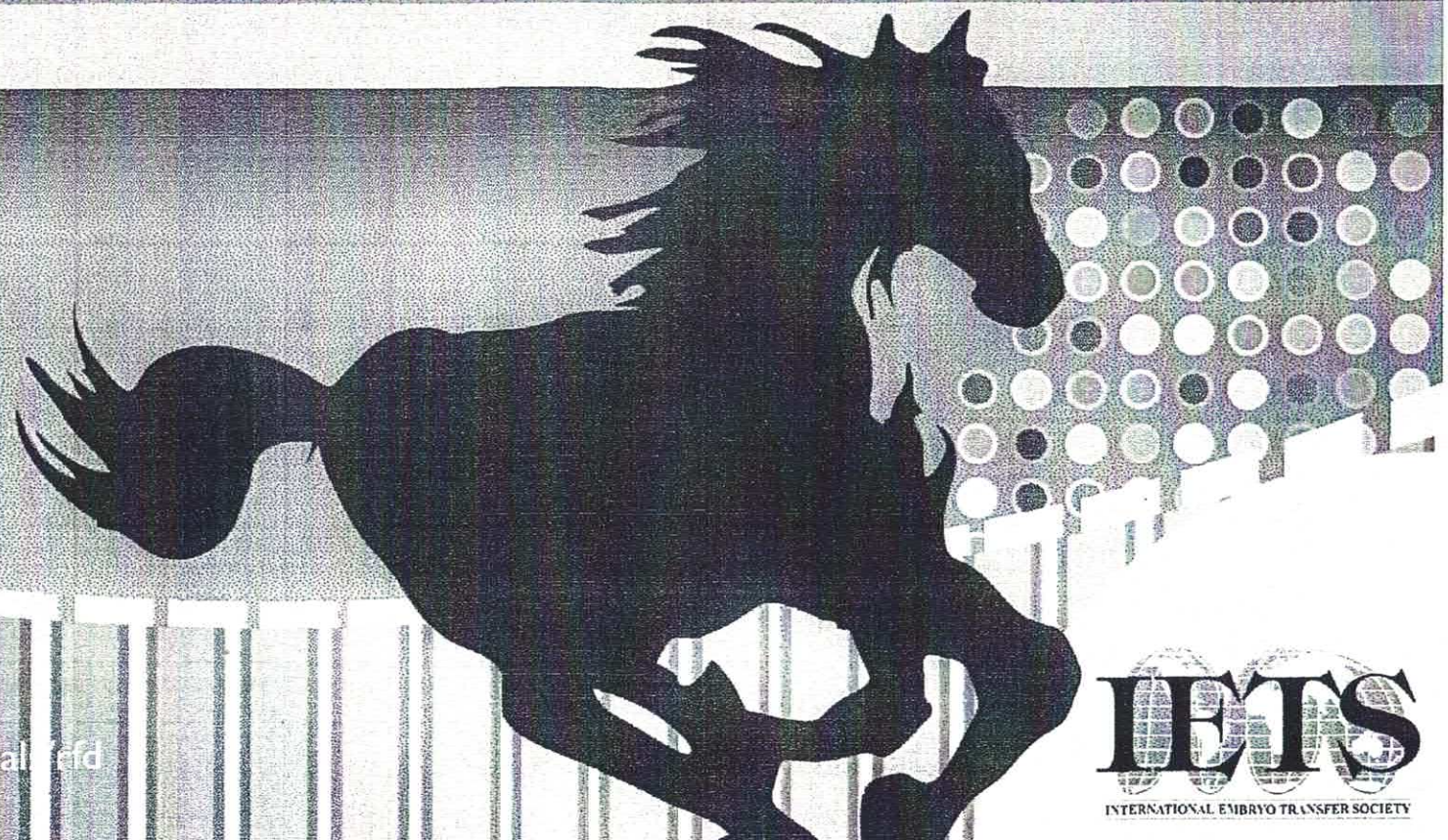

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