program ImageJ 1.14 (Wayne Rasband, National Institutes of Health, Bethesda, MD, USA). The embryos were limited to obtain the area (μm^2), and gray intensity mean (arbitrary units), and gray intensity per area was calculated (arbitrary units/μm^2). Data were analysed by ANOVA with PROC GLM of SAS (SAS Institute, Cary, NC, USA). Sources of variation in the model including treatment and replicas were regarded as fixed and random effects, respectively. Data are presented as mean and standard least-squares error. For all analyzes was adopted the significance level of 5%. There was no difference in blastocyst rate: control (37.0 ± 4.0%), F 2.5 μM (38.6 ± 4.0%), F 5 μM (40.7 ± 4.0%). There were difference in lipids content between all groups: control (136.8 ± 2.2^{ab}); F 2.5 μM (128.5 ± 2.2^{b}), F 5 μM (135.6 ± 2.3^{c}; $P < 0.05$). The F 2.5 μM group showed the higher rate of apoptotic cells compared to other groups: control (12.1 ± 3.5%^{a}), F 2.5 μM (16.7 ± 4.1%^{b}), F 5 μM (11.1 ± 6.5%^{a}; $P < 0.05$). After vitrification, there was no difference in re-expansion: control (71.3 ± 8.9%), F 2.5 μM (73.1 ± 8.9%); F 5 μM (66.1 ± 8.9%) and apoptosis rate: control (22.3 ± 3.1%); F 2.5 μM (37.3 ± 3.8%); F 5 μM (33.2 ± 6.5%) between the groups. The Forskolin was effective at lower concentration to diminish lipids concentrations in embryos. But when we analysed the apoptotic cell, the lower concentration of Forskolin damaged embryos, but this effect could be diminished after vitrification and warming, when the drug did not increase the apoptotic cells. However, we need to study other concentrations of Forskolin.

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166 THE EFFECT OF TEMPERATURE DURING STORAGE OF *IN VITRO*-MATURED BOVINE OOCYTES IN A HEPES-BUFFERED MEDIUM ON DEVELOPMENTAL COMPETENCE

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The objective of this study was to clarify the effect of the temperature during liquid storage in *in vitro* matured (IVM) bovine oocytes. IVM bovine oocytes were stored in Eppendorf tube containing 1 mL HEPES TCM-199 supplemented with 10% (v/v) new born calf serum at different temperatures (4°C, 15°C, 25°C, and 38.5°C) for 20 h. The developmental rates of stored and not stored (control) oocytes to the blastocyst stage, cell numbers in resultant blastocysts, and fertilization normality were evaluated after *in vitro* fertilization and *in vitro* culture. The ATP content, reduced glutathione (GSH) content, and apoptosis rates in oocytes were also determined in stored and control groups. At least 3 replicates were conducted for each experiment. The data were analysed by 1-way ANOVA followed by *post hoc* Fisher's protected least significantly difference test. Percentage data were transformed to arc-sine before analysis. All of the storage groups (4, 15, 25, and 38.5°C groups, respectively) showed significantly lower blastocyst developmental rates (8.5, 14.9, 19.3, and 24.5%, respectively) compared with the control group (39.8%; $P < 0.05$). Within the storage groups, the 25°C and the 38.5°C groups exhibited the greatest rate of blastocyst formation. In contrast, the total cell number of the 38.5°C group was significantly lower than that of control group ($P < 0.05$), whereas that of the 25°C group was similar with the control group. The frequency of normal emission of the second polar body (2PB) was significantly greater in the control group compared with the storage groups ($P < 0.05$). The 2PB emission rate was significantly lower in the 38.5°C group compared with the 4°C group ($P < 0.05$) but not different from those of the 15°C and 25°C storage groups. The percentage of male pronuclear formation in the control group was significantly higher than those in the stored groups ($P < 0.05$) except for the 25°C group. During storage at 4°C, the ATP content was significantly decreased compared with the control group (1.3 *v.* 1.7 pmol; $P < 0.05$); however, in the 25°C and 38.5°C groups, the ATP content (2.0 and 1.9 pmol, respectively) was significantly higher than that in the control group (1.7 pmol; $P < 0.05$); whereas the 15°C group showed the same ATP level compared with the control group. Storage of oocytes for 20 h reduced the GSH content compared with the control group without storage ($P < 0.05$); however, there were no significant differences among storage groups. Annexin-V staining revealed increased incidences of early apoptotic oocytes in the 4°C and 15°C groups ($P < 0.05$) compared with other groups. In conclusion, based on the embryo developmental competence of stored oocytes and quality of resultant blastocysts, 25°C was determined as the most suitable temperature for temporal storage of matured bovine oocytes.

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167 EFFECT OF DNA METHYLATION INHIBITOR ON HETEROCHROMATIN IN BOVINE EMBRYOS DERIVED FROM HEAT-SHOCKED OOCYTES

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The cell response to stress involves epigenetic modifications in order to regulate the gene expression, which is dependent of chromatin structure and DNA methylation status. On the other hand, changes on DNA methylation can have an effect on chromatin organisation (Espada and Esteller 2010 Semin. Cell. Dev. Biol. **21**, 238–246). In this study we evaluated the effect of 5-aza-2′-deoxycytidine (5-aza; Sigma, St. Louis, MO, USA), a DNA methylation inhibitor, on heterochromatin 1 β formation of bovine pre-implantation embryos derived from oocytes that did or did not undergo heat shock during *in vitro* maturation (IVM). Oocytes were IVM under 38.5°C for 24 h (non-heat-shock: NHS group) or under 41.5°C for 12 h followed by

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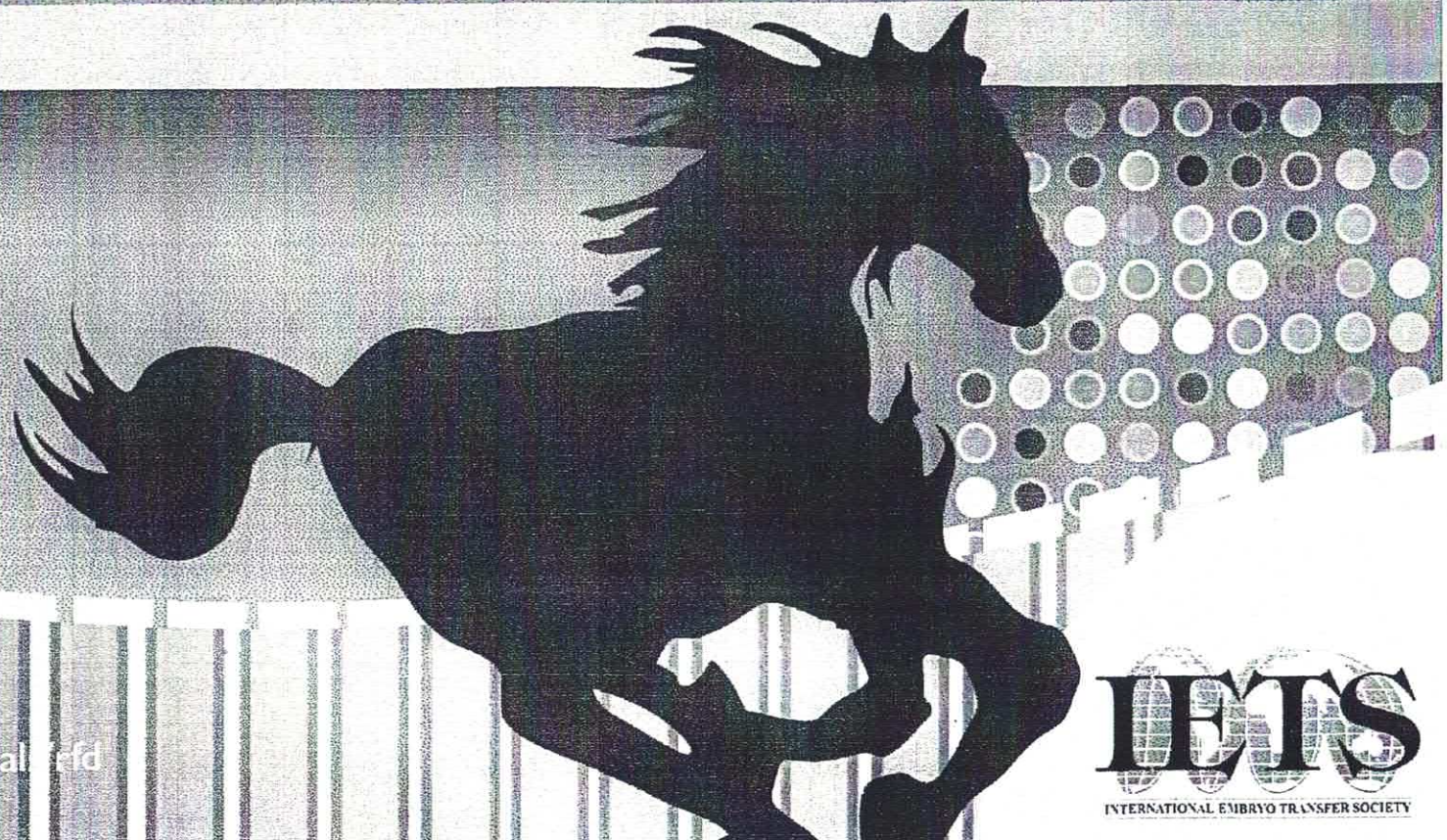

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