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77 EFFECT OF HEAT SHOCK DURING *IN VITRO* MATURATION ON HETEROCHROMATIN COMPACTION IN BOVINE EMBRYOS AT 4- AND 8-CELL STAGES: PRELIMINARY STUDY

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High temperatures cause several reproductive losses in cattle. Under *in vitro* conditions, heat shock decreases oocyte developmental competence and influences embryonic gene expression (Gendelman and Roth 2012 Anim. Reprod. Sci. **134**, 125–134). This preliminary study aimed to evaluate whether heat shock during oocyte *in vitro* maturation (IVM) could have any further effect on chromatin remodelling of fertilized embryos at 4- and 8-cell stages, once such modifications are required for the gene activation in bovine embryos. We evaluated the distribution of heterochromatin 1 (HP1β) and of histone H3 trimethylated at lysine 9 (H3K9me3), both reportedly correlated with heterochromatin formation, in 4- and 8-cell stage embryos derived from control (C) and heat-shocked (HS) bovine oocytes. Immature cumulus-oocyte complexes (COC) collected from crossbred cows in Brazil were exposed for 12 h to 38.8°C (C group) or 41.0°C (HS group) followed by 12 h at 38.8°C, totalizing 24 h of IVM at 5% CO_2 in air. Oocytes were *in vitro* fertilized (IVF) with non-sexed sperm and denuded zygotes were *in vitro* cultured in CR2aa medium at 38.8°C and 5% CO_2, 5% O_2 and 90% N_2. Four- and 8-cell embryos at 44 h post-IVF were fixed in 4% paraformaldehyde and stained with anti-mouse HP1β and anti-rabbit H3K9me3 first antibodies. Immunofluorescence was evaluated by confocal microscopy (Zeiss LSM 700, MIMA platform, INRA) and 3D images processed by ZEN Lite software (Zeiss, Jena, Germany). Three different distribution patterns of fluorescence were identified based on morphological criteria: diffuse, little clusters, and big clusters. Proportions of embryos in every distribution pattern were compared between C and HS groups by Chi-squared test. No difference ($P > 0.05$) on cleavage rate was found between C and HS groups until 44 h post-fertilization. Embryos at the 4-cell stage from HS group displayed an increased ($P < 0.01$) proportion of nuclei with H3K9m3 big clusters (44%, $n = 7/16$ embryos), whereas embryos from C group displayed only few nuclei with this pattern (5%, $n = 1/18$). At the 8-cell stage, distribution of H3K9m3 was similar ($P > 0.05$) between C and HS groups. For HP1β, embryos at the 4-cell stage from HS group displayed an increased ($P < 0.05$) proportion of nuclei with little clusters (81%, $n = 13/16$ embryos), whereas embryos from C group had low proportion of nuclei with this same pattern (40%, $n = 7/18$). Mostly 4-cell stage embryos from C group presented the diffuse pattern (61%, $n = 11/18$ *v.* 18%, $n = 3/16$ in the HS group; $P < 0.05$). At the 8-cell stage, some embryos from the C group (31%, $n = 5/16$) still showed nuclei with diffuse distribution of HP1β, whereas no nucleus with this pattern was found for the HS group. These preliminary data suggest that bovine embryos derived from heat-shocked oocytes can display precocious heterochromatin compaction, represented by the accumulation of H3K9me3 and HP1β at the 4-cell stage, compared with embryos derived from non-heat-shocked oocytes, which may affect embryonic genome activation with consequences for further gene expression.

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78 NONINVASIVE CELL LINEAGE TRACING IN BOVINE EMBRYOS FROM 2-CELL STAGE UP TO BLASTOCYST STAGE

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The first lineage specification occurs during pre-implantation mammalian development. At the blastocyst stage, 2 cell lineages can be distinguished: the inner cell mass (ICM) and the trophectoderm (TE). The exact timing when embryo cells are skewed to these lineages is not clearly determined in mammalian species. In murine embryos, it has been suggested that the first cleavage plane might be related to the embryonic-abembryonic (Em-Ab) axis at blastocyst stage. Thus, the daughter cells of the 2-cell embryo might already be predisposed to a specific cell lineage further on development. The objective of the present study was to observe how the first cleavage in bovine embryos may be related to cell lineage allocation at the blastocyst stage, using a noninvasive tracing approach. Bovine oocytes were harvested, *in vitro* matured, and fertilised. At the 2-cell stage, embryos were injected in one blastomere with the membrane tracer DiI. At the blastocyst stage, embryos ($n = 346$) were classified as orthogonal when the Em-Ab axis was orthogonally divided by the borderline between labelled and non-labelled cells; as deviant if the borderline was overlapping the Em-Ab axis; and as random when the labelled and non-labelled cells were randomly distributed. Total cell count (TCC) and the ICM/TE ratio was allowed by DNA staining with 4′,6-diamidino-2-phenylindole (DAPI) and by immunostaining of the ICM with Sox2 antibody. Analysis of variance was performed by one-way ANOVA employing IBM SPSS v21 (SPSS Inc., Chicago, IL, USA) to determine any difference between the cell lineage allocation patterns, TCC, and the ICM/TE ratio. *P*-values = 0.05 were considered significant. All values are reported as mean ± standard error of mean. Within 40 repetitions, the blastocyst classification was as follows: orthogonal 14.9% (±2.32, $n = 56$), deviant 22.2% (±2.58, $n = 80$), and random 62.9% (±2.64, $n = 210$). A significant difference was found in the incidence between the random group against the orthogonal and deviant, but not between the latter two. Regarding TCC, a significant difference was observed only between the orthogonal (99.6 ± 11.7 cells, $n = 15$) and deviant (135 ± 7.3 cells, $n = 25$) groups, but not with random embryos (116 ± 5.5 cells, $n = 42$). Finally, no significant difference was found among the groups concerning the ICM/TE ratio (0.43 ± 0.07 for orthogonal, $n = 7$; 0.54 ± 0.06 for deviant, $n = 14$; and 0.40 ± 0.03 for random embryos, $n = 26$). In conclusion, bovine embryos present a marked tendency for a random distribution of the daughter cells derived from the 2-cell blastomeres. However, around 37% of the blastocysts present a patterned cell division, where the daughter cells remain together through pre-implantation development. The effect of these cell lineage allocation patterns on implantation and further embryo development needs to be addressed.

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