RESEARCH ARTICLE

69 GLOBAL H3k9ac AND H3k27me3 EXPRESSION IN BLASTOMERES FROM 8- TO 16-CELL STAGE BOVINE EMBRYOS

C. S. Oliveira [A B] , N. Z. Saraiva [B] , L. Z. Oliveira [C] , R. V. Serapião [D] , M. R. de Lima [B] and J. M. Garcia [B]

+ Author Affiliations

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Abstract

Embryonic genome activation is a crucial step in early embryo development, and is accompanied by a dramatic change in the epigenetic profile of blastomeres. Histone modifications related to euchromatin and heterochromatin can be important parameters to infer developmental competence, as they are affected by manipulation and environmental stress conditions. The aim of this study was to characterise permissive (H3k9ac) and repressive (H3k27me3) histone modifications during the embryonic genome activation cell cycle in bovine embryos, regarding correlation between those marks and variance among blastomeres. For that, bovine embryos were produced by IVF and cultured in SOF medium supplemented with 5 mg mL^{-1} of BSA and 2.5% FCS in 5% O_2 in an air atmosphere for 5 days (70 h after IVF). The 8 to 16 cell embryos were fixed in 4% paraformaldehyde and submitted to H3k9ac and H3k27me3 immunofluorescence assay (mouse anti-H3K9ac monoclonal antibody, 1 : 200; Sigma; rabbit anti-H3k27me3 monoclonal antibody, 1 : 200; Upstate, Charlottesville, VA, USA). Nuclei were counterstained with Hoechst 33342. Images of each embryo were captured (AxioCam, Carl Zeiss, São Paulo, Brazil) and measured for nuclear fluorescence intensity in each blastomere using Adobe Photoshop CS3 (Adobe Systems, San Jose, CA, USA). Mean levels were compared using the Mann-Whitney test and variances were compared using *F*-test (SAS 9.1, SAS Institute Inc., Cary, NC, USA; $P = 0.05$). We evaluated 2 replicates and 12 embryos during the transition from the 8 to 16 cell stages, totaling 169 blastomeres. Global H3k27me3 levels varied accordingly to H3k9ac levels, as indicated by a high Pearson correlation coefficient ($r = 0.913$). Levels of each blastomere were normalized to the lowest level obtained within each embryo. Some embryos displayed a high variation between blastomeres, and, for further analysis, we divided the embryos into groups: group A for embryos that presented similar H3k9ac levels between blastomeres (8 embryos, 66%), and group B for embryos that exhibited higher heterogeneity between blastomeres (at least 2 blastomeres presenting a 2-fold increase compared to the lowest blastomere; 4 embryos, 33%). Mean H3k9ac and H3k27me3 normalized levels were lower for group A [H3k9ac: 1.35 ± 0.29 (A), 1.94 ± 1.02* (B); H3k27me3: 1.33 ± 0.24 (A), 1.99 ± 0.77 (B)], and group A displayed lower variance values (H3k9ac: 0.07 (A), 1.05* (B); H3k27me3: 0.06 (A), 0.60 (B)]. Within each embryo, blastomeres were sorted in ascending order for H3k9ac level (1 to 16), and compared between groups A and B. We detected that mean levels differed ($P < 0.05$) between groups from blastomere 9 to 16 for H3k9ac and 10 to 16 for H3k27me3. Therefore, in 8- to 16-cell stage embryos, the H3k27me3 repressive mark is highly correlated with the H3k9ac permissive mark. Also, our results describe the presence of 2 distinguishable populations of bovine embryos at this stage, considering their epigenetic status. One population presented similar levels of repressive and permissive marks among blastomeres, whereas the second one displayed a remarkable variation among their blastomeres. This observation should be further studied, as it might reflect distinct cleavage pattern embryos and blastomere competence.

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