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Zinc Supplementation Influences 8-cell Stage Global Transcriptional Activity And Improves Preimplantation Embryo Development In Cattle.

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One of the unsolved questions in mammalian early embryogenesis relates to the morpho-functional and molecular processes that drive transcriptional reactivation of the embryonic genome after fertilization. This gap of knowledge limits the ability to recapitulate this transition in vitro and further improve the efficiency of in vitro embryo production. In this study, we tested the hypothesis that zinc, emerging as a key component in several conserved processes regulating female germ cell growth, fertility, and pregnancy, is a critical determinant of transcriptional regulation in bovine early embryogenesis. To this end, immature oocytes collected from abattoir-derived ovaries were subjected to standard in vitro maturation and fertilization, and then cultivated in control conditions, which do not include the supplementation of trace elements (control) or in the presence of two concentrations of zinc (0.15 or 1.5 µg/ml) in the form of zinc sulfate. Zinc concentrations were chosen based on the content in the follicular fluid and the female genital tract. In the first set of experiments, the global transcriptional activity was directly assessed by uridine incorporation with a commercial Imaging Kit in embryos cultured in the absence (control) or in the presence zinc after 64 hours of culture when bovine embryos typically reach the 8-cell stage. Whole mount samples were imaged using an EclipseE600 fluorescence microscope equipped with a Sight DS-U3 camera (Nikon) at 40X magnification. For each embryo, multiple images were acquired at different focal planes, using the nuclear staining with DAPI as a reference. Only embryos whose nuclei corresponded to the number of blastomeres observed under bright field conditions were considered, while embryos showing multinucleated or anucleated blastomeres were discarded. A total of 478 blastomeres (of 66 embryos at the 5-8 cell stage) and 312 blastomeres (of 28 embryos at the > 8 cell stage) were included in the analysis. The blastomeres' fluorescence intensity was calculated with ImageJ after background subtraction. Data analysis showed that transcriptional activity levels of the blastomeres did not significantly differ between the control and the treatment with a higher zinc concentration. On the contrary, there was a significant increase in levels of transcriptional activity when low concentrations of zinc were used (Two-way ANOVA followed by Tukey's multiple comparison test, $p<0.05$). Subsequently, we tested the effect of supplementation of the two doses of zinc on the ability to reach the blastocyst stage of development after 8 days of culture. A total of 584 oocytes in 5 biological replicates were included in the study. Data analysis showed that the low concentration promotes a higher blastocyst rate when compared to the higher one (Kruskal-Wallis followed by Dunn's multiple comparison test, $p<0.05$), while the control group showed intermediate blastocyst rates. In conclusion, our studies indicate that zinc supplementation has a dose-dependent effect on the ability of the embryo to develop in vitro.

The increase in developmental competence obtained in the presence of low concentrations of zinc appears to be correlated to a stimulation of the global transcription process of the embryo. Further studies are necessary to identify which molecular mechanisms govern this process.

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