Animal Reproduction

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Zinc sulfate attenuates the negative effects of Roundup on in vitro bovine oocyte maturation

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Roundup (R©) is the most used herbicide; glyphosate, its active ingredient in doses >50μg/ml has anti-reproductive effects acting mainly through promoting endocrine disruptions, oxidative stress and apoptosis. Here, we examined if very low doses of R© (1ppm, equivalent to 0.36μg/ml of glyphosate) affect developmental competence of in vitro matured-fertilized bovine oocytes and if Zn, in the form of ZnSO4, can reverse the negative effects of R© acting as antioxidant. The selected dose of R© was more than 20 times lower than the mean R© levels found in cases of very mild R© intoxications in humans. In 5 replicates, follicles from abattoir derived cow ovaries were aspirated and COCs (n=1324) were allocated in 4 groups. The maturation medium (IVM) was either the standard medium (TCM199, plus 10%FCS and 10ng/ml EGF, group C n=339), or modified with the addition of: 1ppm R© (group R, n=425), 0.82μg/ml of Zn (group Z, n=276), R© and Zn (group RZ, n=284). The COCs were matured for 24 hours at 39oC in 5%CO2 in maximum humidify. Matured oocytes were fertilized with coincubation with frozen-thawed semen (106 sperms/ml), and 20 hours post insemination (pi), the presumptive zygotes were denuded, and the embryos were cultured in 25μl droplets (SOF supplemented with 5% FCS) covered by mineral oil, at 39oC in 5% O2, 5%CO2 and maximum humidity. Cleavage and blastocyst formation rates were evaluated 24 h pi and on days 7,8, and 9, respectively. In each replicate, groups of 5 day7 blastocysts, were snap frozen for molecular studies. The expression of 6 genes related to oxidation (SOD2, GPX1), epigenetic regulation (DNMT1, DNMT3A) and apoptosis (BCL2, BAX) was measured by Real Time PCR. Cleavage and embryo yield were analyzed by ANOVA, while gene expression was analyzed by R Studio using permutation analysis, imbedding in the package lmperm. Cleavage rate in group R (76.7±7.2) was lower ($p<0.01$) than C (87.5±6.6), while in group ZR (77.5±9.7) tended ($p=0.06$) to be lower than C; no other differences were detected among groups. Blastocyst formation rate in group R was steadily lower compared to those of groups C and Z ($p<0.03$). On day 7 blastocyst formation rate in group ZR (17.0±3.2) was lower ($p=0.03$) than in C (23.3±4.1), while on day 8 (21.9±5.3) tended ($p=0.06$) to be higher than that of R (15.4±5.1) and did not differ from C (28.0±8.4). Significant ($p<0.05$) changes were detected in the expression of SOD2, GPX1 and DNMT1. The expressions of antioxidant genes, as well as DNMT3A were the highest ($p<0.05$) in group R, while the combination of Zn with R© (group RZ) mitigated the negative effects, leading to lower expression of the antioxidant genes. These results imply that R©, even at very low doses, induces oxidation disrupting fertilization and embryo development, which can be partly prevented by providing anti-oxidative