Effect of glycine on *in vitro* production of sheep embryos: preliminary results

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Reactive oxygen species (ROS) are involved in a series of degenerative processes, due to the property of generating free radicals. The formation of ROS in the *in vitro* embryo production (IVP) is a significant obstacle, requiring the use of antioxidants such as glycine. Therefore, the objective of this study was to test the effect of three concentrations of glycine (3, 6 and 12 mM) in the *in vitro* maturation medium of ovine oocytes on the *in vitro* production of embryos. Oocytes from ovaries from a local slaughterhouse were collected. For the oocyte collection, the follicular aspiration method was used, with an 18G needle coupled to a vacuum pump. Right after collection, the oocytes were poured into petri dishes, analyzed under a stereomicroscope and forwarded to *in vitro* maturation (IVM), where four experimental groups were tested: CON group, where the COCs were immersed in TCM-199, supplemented with sodium pyruvate, fetal bovine serum, antibiotic-antimycotic solution, FSH / LH and 100 mM cysteamine; GLI3, GLI6 and GLI12 groups, where the COCs were included in the medium of the CON group, replacing cysteamine by 3 (GLI3), 6 (GLI6) and 12 (GLI12) mM of glycine, respectively. The drops of IVM were covered with mineral oil and incubated for 24 hours, at 38.5°C, with 5% of CO_2. Matured oocytes were evaluated for the degree of expansion of the cumulus cells, being classified as: High, Moderate and Mild and then destined for *in vitro* fertilization (IVF), where they were placed in drops with IVF medium, together with selected spermatozoa and capacitated in SOF medium supplemented with 10 µg/mL of sodium heparin and 10%(v/v) of FBS. After the evaluation of the presumptive zygotes, they were switched to *in vitro* culture medium (IVC) composed of SOF medium supplemented with BSA and cleavages in D1 and D2 were evaluated. The conditions of the IVC were the same as for IVM and IVF. Data were submitted to Analysis of Variance, followed by Tukey's Test and Fisher's Exact Test, with a significance level of 5%. Considering the comparison between the treatment groups regarding the presence and absence of expansion of cumulus cells, there was no significant difference. Regarding the comparison within the treatment groups, there was no significant difference regarding the presence and absence of cumulus cells for the CON (with expansion: 11.00±2.82; without expansion: 0.50±0.35), GLI3 (with expansion: 7.50±2.47; without expansion: 3.00±2.12) and GLI12 (with expansion: 9.00±1.41; without expansion: 1.50±1.06) groups. However, within the GLI6 treatment group, a significant increase in oocytes with expansion of cumulus cells was observed (with expansion: 9.50±1.06; without expansion: 1.00±0.70). Analyzing the comparison between the treatment groups regarding the degree of High, Moderate and Mild expansion, there was no significant difference. Regarding the comparison within each treatment group, there was no significant difference in the CON (High: 4.5±1.76; Moderate: 5.0±1.41 and Mild: 1.5±0.35), GLI3 (High: 2.0±0.70; Moderate: 4.5± 1.76 and Mild: 1.0±0.0) and GLI12 (High: 1.5±0.35; Moderate: 6.0±0.70 and Mild: 1.5±1.06) groups. However, within the GLI6 treatment group, a greater number of oocytes with degree of expansion mild was observed (High: 1.5±0.35; Moderate: 2.0±0.0 and Mild: 6.0±0.70). Regarding the number of presumptive zygotes (CON: 5.00±0.70; GLI3: 3.00±0.70; GLI6: 5.00±0.70 and GLI12: 2.00±0.0) and number of cleaved structures (CON: 8.50±2.47; GLI3: 6.00±2.82; GLI6: 6.50±0.35 and GLI12: 2.00±0.0), there was no significant difference. With these results, it can be concluded that, to date, the addition of glycine in the IVM medium does not improve the oocyte rate with expansion of cumulus cells. With regard to presumptive zygotes and cleaved structures, glycine may become an option for improving the quality of oocytes and, consequently, the in vitro production of ovine embryos. Later sessions will likely elucidate some questions that have not yet been answered.

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