Animal Reproduction

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Effects of forskolin supplementation at different stages of IVP on the preimplantation development of bovine embryos

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Resumo

Lipid accumulation is commonly observed in IVP embryos. It is used as a parameter to assess the embryonic quality and has a direct impact on cryosurvival. Supplementing culture media with metabolic regulators is a promising strategy to improve cryopreservation results, however, the ideal period time to supplement these agents in IVP has not yet been defined. The objective of this work was to evaluate the effects of forskolin (FO), a lipolytic agent that increases the levels of cAMP in cells, on cleavage and blastocyst (D7) rates, aiming at future increases in cryopreservation rates, by reducing lipid content. Oocytes obtained from slaughterhouse ovaries were in vitro matured (TCM199 with 10% FCS, hormones and sodium pyruvate) and fertilized (Talp-FIV with 0,6% BSA). Presumptive zygotes were partially denuded and cultured in SOF medium supplemented with 1.5% FCS, forskolin (10mM) and L-carnitine (1mM), following previously defined protocol (Lima, M.R. Thesis (Doctorate) - 74 p., 2015, http://hdl.handle.net/11449/136752) at 38.5°C in 5% CO2, 5% O2, 90% N2. FO was supplemented during IVM and/or at D3 (72h after fertilization) in the followings concentrations: 0mM (control), 10mM (treat.1) and 15 mM (treat.2). Experimental groups were: A: IVM(control)/D3(control); B: IVM(control)/D3(treat.1); C: IVM(control)/D3(treat.2); D: IVM(treat.1)/D3(control); E: IVM(treat.1)/D3(treat.1); F: IVM(treat.2)/D3(control); G: IVM(treat.2)/D3(treat.2). Five replicates were performed, totaling approximately 100 oocytes per group. Statistical analyses were performed in GraphPad Prism 9 software. Proportions were analyzed by Chi-Square Test ($\chi 2$). We detected that groups B (56/64 – 92,2%a) and F (85/95 – 89,5%a), despite being numerically higher in terms of cleavage rate, did not differ from the control group (58/71 – 81,7%ab). However, increased rates were detected for B and F groups in relation to other groups (C: 58/77 – 75,3%b; D: 81/107–75,7%b; E: 94/120 – 78,3%b; G: 63/116 – 54,3%c). Regarding blastocyst rates, B group (39/64 –60,9%a) presented similar rates in comparison to control (42/71 – 59,2%ab), with were higher than other groups (C: 34/77 – 44,2%bc; D: 45/107 – 42,1%c; E: 45/120 – 37,5%c; F: 36/95 – 37,9%c; G: 40/116 – 34,5%c). We concluded that the addition of 10mM forskolin in D3 does not harm embryonic development. Improvement of embryonic quality and cryosurvival is expected, which will be assessed in the following steps of this study.

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