Proceedings of the 29th Annual Meeting of the Brazilian Embryo Technology Society (SBTE); Gramado, RS, Brazil, August 20th to 23rd, 2015, and 31st Meeting of the European Embryo Transfer Association (AETE); Ghent, Belgium, September 11th and 12th, 2015. Abstracts.

A298 Cryopreservation and cryobiology, diagnosis through imaging, molecular biology and "omics"

Effect of handmade biopsy on bovine embryos at the sixth day post *in vitro* fertilization

C.C.R. Quintão, E.D. Souza, C.S. Oliveira, C. Freitas, J.A.S. Oliveira, L.S.A. Camargo

EMBRAPA Gado de Leite.

Keywords: biopsy, embryos, in vitro.

The genomic selection is a useful tool for animal breeding and it can be applied for in vitro produced bovine embryos through the analysis of embryo cells. However, it is necessary to evaluate the viability of the embryos biopsied at early stages. The aim of this work was to evaluate the impact of the biopsy technique without micromanipulation devices on development and further quality of embryos at the sixth day after in vitro fertilization. Oocytes obtained from ovaries collected at slaughterhouse were in vitro matured and fertilized. Followed in vitro fertilization, the presumptive zygotes were denuded and cultured in CR2aa medium supplemented with 2.5% SFB at 38.5ºC in an humidified atmosphere with 5% CO_2, 5% O_2 and 90% N_2. Embryos (morulas and early blastocysts) at the sixth day post-fertilization (D6) were randomly distributed in two groups: G1-control (n=61) and G2-biopsy (n=64). Embryos in drops of 20 L of TALP-HEPES with 2% SFB were sectioned by hand using microblades (Bioniche, Canada) under stereoscope microscope. After that, single embryos were cultured in drops of 20 L CR2aa. The blastocyst rate was evaluated on day seven (D7) and day eight (D8) post fertilization (24h and 48h after biopsy, respectively). On D8, the blastocysts from both groups were fixed and available by TUNEL assay (Promega, USA) for total cell number and number of apoptotic cells and then calculated the apoptotic index. The embryo development (blastocyst rate at D7 and D8) was analyzed by Qui-square. The total cell number, number of apoptotic cells and apoptotic index were analyzed by the t-Student test and their values are shown as mean +SEM. When only biopsied embryos at early blastocyst stage on D6 were analyzed, the blastocyst rate in G2 was lower ($P<0.01$) on D7 (43.5%) and on D8 (30.7%) than in G1 (78.7% and 67.8% on D7 and D8, respectively) but when the data of morula and blastocysts were grouped, the effect of biopsy on embryo viability was only perceived ($P<0.05$) on D8 (78.5% vs 43.5% for G1 and G2, respectively). There was no difference ($P>0.05$) in the total cell number between G1 and G2. However, the number of apoptotic cells was higher ($P<0.05$) in G1 than in G2 (29.4±4.5 vs 10.0±1.5, respectively). Similar finding was observed for the apoptotic index (27.5±3.9 vs 9.7±1.3, for G1 and G2, respectively, $P<0.01$). We concluded that the handmade biopsy in embryos on D6 influences the embryo development, but it doesn't affect the total cell number in blastocysts on D8, i.e, 48h post-biopsy. The lowest apoptotic index found in biopsied embryos may be due to the unnoticed withdrawal of cells from the inner cell mass during the biopsy, since this site display great number of apoptotic cells.

Financial support: CNPQ, Fapemig and Embrapa.