Animal Reproduction

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SUPPORT BIOTECHNOLOGIES CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSIS THROUGH IMAGING, MOLECULAR BIOLOGY, AND "OMICS"

Handmade biopsy for genotyping of *in vitro*-produced bovine embryos

Clara Slade Oliveira[1], Carolina Capobiango Romano Quintão[1], Júlia Medeiros Cruz de Lima Martins[1], Marcos Vinícius Gualberto Barbosa da Silva[1], Agostinho Jorge dos Reis Camargo[2], Luiz Fernando Feres[3], Marco Antônio Machado[1], Celio Freitas[1], Naiara Zoccal Saraiva[1], Luiz Sérgio de Almeida Camargo[1]

[1]Empresa Brasileira de Pesquisa Agropecuária (*EMBRAPA Gado de Leite*)
[2]Empresa de Pesquisa Agropecuária do Estado do Rio de Janeiro (*Pesagro-RIO*)
[3]Universidade José do Rosário Vellano (*UNIFENAS*).

E-mail: clara.oliveira@embrapa.br

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Genomic selection has been used to accelerate the genetic gain in livestock in several countries. In cattle, based on genomic estimated breeding value (GEBV) young bulls and cows can be selected. In order to shorten the generation interval, embryos can also be selected based on their GEVB, enhancing the genetic gain. However, embryo biopsy usually requires equipment for embryo manipulation in a well-established laboratory. This study aimed to evaluate an inexpensive and simple procedure of biopsy performed by hands (herein called handmade embryo biopsy) on *in vitro*-produced embryos using a splitting microblade and a stereoscope. Results were statistically analyzed in SAS 9.1 software, adopting a 95% confidence level. Rates were compared by chi-square and means were compared by analysis of variance among groups. In the first experiment, crossbred *Bos taurus x Bos indicus* blastocysts at day 6 or 7 after *in vitro* fertilization (IVF) were biopsied and cultured *in vitro* for 48 h (n = 223, 28-79 per group, 4 replicates). Biopsy reduced ($P<0.01$) embryo development as noted by the lower blastocoel formation/re-expansion rates on both day 6 (34.2% *vs.* 71.4%) and day 7 (66.6% *vs.* 89.8%) blastocysts, when compared to non-biopsied control embryos, respectively. Biopsy on day 7 after IVF decreased ($P<0.01$) total cell number (112.8 ± 6.3 *vs.* 149.9 ± 5.6) and increased ($P<0.01$) apoptotic index (14.9 ± 1.4 *vs.* 6.2 ± 1.2). In the second experiment crossbred *Bos taurus x B.os indicus* blastocysts on day 7 post IVF were produced and biopsied in the farm and then cultured for 3 h in buffered medium on a warm plate (n = 96, 46-50 per group, 3 replicates). Blastocysts with re-expanded blastocoel were then transferred to synchronized recipients. Blastocoel re- expansion rate after 3h in culture was 78.3 . Pregnancy rate (58.7% *vs.* 62%), birth rate (52.1% *vs.* 56%) and birthweight (29.6 ± 2.0kg *vs.* 30.07 ± 1.2kg) were similar ($P>0.05$) between handmade biopsy and non-biopsied control embryos, respectively. The whole genome amplification of DNA obtained from biopsies samples resulted in 89.6 ± 46.6 ng/µL DNA, with fragment length ranging between 2.3 ± 0.6 and 5.6 ± 0.6 kb. In conclusion, handmade biopsy can be performed in *in vitro*-produced blastocyst without compromising pregnancy, calf delivery and birth weight. Cell samples can be suitable for downstream application as genotyping. This procedure is suitable for small laboratories and at farm level without requiring expensive equipment, which contributes to reducing costs and making embryo genomic selection more affordable for farmers.