141 BIOPSY TECHNIQUE IN BOVINE EMBRYOS PRODUCED *IN VITRO* AT EARLY STAGES OF DEVELOPMENT: EVALUATION OF QUALITY AND DEVELOPMENT CAPACITY

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Reproduction, Fertility and Development 20(1) 151 - 151
Published: 12 December 2007

Abstract

One of the causes of embryo mortality is chromosome abnormalities that occur during gametogenesis, fertilization, and embryo early development. Thus, a combination of morphological standards and techniques of molecular analyses could identify abnormal embryos. Preimplantation genetic diagnosis (PGD) is an emergent technology for use with farm animal embryos. With this procedure, blastomeres are removed by the biopsy of embryos at the 8- to 16-cell stage to provide cells for analyses of chromosome abnormalities prior to transfer. The aim of this study was to evaluate the effect of biopsy in bovine 8- to 16-cell embryos fertilized *in vitro* on embryo quality and subsequent development *in vitro*. A group of 706 oocytes were obtained from slaughterhouse ovaries, matured, and fertilized *in vitro* at 38.8°C with 95% humidified air and 5% CO_2. The zygotes were semi-denuded and cultured in $CR2_{aa}$ medium under the same conditions as for *in vitro* fertilization. The rate of cleavage was 78.20%. Three days after fertilization, part of the 8- to 16-cell (298/706) embryos were distributed randomly across two groups: control (n = 103) and biopsy (n = 92) of blastomeres, and then returned to *in vitro* embryo culture to evaluate development until the blastocyst stage and the capacity to hatch. The amount of cells removed was one-fourth of the embryo. The blastocyst rate was evaluated on Day 8 after fertilization and the hatching rate on Day 10. Embryo morphology and quality were evaluated as previously described in the International Embryo Transfer Society manual (1998). To evaluate overall quality, embryos were stained on the 10th day of culture and the blastomeres were counted with the imaging software AxioVision 3.1 (Carl Zeiss, Feldbach, Switzerland). The blastocyst rate was analyzed by treatment groups with the chi-square test and the number of cells/embryo was analyzed by ANOVA with SAS (SAS Institute, Inc., Cary, NC, USA). The percentage of 8- to 16-cell embryos that developed to the blastocyst stage was similar (P > 0.05) between the control (66.0%, 68/103) and the biopsied (53.3%, 49/92) groups. Furthermore, no difference was noted in the hatching rates between the control group and the biopsied group (42.6%, 29/42 *v*. 44.9%, 22/49, respectively). Overall, no impact was detected on embryo quality from embryo biopsy with no difference in mean (±SE) blastocyst cell number between the control group (blastocysts: 67.1 ± 3.1; expanded blastocysts: 100.7 ± 6.9; hatched blastocysts: 189.9 ± 16.1) and the biopsied group (blastocysts: 61.1 ± 5.5; expanded blastocysts: 121.87 ± 10.6; hatched blastocysts: 187.3 ± 18.5). In conclusion, the biopsy used on 8- to 16-cell bovine IVF-derived bovine embryos does not affect the subsequent embryo development and number of cells/embryo or blastocyst, showing that it can be used to provide genetic material for preimplantation genetic diagnosis without affecting embryo quality.

This work was supported financially by FAPEMIG.

https://doi.org/10.1071/RDv20n1Ab141