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## 141 EFFECT OF LASER-ASSISTED EMBRYO BIOPSY AND DEVELOPMENTAL STAGE ON EMBRYO SURVIVAL AT TERM IN SHEEP

J. L. Alabart <sup>A</sup>, B. Lahoz <sup>A</sup>, P. Sánchez <sup>A</sup>, J. Folch <sup>A</sup>, J. H. Calvo <sup>A B</sup> and M. J. Cocero <sup>C</sup>

<sup>A</sup> CITA de Aragón, Zaragoza, Spain;

<sup>B</sup> ARAID, Zaragoza, Spain;

<sup>C</sup> INIA, Madrid, Spain

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### Abstract

A biopsy procedure causing minimal injury to embryos is essential to exploit the possibilities of the preimplantation genetic diagnosis in sheep biotechnologies. The effect of laser-assisted embryo biopsy on embryo survival at term was studied in ovine *in vivo*-derived embryos recovered at 2 different developmental stages. We used 294 embryos (quality scores 1 and 2; IETS manual, 1998 Edition) at the stages of compact morula ( $n = 136$ ) or blastocyst ( $n = 158$ ) recovered from superovulated Rasa aragonesa ewes at Day 8 after sponge removal. Embryos from each donor ewe were either biopsied ( $n = 160$ ; 76 compact morulas and 84 blastocysts) or directly incubated at 38.5°C in TCM199 and 5% CO<sub>2</sub>/air ( $n = 134$ ; 60 compact morulas and 74 blastocysts) during 18 to 22 hours before transfer. During biopsy, embryos were held by a holding pipette (15 µm internal diameter) in Dulbecco's phosphate-buffered saline without bovine serum albumin (100-µL microdrops). A 40× objective equipped with an infrared laser (1480 nm; 300 mW; XYClone, Hamilton-Thorne, Parallax Labs Ltd, St Albans, UK) was used to open a hole in the zona pellucida by one 1-ms laser pulse. Whenever possible, only extruded cells were aspirated through the hole (usually, 3 to 10 cells) using a rounded-end aspiration pipette (19 µm internal diameter). In embryos without sufficient observable extruded cells, the cell mass was aspirated throughout the hole and a portion of the protruded part was ablated using one or several laser pulses of up to 2.4 ms. When performed in blastocysts, cell ablation was carried out in the trophoblastic region opposite to the inner cell mass. After biopsy, embryos were incubated in the same conditions as non-biopsied embryos. Embryos reaching the expanded or hatching/hatched blastocyst stages after culture were transferred in pairs to synchronized recipient ewes. Percentages were analysed by ANOVA for categorical variables using the CATMOD procedure of SAS. The percentage of arrested embryos tended to be higher ( $P < 0.07$ ) in compact morulas either biopsied (11.8%) or not (10.0%) than in blastocysts (4.8 and 5.4%, respectively). The effects of the biopsy procedure and its interaction with the developmental stage were not significant ( $P < 0.85$  and  $P < 0.70$ , respectively). The survival rate at term of the transferred embryos was similar in biopsied and nonbiopsied embryos ( $P < 0.31$ ), either at the compact morula (62.7 and 72.2%, respectively) or the blastocyst (72.5 and 74.3%) stages. The effects of the developmental stage and its interaction with biopsy were not significant ( $P < 0.29$  and  $P < 0.49$ , respectively). The effects of biopsy procedure and its interaction with the developmental stage were not significant ( $P < 0.33$  and  $P < 0.44$ , respectively). In conclusion, the procedures presented here allow performing embryo biopsy with minimal injury, either in the compact morula or blastocyst stages. These results highlight the usefulness of laser in embryo biopsy.

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