Effect of laser-assisted embryo biopsy and developmental stage on embryo survival at term in sheep

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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 two different developmental stages.

- A total of 294 embryos were recovered from superovulated Rasa aragonesa ewes at the compact morula or blastocyst stages.
- About half of the embryos from each donor ewe were either:
  - biopsied and incubated at 38.5 ºC in TCM199 + 10% FBS and 5% CO2/air for 18-22 h before transfer
  - or not biopsied and directly incubated in the same conditions.
- During biopsy, embryos were held by a holding pipette (15 µm ID) in Dulbecco's PBS without BSA (100 µl microdrops).
- A hole was opened in the zona pellucida by one-1 ms laser pulse using a 40X objective equipped with an infrared laser (1480 nm; 300 mW; XYClone, Hamilthon-Thorne, Parallabs Ltd., St Albans, UK) as shown in Figures 1 and 2.
- Whenever possible, only cells excluded from the compaction (extruded cells) were aspirated through the hole (usually, 1 to 10 cells) using a rounded-end aspiration pipette (19 µm ID) as shown in Figure 2.
- 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.
- Embryos reaching the expanded or hatching/hatched blastocyst stages after culture (Figure 3) were transferred to synchronized ewes. Results are shown in the following table:

Stage / Biopsy status	Lambs born / Total embryos	Lambs born / Transferred embryos	Arrested / Total embryos
Compact morula			
Not biopsied	65.0 (39/60)	72.2 (39/54)	10.0 (6/60)
Biopsied	55.3 (42/76)	62.7 (42/67)	11.8 (9/76)
Blastocyst			
Not biopsied	70.3 (52/74)	74.3 (52/70)	5.4 (4/74)
Biopsied	69.0 (58/84)	72.5 (58/80)	4.8 (4/84)
Significance (P<)			
BIOPSY	0.33	0.31	0.85
STAGE	0.09	0.29	0.07
BIOPSY x STAGE	0.44	0.49	0.70

Figure 1. Laser objective.

Figure 2. Laser-assisted biopsy in a compact morula. Arrow: hole performed by the laser beam. Dashed area: cells excluded from compaction.

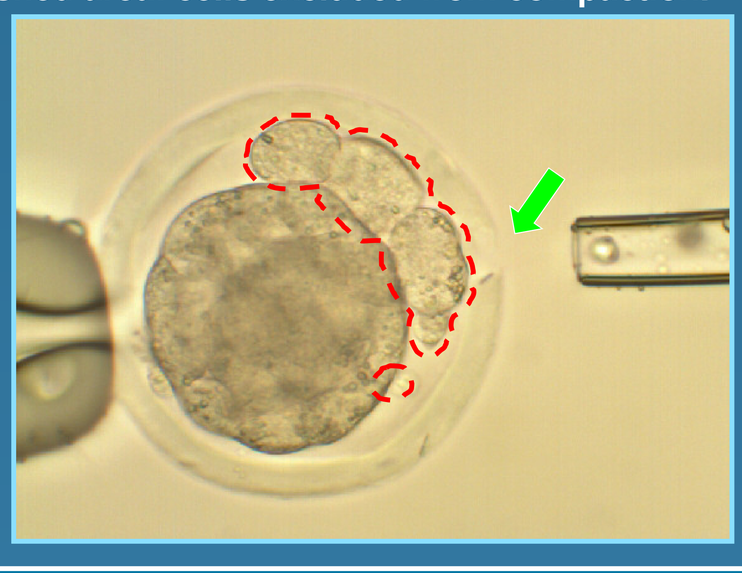
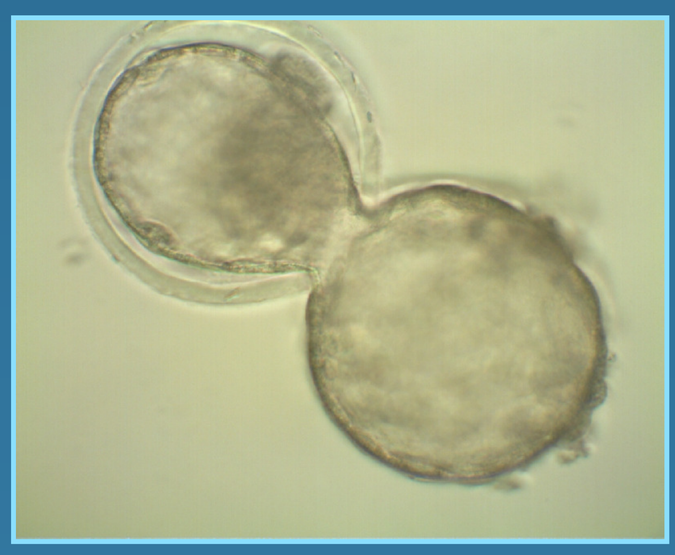


Figure 3. Biopsied embryo after 20 h of culture.



The survival rate of either the compact morulas or the blastocysts was not significantly decreased by the biopsy procedures described. These results are noticeably better than we previously reported without using laser-assisted procedures (Alabart et al., 2007. *23rd Scientific Meeting of the E.E.T.A. Alghero, Sardinia, 7th - 8th September*, p. 122), where 15.5% of the biopsied embryos were severely injured and not transferred, while the survival rate of the transferred embryos was decreased by 15.6% due to the biopsy procedures.

In conclusion, the procedures presented here allow performing embryo biopsy with minimal injury, either in the compact morula or in the blastocyst stages. These results highlight the usefulness of laser in embryo biopsy.

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