

# IN VITRO DEVELOPMENT OF BOVINE EMBRYOS SUBMITTED TO LASER ASSISTED HATCHING ON DAY 6 AND DAY 7 OF EMBRYO CULTURE

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## INTRODUCTION

- ✓ *In Vitro* embryo production (IVP) is a valuable tool in bovine assisted reproduction;
- ✓ Embryos produced *in vitro* show lower pregnancy rates than *in vivo* produced embryos;
- ✓ The inability of IVP embryos to hatch from the zona pellucida is believed to be one contributing factor for lower pregnancy rates.

## OBJECTIVES

To verify the ability of IVP embryos to hatch and develop *in vitro* after being submitted to laser assisted hatching (LAH) at Day 6 and Day 7 of embryo culture.

## MATERIALS AND METHODS

- ✓ Slaughterhouse oocytes were fertilized after 24h of *in vitro* maturation and cultured in G1/G2 version 5 medium supplemented with 8 mg/mL of BSA at 38.5° C, 5% CO<sub>2</sub>, 5% O<sub>2</sub> and 90% N<sub>2</sub>, in a humidified atmosphere.
- ✓ Day 6 after IVF- viable embryos (251) were divided into four treatment groups:
  - 1) LAHD6- ZP was submitted to a laser beam on Day 6;
  - 2) CD6- ZP was not submitted to a laser beam (Control);
  - 3) LAHD7- ZP was submitted to a laser beam on Day 7;
  - 4) CD7- ZP was not submitted to a laser beam (Control).
- ✓ ZP was exposed to a laser beam, using XY Clone® laser system, with a pulse strength of 90% and a pulse length of 600 μs (figure 1).
- ✓ Embryos from all groups were cultured *in vitro* and evaluated on Day 8 and Day 9 of culture for stage of development (figure 2). On Day 9, a random sample of embryos from each treatment group (n = 48) was stained with Hoechst 33342 (2.5 μg/mL) and evaluated under UV light to determine the total number of cells.



XY Clone® laser system attached to microscopy objective (40 x)

## RESULTS

The number of hatched blastocysts was not different among the groups on Day 9 of culture;

On Day 8 of culture, LAHD6 showed a higher number of hatched blastocysts, when compared to group CD6.

There was no difference between groups LAHD7 and CD7 on Day 8.

See Table 1 for results.

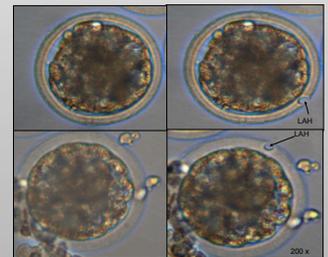


Figure 1. Day 6 morulas before (left) and after (right) LAH treatment.

Table 1. Number of hatched blastocysts on Day 8 and Day 9 of embryo culture, after being submitted to LAH on Day 6 and Day 7

Treatment Group	Hatched Blastocysts Day 8 (%)	Hatched Blastocysts Day 9 (%)	Total Number of Embryos
LAHD6	40 <sup>a</sup> (60%)	49 <sup>b</sup> (74%)	66
CD6	26 <sup>b</sup> (42%)	38 <sup>b</sup> (62%)	61
LAHD7	33 <sup>ab</sup> (55%)	42 <sup>b</sup> (71%)	59
CD7	31 <sup>ab</sup> (47%)	47 <sup>b</sup> (72%)	65

<sup>a-c</sup> Values with different superscripts differ significantly (Chi-square, p < 0.05).

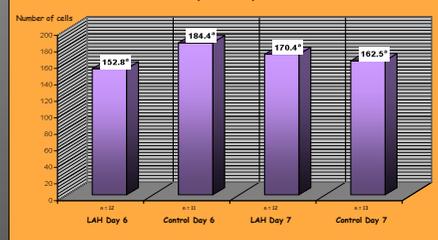


Figure 2. Embryos on Day 9 of culture, after being submitted to LAH.

Comparison of the total number of cells showed no difference among the groups. (Student's *t*-test, *p* > 0.05)

See Graph 1 for results.

Graph 1: Mean number of total embryonic cells evaluated on Day 9 of *in vitro* culture, with or without LAH on Day 6 and Day 7.



## CONCLUSIONS

✓ These data show that LAH on Day 6 of culture improves *in vitro* hatching rates on Day 8, while LAH on Day 7 shows no improvement on either Day 8 or 9.

✓ LAH does not have a detrimental effect on mean cell production throughout embryo development *in vitro*.

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