

# **Utilization of Laser Assisted Hatching to Improve the Pregnancy Rate of In Vitro Fertilized Bovine Embryos**

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## **INTRODUCTION**

In vitro fertilization can prove to be a valuable instrument in the ability to assist genetic selection strategies and breeding plans for cattle production systems. In vitro produced bovine embryos are known to produce a lower pregnancy rate when compared to conventional in vivo produced embryos. Fertilization and culture in vitro may lead to failure of the bovine embryo to hatch following transfer, resulting in lower pregnancy rates.

## **OBJECTIVE**

This study was designed to evaluate the utilization of the XYClone® laser system to cut the zona pellucidae of the developing in vitro produced embryos to assist hatching. Preliminary data were acquired to evaluate the effect of the laser treatment on in vitro development and blastomere survival following the treatment. Timing of treatment and laser settings were evaluated for optimum embryo survival and commercially produced embryos were randomly treated prior to transfer into an estrus synchronized recipient. Subsequent pregnancy rates were evaluated.

## **EXPERIMENT 1**

### **Materials and Methods**

Oocytes were in vitro matured, fertilized and cultured as per standard laboratory procedures in six replicates. On days 5, 6, and 7 of in vitro culture, embryos were randomly divided into 3 treatment groups: no treatment, sham zona pellucida cut, or zona pellucida cut.

- ❑ Control embryos were immediately returned to the incubator following selection.
- ❑ Sham embryos were exposed to all conditions as Cut except laser assisted hatching.
- ❑ The XYClone system is a 300 milliwatt, class 1 laser that emits a 3.5  $\mu\text{m}$  beam at a wavelength of 1480 nm (Hamilton Thorne Biosciences). The laser was used to produce the Cut group using pulse strength of 90% and pulse length of 600  $\mu\text{sec}$ .

Embryos were returned to culture until day 8 when the percentage of live cells was determined. Chi Square was used to analyze all data.

### **Preliminary Data: Mean Proportion of Live Cells of In Vitro Produced Embryos at Day 8**

	<b>TOTAL EMBRYOS</b>	<b>TREATED DAY 5</b>	<b>TREATED DAY 6</b>	<b>TREATED DAY 7</b>
<b>CUT</b>	<b>70</b>	<b>92.8%</b>	<b>91.9%</b>	<b>92.9%</b>
<b>SHAM</b>	<b>68</b>	<b>84.4%</b>	<b>92.9%</b>	<b>89.8%</b>
<b>CONTROL</b>	<b>63</b>	<b>96%</b>	<b>89.9%</b>	<b>93.2%</b>

## **EXPERIMENT 2**

### **Materials and Methods**

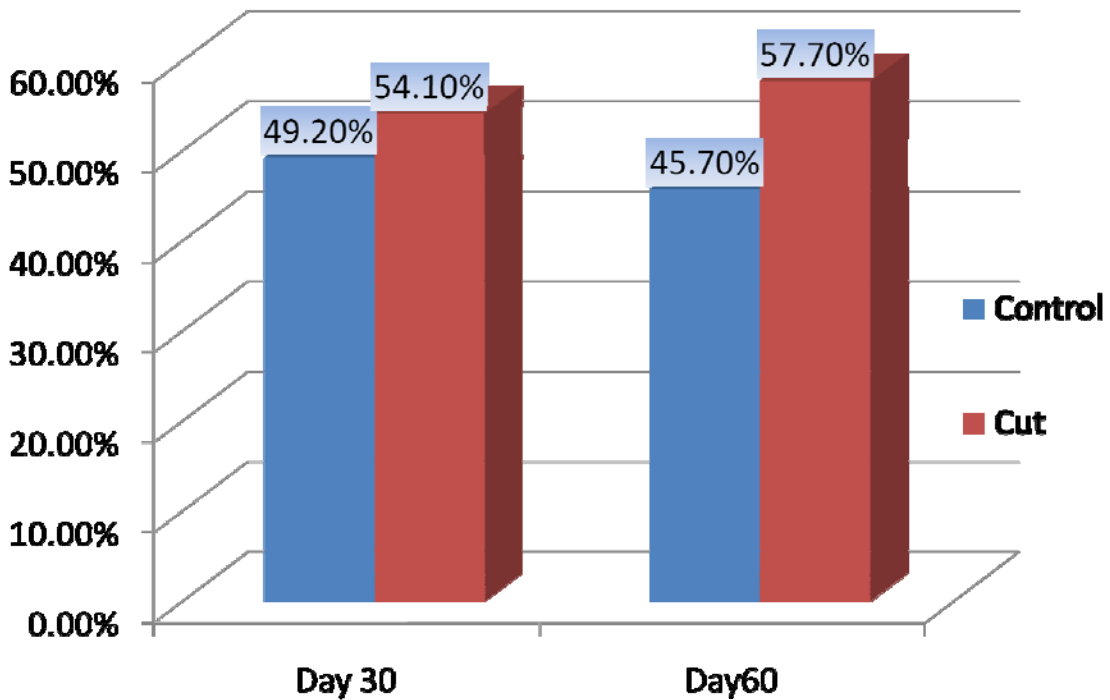
Commercially in vitro produced embryos (TransOva Genetics) were randomly divided into two groups, Control and Cut, on each day of transfer. The zona pellucidae of treated embryos were cut with the laser using 80% pulse strength and pulse length of 500  $\mu\text{sec}$  on day 7, immediately prior to transfer into estrus synchronized recipients. Pregnancy rates were determined via ultrasonography at days 30 and 60. Chi Square was used to analyze all data.

### Pregnancy Rate Results from Assisted Hatching

	# of Recipients Evaluated; d 30	Day 30 Pregnancy Rates	# of Recipients Evaluated; d 60	Day 60 Pregnancy Rates
<b>CONTROL</b>	<b>189</b>	<b>49.2%</b>	<b>166</b>	<b>45.7%*</b>
<b>CUT</b>	<b>148</b>	<b>54.1%</b>	<b>123</b>	<b>57.7%</b>

\*Values within this column are statistically different  $P < 0.05$ .

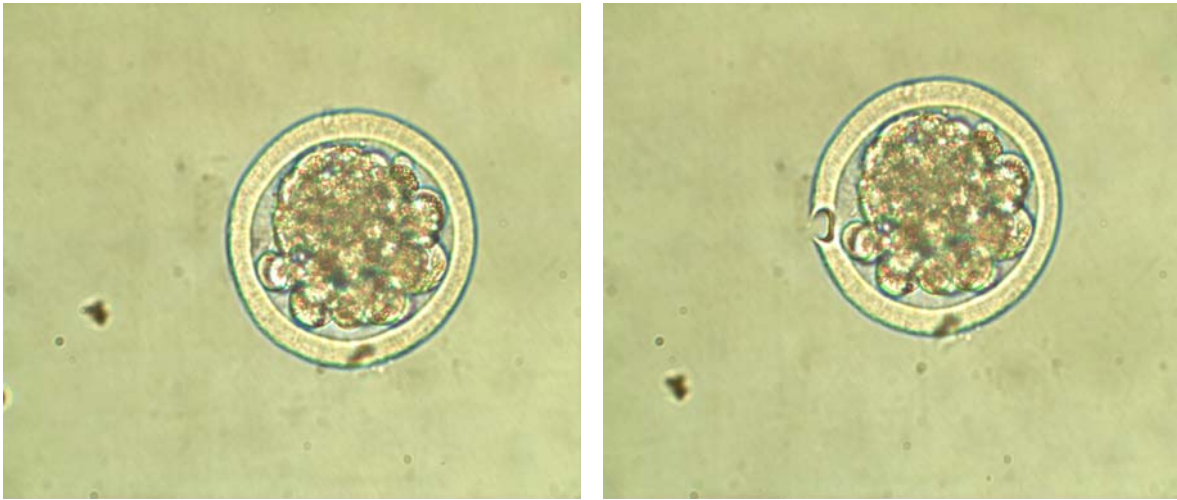
### Pregnancy Rates at Day 30 and Day 60 with Laser Treated Assisted Hatching



## **CONCLUSION**

These results demonstrate that laser assisted hatching using the XYClone system does not inhibit in vitro embryo viability and development. A significant difference was found in the 60 day pregnancy rate between control and cut embryos. This difference exhibits a large potential for this system to improve 60 day pregnancy rates of in-vitro produced embryos. It remains to be determined if this difference influences long term pregnancy rate.

### **Representative embryo before and after laser treatment.**



*Courtesy of Kathy Bradley, Hamilton Thorne Biosciences.*

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