

# IN VITRO PRODUCTION OF BOVINE EMBRYOS BY USING SORTED SEMEN

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## Abstract

*The objective of this study was aimed to produce in-vitro bovine embryo in the laboratory of IAS and produce foreseen sexual embryo by first application of sorted semen (only sperm with X chromosome). Fresh ovaries were collected immediately after slaughtering and sorted semen was imported from O'Connor Land & Cattle Company (Canada), procedure of IVM, IVF and IVC were implemented by NLBC's guide book (Japan). The initial results showed suitable storage conditions for ovary (25°C in 4 hours), 23.1% of IVF and 19.6% of blastocyte by using usual semen; 29.9% of IVF and 35.0% of blastocyte by using sorted semen.*

Key words: IVM, IVF, IVC, embryo, sorted semen

## 1. INTRODUCTION

The use of embryo transfer (ET) technology in cattle breeding has continuing to increase (especially within the dairy cattle) over the past 30 years with the movement toward to genetic improvement as opposed to the production of desirable phenotypes. In Canada, approximately 70% of the ET work is now being done on dairy cattle, and approximately 15,000 embryos are being frozen annually for export. Throughout the world over the past year, more than 100,000 donor cows have been super-stimulated and more than 500,000 bovine embryos have been transferred. This technology is influencing the direction of cattle breeding industries; the numbers are small but the impact is high. Being like ET, technology for *in vitro* production (IVP) of bovine embryos has also encountered many challenges on the path toward widespread commercial application. However, IVP technology will be more useful when combined with sperm-sorting technology. This combination will produce the foreseen sexual embryos which

create suitable calves for different objectives of production (beef or dairy industry). IVP of bovine embryos by using sorted semen within conditions of new lab. is also a primary objective of this study.

## 2. CONTENTS AND METHODS

- Observe the effect of preservative conditions (temperature 10°C-25°C-37°C and time 4h-8h-12h) on IVM-IVF and IVC.
- Compare the efficiency of IVP by using two different semen types, normal and sorted semen (only sperm with X chromosome).
- Oocytes were collected from ovary after slaughtering.
- Sorted semen was imported from O'Connor Land & Cattle company (Canada)
- Procedure of IVM, IVF and IVC were implemented by NLBC's guide book (Japan).

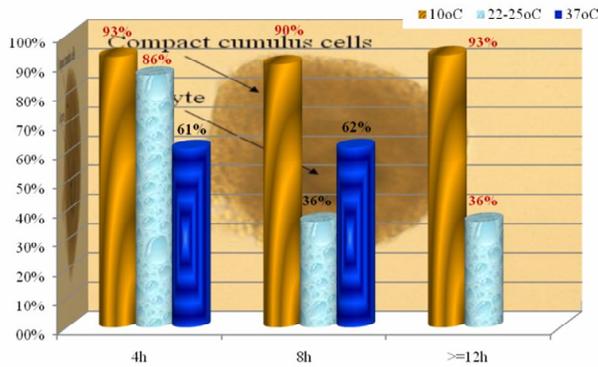


Figure 1. Effect of preservative conditions (temperature & time) on IVM

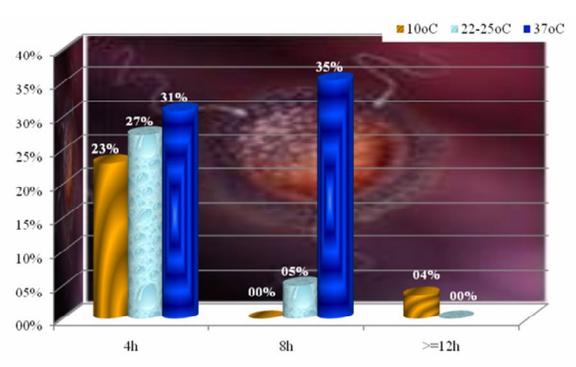


Figure 2. Effect of preservative conditions on IVF

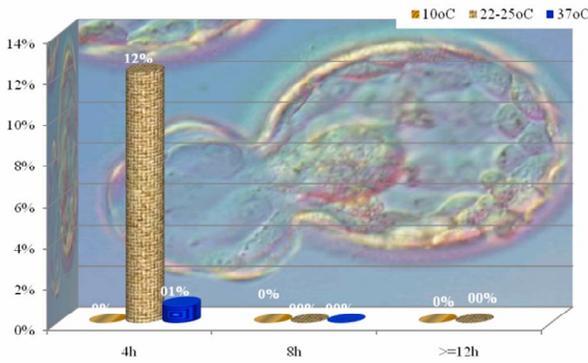


Figure 3. Effect of preservative conditions on IVC

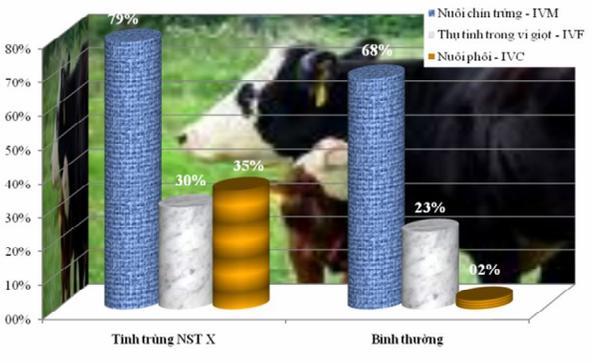


Figure 5. Effect of type of semen on IVP

### 3. RESULTS AND DISCUSSION

#### 3.1. Effect of preservative conditions on IVM-IVF and IVC

The results were presented on figures 1, 2, 3.

- The best preservative duration of ovary from slaughter-house to lab was 4 hours. Results of IVM, IVF and IVC were highest within this preservative duration.

- Rate of maturity oocyte (IVM) was highest on the lowest preservative temperature (10°C). However, the final aims were highest IVF and IVC, especially highest rate of blastocyte. So, 25°C was the most suitable temperature of preservation of ovary from slaughter-house to lab.

In the general, 25°C and 4 hours was the good combination of preservative conditions of ovary from slaughter-house to lab in order to get high results of IVM, IVF and IVC

#### 3.2. Efficiency of IVP by using two different semen types

Although concentration of sperm in normal semen was higher than sorted semen, rate of IVF was equivalent (23.1% compared with 29.9%,  $P > 0.05$ ). It was due to adjustment of sperm concentration before IVF.

However, the rate of morula and blastocyte by using sorted semen was higher than normal semen (35.0% vs. 19.6% respectively,  $P < 0.001$ ). Wilson *et al* (2005) obtained the same results when using both sorted semen and normal semen for IVP, IVF (65.0% and 67.3%) but there were different results on rate of blastocyte: 20.1% vs 12.2%, respectively.

### 4. CONCLUSIONS

- The study results proved that IVP can be done successfully in conditions of IAS Lab.

- The good preservative conditions for ovary from slaughter-house to lab were 25°C and 4 hours.
- Sorted semen can be used for IVP with rather good rate of blastocyte.

## **5. SUGGESTION**

Conditions of preservation of ovary, IVM, IVF and IVC, using sorted semen for IVP should be studied continuously.

## **REFERENCES**

Wilson R.D.; Fricke P.M.; Leibfried-Rutledge M.L.; Rutledge J.J.; Penfield C.M.; Weigel K.A. 2005. In vitro production of bovine embryos using sex-sorted sperm. *Theriogenology*, 2005