# TRANSPLANTATION OF BIOPSIED, SEXED AND CRYOPRESERVED BOVINE EMBRYOS

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***Abstract***

*Embryo biopsy performed in order to obtain a small amount of blastomeres needed for embryo sexing is an invasive method that damages the zona pellucida and therefore decreases the survival capacity of the embryo that is subsequently submitted to cryopreservation. The aim of the present study was to evaluate three biopsy techniques applied in bovine embryos according to their capacity to maintain embryo viability after cryopreservation. Three embryo biopsy techniques (needle, blade and aspiration) were applied on 120 bovine embryos divided into 3 batches (n=40) in order to harvest the blastomeres needed for polymerase chain reaction (PCR) embryo sexing. After the biopsy, the embryos were frozen/thawed using the one step method with ethylene glycol and then transferred into synchronized recipients. DNA was extracted from blastomeres and amplified using bovine Y-chromosome specific primers, in order to determine the sex of the embryo. The pregnancy diagnosis and the assessment of pregnancy rate were performed 30 days after transfer using an ultrasound scanner. There was a significant difference in pregnancy rates according to the biopsy method used: 55% for the needle biopsy, 45% for the aspiration method and 30% for the microblade technique. The accuracy of the PCR sexing method was comparable in all batches, and therefore was not influenced by the biopsy method. The needle method of embryo biopsy proved to be the most suitable as it yielded the highest pregnancy rates and can be successfully applied when harvesting blastomeres for embryo sexing.*

***Key words***: *bovine, embryo sexing, cryopreservation, biopsy*.

## INTRODUCTION

Embryo gender determination is one of the biotechnologies that created great emulation among the specialists studying in vitro fertilization and embryo transfer. Embryo biopsy performed in order to obtain a small amount of blastomeres needed for embryo sexing is an invasive method that damages the zona pellucida and therefore decreases the survival capacity of the embryo that is subsequently submitted to cryopreservation. The aim of the present study was to evaluate three biopsy techniques applied in bovine embryos according to their capacity to maintain embryo viability after cryopreservation.

## MATERIALS AND METHODS

### Embryo production

Donor selection was made according to the general criteria accepted by the International Society for Embryo Transfer (IETS). Only healthy individuals, with no history of reproductive disorders and with regular sexual cycle were taken into consideration. Superovulation was induced with a total dose of 1000 IU porcine FSH-LH (Pluset, Carlier), administered according to the manufacturer's indications. Artificial insemination was performed 48h after the last administration of Pluset, followed by a second and third insemination, 12h and 24h later respectively. Embryo recovery was made using the non-surgical flushing method 6.5-7.5 days later. Morphologic evaluation of embryos was done taking into consideration the IETS criteria and only grade 1 morulae and blastocysts were used for biopsy and further study.

### Embryo biopsy

Embryo biopsy was performed using a Nikon Eclipse TS100 inverted microscope, equipped with an Olympus Narishige ONO-131 three axis hydraulic micromanipulator. The embryos were divided into 3 batches (n=40) according to the biopsy method used. The first batch was biopsied using the needle technique (Tominaga and Hamada, 2004), the second batch by blastomere aspiration (Lopatarova et al. 2010), while the third batch was biopsied using a fine microblade (Akiyama et al., 2010).

### Cryopreservation, transplantation, sexing and pregnancy diagnosis

Embryo cryopreservation was achieved using the one step method with 1.5 M ethylene glycol described by Voelkel and Hu, 1992. Thawing of embryos was made in 37ºC water bath for 10 seconds, followed by direct transfer into synchronized recipients. Embryo sexing was made using the PCR method described by Peura et al., 1991 using the BRY4a primers. Pregnancy diagnosis was carried out 30 days after embryo transfer using an ultrasound scanner and the pregnancy rate was assessed on this occasion. The results were statistically analyzed using the In Stat Graph Pad software and the unpaired t-test.

## RESULTS AND DISCUSSIONS

The pregnancy rate obtained after the transfer of biopsied, sexed and cryopreserved embryos was 57% for batch 1 in which the needle biopsy technique was applied, 43% for batch 2 in which the aspiration biopsy

method was used and 31% for batch 3 in which the microblade biopsy technique was applied.
All pregnancies progressed to term and resulted in healthy calves. The accuracy of the sexing method was of 100% in all batches, all calves presenting the same morphological sex as predicted earlier by PCR.
The statistical analysis showed the following significant differences between batches:
Batch 1 (needle biopsy) vs. Batch 2 (aspiration biopsy): $p=0.0010$, extremely significant; Batch 1 (needle biopsy) vs. Batch 3 (microblade biopsy): $p<0.0001$, extremely significant; Batch 2 (aspiration biopsy) vs. Batch 3 (microblade biopsy): $p=0.0030$, very significant.
Various authors, performing embryo biopsy for different reasons, obtained comparable results. The pregnancy rate after the transfer of fresh, biopsied embryos varied from 53 to 62%, the microblade biopsy method being usually preferred (Bredbacka et al., 1996, Herr and Reed, 1991, Roschlau et al., 1997, Thibier and Nibart, 1995) and is comparable to the one obtained for fresh, intact embryos. Sex determination can also be performed in bisected embryos if transferred freshly, without cryopreservation, with good conception rates, up to 56.5% (Lopatarova *et al.*, 2008).
The results obtained for frozen/thawed biopsied embryos varied quite a lot among various researchers and ranged from 33 to 66% when needle or aspiration was used, but was of only 23% to 28% when a microblade was used (Nibart *et al.*, 1997, Shea 1999).
Other data shows that the efficiency of producing bovine embryos, transferable after vitrification on day 7 was higher when the biopsy was performed on day 4 rather than day 7.5 (Vajta et al., 1997).

## CONCLUSIONS

Biopsied frozen/thawed bovine embryos have a better chance to survive the cryopreservation process if damage of the zona pellucida is minimized as much as possible.
This can be achieved by carefully choosing the embryo biopsy method, the needle technique showing obvious advantages, as presented above.
As biopsied embryos are rarely transferred directly into recipients, and are usually cryopreserved for later use, the choice of an adequate biopsy method has a direct influence on embryo viability after thawing.

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## REFERENCES

Akiyama K., Kobayashi J., Sato Y., 2010. Calf production from vitrified bovine sexed embryos following in-straw dilution. Animal Science Journal, 81 (4), 461–466.

Bredbacka P, Peippo J, Jaakma U., 1996. A simplified protocol for PCR-sexing of bovine embryos: a field trial. Theriogenology, 45, 218 abst.

Herr C.M., Reed K.C., 1991. Micromanipulation of bovine embryos for sex determination. Theriogenology, 35, 45-54.

Lopatarova M., Cech S., Krontorad P., Holy L., Hlavicova J., Dolezel R., 2008. Sex determination in bisected bovine embryos and conception rate after the transfer of female demi-embryos**.** Veterinarni Medicina, 53 (11), 595–603.

Lopatarova M., Cech S., Krontorad P., Holy L., Lalova H., Dolezel R., 2010. Conception rate after sex determination and cryopreservation of D7 bovine embryos. Veterinarni Medicina, 55, 10–18,.

Nibart M, Marquant Le Guienne B, Humblot P, Guerin B., 1997. The application of new reproductive technologies in France. Arq. Fat. Vet. UFRGS 25 (Suppl 1), 21-35.

Peura T., Hyttinen J. M., Turunen M., Janne J., 1991. A reliable sex determination assay for bovine preimplantation embryos using the polymerase chain reaction. Theriogenology, 35, 547–555.

Roschlau K., Roschlau D., Roselius R., Dexne U., Michaelis U., Strehl R., Unicki P., Rink N., 1997. Over 5 years experience in sexing of bovine morulae and blastocysts during routine embryo transfer. Theriogenology, 47, 273 abst.

Shea B.F., 1999. Determining the sex of bovine embryos using polymerase chain reaction results: a six-year retrospective study. Theriogenology, 51, 841-854.

Thibier M., Nibart M., 1995. The sexing of bovine embryos in the field. Theriogenology, 43, 71-80.

Tominaga K., Hamada Y., 2004. Efficient production of sexidentified and cryosurvived bovine in-vitro produced blastocysts. Theriogenology, 61 (6), 1181–1191.

Vajta G., Booth P.G., Holm P., Greve T., Callesen H., 1997. Comparison of two manipulation methods to produce in vitro fertilized, biopsied and vitrified bovine embryos. Theriogenology, 47, 501–509.

Voelkel S. A., Hu Y. X., 1992. Use of ethylene glycol as a cryoprotectant for bovine embryos allowing direct transfer of frozen-thawed embryos to recipient females. Theriogenology, 37, 687–697.