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Technical Note

Use of Biotechnologically-Manipulated Semen in the *In Vitro* Production of Bovine Embryos

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INTRODUCTION

In most mammals, such as cattle, the birth rate of both sexes is equivalent. In the dairy industry, this restriction reduces profits, as only females and a few males are productive (Krishna *et al*., 2022).

The use of sexed semen allows for controlling the sex of the offspring; this is possible through the technique of flow cytometry, which separates X sperm from Y sperm (Nag *et al*., 2022). Its use, combined with other technologies like *in vitro* embryo production (IVP), allows for obtaining a higher number of pregnancies; especially if oocytes from slaughtered cows and females up to three months of gestation, obtained through ultrasound-guided follicular aspiration (OPU), are used. Additionally, it requires a lower number of sperm (Ferré *et al*., 2020).

Semen sexing biotechnology is costly and difficult to access for countries like Cuba, which is why the Tropical Livestock Improvement Research Center (CIMAGT) developed an alternative: biotechnologically manipulated semen. The aim of this preliminary study was to evaluate the obtaining of *in vitro* embryos using biotechnologically manipulated semen.

DEVELOPMENT

Crossbred cows with an average body condition of 2.5 were sacrificed by electric shock and bleeding by jugulation in a slaughterhouse in the province of Artemisa, Cuba. The ovaries were collected directly from the animal within an average time of 20 minutes after sacrifice. Ten

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ovaries were obtained and placed in one liter of transport medium [Phosphate Buffer Solution (PBS) + 0.1 mg/ml Kanamycin (SIGMA, K1377-25G)] at 38.5ºC for transport to the laboratory, within 1:30h after collection.

The ovaries were processed in the CIMAGT Reproductive Biotechnology Laboratory. They were washed three times in transport medium and the follicles with a diameter between two and six millimeters were aspirated using the aspiration pump (Minitube, 23362/0000) and short needle 18G, at a pressure corresponding to 20 drops per minute. The resulting cumulus-oocyte complexes (COCs) were deposited in vials with five milliliters of aspiration medium [PBS + 1% Bovine Fetal Serum (FBS) (Capricorn, FBS-12B) + 0.1mg/ml Kanamycin (SIGMA, K1377-25G) + $2{,}4*10^3$ mg/ml Heparin (SIGMA, H3149-25KU)] at 38.5ºC.

The contents of the vials were diluted in TCM 199 [TCM199-Hepes (SIGMA, M-2520) + 0.1mg/ml Kanamycin + 1% FBS (Capricorn, FBS-12B) + 0.336 mg/ml $NaHCO_3$ (SIGMA, S5761-500G)] in 120 mm Petri dishes (Greiner, 688102). The work was done on thermal plates (12055/0003) at 38.5°C, in the laminar flow hood (Labconco, 64132). The COCs were classified under the stereomicroscope (Olympus, ZS51) according to De Loos *et al*. (1989). Category 1 and 2 were washed five times in TCM 199, in 35 mm Petri dishes (Nunc, 174943). They were randomly distributed into two groups and transferred to 500 µl of commercial maturation medium BO-IVM (IVF Bioscience, 71002) in a four-well plate (Thermo Scientific, 10404532). They were placed in the CO_2 incubator (Nuaire, NU-5100E) at 38.5°C, 5% CO_2, and 95% relative humidity (RH) for 20 hours.

For fertilization, non-manipulated and biotechnologically manipulated semen obtained from ejaculates of a Holstein bull from CIMAGT was used. Sperm separation was performed with commercial medium BO-SemPrep (IVF Bioscience, 71003). Sperm motility was evaluated under the phase contrast inverted microscope (Axiovert, 35M), and sperm counting was performed in a Bürker chamber (Marienfeld, PM-0610230). Mature COCs were transferred to 500 µl of commercial fertilization medium BO-IVF (IVF Bioscience, 71003), along with $2*10^6$ sperm per milliliter. They were placed in the CO_2 incubator for 21 hours. After this time, they were decumulated with vortex (Heidolph, 94323) for three minutes and 30 seconds at 323 g, in 15 ml vials containing two milliliters of TCM 199. They were washed five times in 35 mm Petri dishes with TCM 199, and transferred to 500 µl of commercial culture medium BO-IVC (IVF Bioscience, 71005). They were placed in the CO_2 incubator at 38.5°C, 5% CO_2, and 95% RH. On the fifth day, the stage was evaluated.

Data were plotted in Microsoft Office Excel 2007 and a proportion comparison test (α=0.05) was performed in Minitab 14. They are shown in Fig.1.

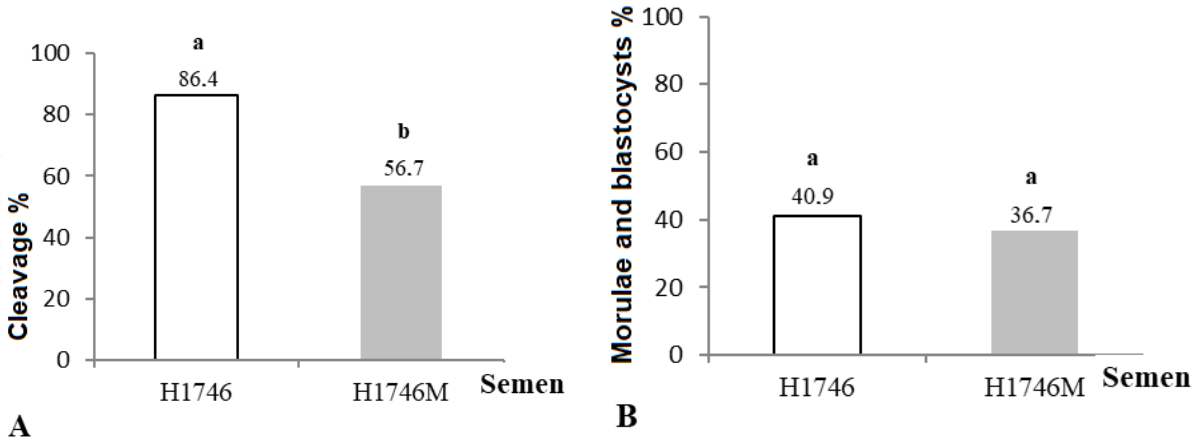

Figure 1: Percentage of A: cleavage and B: morulae and blastocysts, obtained by *in vitro* production using Holstein bull semen (H1746) and biotechnologically manipulated Holstein bull semen (H1746M). n=52. Comparison of proportions A: *P<0.05, gl=1) B: (*P>0.05, gl=1).

A lower number of divided zygotes were obtained with manipulated semen in terms of cleavage percentage (Fig. 1A). Meanwhile, the rate of morulae and blastocysts showed no differences (Fig. 2B). However, when manipulated semen was used, a lower number of hatched blastocysts were observed, suggesting slower development. These results differ from those proposed by Magata *et al*. (2021) when using sexed and non-sexed semen from the same Holstein bull. The authors found no differences in the cleavage rate between the two treatments, but a higher proportion of blastocysts when using non-sexed semen.

CONCLUSIONS

Biotechnologically manipulated semen can be used for cattle IVP, with 36.7% embryos obtained.

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AUTHOR CONTRIBUTION STATEMENT

Research conception and design: HNQ, JMSP, IAP; data analysis and interpretation: HNQ, JMSP, IAP, redaction of the manuscript: HNQ.

CONFLICT OF INTEREST STATEMENT

The authors state there are no conflicts of interest whatsoever.