Technical Note

Evaluation of Bovine Embryos Cultivated in an Intravaginal Device

Helena Navarro Quevedo *, Ingrid Avila Pérez *, José Miguel Sánchez Pellitero *, Luis Enrique Dihigo Cuttis *

*Center for Tropical Animal Improvement Research, Havana, Cuba.
Correspondence: hnavarroquevedo@gmail.com

Received: February 2025, Accepted: February 2025; Published: March 2025.

INTRODUCTION

The *in vitro* production of embryos (IVP) involves the stages of maturation, fertilization, and culture. The culture stage includes the development of presumptive zygotes to the morula and blastocyst stages under conditions resembling those *in vivo*. It is common for a percentage of structures obtained during culture to experience a cessation or blockage of embryonic development (Zhang *et al*., 2023).

Among the factors influencing embryonic blockage is stress caused by the environment the embryos, which are subjected to temperature, humidity, pH, and gas concentrations, among others (Hurtado, 2021). Therefore, techniques that can simulate the environment and processes occurring in the female reproductive tract have been explored. One such technique is the intravaginal culture of embryos using a device that, when introduced into the vagina, creates the temperature, humidity, and oxygen and carbon dioxide concentrations necessary for embryo development (Ranoux, 2012).

This system was initially used in humans, and no reports of its application in bovine IVP existed until 2015, when Pinzón *et al.* (2015) conducted research that demonstrated the system's feasibility for producing 8- and 16-cell bovine embryos capable of further development.

Another benefit of using this device is cost reduction, as it lowers incubation and laboratory monitoring fees during the culture stage. Additionally, unlike *in vivo* culture in oviducts, it does

Citations (APA) Navarro Quevedo, H., Avila Pérez, I., Sánchez Pellitero, J.M., & Dihigo Cuttis, L.E. (2025). Evaluation of Bovine Embryos Cultivated in an Intravaginal Device *Journal of Animal Prod, 37*. https://apm.reduc.edu.cu/index.php/rpa/article/view/e166

ISSN 2224-7920

not require surgical methods or animal sacrifice. Therefore, the aim of this preliminary study was to evaluate the production of bovine embryos cultured in a prototype Cuban intravaginal device.

DEVELOPMENT

Mestizo-Cebu female cattle, with an average body condition score (BCS) of 2.5 on a 5-point scale for dairy cattle, were used. The animals were sacrificed via electric shock and bleeding by jugulation at the sanitary slaughterhouse located in Nueva Paz, Mayabeque province. Ovaries were recovered directly from each animal within an average of 20 minutes after sacrifice. A total of 36 ovaries were collected and placed in one liter of transport solution [Phosphate-buffered saline (PBS) + 0.1 mg/mL Kanamycin (SIGMA, K1377-25G)] at 36.5–38.5°C for transport to the laboratory within two hours of collection.

Ovaries were processed at the Biotechnology and Assisted Reproduction Laboratory of the Tropical Livestock Improvement Research Center (CIMAGT). They were washed three times with transport solution, and follicular aspiration was performed using an aspiration pump (Minitube, 23362/0000), a short 18G needle, and pressure corresponding to a flow rate of 20 drops per minute. Follicles with diameters of two to six millimeters were aspirated, and the resulting cumulus-oocyte complexes (COCs) were deposited in 50 mL vials preloaded with five milliliters of aspiration medium [PBS + 1% Fetal Bovine Serum (FBS) (Capricorn, FBS-12B) + 0.1 mg/mL Kanamycin (SIGMA, K1377-25G) + 2.4 × 10^3 mg/mL Heparin (SIGMA, H3149-25KU)] tempered at 38.5°C.

The COCs and follicular fluid in the vials were diluted in collection medium [TCM199-Hepes (SIGMA, M-2520) + 0.1 mg/mL Kanamycin + 1% FBS (Capricorn, FBS-12B) + 0.336 mg/mL $NaHCO_3$ (SIGMA, S5761-500G)] and identified under stereoscopic magnification 4x (Olympus, ZS51) in 120 mm plates (Greiner, 688102). The work was conducted on thermal plates (12055/0003) tempered to 38.5°C in a horizontal laminar flow hood (Labconco, 64132). COCs were classified and separated based on their morphological quality following the methodology described by De Loos *et al*. (1989) for membrane, cytoplasm, and cumulus characteristics observable under a stereomicroscope.

COCs suitable for maturation (categories 1 and 2) were washed five times in 35 mm Petri dishes (Nunc, 174943) with collection medium. The same number of quality 1 and 2 oocytes were distributed into two groups. The maturation process was conducted in BO-IVM (IVF Bioscience, 71002), with 500 µL of medium in four-well plates (Thermo Scientific, 10404532). They were incubated in a CO_2 incubator (Nuaire, NU-5100E) at 38.5°C, 5% CO_2, and >90% relative humidity for 20 hours.

Frozen semen straws from a Holstein bull were used for fertilization. Sperm separation was performed using BO-SemPrep (IVF Bioscience, 71003). Sperm motility and count were

evaluated in a Bürker chamber (Marienfeld-Superior, PM-0610230), both steps under an inverted phase-contrast microscope (Axiovert, 35M).

Mature COCs were transferred to 500 µL of BO-IVF medium (IVF Bioscience, 71003) in four-well plates, along with 2×10^6 sperm per milliliter. They were incubated in a CO_2 incubator at 38.5°C, 5% CO_2, and >90% relative humidity for 21 hours. After this time, presumptive zygotes were decumulated using a vortex (Heidolph Top-Mix, 94323) for three minutes and thirty seconds at 323 g in 15 mL vials with two milliliters of TCM199. They were then washed five times in 35 mm Petri dishes with the same medium. Presumptive zygotes were randomly distributed into two groups.

The control group consisted of 500 µL of BO-IVC culture medium (IVF Bioscience, 71005) in a four-well plate placed in a CO_2 incubator at 38.5°C, 5% CO_2, and >90% relative humidity. The treatment group consisted of 500 µL of BO-IVC culture medium (IVF Bioscience, 71005) in a 1 mL cryovial placed inside a prototype Cuban intravaginal device. This device was inserted into the vagina of a non-pregnant female bovine, attached to a securing system. The developmental stage was evaluated after five days.

Data were processed and graphed in Microsoft Excel 2007, and a binomial proportion comparison test (α=0.05) was conducted in Minitab 14 to determine whether significant differences existed.

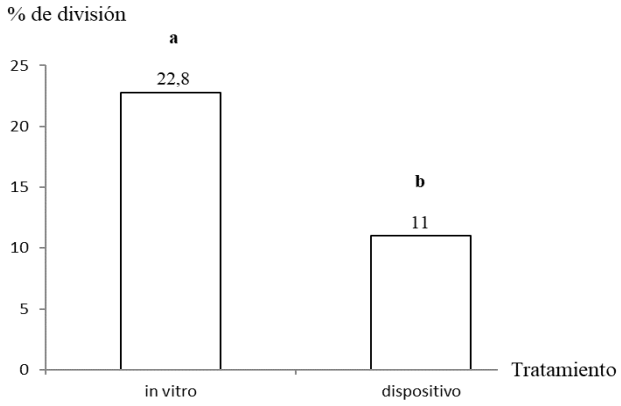

Figure 1: Percentage of bovine zygotes obtained through *in vitro* culture and using the INVO-CIMAGT intravaginal device. n=95. Proportion comparison (P<0.05, df=1).

The animal in which the device was used showed no reaction to its application. A 77% recovery rate of the structures deposited in the cryovial was achieved, due to some loss during the process. In contrast, all structures were recovered from the four-well plate. These values are lower than those obtained by Pinzón *et al*. (2015), who reported a recovery rate of 94%.

Regarding the percentage of zygotes obtained, a lower cleavage rate was observed in those cultured in the intravaginal device (Fig. 1). This may be because the technique still needs improvement. These results differ from those reported by Pinzón *et al*. (2015), who achieved cleavage rates similar to those obtained *in vitro*.

Those authors demonstrated the development of bovine embryos using an intravaginal culture device; however, their experiment was terminated at 72 hours of culture. In contrast, the preliminary study conducted with the Cuban intravaginal device prototype allowed the development of embryos to the morula and blastocyst stages. These stages are internationally recommended for cryopreservation and subsequent transfer in this species.

CONCLUSIONS

In this preliminary study, bovine embryos were obtained for the first time in Cuba using this technique. It was demonstrated that embryonic development to transferable stages is possible with a Cuban intravaginal device prototype. However, this prototype still requires optimization to reduce the loss of structures during the process and achieve cleavage rates comparable to those of *in vitro* culture.

REFERENCES

De Loos, F., Van, C., Van, P., & Kruip, T. A. M. (1989). Morphology of immature bovine oocytes. Gamete Research, *24*(2), 197-204. https://doi.org/10.1002/mrd.1120240207

Hurtado, M. V. (2021). Bloqueo embrionario ¿en qué consiste? España. Retrieved on January 29, 2025 from: www.victoriainvitro.com/bloqueo-embrionario-en-que-consiste

Pinzón, C. A., Acosta, P., Cristancho, R., Vélez, C., Pinzón, J., Zambrano, J., Jiménez, C. & Jiménez, C. (2015). Uso de la vagina como alternativa para la producción de embriones de bovinos. *Veterinaria y Zootecnia*, *9*(2), 54-64. https://www.researchgate.net/publication/308937560

Ranoux C. *In vivo* embryo culture device. Z.P. Nagy *et al*. (eds.), Practical manual of *in vitro* fertilization: Advanced methods and novel devices, Springer Science, Luxemburgo. 2012, p.161-169. https://doi.org/10.1007/978-1-4419-1780-5

Zhang, J., Lyu, Q., Li, J., Ma, Z., Yang, R., Yin, X., Yang, L., & Gao, S. (2023). Dissecting the molecular features of bovine-arrested eight-cell embryos using single-cell multi-omics sequencing. *Biology of Reproduction*, *108*(6), 871-886. https://doi.org/10.1093/biolre/ioad038

AUTHOR CONTRIBUTION STATEMENT

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

CONFLICT OF INTEREST STATEMENT

The authors state there are no conflicts of interest whatsoever.