Technical Note

Comparison of Two Oocyte Retrieval Methods Using Ovaries from Slaughtered Water Buffaloes *Bubalus bubalis*

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INTRODUCTION

In well-managed natural conditions, the water buffalo exhibits favorable reproductive and productive performance. *In vitro* embryo production (IVP) has been used commercially to enhance these outcomes by maximizing the number of offspring from selected females and shortening generation intervals. Moreover, IVP enables fertilization of oocytes from prepubertal, pregnant, or slaughtered females (Kumar *et al*., 2023).

The number of high-quality oocytes per ovary is a critical consideration in IVP; however, obtaining them requires selecting an appropriate technique under specific conditions (Kumar *et al*., 2023). Among the most commonly used methods for recovering oocytes from ovaries of slaughtered water buffaloes are syringe follicular aspiration and ovarian slicing, with slicing frequently reported as the preferred method because it yields a greater number of oocytes of superior quality (Parmar *et al*., 2022). Nevertheless, aspiration is a faster and more practical technique that permits processing a larger number of ovaries (Pitroda *et al*., 2021).

No previous studies have examined follicular puncture performed on ovaries recovered from slaughtered water buffaloes using a vacuum pump, whose advantage is that it provides a measurable and constant aspiration pressure throughout the procedure. Based on the foregoing, it was hypothesized that using a vacuum pump would improve the oocyte recovery rate. Therefore, the objective of this study was to compare the effects of slicing and vacuum-assisted follicular

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puncture on the number and viability of oocytes obtained from ovaries of slaughtered water buffaloes *Bubalus bubalis*.

DEVELOPMENT

The ovaries were collected at the sanitary slab of the Bacuranao Livestock-Genetics Company, located in Havana Province. River buffaloes (*Bubalus bubalis*) were slaughtered by electric stunning and exsanguination via severance of the jugular vein. Ovaries were recovered directly from the carcass and placed in 500 mL of 0.9% saline warmed to 38.5 °C, then transported in temperature-controlled coolers within two hours of collection.

The ovaries were processed at the Laboratory of Biotechnology and Assisted Reproduction of the Center for Research for the Improvement of Tropical Livestock (CIMAGT). They were rinsed three times with phosphate-buffered saline (PBS) supplemented with 0.1 mg/mL kanamycin (SIGMA, K1377-25G), warmed to 38.5 °C. Ovaries were randomly assigned to one of two treatments: oocyte retrieval by vacuum-assisted aspiration or oocyte recovery by slicing.

The follicular aspiration using a vacuum pump (Minitube, 23362/0000) was performed with a short 18-gauge needle and an aspiration pressure adjusted to yield a drip rate of 20 drops per minute. Follicles measuring 2–6 mm in diameter were aspirated, and the resulting cumulus-oocyte complexes (COCs) were deposited into 50-mL tubes prefilled with 5 mL of oocyte collection medium [PBS supplemented with 1% fetal bovine serum (FBS; Capricorn, FBS-12B), 0.1 mg/mL kanamycin (SIGMA, K1377-25G), and 2.4\times 10^3 mg/mL heparin (SIGMA, H3149-25KU)], warmed to 38.5 °C.

For the slicing method, ovaries were held with forceps and incised across the ovarian surface using a No. 22 scalpel blade to expose follicles measuring 2–6 mm in diameter. During and immediately after the procedure, each ovary was rinsed with 100 mL of oocyte collection medium prewarmed to 38.5 °C, and the resulting cumulus–oocyte complexes (COCs) and follicular fluid were collected into a beaker.

The COCs and follicular fluid collected in the tubes and beaker were diluted in wash medium [TCM-199 HEPES (SIGMA, M-2520) supplemented with 0.1 mg/mL kanamycin, 1% FBS (Capricorn, FBS-12B), and 0.336 mg/mL NaHCO{}_3 (SIGMA, S5761-500G)] and examined under a stereoscopic microscope at 4× magnification (Olympus ZS51) in 120-mm Petri dishes (Greiner, 688102). All manipulations were performed on heated stages (12055/0003) prewarmed to 38.5 °C inside a horizontal laminar-flow hood (Labconco, 64132). COCs were classified and separated according to morphological quality based on the appearance of the membrane, cytoplasm, and cumulus as observed under the stereomicroscope, following the criteria adapted for buffaloes by Dubeibe (2021).

COCs were washed five times in 35-mm Petri dishes (Nunc, 174943) with wash medium. Maturation was carried out in 500 µL of BO-IVM medium (IVF Bioscience, 71002) in four-well plates (Thermo Scientific, 10404532). Cultures were incubated in a CO_2 incubator (Nuaire, NU-5100E) at 38.5 °C with 5% CO_2 and maximal humidity (>90% relative humidity) for 20 hours.

Frozen buffalo semen straws were used for fertilization. Sperm separation was performed using the commercial BO-SemPrep medium (IVF Bioscience, 71003). Sperm motility and concentration were subsequently assessed in a Bürker counting chamber (Marienfeld-Superior, PM-0610230) under an inverted phase-contrast microscope (Axiovert 35M).

Mature COCs were transferred to 500 µL of BO-IVF medium (IVF Bioscience, 71003) per well in four-well plates, together with spermatozoa at a final concentration of 4\times 10^6 sperm/mL, and incubated at 38.5 °C in a CO_2 incubator with 5% CO_2 and maximal humidity for 21 hours. Thereafter, presumptive zygotes were denuded by vortexing (Heidolph Top-Mix, 94323) for 3 minutes 30 seconds at 323 g in 15-mL tubes containing 2 mL of TCM-199. Zygotes were then washed five times in 35-mm Petri dishes with the same medium and transferred to 500 µL of BO-IVC medium (IVF Bioscience, 71005) per well in four-well plates. Cultures were maintained at 38.5 °C with 5% CO_2 and maximal humidity. Embryonic stage was evaluated on day 5.

Data were processed and plotted using Microsoft Excel 2007. A binomial test for comparison of proportions (α=0.05) was performed in Minitab 14 to determine the presence of statistically significant differences.

Application of both slicing and vacuum-assisted follicular aspiration yielded an equivalent number of cumulus–oocyte complexes (COCs). No significant differences were detected in their viability for *in vitro* maturation (Table 1). Stereoscopic examination revealed a greater number of cumulus cell layers in oocytes recovered by slicing; however, this technique also produced COCs with excessively expanded cumuli that were unsuitable for initiating IVP. Following the criteria proposed by Dubeibe (2021) for buffaloes, COCs classified as categories I–III were considered viable, whereas those with poorly compacted cumuli (category IV) were excluded.

Table 1. Classification of buffalo COCs obtained by each technique. Percentage of COCs viable for maturation (Category I to III). Comparison of proportions (p>0.05, df=1).

COCs by category	Method	
	Vacuum-assisted follicular puncture	Slicing
I	0	1
II	2	4
III	9	4
IV	0	2
viable percentages for maturation	100% [(a)]	81.8% [(a)]

In contrast, Parmar *et al*. (2022) proposed the slicing technique as the most effective method for oocyte retrieval; however, the authors compared it with follicular aspiration using a syringe and not with vacuum-assisted aspiration. This is the first study reporting the use of this technique on ovaries from buffaloes recovered after slaughter.

Regarding the cleavage rate (75% for the vacuum-pump method and 50% with slicing), no significant differences were found for either technique (Fig. 1A). Nor were there differences in the rate of transferable embryos obtained — morulae and blastocysts (62.5% for the vacuum-pump method and 50% with slicing) (Fig. 1B).

These values are higher than those reported by Quintana *et al*. (2018), who obtained a 38.79% cleavage rate and 17% transferable embryos in our country using the syringe-aspiration method. Ávalos *et al*. (2022) reported similar cleavage rates (63.15%) when using the same media and protocols applied in this work and the follicular aspiration by syringe technique.

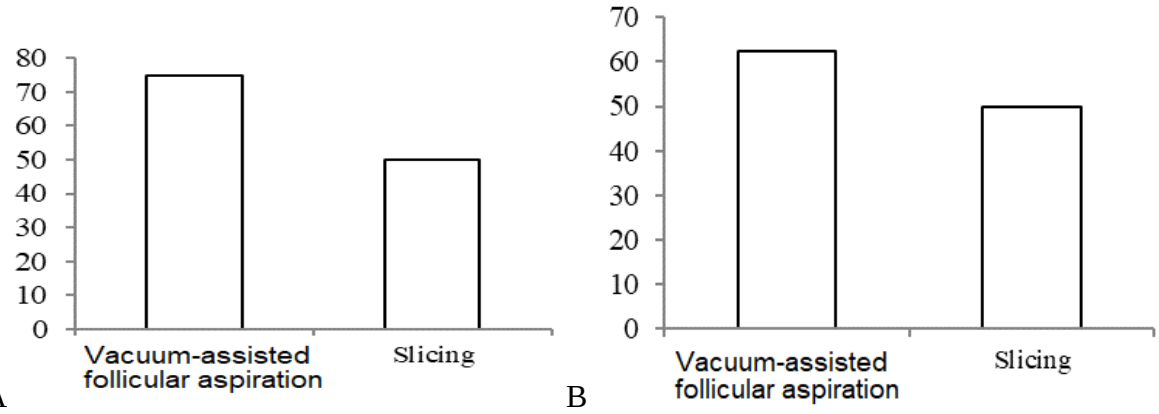

Figure 1: Percentage of A: cleavage and B: transferable embryos obtained by *in vitro* production in buffalo using two oocyte collection techniques, vacuum-assisted follicular aspiration and slicing. Comparison of proportions (*p > 0.05, df = 1).

CONCLUSIONS

The vacuum pump can be used for follicular aspiration in ovaries from slaughtered buffaloes, achieving results similar to those obtained with the slicing method in terms of the number and viability of COCs, and reaching transferable stages.

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AUTHOR CONTRIBUTION STATEMENT

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

CONFLICT OF INTEREST STATEMENT

The authors state there are no conflicts of interest whatsoever.