

Parthenogenetic activation of buffalo oocytes aspirated by ultrasound guided transvaginal ovum pick up from less fertile Murrah buffaloes (*Bubalus bubalis*)

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INTRODUCTION

Parthenogenetic activation means the production of embryos from oocytes without sperms. This way of embryo production can help to understand the potential of oocytes or reproductive efficiency of females because it only has maternal chromosomes. In the previous studies, the different methods or quality of oocytes was accessed by activation of buffalo's oocytes using the slaughter ovaries. To our knowledge, this is the first study that compares the competence of the less and normal fertile Murrah buffalo's oocytes (obtained by ovum-pick-up: OPU) by parthenogenetic *in vitro* embryo production.

METHODOLOGY

A total of 15 females belonging to the Laguna farm located in the municipality of Paracuru-CE (Brazil) were selected based on their reproductive history. Females that became pregnant after 100 days of delivery were classified as low fertile (LF, n=8) and less than 100 days with normal fertile (Cr, n=7). The average body condition score of the Cr and LF group animals was 3.57 (range 2.75 - 4) and 3.34 (range 2.75 - 4.5), respectively. The oocytes were collected by transvaginal OPU technique and classified as grade A, B, C and D. Grade A, B and C were submitted to *in vitro* maturation at 38.5 °C, 5% CO₂, 5% O₂ and 90% N₂ in a portable incubator. After 24 hours of *in vitro* maturation, the oocytes were denuded and activated by ionomycin (5 µM) for 5 minutes followed by incubation with 6-DMAP (2 mM) for 4 hours in SOF medium. Finally, the presumptive parthenotes were *in vitro* cultured in synthetic oviductal fluid (SOF) medium at 38.5 °C in 5% CO₂, 5% O₂ and 90% N₂ for 6 or 7 days. The recovery rate, morphological classification of oocytes, maturation, cleavage and blastocysts rates were observed in the groups and compared using the chi-square test, considering a 5% significance level, using the SPSS software, version 22.0.

RESULTS AND DISCUSSION

The aspiration rate of oocytes and their grades of Cr were similar (P>0.05) to the LF group (Table 1). Also, the maturation cleavage and blastocyst rates were similar (P>0.05) between the Cr (51.1, 62.1 and 45.9%, respectively) and LF (44.4, 45.2 and 30.1%, respectively) groups (Figure 1). The images of cleavage and blastocyst of parthenotes are presented in Figure 2. Studies revealed that the genetics and bull factor affect the fertility of buffalo. Perhaps these factors may have influenced the results observed.

Table 1. Aspiration rates of oocytes and their grades after OPU

Groups	Aspiration rate (%)	Grade A (%)	Grade B (%)	Grade C (%)	Grade D (%)
Cr	66.0 (72/109)	5.5 (4/72)	31.9 (23/72)	33.3 (24/72)	29.1 (21/72)
LF	73.7 (118/160)	5.9 (7/118)	35.5 (42/118)	32.2 (38/118)	26.2 (31/118)

P<0.05., Cr: Control., LF: Low fertile.

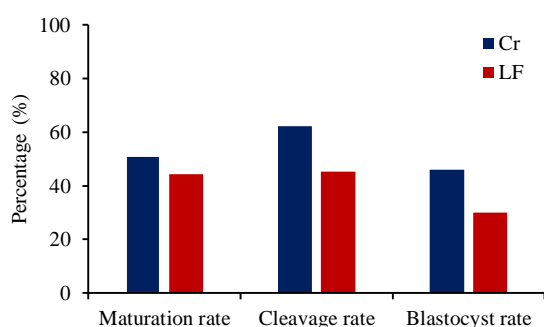


Figure 1. Representing maturation, cleavage and blastocyst rates of Cr and LF groups. P<0.05., Cr: Control., LF: Low fertile.

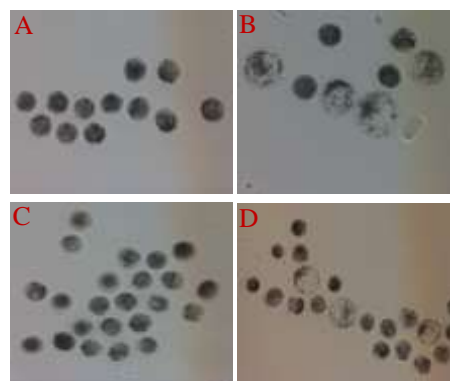


Figure 2. Observed cleavage on day 2 and blastocyst on day 6 of *in vitro* culture after activation of buffalo oocytes for Cr (A and B) and LF (C and D) groups, respectively.

FINAL CONSIDERATIONS

It was concluded that oocytes obtained from less fertile buffaloes could develop into blastocysts after *in vitro* chemical activation. However, additional studies are needed to better understand the factors that affect the fertility of these female buffaloes.

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