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Effect of Media on *in vitro* Maturation, Fertilization and Early Embryonic Development in Nili-Ravi Buffaloes

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ABSTRACT

Effects of TCM-199, Ham's F-10, BSFF and DPBS media on *in vitro* maturation of Nili-Ravi buffalo follicular oocytes and subsequently *in vitro* fertilization and early embryonic development in IVF-Tl and CZB media have been reported. TCM-199, Ham's F-10 and BSFF differed non-significantly for *in vitro* maturation (81.06, 75.29 and 79.36%, respectively), *in vitro* fertilization (60.39, 55.62 and 52.85%, respectively) and early embryonic development (48.38, 44.94 and 41.89%, respectively). Whereas, DPBS showed significantly poor results for these parameters.

Key Words: Media; *In vitro* maturation; *In vitro* fertilization; Early embryonic development; Buffalo

INTRODUCTION

For an effective *in vitro* system to be developed in buffaloes, a great deal of study is required to establish various factors responsible for optimum oocyte maturation, fertilization and culture of embryos. At present, the efficiency of buffalo embryo production is not satisfactory and has resulted in embryos which often display low fertilization and subsequent embryonic development. This may indicate some fundamental problems such as inadequacy of the culture medium or possibly inadequate oocyte maturation that subsequently influences fertilization and embryo development *in vitro*. In the current study, various maturation media were compared for their effect on *in vitro* maturation (IVM), fertilization (IVF) and early embryonic development of Nili-Ravi buffalo follicular oocytes.

MATERIALS AND METHODS

Collection of ovaries. Buffalo ovaries were collected from a local abattoir within 1-2 hours post-slaughtering and were transported immediately to the laboratory in a thermos containing sterile normal saline with added antibiotics (100 IU/ml penicillin G, 100 g/ml streptomycin sulphate and 0.25 g/ml amphotericin B) held at 30-35°C. Extraneous tissue was removed and the ovaries were cleaned in normal saline. Prior to oocyte collection the ovaries were rinsed in 70% ethanol to minimise the risk of contamination followed by three rinses with sterile normal saline to remove the traces of ethanol.

Recovery of oocytes. Follicular oocytes were recovered from 2-6 mm diameter follicles by scoring (slicing) the ovarian surface with a sterile surgical blade, with instant rinsing and tapping the ovary to release oocytes in a sterile 60 x 15 mm petridish. The petridish contained modified tyrode-lactate medium, Tl-Hepes (Bavister, 1989), supplemented with 20% oestrus buffalo serum, sodium pyruvate (0.20 mM) and gentamycin sulphate (10 g/ml). The pH of the medium was adjusted to 7.4. For classification of the oocytes, the criteria of De Loos *et al.* (1989) and Lonergan *et al.* (1991) based on their cumulus investment and ooplasm homogeneity were adopted. Category A, B and C oocytes were considered morphologically good for IVF.

***In vitro* studies.** Four maturation media viz. Tissue culture medium-199 (TCM-199; Sigma, USA), Ham's F-10 (Sigma, USA), Bovine synthetic follicular fluid (BSFF; Sirard & Coenen, 1993) and Dulbecco's phosphate buffered saline (DPBS; Gibco, USA) each containing 20% heat inactivated (56°C) oestrus buffalo serum were compared for their effect on IVM, IVF and early embryonic development of Nili-Ravi buffalo embryos. A total of 18-20 oocytes were placed in each 100 (l drop, covered by paraffin oil, cultured in an atmosphere of 5% CO_2 in humidified air at 39°C for 4 hours. Maturation of oocytes were monitored through dispersion of cumulus cells surrounding the oocytes reaching at metaphase II with one polar body extruded (Fig. 1). Each drop was inseminated with 40 µl of 2.9% sodium citrate washed spermatozoa with 1 x 10^6 live sperm in 200 (l IVF-Tl). Fertilization was defined as the number of ova that cleaved to 2-cell stage.

After 24 hours of gametes co-culture in IVF-Tl, the fertilized oocytes were removed, washed with equilibrated Tl-Hepes and transferred to Chatot Ziomek Bavister (CZB) medium drops of 100 µl size already equilibrated at 39°C under 5% CO_2 in humidified air for 4 hours prior to use. The oocytes were cultured in CZB

medium for another 48 hours at 39°C in 5% CO_2 in humidified air in an incubator for early embryo development (Fig. 2).

Statistical analysis. In order to see the magnitude of variation among four media, the data were subjected to analysis of variance using completely randomised design (Steel & Torrie, 1980).

Fig. 1. *In vitro* matured buffalo follicular oocytes

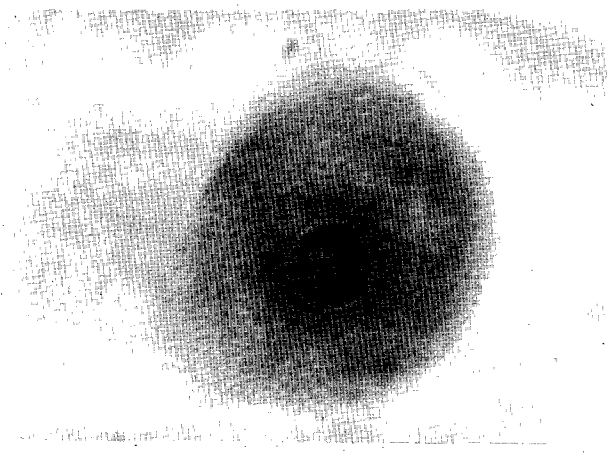
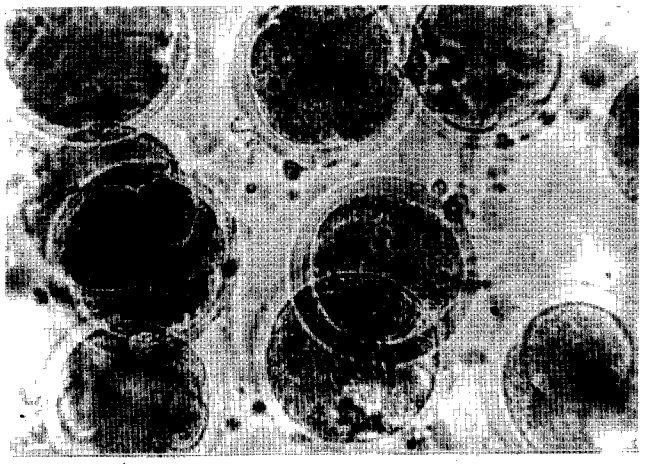


Fig. 2. IVM–IVF buffalo embryos at 4–8 cell stage



RESULTS AND DISCUSSION

It is evident from the results (Table I) that there was no difference ($P<0.05$) in the IVM, IVF and embryonic development among TCM-199, BSFF and Ham's F-10 media. However, significantly lower ($P<0.05$) values of all these parameters were recorded for DPBS medium. A wide variety of media have been used for IVM in domestic animals, ranging from simple salt solutions to complex culture media containing amino acids, vitamins, purine and other compounds regarded as essential for general cell culture. Although acceptable rates of maturation have been obtained with most of the media but TCM-199 has emerged as the most commonly used media for bovine oocytes (Stagmiller, 1988). Varying effects of composition of media on IVM, IVF or its further development has also been demonstrated by Bavister *et al.* (1992). Findings of the current study indicate that the buffalo oocytes can be successfully matured in TCM-199, Ham's F-10 and BSFF and subsequently cleaved in IVF-Tl and CZB media. Similar results have been reported by Sirard and Coenan (1991). The lower maturation as well as embryonic development rates of oocytes in DPBS support the findings of Minhas *et al.* (1989), who reported that there was marked reduction in zygotes development and blastocyst formation of murine oocytes following exposure to phosphate buffered saline. Such differential effects of maturation media, although in mouse, have also been reported previously (Van de Sandi *et al.*, 1990).

Table I. Effect of different media on maturation, fertilization and embryonic development of Nili-Ravi buffalo follicular oocytes

Medium	Oocytes matured	Oocytes fertilized 2 cell	Embryonic Development
TCM-199	81.06a (137/169)	60.39a (93/154)	48.38a (45/93)
BSFF	79.36a (150/189)	52.85a (74/140)	41.89a (31/74)
DPBS	59.11b (94/159)	42.02b (58/138)	25.86b (15/58)
Ham's F-10	75.29a (128/170)	55.62a (89/160)	44.94a (40/89)

Values with similar letters do not differ; Figures in the parenthesis are the numbers.

CONCLUSION

It was concluded that the maturation media TCM-199, BSFF and Ham's F-10 can be successfully used for IVM-IVF for buffalo follicular ooocytes. Moreover, the culture media used for *in vitro* maturation not only affect the proportion of oocytes capable of undergoing fertilisation, but also their subsequent early embryonic development.

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