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THE USE OF TRYPAN BLUE STAINING TO SELECT THE DEVELOPMENTALLY COMPETENT BOVINE OOCYTES AND ITS EFFECT ON IN VITRO MATURATION RATE

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ABSTRACT Trypan blue staining is a rapid, non-cytotoxic oocyte viability test. Ovaries were collected from 24 slaughtered cattle and were randomly allotted to two groups. Group culture grade oocytes were selected using morphological grading, whereas live oocytes from group were selected by trypan blue staining method. Cumulus expansion rate on in vitro maturation was 85.71 per cent in group , whereas in group the value was 84.62 per cent. Maturation rate of bovine oocytes did not differ (P > 0.05) significantly between two groups. The results indicated that trypan blue staining has no adverse effect on bovine oocyte maturation in vitro.

KEYWORDS : Non-cytotoxic, cumulus expansion, bovine oocytes, *in vitro*

SUMMARY

Determination of oocyte viability is useful in evaluating further developmental competence. Trypan blue staining can be used as cost effective and reliable technique for evaluating oocyte viability.

INTRODUCTION

The relatively low level of efficiency achieved using *in vitro* embryo production is almost related to the quality of oocyte at the beginning of the maturation (Khatir *et al.*, 2004). Trypan blue has the potential to be used in non-invasive assessments of oocyte quality (Isayeva *et al.*, 2004). Trypan blue staining is rapid, non-cytotoxic and based on plasma membrane integrity (Schrek, 1936). Trypan blue staining was used to assess the viability of fetal germ cells, pre-antral follicles, oocytes, spermatozoa and embryos as dead cells are permeable to trypan blue (Hutz *et al.*, 1985; Kovacs and Foote, 1992; Fauque *et al.*, 2007).

Perusal of literature did not reveal any study on the effect of trypan blue staining on *in vitro* maturation bovine oocyte. Hence the present study was conducted to evaluate the usefulness of trypan blue (TB) dye exclusion test in selection of more competent bovine oocytes and to study its effect on *in vitro* maturation.

MATERIALS AND METHODS

Ovaries were collected from slaughtered cattle of various South Indian breeds like Kangayam, Khillari, Hallikar and crossbred cattle from Thrissur corporation slaughter house and transported to the laboratory in a thermos flask containing freshly prepared normal saline solution at 36-38°C within 2 hour. The ovaries were washed several times in sterile normal saline solution at 37°C to remove excess blood and tissue debris. The ovaries were allotted randomly into two groups containing 12 animals each.

Oocytes were retrieved from surface follicles of 2 to 8mm size by aspiration in Cumulus Oocyte Complex (COC) handling media prepared with Dulbecco's Phosphate Buffered Saline (DPBS) enriched with five per cent heat inactivated (56°C for 30 min) day zero estrus cow serum and 0.5 per cent BSA maintained at 39°C.

Culture grade oocytes obtained by morphological evaluation from the group were subjected to *in vitro* maturation (Spricigo *et al.*, 2012). Live oocytes from group were selected by trypan blue staining and were also subjected to maturation.

In vitro maturation of oocytes

Medium used for *in vitro* maturation of oocytes was TCM-199 supplemented with 10 per cent fetal calf serum (v/v), 10 mg/ml of FSH and antibiotics (100 IU/ml of penicillin and 50 mg/ml of streptomycin). Fifty microliter maturation drops were prepared with this media in 35mm sterile petri dishes and sterile mineral oil was layered over these drops. After repeated washing, five to seven oocytes were loaded gently into separate 50 μl maturation drops and incubated for 22 h at 39°C temperature, five per cent CO_2 tension and maximum humidity in a standard air jacketed CO_2 incubator.

Assessment of maturation

After 22 h of culture, all oocytes in the culture drop were examined under inverted phase contrast microscope for maturation changes such as expansion and mucification of cumulus cells. Those oocytes showing expansion of cumulus cells in a radiating fashion with mucification were graded as matured oocytes.

Trypan blue staining technique

Oocytes were stained with 0.05 per cent trypan blue to assess viability of oocyte as described by Gupta *et al.* (2002). Working solution of stain was prepared by dissolving trypan blue dye in phosphate buffer saline and pH was adjusted to 7.0. Different grades (A, B, C & D) of cumulus oocyte complexes (COCs) were then placed individually in 50 μl droplet of trypan blue solution for 2 minutes. Cumulus oocyte complexes were then washed in phosphate buffer saline to remove the residual stain and observed under inverted microscope at 200X magnification. Cumulus oocyte complexes (COCs) whose ooplasm got stained were considered as dead. COCs with fully unstained or with partial or fully stained cumulus cells and zona pellucida but with unstained ooplasm were considered as alive. Live oocytes obtained were subjected to *in vitro* maturation.

RESULTS AND DISCUSSION

Among 35 oocytes retrieved in group 30 (85.71%) oocytes showed cumulus expansion on *in vitro* maturation, whereas in group 33 (84.62%) oocytes showed cumulus expansion out of 39 oocytes recovered (Table 1 and Fig. 1). Eventhough the proportion of matured oocytes was higher in group compared to group , there was no significant difference between these two groups.

The result of the present study was in agreement with the results obtained by Gupta *et al.* (2002), who obtained higher proportion of cumulus expansion for buffalo oocytes in control

group compared with trypan blue treated group. Abd-Allah *et al*. (2008) obtained significantly higher cumulus expansion rate for trypan blue treated camel oocytes when compared with morphologically selected COCs on *in vitro* maturation.

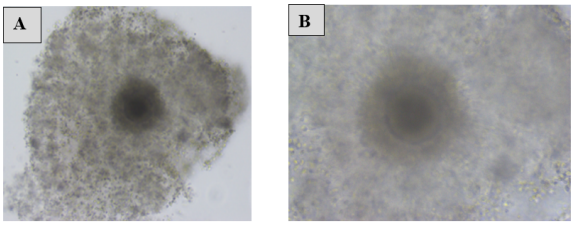
Table 1: Cumulus expansion rate of oocytes selected using morphological grading and trypan blue staining

Group	No. of ovaries collected	No. of oocytes recovered by aspiration	No. of oocytes kept for maturation	No. of oocytes showing cumulus expansion
Group (Morphological grading)	24	59	35	30 (85.71)
Group (Trypan blue staining)	24	61	39	33 (84.62)

Any value in the same column between two groups differ non-significantly (χ^2 test)

Figures in parentheses indicate percentage

Fig. 1. Oocyte showing cumulus expansion (100x) and mucification (200x)



A.Cumulus expansion **B. Mucification**

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