

**SEXING BY POLYMERASE CHAIN REACTION OF *IN VITRO*  
PRODUCED PREIMPLANTATION BUFFALO EMBRYOS  
BASED ON EARLY CLEAVAGE RATES**

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**CERTIFICATE**

This is to certify that the thesis entitled "SEXING BY POLYMERASE CHAIN REACTION OF *IN VITRO* PRODUCED PREIMPLANTATION BUFFALO EMBRYOS BASED ON EARLY CLEAVAGE RATES" submitted in partial fulfilment of the requirements for the award of the degree of **MASTER OF VETERINARY SCIENCE in ANIMAL REPRODUCTION GYNAECOLOGY AND OBSTETRICS** to the Tamil Nadu Veterinary and Animal Sciences University, Chennai-51, is a record of bonafide research work carried out by **D. GOPIKRISHNAN**, under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

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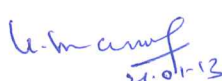
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## ABSTRACT

### SEXING BY POLYMERASE CHAIN REACTION OF *IN VITRO* PRODUCED PREIMPLANTATION BUFFALO EMBRYOS BASED ON EARLY CLEAVAGE RATES

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The aim of the present study was to determine the sex of *in vitro* produced preimplantation buffalo embryos in relation to the time of first cleavage, by polymerase chain reaction (PCR) and to study whether the presence of glucose in the culture medium caused more growth of male embryos than of female embryos. The entire experimental study consisted of three experiments. In Experiment I (n=6), abattoir derived buffalo oocytes were subjected to *in vitro* maturation and *in vitro* fertilization and were monitored from 18 hours post insemination (hpi) to establish the time of first cleavage. Of the 113 embryos cleaved, the percentage of embryos that cleaved at 18, 24 and 36 hpi was 2.65 (3/113), 6.19 (7/113) and

91.15(103/113) per cent respectively. Based on the results of experiment I, experiment II (n=6) was carried out wherein early cleaved embryos were collected at specific time points viz. 18, 24 and 36 hpi, snap frozen and sexed to study the time cleavage versus sex of *in vitro* produced buffalo embryos. Out of the 3 embryos that cleaved at 18 hpi 66.67 per cent (2/3) were males and 33.33 per cent (1/3) were females. Among all the 8 embryos that cleaved at 24 hpi and sexed, 50 per cent (4/8) were males and 50 per cent (4/8) females. Only 20 embryos out of 98 embryos that cleaved at 36 hpi were sexed, of which 40 per cent (8/20) were males and 60 per cent (12/20) females. Experiment III was designed to establish the influence of glucose supplementation on growth rate differences between sexes. In this experiment (n=6), presumptive zygotes were subjected to *in vitro* culture with glucose supplemented and glucose free Synthetic Oviductal Fluid (SOF) and the influence on cleavage rate and sex of buffalo embryos was studied. Of the 220 presumptive zygotes cultured in SOF medium plus glucose (0.5mM) 48 cleaved resulting in a cleavage rate (Mean $\pm$  SE) of 21.85 $\pm$  0.84. On sexing of these embryos, 29 were found to be males and 19 females with a sex ratio of 1.5:1. Of the 221 presumptive zygotes cultured in SOF medium minus glucose 49 cleaved resulting in a cleavage rate (Mean $\pm$  SE) of 22.21 $\pm$  1.14. On sexing of these embryos, 25 were found to be males and 24 females with a sex ratio of 1:1. Although the addition of glucose resulted in an increase in number of males (29 vs. 25) this difference was not statistically significant (P = 0.87). In conclusion, the results of the present study showed that the time of first cleavage in buffalo embryos occurred as early as 18 hpi with male embryos cleaving at a faster rate than the females. The presence of glucose in the culture medium when buffalo embryos were cultured for 24 to 48 hpi, lead to a higher percentage of cleavage among the male embryos. These results suggest that manipulating the metabolic profile of the embryos during culture may have an impact on sex ratio.

Key words: Sexing-buffalo embryos-early cleavage-PCR-glucose.