INTRODUCTION
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Buffaloes (*Bubalus bubalis*) are among the most important domestic ruminants and are valuable draught animals as source of milk and meat worldwide. However, the lower reproductive capacity of domestic buffalo when compared to cattle is a major impediment in the commercial exploitation of buffaloes. This problem is more acute in Asia, which has 95% of the world population of domestic buffalo. The buffalo is the mainstay of the Indian dairy industry where 55% of the milk production is contributed by only 19 million buffaloes.

Recent advances, such as embryo transfer technology has progressed allowing increase in the number of offspring produced from genetically superior buffaloes. Studies have unequivocally shown that the application of bovine embryo transfer technology to buffalo has only limited success (Vlahov et al., 1985; Karaivanov et al., 1990; Madan, 1990; Misra et al., 1990). However, there is a slow yet steady progress in this field with better understanding and application of technology.

Development of *in vitro* techniques for the laboratory production of farm animal embryos has excellent potential, both for basic research and for practical application on the farm. The first offspring produced from *in vitro* maturation (IVM), *in vitro* fertilization (IVF), and *in vitro* culture (IVC) of buffalo oocytes was reported by Suzuki *et al.* (1992). The birth of buffalo calves using IVM/IVF/IVC of buffalo oocytes were also reported by Madan *et al.* (1994) and Totey *et al.* (1996). Studies have shown that addition of gonadotropins (LH, FSH and estrodiol) resulted in a significant increase in the maturation rate (Totey *et al.*, 1992; Totey *et al.*, 1993). The beneficial effects of buffalo sera recovered at standing estrous, in oocyte maturation media was also noted (Madan *et al.*, 1994).
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Although the technique of IVM/IVF/IVC of buffalo oocytes have been well studied in recent years the number of transferable embryos recovered have been low.

The problem with these techniques is the lack of knowledge of the *in vivo* mechanisms of maturation, fertilization and development of early stage embryos. The ultimate aim of culturing embryos *in vitro* is to stimulate the microenvironment in which the embryo grows in the reproductive tract. Development of the early embryo is controlled by secretion of several factors which are secreted in the follicle and the female reproductive tract (Robert and Bazer, 1988). For the optimal production of viable embryos which can be transferred, it is necessary to mimic secretions *in vitro* and explore the nature of these factors.

Peptide growth factors are potential regulators of ovarian function and growth of early embryos (Carson *et al.*, 1989; Harvey and Kaye, 1988; Paria and Dey, 1990). Many growth factors are known to exist in the maternal tract (Munson *et al.*, 1992; Simmen and Simmen, 1991; Heyner *et al.*, 1993a, b). Growth factor ligand and receptor genes are expressed before and after activation of the embryonic genome. During embryogenesis, a number of growth factors and their cognate receptors have been shown to be expressed by preimplantation embryos (Schultz and Heyner, 1993). The effects noted *in vitro* include stimulated cell proliferation (Harvey and Kaye, 1992), enhanced differentiation and morphological development (Dardik and Schultz, 1991; Smith *et al.*, 1993) and an increased rate of DNA, RNA and protein synthesis (Heyner *et al.*, 1989; Harvey and Kaye, 1991). The goal of this study is to provide information about the role of growth factors during oocyte maturation, embryogenesis and *in vitro* development.
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of buffalo embryos.

The first objective of this study describes the transcription of various growth factor ligand and receptor mRNAs at the different stages of preimplantation buffalo embryos. This study was carried out using the mRNA phenotyping method in which consists of three linked techniques, (1) microadaptation of RNA isolation, followed by (2) reverse transcription (RT) and (3) polymerase chain reaction (PCR).

The second objective describes embryo culture in chemically defined media to study the effect of insulin and IGF-I on embryo development.

The third objective describes the developmental rate of buffalo embryos based on early and late cleavage; their relationship with cell number so as to determine a valid indicator for viability of preimplantation embryos and to study the correlation between development rate and the sex ratio of buffalo embryos.