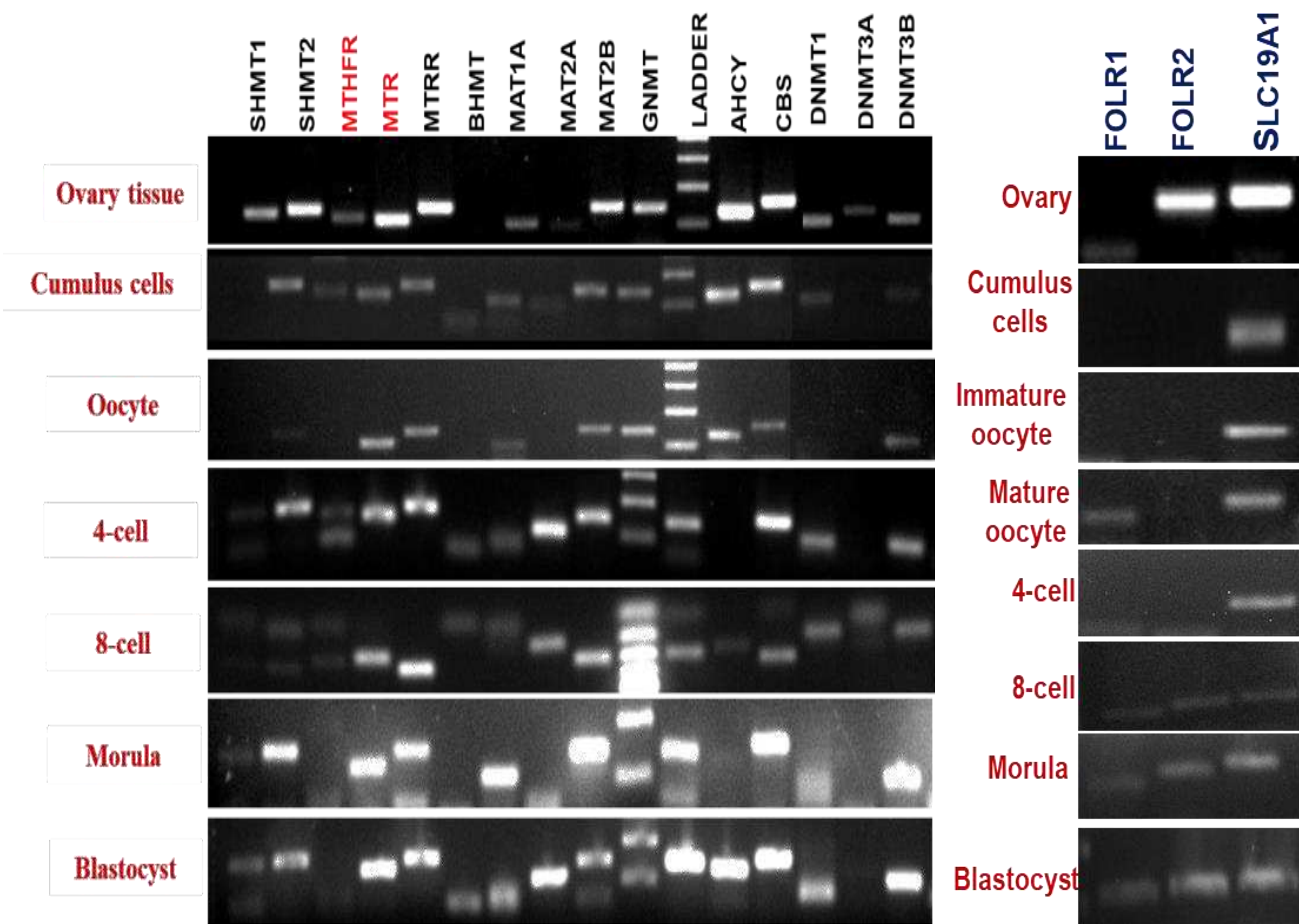


Introduction

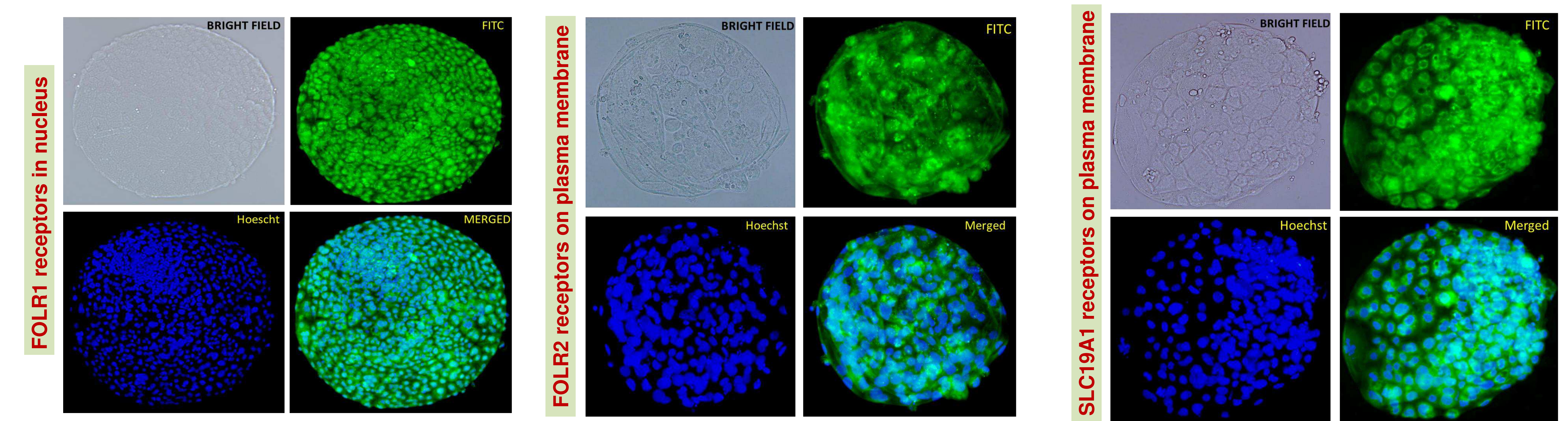
5-methyltetrahydrofolate (5-MTHF) is the predominant form of dietary folate and plays a key role in one-carbon metabolism through Folate-Methylation cycle. Folate is essential for amino acid metabolism, biosynthesis of DNA and RNA, and formation of S-adenosyl methionine (SAM) which is the universal methyl donor for DNA, histones, proteins and lipids. Folate have an important role in prevention of neural tube defects (NTD) and aiding rapid cell division furthermore growth of fetus. However, till date, Folate-Methylation cycle and effect of *in vitro* 5-MTHF supplementation in embryonic development of buffalo have not been studied. The present study was focussed to analyse the transcript expression key enzymes in the linked Folate-Methylation cycle in pre-implantation embryos. Further, to observe the effect of 5-MTHF supplementation on blastocyst production rate and global DNA methylation level. Using RT-PCR, we examined the gene expression pattern of the Folate-Methylation pathway enzymes in pre-implantation embryos. The *in vitro* blastocyst production rate was observed at different conc. of 5-MTHF supplementation in *in vitro* culture medium and also global DNA methylation was detected by immunofluorescence analysis of blastocysts.

Results

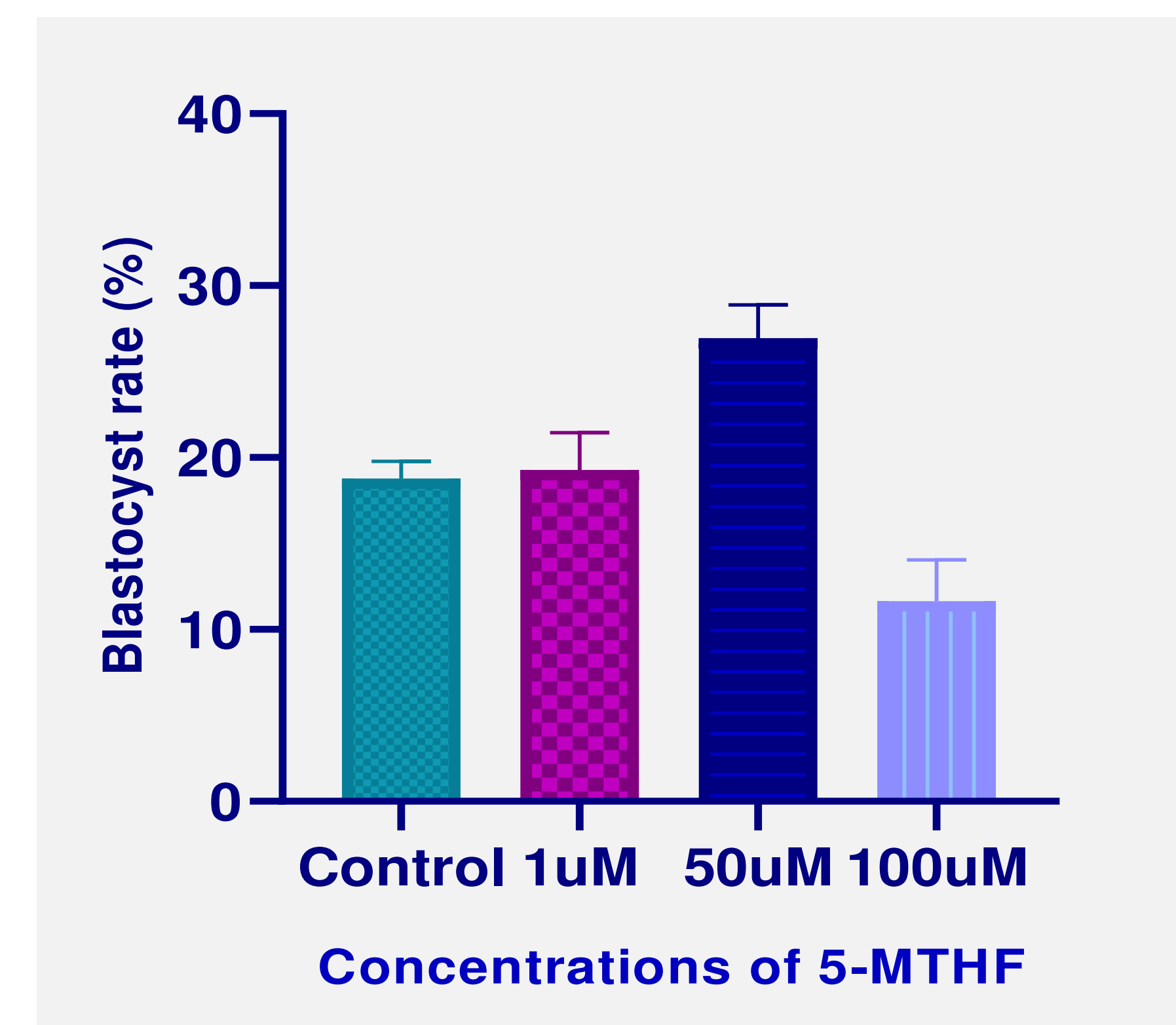
Expression of transcripts for Folate-Methylation pathway and Folate transporters



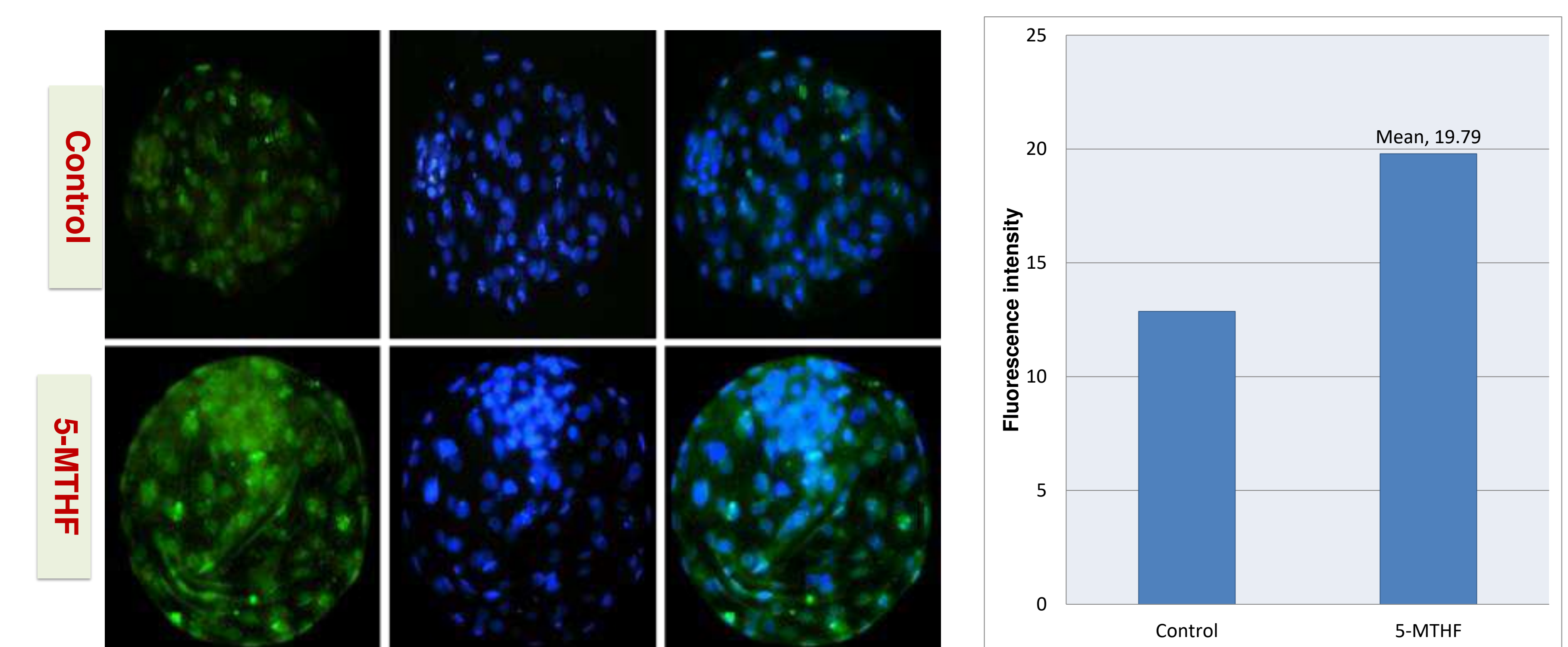
Immunolocalization of Folate transporter in blastocyst



Effect of 5-MTHF on blastocyst production



Effect of 5-MTHF on global DNA methylation



Conclusion

This study advocates the necessity for 5-MTHF supplementation throughout pre-implantation *in vitro* embryo production for improving the quantity and quality of the transferable blastocysts which may subsequently help in large scale breeding programs in buffalo.

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