

Quantification of mRNA in buffalo oocytes and different stages of developing embryos produced *in vitro*

ASHISH MISHRA¹ and G TARU SHARMA²

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

Received: 26 March 2009; Accepted: 22 September 2009

Key words: Buffalo, *In vitro* embryos, mRNA, Oocytes

The emerging array of new methods in biology such as differential display reverse transcription-polymerase chain reaction (DDRT-PCR) (Natale *et al.* 2001), subtractive cDNA libraries (Mohan *et al.* 2004) and quantitative real time polymerase chain reaction (RT-PCR) (Wrenzycki *et al.* 2004) significantly extend the possibilities of revealing differences in mRNA expression patterns throughout the preimplantation development. The expression pattern of specific genes critically involved in preimplantation development, will aid in selecting markers for determining quality embryo and acts as a useful tool to assess the normality of the embryos (Wrenzycki *et al.* 2005). Using qualitative and quantitative RT-PCR assays, expression pattern of several classes of genes were studied in preimplantation stages of murine and bovine embryos (Wrenzycki *et al.* 2004, Nieman *et al.* 2002). In bovine embryos, major embryonic genomic activation occurs at 8–16 cell stage (Telford *et al.* 1990) whereas minor activation is seen at the 2–4 cell stage (Viuff *et al.* 1996). Protein synthesis in the early pre implanted embryo was investigated extensively (Thompson *et al.* 1996). The protein content increased 2-fold from the morula to the expanded blastocyst stage followed by a 160-fold increase to the hatched blastocyst stage on day 13 (Grealy *et al.* 1996). But there are few reports regarding the transcription and translational control during preimplantation stages of buffalo embryo (Rajhans *et al.* 2006). These rapid changes in morphology require well-orchestrated expression of genes from the maternal and embryonic genome (Kidder 1992).

The present study was undertaken to quantify the mRNA in buffalo oocytes and different stages of developing embryos produced *in vitro*.

Buffalo ovaries were collected from local slaughterhouse. Oocytes were aspirated from follicle and the embryos were

produced by the standard *in vitro* maturation, fertilization and culture (IVMFC) as per Rajhans *et al.* (2006).

Immature oocytes, matured oocytes (oocytes with an extruded polar body) and preimplantation embryos generated *in vitro* were used for isolation of RNA. Pools of immature oocytes, *in vitro* matured oocytes, 8–16 cell embryos, morula and blastocysts were collected at the appropriate developmental times. Pools of oocytes (immature and *in vitro* matured) and embryos (8–16 cell, morula, blastocyst) were prepared for RNA isolation. Immature and matured oocytes were treated with 1X trypsin – EDTA solution and vortexed for 2 min to remove the attached cumulus cells, and were then washed 5–10 times in the handling medium containing TCM–199 and 10% FCS. mRNA was extracted from pools of oocytes, immature (n=200), *in vitro* matured (n=200) and embryos of 8–16 cell (n=80), morula (n=77) and blastocyst (n=40) by using m-RNA isolation kit as per manufacture guidelines.

Quantification of mRNA was done spectrophotometrically. 1 µl of mRNA was dissolved in 9 µl of nuclease free water to achieve a final dilution of 1: 10 and absorbance at 260 nm was recorded against nuclease free water as blank. Quantification of mRNA was based on the following calculation:

$$\text{mRNA (ng/}\mu\text{l)} = A_{260} \times 40 \times \text{dilution factor.}$$

Concentration of mRNA (ng/oocyte or embryo) from the pools of immature, *in vitro* matured and embryos of 8–16 cell, morula and blastocyst were quantitated spectrophotometrically. The concentration of mRNA in oocytes and in developing embryos of this study showed a decline from immature to matured oocytes followed by an increase from zygote to blastocyst stage. There exists a stockpile of maternally derived mRNAs in oocyte, which govern embryogenesis from cleavage to the blastocyst stage. Maternal mRNAs are translated during oocyte maturation, after which there is sufficient protein synthesis to carry the embryo throughout early development (Alizadeh *et al.* 2005). mRNA may need to be stable and translated a number of times throughout cleavage. As development continues beyond fertilization, there is a switch of control from the

Present address: ¹Assistant Professor, Department of Veterinary Physiology, College of Veterinary Science and Animal Husbandry, Kuthuliya, Rewa, Madhya Pradesh 486 001 (Email: ashishvet1@rediffmail.com).

²Head and Director, Centre of Advanced Studies, Division of Physiology and Climatology.

maternal genome (maternal mRNA) to the zygotic genome called maternal zygotic transition (MZT) (Bilodeau-Goeseels 1996). It has been reported that major activation of bovine embryonic genome occurs at 8–16 cell stage (Telford *et al.* 1990) but minor activation is seen at the 2–4 cell stage (Viuff *et al.* 1996). In this study the mRNA concentration was found to be 0.63 ng/immature oocyte, 0.37 ng/matured oocyte, 1.52 ng/8–16 cell, 1.76 ng/morula and 1.89 ng/blastocyst. It was observed that there is around 50% decrease in mRNA level in matured oocyte than that of immature oocyte which agrees with previous reports with a suggestion that mRNA is used for protein synthesis during oocyte maturation, which carry the embryo throughout early development (Schultz 1993, Alizadeh *et al.* 2005). We observed that there is a gradual increase in mRNA level from early embryo to blastocyst stage, which supports the previous observations (Browder *et al.* 1991). Maternal–zygotic transition normally occurs at the 8–16 stage embryos generated *in vivo* (Telford *et al.* 1990) but in embryos generated *in vitro*, embryonic transcription is thought to begin earlier at the 2–4 cell stage (Iwasaki *et al.* 1995). The length of poly (A) tails of various gene transcripts in oocytes is an important criterion for functional mRNA to assess gene expression. During *in vitro* maturation there is significant shortening of poly (A) tail (Brevin-Gandolfi *et al.* 1999) and there is degradation of mRNA during embryo development (Carpousis *et al.* 1999) but *in vitro* culture systems used in this study lead to enhance the expression of mRNA throughout the critical phase of embryo development. From this study it can be concluded that in buffalo, the degradation rate of maternal mRNA may be slow than other reported species with sufficient embryonic mRNA synthesis, which continues up to blastocyst stage.

SUMMARY

This study was conducted to find out the quantity of messenger ribonucleic acid (mRNA) in buffalo (*Bubalus bubalis*) oocytes and different stages of developing embryos produced *in vitro*. The mRNA content (ng/oocyte or embryo) in this study showed a decline from immature to matured oocytes followed by an increase from zygote to blastocyst stage. 50% decrease in mRNA level in matured oocyte than that of immature oocyte in this study suggested that mRNA is used for protein synthesis during oocyte maturation, which carry the embryos throughout the early development. The gradual increase in mRNA level from early embryo to blastocyst stage of this study suggested that mRNA transcription increases from cleavage to the blastocyst stage in buffalo embryos produced *in vitro*.

REFERENCES

- Alizadeh Z, Kageyama S I and Aoki F. 2005. Degradation of maternal mRNA in mouse embryos: Selective degradation of specific mRNAs after fertilization. *Molecular Reproduction and Development* **72**: 281–90.
- Bilodeau-Goeseels S. 1996. 'Changes in RNA abundance in early bovine embryos.' Ph.D. thesis, Department of Biochemistry and Molecular Biology, The University of Calgary, Canada.
- Brevin-Gandolfi T A L, Favetta L A, Mauri L, Luciano A M, Cilio F and Gandolfi F. 1999. Changes in poly A tail length of maternal transcripts during *in vitro* maturation of bovine oocytes and their relation with developmental competence. *Molecular Reproduction and Development* **47**: 248–54.
- Browder L W, Erickson C A and Jeffery W R. 1991. *Developmental Biology*. 3rd edn. Saunders College Pub. Philadelphia.
- Carpousis A J, Vanzo N F and Raynal L C. 1999. mRNA degradation: A tale of poly A and multiprotein machines. *Trends in genetics* **15**: 24–28.
- Grealy M, Diskin M G and Sreenan J M. 1996. Protein content of cattle oocytes and embryos from the two cell to the elongated blastocyst stage at day 16. *Journal of Reproduction and Fertility* **170**: 229–33.
- Iwasaki S, Witmut I and Campbell K H S. 1995. Time dependent RNA synthesis in early bovine embryos derived from *in vitro* fertilization. *Journal of Reproduction and Fertility Abstract series* **15**: 59–60.
- Kidder G M. 1992. The genetic program of preimplantation development. *Developmental Genetics* **13**: 319–25.
- Mohan M, Hurst A G and Malayer J R. 2004. Global expression analysis comparing bovine blastocysts flushed on day 7 or produced *in vitro*. *Molecular Reproduction and Development* **68**: 288–98.
- Natale D R, De Sousa P A, Westhusin M E and Watson A J. 2001. Sensitivity of bovine blastocysts expression patterns to culture environments assessed by different display RT-PCR. *Reproduction* **122**: 687–93.
- Niemann H, Wrenzyckie C, Lucas-Hahn A, Brambrink T, Kues W A and Carnwath J W. 2002. Expression patterns in bovine *in vitro*-produced and nuclear transferred-derived embryos and their implications for early development. *Cloning Stem Cell* **4**: 29–38.
- Rajhans R, Kumar G S and Sharma G T. 2006. Expression profiles of stress and metabolic marker genes during *in vitro* production of buffalo *Bubalus bubalis* embryos. *Journal of Reproduction and Fertility Development* **18** (2): 253–54.
- Schultz R M. 1993. Regulation of zygotic gene activation in the mouse. *Bioessay* **15**: 531–38.
- Telford N A, Watson A J and Schultz G A. 1990. Transition from maternal to embryonic control in early mammalian development: A comparison of several species. *Molecular Reproduction and Development* **26**: 90–100.
- Thompson J G, Sherman A N M, Allen N W, Mc Gowan L T and Tervit H R. 1996. Total protein content and protein synthesis within pre-elongation stage bovine embryos. *Molecular Reproduction and Development* **50**: 139–45.
- Viuff D, Avery B, Greve T, King W A and Hyttel P. 1996. Transcriptional activity in *in vitro* produced bovine 2 and 4 cell embryos. *Molecular Reproduction and Development* **43**: 171–79.
- Wrenzyckie C, Hermann D, Lucas-Hahn A, Korsawe K, Lamme E and Niemann H. 2005. Messenger RNA expression patterns in bovine embryos derived from *in vitro* procedures and their implications for development. *Reproduction Fertility and Development* **17**: 23–35.
- Wrenzyckie C, Hermann D, Lucas-Hahn A, Lamme E, Korsawe K and Niemann H. 2004. Expression pattern in *in vitro* produced and somatic nuclear transfer-derived preimplantation bovine embryos: relationship to the large offspring syndrome? *Animal Reproduction Science* **82–83**: 593–603.