6. Lin, Y. M., Lin, P. H., Chen, C. J. and Huang, C. C., Measurement of terrestrial gamma radiation in Taiwan, Republic of China. *Health Phys.*, 1987, **52**, 805–811.
7. Yu, K. N., Guan, Z. J., Stokes, M. J. and Young, E. C. M., The assessment of the natural radiation dose committed to the Hong Kong people. *J. Environ. Radioact.*, 1992, **17**, 31–48.
8. Chen, C. J., Weng, P. S. and Chu, T. C., Evaluation of natural radiation in houses built with black schist. *Health Phys*., 1993, **64**, 74–78.
9. Baeza, A., Rio, M. D., Miro, C. and Paniagua, J., Natural radionuclide distribution in soils of Caceres (Spain): dosimetry implications. *J. Environ. Radioact.*, 1994, **23**, 19–37.
10. Miah, F. K., Roy, S., Touhiduzzaman, M. and Alam, B., Distribution of radionuclides in soil samples in and around Dhaka City. *Appl. Radiat. Isot*., 1998, **49**, 133–137.
11. Hill, P. *et al.*, The use of TLD-700H dosimeters in the assessment of external doses at the former Semipalatinsk nuclear test site. *Radiat. Prot. Dosim*., 2002, **101**(1–4), 297–300.
12. Rybach, L., Bachler, D., Bucher, B. and Schwarz, G., Radiation doses of Swiss population from external sources. *J. Environ. Radioact.*, 2002, **62**, 277–286.
13. Tavakoli, M. B., Annual radiation background in the city of Isfahan. *Med. Sci. Monit.*, 2003, **9**(7), PH7–PH10.
14. Fayez, H. H. and A1-Ghorabie, Measurements of environmental terrestrial gamma radiation dose rate in three mountainous locations in the western region of Saudi Arabia. *Environ. Res.*, 2005, **98**, 160–166.
15. Chougaonkar, M. P., Eappen, K. P., Ramachandran, T. V., Shetty, P. G., Mayya, Y. S., Sadasivan, S. and Venkat Raj, V., Profiles of doses to the population living in the high background radiation areas in Kerala, India. *J. Environ. Radioact.*, 2003, **71**, 275–297.
16. Selvasekarapandian, S., Lakshmi, K. S., Brahmanandhan, G. M. and Meenakshisundaram, V., Indoor gamma dose measurement along the east coast of Tamil Nadu, India using TLD. In International Congress Series, 2005, vol. 1276, pp. 327–328.
17. Nambi, K. S. V., Mehta, N. K., Muraleedharan, T. S., Mayya, Y. S. and Saha, S. C., Preliminary dose estimates in the high background radiation areas of Orissa coastal region near Chhatrapur. In Proceedings of the Third National Symposium on Environment with Special Emphasis on High Background Radiation Areas, Thiruvananthapuram, Kerala, 1994.
18. UNSCEAR, Sources and effects of ionizing radiation, United Nations Scientific Committee on the Effects of Atomic Radiation Report to the General Assembly, with Scientific Annexes, New York, 2000.

Received 5 February 2015; revised accepted 18 May 2015

In vitro embryo production in buffalo: effects of culture system on pre-implantation development and gene expression pattern

Sadeesh E. M.*

Division of Animal Physiology and Reproduction,
ICAR-Central Institute for Research on Buffaloes, Hisar 125 001, India

Expression profile of developmentally important genes can be used to optimize the *in vitro* culture system to produce superior quality buffalo embryos. In nearly all the studies on *in vitro* embryo production of buffaloes, the presumptive zygotes are subjected to an *in vitro* culture system which involves use of TCM-199 or simple media like charles rosenkrans and synthetic oviductal fluid with or without serum; however, these media do not fully mimic the *in vivo* conditions. The inhibitory or stimulatory effects of culture conditions on the expression of candidate genes involved in buffalo embryo development, quality and stress response will help identify the post-fertilization culture environment effects on *in vitro* developmental characteristics of embryo. Further, identification of genes whose expression profiles are frequently abnormal in *in vitro* fertilized (IVF) embryo derived from different culture systems will help provide markers for the diagnosis of IVF embryo viability prior to embryo transfer, and thus negate the time and money-consuming transfer of non-viable embryos to recipient animals. The studies reported here explore the possibility of establishing a suitable culture system which provides greater *in vitro*-development of embryos in buffalo.

Keywords: Buffalo, culture media, embryo, gene expression, *in vitro* production.

SUBSTANTIAL progress has been achieved in assisted reproductive technology (ART) in animals during the last decade. However, the overall efficiency of some of these techniques is less than expected. For example, *in vitro* culture of oocytes and embryos, composition of the media and environmental conditions can have a profound effect on the final outcome in *in vitro* fertilization. In spite of progress made in procedures for *in vitro* maturation, fertilization and culture of bovine oocytes, the percentage of embryos which are able to develop normally *in vitro* is less than that under *in vivo* conditions[1–3]. Several differences have been shown between these two types of embryos such as cell number, lipid content, tolerance to cryopreservation and chromosomal abnormalities[4–6]. Despite a similar maturation rate (87% versus 94%), a significantly lower cleavage rate (65% versus 84%) has been observed in buffaloes than in cattle[7,8]. It is, therefore, a

e-mail: sadeeshcirb@gmail.com

matter of concern to further improve *in vitro* embryo production (IVEP) so that it can be extensively used in buffaloes.

Abundant evidence has been presented over the last decade indicating that the developmental potential of embryos *in vitro* depends mainly on the quality of the oocyte from which it originates[3,9,10], as well as the culture environment to which the embryos are exposed[11–14]. The higher rates of pre-implantation development of bovine embryos observed in commercially available sequential medium (G1.3/G2.3), indicate the importance of quality control in the preparation of culture media. Sub-optimal culture conditions in the laboratory for media preparation have been documented for buffalo *in vitro* fertilized (IVF) embryos, and it has been reported that the quality of water and chemicals used for preparation of media like modified Charles Rosenkrans 2 (mCR2) and modified synthetic oviductal fluid (mSOF) will affect *in vitro* embryo development[15]. Addition of hormones like FSH and estradiol improved *in vitro* maturation and further development of buffalo oocytes, irrespective of the source of sera supplement[16]. Media like synthetic oviductal fluid (SOF) and Charles Rosenkrans (CR) for *in vitro* culture of buffalo embryo have been used[17], but there is no report in which the comparison of these media to support the development of buffalo zygotes to morula and blastocysts stages has been made. Different media for buffalo *in vitro* embryo culture with or without serum have been compared[18] and it has been shown that the SOF medium is the most effective for supporting the development of buffalo zygotes to morula and blastocyst stages. There is no published report related to the effects of different culture media for the mRNA expression of genes affecting embryo development, quality and stress response.

Gene expression has a fundamental role in the coordination of homeostatic and metabolic mechanisms throughout life. Precise control of gene expression during the pre-implantation phase of development is particularly important. Several major developmental events occur during this period, including the first cleavage division, the timing of which is important, embryonic genome activation, morula compaction which involves the establishment of the first intimate cell-to-cell contacts in the embryo and blastocyst formation involving the differentiation of two cell types, the trophectoderm and the inner cell mass[11,19]. Thus, analysis of expression patterns of developmentally important genes essential in early development provides a useful tool to assess the normality of the produced embryos and to optimize assisted reproduction technologies. There are many reasons behind the quality and biochemical aspects of IVF embryos to be distinct as compared to *in vivo* counterparts. Studies of gene expression in cells and embryos provide a better understanding of several biochemical pathways at the molecular level ('molecular phenotype') and can contribute to the development of more efficient protocols for IVEP. Findings from bovine embryonic genome studies indicate that the currently used *in vitro* embryo development conditions cannot fully mimic the *in vivo* conditions with regards to mRNA expression[20–22].

In domestic species, there is a large body of evidence demonstrating that the culture media can perturb gene expression in the developing embryo[23]. This is the case, not only when one compares *in vitro* and *in vivo* culture systems, but also when comparisons of different *in vitro* culture systems are made[24–27]. It is clear from the above discussion that the post-fertilization culture environment can have a dramatic effect on the pattern of mRNA abundance of many developmentally important genes in the embryo. This effect has been generally measured in blastocysts at the end of the culture period. Evidence for a temporal association between culture environment and gene expression is scarce. In nearly all the studies on IVEP of buffalo, the presumptive zygotes are subjected to an *in vitro* culture (IVC) system which involves use of TCM-199, a complex medium co-culture with buffalo oviductal epithelial cells or granulosa cells and supplemented with serum; even though it has been demonstrated that primary oviduct[28–30] and granulosa cell[31,32] cultures secrete a series of factors which act in a paracrine manner stimulating development to the blastocyst stage. This system is quite complex and blastocyst yield associated with this is very low. Simultaneously, many studies indicate that varying concentrations of serum in culture media inhibit the expression of many genes related to embryo development and quality. For IVEP, no particular culture environment has been adjudged as the best or optimal medium and simple media like SOF and CR were found capable of supporting the developments of buffalo zygotes to blastocyst stage even in the absence of co-culture with somatic cells. Yet these media do not fully mimic the *in vivo* conditions[18]. Morula and blastocysts cultured in TCM-199 supplemented with serum carry a large number of high-density lipid droplets, suggesting the presence of polyunsaturated fatty acids (PUFAs)[33]. This increase in PUFA, which has been described to down-regulate mRNA expression of proteins involved in lipid metabolism can predispose the embryo to oxidative injuries triggering lipid peroxidation chain reaction and consequent membrane damage[34]. Higher serum content in culture media leads to alterations in membrane function and permeability, especially in mitochondrial membranes and may cause irreversible loss of cellular respiration, oxidative phosphorylation and ion transport compromising embryonic metabolism and messenger RNA expression[35]. Serum supplementation has been shown to increase the number of immature mitochondria[36], inhibit cell division[37] and induce mitochondria-mediated apoptosis[38]. Foetal calf serum (FCS) also increases the percentage of apoptosis, reducing the number of cells per blastocyst and the blastocyst rate[39].

Gene expression studies might help assess the quality of embryos and optimize IVEP and related assisted reproduction technologies[40,41]. It has been reported that many of the differences in quality can be attributed to the culture environment medium-induced changes in mRNA abundance[42]. Studies on the embryonic genome indicate that the currently used *in vitro* embryo development conditions cannot fully mimic the *in vivo* conditions with regard to mRNA expression[20–22]. During the culture period, the presence of serum (10% FCS) can induce a number of subtle modifications in blastocyst development, including the increased expression of stress responsive genes (*MnSOD*, *GPX-4*) and decreased expression of embryo quality genes like interferon-tau[6,43]. As far as gene expression in *in vitro*-produced buffalo embryos in relation to culture environment is concerned, the literature is scarce. It has been reported that the total cell number and expression profile of developmentally important genes should be employed to optimize the *in vitro* culture system to produce superior quality buffalo embryos[44] and housekeeping genes such as *GAPDH*, *RPS15* and *RPS18* can be used as a reliable normalizer in gene expression studies associated with *in vitro* early embryonic development in buffalo[45]. Expression pattern of metabolism genes such as *G6PDH* and *LDH*; and developmentally important genes such as *HSPA-1A*, *LIF* and *LIFRβ* can be used as a biomarker during embryonic development as a tool to optimize *in vitro* culture conditions for buffalo embryos[46–48]. Although IVEP would be an effective technique to improve the efficacy of transferable embryo production in buffalo, IVEP improvement is important for high-quality embryos[49]. The identification of genes whose expression profiles are frequently abnormal in IVF embryo from different culture systems will provide markers for the diagnosis of IVF embryo viability prior to embryo transfer and, therefore, potentially negate the time- and money-consuming transfer of non-viable embryos to recipient animals. From such a study, it is possible to establish a suitable culture system which encourages greater *in vitro* development of embryos in buffalo.

Compared with conventional superovulation and embryo transfer, production of embryos in the laboratory has several advantages, but the success rate is low. It is, therefore, a matter of concern to further improve IVEP so that it can be widely used for buffaloes. A proper expression of all developmentally important genes in a well-orchestrated manner is essential for appropriate development of an embryo. Findings from bovine embryonic genome studies indicate that the currently used *in vitro* embryo development conditions cannot fully mimic the *in vivo* conditions with regards to mRNA expression. The culture medium has a significant role to play in the development of embryos in most of the species, including buffalo. Many of the differences in the quality of *in vitro*-produced embryos can be attributed to culture environment-induced changes in mRNA abundance. Different culture media like TCM-199, mCR2 or mSOF have been used for the *in vitro* culture of buffalo embryos. Yet these media do not fully mimic the *in vivo* conditions and most of them have supported embryo development to varying rates. The quality of water and chemicals used for the preparation of such formulations with respect to toxicity levels and batch-to-batch variation is a concern for laboratory-made media like mCR2 or mSOF. Such effects resulting from suboptimal culture conditions in the laboratory for media preparation have also been documented for buffalo IVF embryos. High concentrations of serum in culture media inhibit the expression of many genes related with embryo development and quality. The study of inhibitory/stimulatory effects of different culture conditions on the expression of candidate genes involved in buffalo embryo development, quality and stress response will help elucidate the role of post-fertilization culture environment on *in vitro* developmental characteristics of the embryo. Thus it may be possible to establish a suitable culture system which encourages greater *in vitro* development of embryos for commercial application of this technology in buffaloes.

1. Khurana, N. K. and Niemann, H., Effects of oocyte quality, oxygen tension, embryo density, cumulus cells and energy substrates on cleavage and morula/blastocyst formation of bovine embryos. *Theriogenology*, 2000, **54**, 741–756.
2. Takahashi, M., Keicho, K., Takahashi, H., Ogawa, H., Schultz, R. M. and Okano, A., Effect of oxidative stress on development and DNA damage in *in vitro* cultured bovine embryos by comet assay. *Theriogenology*, 2000, **54**, 137–145.
3. Lonergan, P., Rizos, D., Gutierrez-Adan, A., Moreira, P. M., Pintado, B. and Dela, F. J., Temporal divergence in the pattern of messenger RNA expression in bovine embryos cultured from the zygote to blastocyst stage *in vitro* or *in vivo*. *Biol. Reprod*., 2003, **69**, 1424–1431.
4. Viuff, D., Greve, T., Avery, B., Hyttel, P., Brockhoff, P. B. and Thomsen, P., Chromosome aberrations in *in vitro* produced bovine embryos at days 2–5 post-insemination. *Biol. Reprod*., 2000, **64**, 1143–1148.
5. Ward, F., Rizos, D., Corridan, D., Quinn, K., Boland, M. and Lonergan, P., Paternal influence on the time of first embryonic cleavage post insemination and the implications for subsequent bovine embryo development *in vitro* and fertility *in vivo*. *Mol. Reprod. Dev*., 2001, **60**, 47–55.
6. Rizos, D., Guti´errez-Adan, A., P´erez-Garnelo, J., De La Fuente, J., Boland, M. P. and Lonergan, P., Bovine embryo culture in the presence or absence of serum: implications for blastocyst development, cryotolerance, and messenger RNA expression. *Biol. Reprod*., 2003, **68**, 236–243.
7. Gasparrini, B., *In vitro* embryo production in buffalo species: state of art. *Theriogenology*, 2002, **57**, 237–257.
8. Neglia, G., Gasparrini, B., Di Brienza, V. C., Palo, R. D., Campanile, G., Presicce, G. A. and Zicarelli, L., Bovine and buffalo *in vitro* embryo production using oocytes derived from abattoir ovaries or collected by transvaginal follicle aspiration. *Theriogenology*, 2003, **59**, 1123–1130.
9. Sirard, M. A., Dufort, I., Coenen, K., Tremblay, K., Massicotte, L. and Robert, C., The use of genomics and proteomics to understand oocyte and early embryo functions in farm animals. *Reprod. Suppl*., 2003, **61**, 117–129.

10. Rodriguez, K. F. and Farin, C. E., Gene transcription and regulation of oocyte maturation. *Reprod. Fertil. Dev*., 2004, **16**, 55–67.
11. Lonergan, P., O'Kearney-Flynn, M. and Boland, M. P., Effect of protein supplementation and presence of an antioxidant on the development of bovine zygotes in synthetic oviduct fluid medium under high or low oxygen tension. *Theriogenology*, 1999, **51**, 1565–1576.
12. Van Soom, A., Yuan, Y. Q., Peelman, L. J., De Matos, D. G., Dewulf, J., Laevens, H. and De Kruif, A., Prevalence of apoptosis and inner cell allocation in bovine embryos cultured under different oxygen tensions with or without cysteine addition. *Theriogenology*, 2002, **57**, 1453–1465.
13. Rizos, D., Lonergan, P., Boland, M. P., Arroyo-Garcia, R., Pintado, B., De La Fuente, J. and Gutierrez-Adan, A., Analysis of differential mRNA expression between bovine blastocysts produced in different culture systems: implications for blastocyst quality. *Biol. Reprod*., 2002, **66**, 589–595.
14. Yuan, Y. Q. *et al.*, Influence of oxygen tension on apoptosis and hatching in bovine embryos cultured *in vitro*. *Theriogenology*, 2003, **59**, 1585–1596.
15. Palta, P. and Chauhan, M. S., Laboratory production of buffalo (*Bubalus bubalis*) embryos. *Reprod. Fertil. Dev*., 1998, **10**, 379–391.
16. Yadav, P. S., Singh, I. and Pathk, N. N., Effect of sera and hormone on oocyte maturation, cleavage and development of buffalo embryos. *Bubalus bubalis*, 2002, **4**, 57–60.
17. Nandi, S., Ravindranatha, B. M., Gupta, P. S., Raghu, H. M. and Sarma, P. V., Developmental competence and post-thaw survivability of buffalo embryos produced *in vitro*: effect of growth factors in oocyte maturation medium and of embryo culture system. *Theriogenology*, 2003, **60**, 1621–1631.
18. Kumar, D., Manik, R. S., Singla, S. K. and Chauhan M. S., Effect of culture media and serum supplementations on the development of *in vitro* fertilized buffalo embryos. *Indian J. Anim. Rep*., 2007, **77**, 697–701.
19. Boni, R., Tosti, E., Roviello, S. and Dale, B., Intercellular communication in *in vivo*- and *in vitro*-produced bovine embryos. *Biol. Reprod*., 1999, **61**, 1050–1055.
20. Wrenzycki, C., Herrmann, D., Carnwath, J. W. and Niemann, H., Expression of the gap junction gene connexin-43 (Cx-43) in pre-implantation bovine embryos derived *in vitro* or *in vivo*. *J. Reprod. Fertil*., 1996, **108**, 17–21.
21. Lequarre, A. S., Feugang, J. M., Malhomme, O., Donnay, I., Massip, A., Dessy, F. and Van Langendonckt, A., Expression of Cu/Zn and Mn superoxide dismutases during bovine embryo development: influence of *in vitro* culture. *Mol. Reprod. Dev*., 2001, **58**, 45–53.
22. Corcoran, D., Rizos, D., Fair, T., Evans, A. C. and Lonergan, P., Temporal expression of transcripts related to embryo quality in bovine embryos cultured from the two cells to blastocyst stage *in vitro*. *Mol. Reprod. Dev*., 2007, **74**, 972–977.
23. Wrenzycki, C., Herrmann, D., Lucas-Hahn, A., Korsawe, K., Lemme, E. and Niemann, H., Messenger RNA expression patterns in bovine embryos derived from *in vitro* procedures and their implications for development. *Reprod. Fertil. Dev*., 2005, **17**, 23–35.
24. Fair, T., Lonergan, P., Dinnyes, A., Cottell, D. C., Hyttel, P., Ward, F. A. and Boland, M. P., Ultra structure of bovine blastocysts following cryopreservation: effect of method of blastocysts production. *Mol. Reprod. Dev*., 2001, **58**, 186–195.
25. Rizos, D., Ward, F., Duffy, P., Boland, M. P. and Lonergan, P., Consequences of bovine oocyte maturation fertilization or early embryo development *in vitro* versus *in vivo*: implication for blastocyst yield and blastocyst quality. *Mol. Reprod. Dev*., 2002, **61**, 234–248.
26. Natale, D. R., De Sousa, P. A., Westhusin, M. E. and Watson, A. J., Sensitivity of bovine blastocyst gene expression patterns to culture environments assessed by differential display RT-PCR. *Reproduction*, 2001, **122**, 687–693.
27. Doherty, A. S., Mann, M. R., Tremblay, K. D., Bartolomei, M. S. and Schultz, R. M., Differential effects of culture on imprinted H19 expression in the pre-implantation mouse embryo. *Biol. Reprod*., 2000, **62**, 1526–1535.
28. Ando, H., Kobayashi, M., Toda, S., Kikkawa, F., Masahashi, T. and Mizutani, S., Establishment of a ciliated epithelial cell line from human fallopian tube. *Hum. Reprod*., 2000, **5**, 1597–1603.
29. Jang, H. Y., Jung, Y. S., Cheong, H. T., Kim, J. T., Park, C. K. and Kong, H. S., Effects of cell status of bovine oviduct epithelial cell on the development of bovine IVM/IVF embryos and gene expression in the BOEC used or not used for the embryo culture. *Asian Australas. J. Anim. Sci*., 2008, **21**, 980.
30. Mugnie, S., Kervella, M., Douet, C., Canepa, S., Pascal, G. and Deleuze, S., The secretions of oviduct epithelial cells increase the equine *in vitro* fertilization rate: are osteopontin, atrial natriuretic peptide A and oviductin involved? *Reprod. Biol. Endocrinol*., 2009, **7**, 129.
31. Taniguchi, F., Harada, T., Nara, M., Deura, I., Mitsunari, M. and Terakawa, N., Co-culture with a human granulosa cell line enhanced the development of murine pre-implantation embryos via SCF/c-kit system. *J. Assist. Reprod. Genet*., 2004, **21**, 223–228.
32. Nottola, S. A., Heyn, R., Camboni, A., Correr, S. and Macchiarelli, G., Ultra structural characteristics of human granulosa cells in a co-culture system for *in vitro* fertilization. *Microsc. Res. Tech*., 2006, **69**, 508–516.
33. Abe, H., Yamashita, S., Itoh, T., Satoh, T. and Hoshi, H., Ultrastructure of bovine embryos developed from *in vitro* matured and fertilized oocytes: comparative morphological evaluation of embryos cultured either in serum-free medium or in serum-supplemented medium. *Mol. Reprod. Dev*., 1999, **53**, 325–335.
34. Wakefield, S. L., Lane, M., Schulz, S. J., Hebart, M. L., Thompson, J. G. and Mitchell, M., Maternal supply of omega-3 polyunsaturated fatty acids alter mechanisms involved in oocyte and early embryo development in the mouse. *Am. J. Physiol. Endocrinol. Metab*., 2008, **294**, 425–434.
35. Kowaltowiski, A. J. and Vercesl, A. E., Mitochondrial damage induced by conditions of oxidative stress. *Free Radic. Biol. Med*., 1999, **26**, 463–471.
36. Pegoraro, L. M., Thuard, J. M., Delalleau, N., Guerin, B., Deschamps, J. C., Marquant-Le Guienne, B. and Humblot, P., Comparison of sex ratio and cell number of IVM-IVF bovine blastocysts co-cultured with bovine epithelial cells or with vero cells. *Theriogenology*, 1998, **49**, 1579–1590.
37. Crosier, A., Farin, P. W., Dykstra, M. J., Alexander, J. E. and Farin, C. E., Ultrastructural morphometry of bovine blastocysts produced *in vivo* or *in vitro*. *Biol. Reprod.*, 2001, **64**, 1375–1385.
38. Liu, L., Trimarchi, J. R. and Keefe, D. L., Involvement of mitochondria in oxidative stress-induced cell death in mouse zygotes. *Biol. Reprod*., 2000, **62**, 1745–1753.
39. Cui, X. S., Lee, J. Y., Choi, S. H., Kwon, M. S., Kim, T. and Kim, N. H., Mouse granulocyte–macrophage colony-stimulating factor enhances viability of porcine embryos in defined culture conditions. *Anim. Reprod. Sci*., 2004, **84**,169–177.
40. Khurana, N. K. and Niemann, H., Energy metabolism in preimplantation bovine embryos derived *in vitro* or *in vivo*. *Biol. Reprod*., 2000, **62**, 847–856.
41. Rizos, D., Clemente, M., Bermejo-Alvarez, P., de La Fuente, J., Lonergan, P. and Gutierrez-Adan, A., Consequences of *in vitro* culture conditions on embryo development and quality. *Reprod. Domest. Anim*., 2008, **43**, 44–50.
42. Corcoran, D. *et al.*, Suppressed expression of genes involved in transcription and translation in *in vitro* compared with *in vivo* cultured bovine embryos. *Reproduction*, 2006, **131**, 651–660.
43. Tavares, L. M. T., Feitosa, W. B., Mello, M. R. B., Nicácio, A. C., Lima, A. S., Assumpçao, M. E. O. A. and Visintin, J. A., Is the

early reduction of fetal calf serum concentration in bovine *in vitro* embryo culture beneficial? *Anim. Reprod*., 2008, **5**, 34–38.
44. Rajhans, R., Kumar, G. S., Dubey P. K. and Sharma, G. T., Effect of timing of development on total cell number and expression profile of HSP-70.1 and GLUT-1 in buffalo (*Bubalus bubalis*) oocytes and pre-implantation embryos produced *in vitro*. *Cell Biol. Int*., 2010, **34**, 463–468.
45. Kumar, P., Yadav, P., Verma, A., Singh, D., De, S. and Datta. T. K., Identification of stable reference genes for gene expression studies using quantitative real time PCR in buffalo oocytes and embryos. *Reprod. Domest. Anim.*, 2012, **47**, e88–e91.
46. Kumar, P., Verma, A., Kumar, M., De, S., Kumar, R. and Datta, T. K., Expression pattern of glucose metabolism genes correlate with development rate of buffalo oocytes and embryos *in vitro* under low oxygen condition. *J. Assist. Reprod. Genet*., 2015, **32**, 471–478.
47. Sharma, G. T. *et al.*, Expression and characterization of constitutive heat shock protein 70.1 (HSPA-1A) gene in *in vitro* produced and *in vivo*-derived buffalo (*Bubalus bubalis*) embryos. *Reprod. Domest. Anim*., 2012, **47**, 975–983.
48. Eswari, S., Sai, K. G. and Sharma, G. T., Expression of mRNA encoding leukaemia inhibitory factor (LIF) and its receptor (LIFRβ) in buffalo pre-implantation embryos produced *in vitro*: markers of successful embryo implantation. *Zygote*, 2013, **21**, 203–213.
49. Sadeesh, E. M., Shah, F., Balhara, A. K., Thirumaran, S. M. K., Yadav, S. and Yadav, P. S., Effect of growth factor and antioxidant on *in vitro* maturation of oocytes and cleavage rates of *in vitro* produced Indian buffalo (*Bubalus bubalis*) embryos. *Vet. Archiv*., 2014, **84**, 459–474.

ACKNOWLEDGEMENTS. I thank Dr Inderjeet Singh (Director, ICAR-Central Institute for Research on Buffaloes, Hisar) for providing the necessary facilities to carrying out this work and Dr B. S. Punia (ICAR-Central Institute for Research on Buffaloes, Hisar) for reviewing the manuscript. I also thank Indian Council of Agricultural Research, New Delhi for providing funds.

Received 30 October 2014; revised accepted 1 May 2015

Estimation of fluxes across boundaries for groundwater flow model using GIS

Samanpreet Kaur[1,*], Rajan Aggarwal[1], S. K. Jalota[2] and Anil Sood[3]

[1]Department of Soil Water Engineering,
[2]Department of Soil Science, and
[3]Punjab Remote Sensing Centre, Punjab Agricultural University, Ludhiana 141 004, India

The present study aims at using GIS hydrology tool for calculating inflow/outflow fluxes across the boundary of a study area in a situation where physical boundaries in the vicinity of the study area cannot be identified. This approach has an edge over the simplest approach of no flow or constant head boundaries alone, which may be far from reality. The reported methodology will improve groundwater modelling in the areas where the hydrological cycle is predicted because of climate change.

Keywords: Climate change, groundwater flow models, hydrological boundaries, lateral fluxes.

RECENT analyses using the terrestrial water storage-change observations from the NASA Gravity Recovery and Climate Experiment satellites have reported that groundwater is being dramatically depleted in the Indian states of Rajasthan, Punjab and Haryana (including Delhi). During the 2002–2008 study period, 109 km^3 of groundwater has been lost. The decreasing water levels in these regions are largely attributed to unsustainable consumption of groundwater for irrigation and other uses along with increased run-off and/or evapotranspiration, which may further be exacerbated by climate change[1]. In such regions, groundwater management is important to combat the emerging problem of its overexploitation and contamination[2]. Groundwater modelling in such regions can aid in its management. Numerical codes such as MODFLOW, FEFLOW, etc. are most commonly used for simulation of groundwater flow processes. However, there are many methodological challenges like data scarcity, especially unknown fluxes (boundary fluxes, recharge, leakage, evapotranspiration), heterogeneity and resulting parameter uncertainty and non-uniqueness of model calibration, which the researchers need to overcome in order to use a robust and dependable groundwater model. Considerable research has been undertaken for estimating the parameters of transmissivity and storage coefficient and recharge in deterministic groundwater models[3–10]. However, less research has been directed on estimation of boundary fluxes as these require both an accurate physical representation of the system and its differentiation from the adjacent groundwater system and appropriate specified boundary conditions. Attempts are generally made to use no flow or constant head boundaries alone, but this may not be always true and is also far from reality[11]. The absence of well-defined physical boundaries in the near vicinity of zone of interest and necessitate a methodology to compute influx and outflux at the boundaries. GIS is an important tool which offers facilities for creating profile graphs, extract values to a point, creating buffer, interpolation tools, etc. that could be used along with Darcy's law to estimate the average flow across a given boundary. In a groundwater flow and transport modelling of Pali district, Rajasthan, India, a GIS-based methodology was demonstrated to calculate the average flux across the boundary[11]. The study area boundary was grouped into eight segments on the basis of average values of gradients for individual line segments and the mean gradient values for these line segments

*For correspondence. (e-mail: samanpreet1974@gmail.com)