Buffalo cloning: what we have achieved so far

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Buffalo husbandry is an important source of income and means of employment for farmers in India. Buffaloes contribute over half of the total milk produced in the country, despite the fact that buffalo population is nearly half that of cattle. With such a huge contribution by buffaloes, today India is the largest milk producer in the world producing about 140 million tonnes during 2013–14. In addition to milk production, buffalo meat is the major part of Indian meat export generating huge revenue in the animal products sector. Despite its large contribution in total milk and meat production of the country, little efforts have been made to improve the genetic potentiality of buffaloes. In this context, the National Dairy Research Institute (NDRI), Karnal has set a target to produce elite male and female animals with the application of advance reproductive techniques and to disseminate elite buffalo germplasm throughout the country.

Apart from the increasing application of embryo transfer, ovum pickup and *in vitro* fertilization in buffalo breeding, the most important but so far underexploited possibility is the application of somatic cell nuclear transfer in buffalo breeding. This technique enables production of a infinite (theoretically) number of copies of an existing animal of proven genetic values and consequently dramatic improvement of the genetic value of livestock in just one generation. We initiated work on buffalo cloning nearly two decades ago using the traditional micromanipulation-based approach. However, we were able to produce only a few transferable quality blastocysts due to technical limitations at that time[1]. In 2005, we standardized a simplified cloning technique called handmade cloning (HMC) that requires only a stereomicroscope and a simple electrofusion machine for manipulations. The technique was based on the report of Vajta *et al.*[2]. It has opened up a new perspective for the large-scale breeding application.

After the birth of the first cloned buffalo produced by conventional micromanipulation-based SCNT[3], a major breakthrough was made in February 2009 by producing the world's first cloned buffalo calf through the handmade/hand-guided cloning technique. With this advancement, India has entered an era of buffalo cloning, an important dairy and meat animal, through a simplified technique. After the birth of the first cloned buffalo, we hastened the work on this approach which led to the birth of offspring produced using ear skin-derived somatic cells[4], embryonic stem cells[5], semen-derived somatic cells[6] and recently, urine-derived somatic cells[7] as nuclear donors, or that produced from a vitrified–warmed cloned embryo[8]. Recently, we have produced superior quality male cloned calf. An advantage of this calf over others is that it is the clone of a progeny tested bull with superiority of >22% over contemporary daughters. Body cells (somatic cells) were cultured from frozen semen of bull stored at NDRI, which had died a decade ago and was used for cloning. If this bull-calf born matures well and donates semen, it can do so worth about Rs 3 crores in its lifetime, according to rough calculations. We are heading closer to developing a xerox machine for duplicating outstanding buffaloes and/or bulls.

In addition to faster multiplication of domestic buffalo populations, we currently use this technology to conserve and manage population of endangered species, specially Bovidae family. Wild buffalo (*Bubalus arnee*; chromosome number 50), belongs to the Bovidae family and found in India, Nepal, Bhutan, Cambodia and Thailand. In central India, wild buffaloes are found only in the Indravati Tiger Reserve Udanti, Chhattisgarh. In Udanti Wildlife Sanctuary only

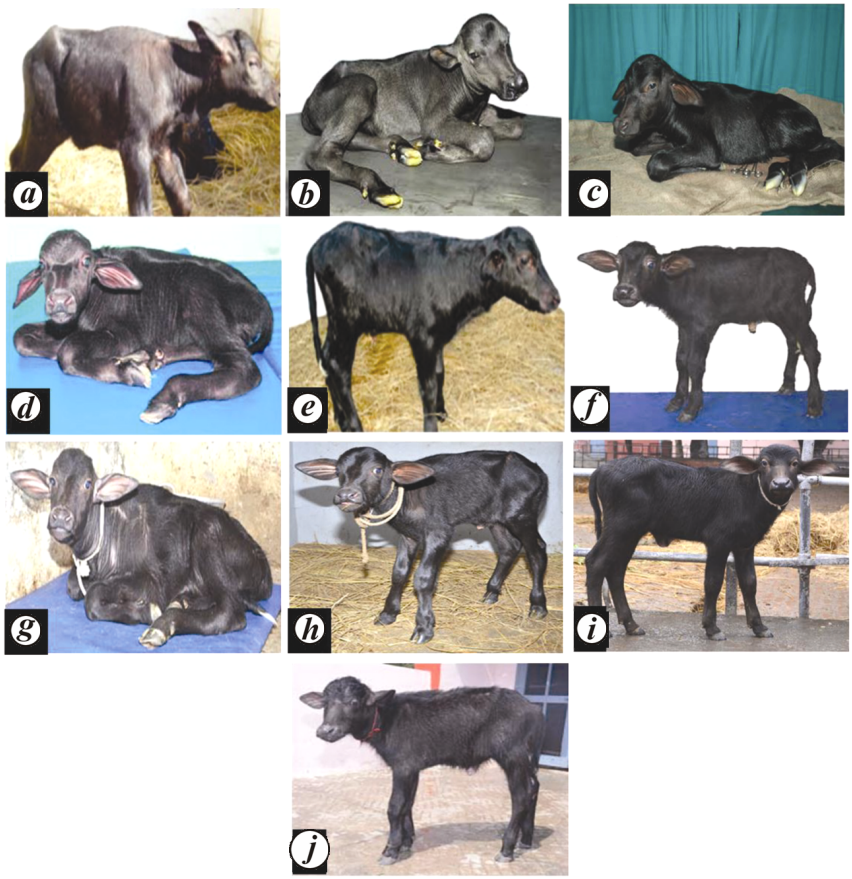

Figure 1. Clones that were born at the National Dairy Research Institute, Karnal (date of birth is indicated with in brackets). ***a***, Sammrupa (6 February 2009); ***b***, Garima (6 June 2009); ***c***, Garima-II (22 August 2010); ***d***, Shrestha (26 August 2010); ***e***, Swaran (18 March 2013); ***f***, Purnima (6 September 2013); ***g***, Lalima (2 May 2014); ***h***, Rajat (23 July 2014); ***i***, Wild buffalo Deepasha (12 December 2014); ***j***, Apurva (5 February 2015).

ten buffaloes, including eight male and two female (http://www.wti.org.in) are left; hence there is an urgent need to restore their population. In collaboration with Chhattisgarh Wildlife Sanctuary, we are currently working on wild buffalo cloning and recently reported the successful production of cloned calf (Deepasha; Figure 1) using somatic cells from wild buffalo and recipient oocyte from domestic buffalo[9].

Apart from buffalo cloning, Sher-e-Kashmir University of Agriculture Sciences and Technology of Kashmir, Srinagar successfully cloned the first Pashmina goat through our scientific collaborations. These goats are found in the cold desert of Ladakh and people in the region rear them for their wool, which is used for making the exquisite Pashmina shawls that are in great demand both in and outside Kashmir.

In our studies we observed that more than 40% birth rate could be obtained with embryos produced by *in vitro* fertilization. With buffalo cloning, we obtain <5% of calves, the rate of live offspring obtained from cloned blastocysts in buffalo. Our priority for future work is to achieve a consistent, predictable outcome and further improvement in the overall efficiency of cloning technique in this species. During somatic cell nuclear transfer we need to consider the molecular mechanisms that have evolved to regulate embryonic development and pregnancy. Although some improvements in efficiency are to be expected from optimization of the present procedures, greater benefits might be expected from intervention to assist reprogramming of the transferred nucleus.

However, crucial questions that remain to be answered include the following:

- What would be an alternative technique to produce quality cloned embryos?
- How does a zona-free situation influence *in vivo* survival and development?
- Why genomic reprogramming is important in cloning success or else one having a major impact?
- What will be the suitable method for efficient cryopreservation of zona-free cloned embryos?
- Does the in-straw dilution and direct transfer of cloned embryos a realistic option for embryos transfer?

We need to focus on both technical and cellular issues, which might have an impact on the future applications of the technique for the production of elite animals, recreation of dead progeny tested bulls, conservation of endangered species/breeds, transgenic animal production and human therapeutic cloning.

1. Singla, S. K. *et al.*, *Indian J. Exp. Biol.*, 1997, **35**, 1273–1283.
2. Vajta, G. *et al.*, *Cloning*, 2001, **3**, 89–95.
3. Shi, D. *et al.*, *Biol. Reprod.*, 2007, **77**, 285–291.
4. Shah, R. A. *et al.*, *Theriogenology*, 2009, **71**, 1215–1219.
5. George, A. *et al.*, *Cell. Reprogram.*, 2011, **13**, 263–272.
6. Selokar, N L. *et al.*, *PLoS ONE*, 2014, **9**, e90755.
7. Madheshiya, P. K. *et al.*, *Cell. Reprogram.*, 2015, **17**, 160–169.
8. Saha, A. *et al.*, *Reprod., Fertil. Dev.*, 2012, **25**, 860–865.
9. Saini, M. *et al.*, *Theriogenology*, 2015, **84**, 101–108.

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Smile with Science

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