



Review Article

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Cloning of breeding buffalo bulls in India: Initiatives & challenges

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The term animal cloning refers to an asexual mean of reproduction to produce genetically identical copies of any animal without the use of sperm. In India, the cloning of buffalo is well established and clones of the Murrah, the best dairy breed of buffalo, have been produced. The most acclaimed example is the restoration of progeny-tested breeding bull by isolating somatic cells from frozen doses of semen, which were stored for more than a decade in the semen bank. Buffalo bull cloning is considered the best available option to reproduce declared proven bulls and their semen would contribute to accomplishing the demand of ever-growing frozen semen, which is the prime requirement of conventional breeding. This article highlights the importance of buffalo bull cloning and its current status in India.

Key words Animal cloning - buffalo - bull - cloned embryo - semen

Introduction

In India, domestic buffalo (*Bubalus bubalis*) has been contributing to country's milk and meat production and is a source of employment to the millions of farmers. In 2016-2017, India produced 165.4 million tons of milk, of which approximately 49 per cent (81 million tons) was contributed by the buffalo (Annual Report 2017-2018, Department of Animal Husbandry Dairying and Fisheries (DADF) of India¹. In addition to milk, the country had exported buffalo meat and meat products to the world worth of ₹4036.89 USD millions in 2017-2018, which makes buffalo the preferred bovine animal to boost the Indian meat industry (data were obtained from the Agricultural and Processed Food Products Export Development Authority of India². Overall, buffalo is considered as the best animal of choice for India's dairy and meat sector. In spite of its huge contribution to milk production and

meat export, less efforts have been invested to improve buffalo's genetic potential for higher milk and meat productivity, particularly the use of assisted reproductive techniques (ARTs) such as superovulation, embryo transfer technology, ovum pickup, *in vitro* fertilization (IVF) and somatic cell nuclear transfer (SCNT)^{3,4}. Over the many decades, the artificial insemination (AI) has been used to improve the buffalo germplasm, and it will continue to contribute because there is no replacement of AI which can easily be adopted at the village level. In the last decade, there has been increasing interest to explore *in vitro* embryo production (IVEP) technologies and SCNT for the faster propagation of superior buffalo germplasm. In the buffalo breeding, advanced ARTs are already in place coupled with conventional breeding programmes to improve the germplasm.

SCNT, commonly known as animal cloning, is an advanced ART technique used to multiply the best

productive animals. Many species of domestic animals (cattle, buffalo, pig, sheep, goat, camel and horse) have already been cloned using SCNT methods⁵. In the last few years, the clones of elite breeding bulls were produced by our group and their semen was used for AI and produced normal healthy calves^{4,6}. We earlier reported birth of a cloned bull by isolating somatic cells from frozen semen that were stored for a decade in the semen bank⁷. This report has opened a new way to restore the population of superior progeny-tested bulls, who died or culled, but their semen was available in the semen banks. Despite tremendous application of buffalo cloning, the technique is suffered from lower pregnancy rates, high abortion rates and poor survival of clones⁶. This article highlights the present status of buffalo bull cloning in India and its challenges.

Why breeding bulls only to be cloned?

The importance of bulls is often underestimated in breeding programmes and more focus has been given to female animals to boost herd productivity. In the herd, one female is responsible for half genetic of one calf per year, whereas one bull can be responsible for half genetic of a large number of calves. Conventionally, the genetic improvement in domestic animals has been achieved by inseminating females with the semen of desirable genotype bulls to produce higher productive calves⁸. Therefore, availability of best genotype bulls is a fundamental requirement to boost genetic gains. In India, the National Dairy Plan (NDP) has been initiated to increase the productivity of dairy animals, and it has been anticipated that higher productivity can be achieved by expanding coverage of AI. According to the NDP strategy, during the first phase, around 35 per cent of breedable bovines (cattle and buffalo) need to be covered under AI (ongoing), and 50 per cent population need to be covered by the end of the second phase (2021-2022), from an existing 15 to 20 per cent coverage of AI⁹. According to the NDP estimates, the requirement of semen doses to achieve targets would be approximately 100 million frozen doses annually (900 bulls) during the first phase and 140 million frozen doses annually (1200 bulls) by the end of the second phase⁹.

In India, the majority of domestic animal bulls, particularly buffalo and cattle, are selected on the basis of their pedigree records followed by extensive progeny testing programmes (a bull to be declared proven requires the span of 8 to 10 years, and by that time either bull is culled or limited semen doses are

available in the semen bank). In addition to in-house bull production, the country has also been importing exotic bulls and equivalent embryos to fulfill the demand of bulls for breeding. During 2016-17, 400 exotic bulls and/or equivalent embryos were imported to accomplish demand of dairy bulls in the country¹.

SCNT can also be used to produce breeding bulls to reduce dependency on other methods of bull production and procurement (a schematic presentation of bull cloning for buffalo breeding is depicted in the Figure). Advantages of the bull cloning over to the female cloning are (i) bulls have a significant direct impact on the genetic gain in the population within a short period of time; (ii) a number of identical clone copies can be produced from a proven bull to compensate demand of its semen; and (iii) due to lower efficiency of cloning, approximately <2 per cent, it is practically impossible to produce a huge number of females, but hundreds of elite bulls can be cloned. If the best genetic merit breeding bull was culled from semen production due to ageing or infection or lameness or any reason and limited semen doses are available in the semen bank, cloning can be done.

Previous studies in cattle and pigs suggested that cloned bulls have normal production and reproduction performances such as the production of good quality semen and ability to impregnate females^{10,11}. Furthermore, several reports demonstrated that the progenies of clones exhibited normal growth, health, reproduction and production similar to animals produced through conventional breeding¹²⁻¹⁵. By considering the requirement of buffalo bulls in the country, more efforts are needed to produce more cloned copies of bulls, particularly declared proven bulls.

Current status of buffalo bull cloning in India

Animal cloning in India was first attempted in buffalo 1990s¹⁶. During that period, the technique used to clone the buffalo was similar to micromanipulator-assisted SCNT. Despite the multiple attempts, researchers could not produce the blastocyst stage embryos¹⁶. Later the micromanipulator-free SCNT technique called handmade cloning (HMC) was comprehensively demonstrated in cattle by Vajta *et al*¹⁷, India's first live cloned buffalo was born in 2009, and afterward, more than 20 buffalo clones, including breeding bulls, were produced in India^{4,6}. Saini *et al*⁶ reviewed that cloned male buffaloes donated semen was used for AI which resulted in the production of

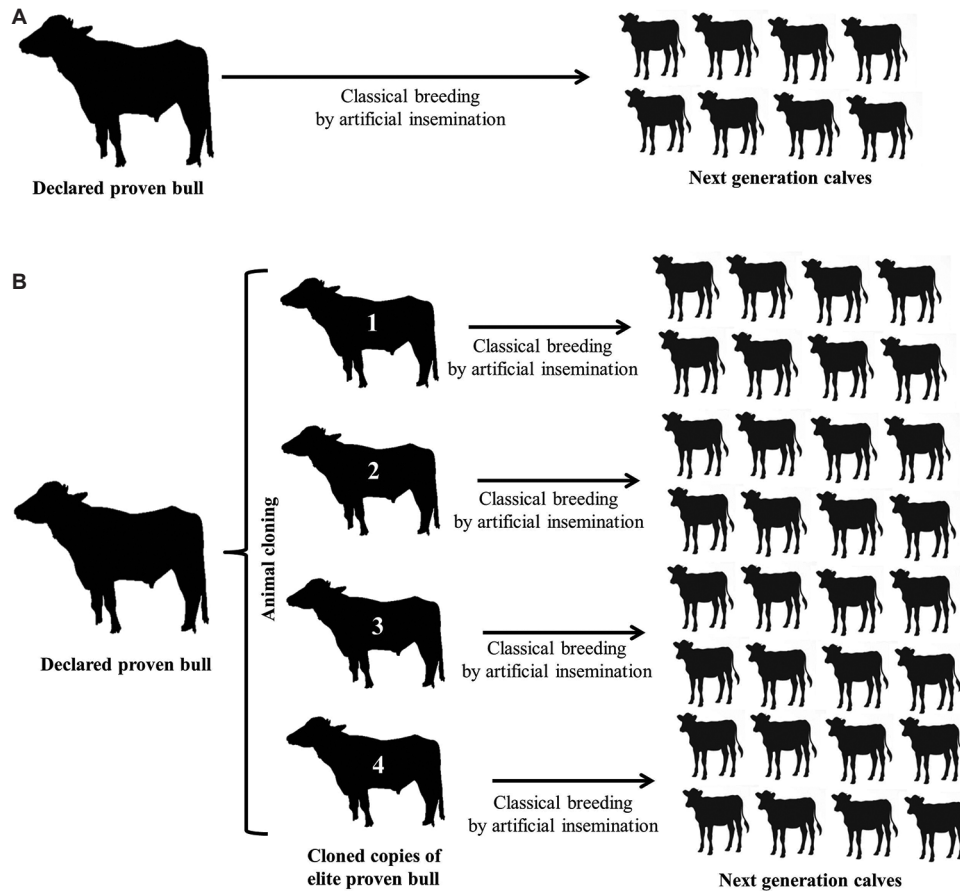


Figure. Schematic presentation of how the buffalo bull cloning can be included in the buffalo breeding programme along with the classical breeding. Conventionally, the bull is nominated for breeding after rigorous and exhaustive progeny testing studies, which takes 8-10 years to declare the bull is proven, after the nomination, semen doses of the proven bull are used to inseminate females to produce future elite calves (A). If such proven bull (s) died or culled and have a very few doses of semen, then farmers and breeders could not have a constant supply of proven semen for breeding. Through animal cloning, it is possible to restore the genotype of declared proven bulls and the semen of such restored bulls can mitigate the demand of proven semen for classical breeding (B). This way, farmers and breeders can speed up the genetic gain. Also, multiple locations or herds can be targeted by cloned copies of one bull to disseminate best genetic at large scale.

healthy calves. This indicates that cloned bulls can be used along with conventionally produced bulls to disseminate elite germplasm using application of ARTs (AI, IVF and multiple ovulation and embryo transfer). In addition, the female clones delivered the progenies following AI⁶.

Challenges of buffalo cloning research

Buffalo cloning offers a great hope for conventional breeding to speed up the dissemination of elite germplasm, particularly bulls; however, the results are not always promising because the cloning efficiency has improved marginally over the past decade, and poor conception rates, early pregnancy losses and mortality of born clones are the major challenges. Extensive research of cattle and pig cloning indicates that major factor which affects the efficiency of cloning is the abnormal nuclear reprogramming.

Abnormal epigenetic modifications such as aberrant DNA methylation and histone modifications were also observed in cloned buffalo embryos⁶. Therefore, epigenetic errors need to be controlled to improve the buffalo cloning success rates. Our research team has investigated the faulty reprogramming of donor nucleus to better understand the cellular and molecular mechanisms of cloned embryonic development in buffalo¹⁸⁻²¹. However, the exact cause of faulty reprogramming is not yet identified and *in vitro* improvements did not translate to *in vivo* success; therefore, more efforts in this direction are needed. On the basis of our previous experiments^{4,6}, several approaches such as selection of competent oocytes using Brilliant Cresyl Blue staining, culture of embryos under lower oxygen tension, treatment of embryos with epigenetic modifiers and modification in embryo production method like single ooplasm

to avoid mitochondrial heteroplasmy, can be used to improve embryo quality to achieve more pregnancies.

It was noticed that approximately 50 per cent of buffalo clones died during the first three months after birth and they displayed various abnormalities such as enlarged umbilical cord, abnormal respiration, abnormal large size body organs and succumbed to infections⁶. Therefore, the practicing veterinarians can play an important role to develop preventive measures and care for newborn clones, so that they can survive long.

In addition to IVEP and refinement of the technique, the stringent selection of best embryo and best recipient for ET is needed to achieve higher pregnancy rates and overall success. A previous study suggested that the selection of healthy reproductive animals for embryo transfer is crucial and necessary to rule out the possibilities of lower conception rates due to inferior recipients²². At present, in India we neither have a large number of cloned embryo transfer data to predict conception rates nor many cloned animals to predict their performances such as milk production ability of females and fertility of bulls; therefore, it would be premature to interpret the exact efficiency and success of buffalo cloning in India. Therefore, consistent efforts are needed to generate buffalo specific data, which can be explored to improve buffalo cloning efficiency.

Conclusion

Breeding bulls can multiply using SCNT techniques, including HMC. Demand for semen doses of progeny-tested bulls is high among the farmers and breeders due to their high breeding merits. Some of the tested bulls have died or culled, and their limited semen doses are available in the semen banks. It is possible to clone these bulls through SCNT by isolating somatic cells from frozen semen.

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Conflicts of Interest: None.

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