

## Sach-Gaurav: world's first cloned buffalo born in the field at an Indian dairy farm

Recently, the ICAR-Central Institute for Research on Buffaloes has produced world's first cloned buffalo in the field, named Sach-Gaurav, on 22 December 2017. In addition to being the world's first cloned buffalo born in the field, this is also India's first cloned calf of Assamese buffalo; earlier only Murrah breed of buffaloes was cloned. A small tail tissue of Assamese buffalo was airlifted from the College of Veterinary Science, Guwahati (which is 2000 km away from our cloning laboratory) to establish the cultures of donor cells. This proves that distant and remote animals can be cloned. A singlet method of embryo reconstruction has been used, with one recipient oocyte instead of two (a standard Indian version of animal cloning method). It is a step towards simplification of animal cloning technique, and this approach can reduce the mitochondrial heteroplasmy in cloned calves. Ovaries of Murrah buffalo were used as a source of recipient oocytes, indicating that oocytes from one breed of buffaloes can reprogramme donor cells of other breeds. Also, the Murrah buffalo can serve as a surrogate mother for other buffalo breeds.

India is distinguished for its achievements in the field of buffalo cloning research and the country has produced

more than 20 cloned buffaloes. The buffalo, popularly called India's Black Gold, is the only species in the country that has benefited from an emerging animal cloning technique. In 2009, India produced the first cloned buffalo at the ICAR-National Dairy Research Institute (NDRI), Karnal, Haryana. In 2015, the ICAR-Central Institute for Research on Buffaloes (CIRB), Hisar, Haryana, cloned a buffalo-breeding bull to become the second centre to produce cloned buffalo. At present, CIRB and NDRI are the two institutes in India, which have demonstrated the success of animal cloning technique through which the world's first mammal (Dolly) was cloned.

Buffalo cloning is an advanced assisted reproductive technique, in which a single somatic cell is electrically fused with an enucleated oocyte to produce the transferable blastocyst stage embryos. The laboratory-produced cloned blastocysts need to be transferred into recipient buffaloes to produce viable cloned calves. Earlier, all cloned buffaloes were produced in the animal farm associated with the research institute, close to the embryo production laboratory; therefore, stress-free transportation of highly sensitive cloned embryos was easy, before

they were actually transferred into the womb of recipient buffaloes.

In addition, the monitoring of recipients and pregnant buffaloes in the institute animal farm was easy as they are usually located close to the institute campus. Since the initiation of buffalo cloning in India from 2009 onwards, research is underway to transfer cloning technology to the farmer's doorstep. Keeping this in mind, we have worked on producing cloned buffaloes in the field. And, recently, we have produced a cloned buffalo at the Hi-tech Sach Dairy Farm, Sirsa, Haryana, which is about 100 km from our cloning laboratory (Figure 1). The produced clone maintains good health and its physiological parameters such as blood hematological parameters, respiration rate, body temperature and heart rate are within the recommended healthy range for buffalo. The calf's genotype was confirmed by microsatellite analysis (parentage verification) and no numerical chromosome abnormalities were detected ( $n = 50$ ).

The Hi-tech Sach Dairy Farm played a vital role by providing a sufficient number of recipient buffaloes for cloned embryo transfer, and also in the management of pregnant buffaloes and care of the cloned calf. This success has realized the concept of 'Lab-to-Land' to transfer agricultural and allied technologies to the farmer's field. Thus cloning technology can be used as a valuable breeding tool to complement the artificial insemination to improve buffalo germplasm in the country.



**Figure 1.** **a**, Sach-Gaurav the cloned Assamese buffalo calf with its surrogate Murrah mother at the Hi-tech Sach Dairy Farm, Sirsa, Haryana, India; **b**, Cloned calf at 6 months of age.

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