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'Holi': India's first cattle calf produced through Ovum pick-up – IVF technology– from an aged Sahiwal cattle

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Cattle are an important species of our dairy industry. The milk yield of cattle has enabled India to become the world's largest milk producing nation. Application of superovulation and ETT has given satisfactory results in cattle. However, conventional superovulation and ETT cannot be applied to animals that are clinically subfertile, infertile or aged or those which do not respond to superovulation but which are otherwise of high genetic merit. Furthermore, the development and refinement of techniques for *in vitro* embryo production (IVEP) has made available a tool for utilization of the female gamete pool for enhancing the maternal contribution to genetic improvement. However, if the source of oocytes is slaughterhouse ovaries, the offspring produced cannot be used for breeding purpose due to lack of information of pedigree of the dam. Ovum Pick-Up (OPU) or ultrasound-guided transvaginal oocyte retrieval (TVOR) is the only means available for obtaining oocytes from live animals of known pedigree which enables repeated collection of oocytes from live animals, on a weekly or biweekly basis over long periods of time. Moreover, there has been no progress in India towards development of various other advanced reproductive technologies like cloning, transgenesis, production of embryonic stem cells etc. primarily because of the lack of availability of cattle oocytes due to ban on cow slaughter nearly all over India due to religious reasons. The use of high genetic merit animals that are clinically subfertile, infertile or aged or those which do not respond to conventional superovulation procedures, as oocyte donors can result in addition of another source for obtaining high value oocytes. Here, we report the success of this technique in giving birth to India's first female Sahiwal calf named 'Holi' from aged animal.

The oocytes were collected from aged Sahiwal cow (after 9th lactation), maintained at the Cattle Yard of the National Dairy Research Institute, Karnal. Follicular aspiration was

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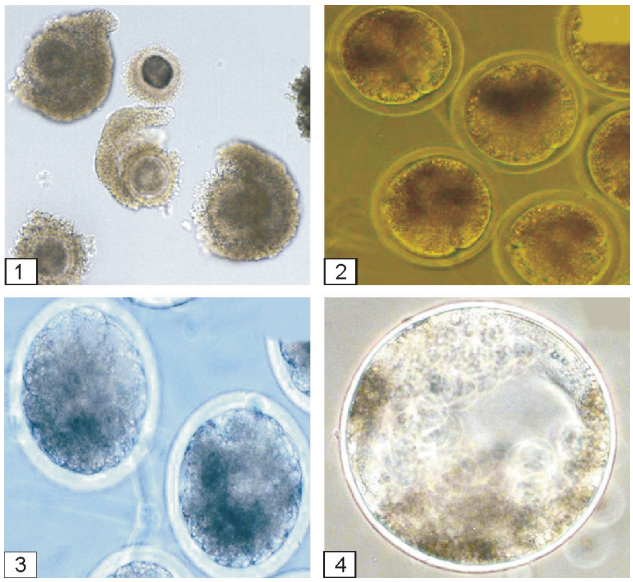

Figs 1–4. **1.** Bovine cumulus oocyte complexes obtained through OPU (100×). **2.** *In vitro* fertilized and cleaved oocytes on day 3 of *in vitro* culture (100×). **3.** Morulae obtained from IVM/IVF bovine oocytes on day 7 of *in vitro* culture (200×). **4.** Expanded blastocyst obtained from IVM/IVF bovine oocytes on day 8 of *in vitro* culture (200×).

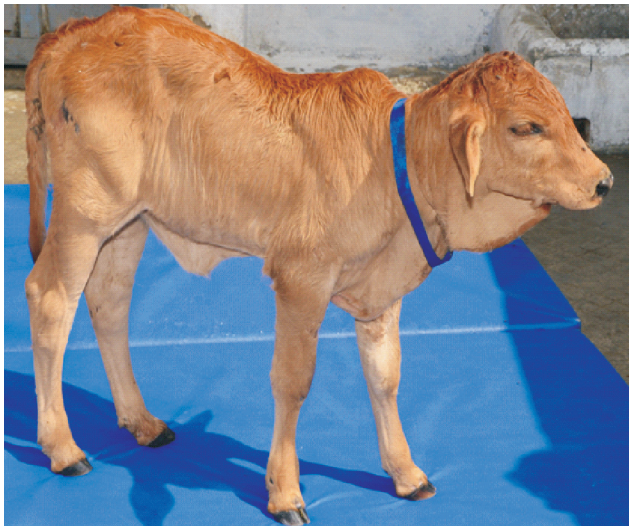

Fig. 5. India's first Ovum Pick-Up – IVF cattle calf 'HOLI' at the time of birth.

performed, twice a week for 2 weeks, using an ultrasound machine with a 5 MHz transvaginal convex transducer, equipped with a needle guide, single lumen 18G 55cm long sterile needle with an ultrasound echo tip and a vacuum pressure of 90 mm Hg as reported earlier (Chauhan *et al.* 1999). The oocyte retrieval was performed under general (Rompun, 0.8 ml/animal) and epidural (2% xylocaine, 3–5 ml/animal) anesthesia, administered 15 min prior to the start. The contents (oocytes, follicular fluid, granulosa cells and tissue debris) retrieved were allowed to settle down for 10–15 min in 50 ml falcon tube, the sediment was collected and searched for oocytes under a zoom stereomicroscope at 20× magnification (Manik *et al*. 2002). The collected oocytes were classified into 4 grades: grade A, B, C and D as per Chauhan *et al*. (1998). Only A and B grade cumulus oocyte complexes (COC; Fig. 1) obtained through OPU were washed separately 4–6 times in washing medium (TCM 199 + 10% FBS + 0.81 mM sodium pyruvate + 50 µg/ml gentamicin sulfate), followed by 2 washes in maturation medium (TCM 199 + 10% FBS + 5 µg/ ml pFSH + 10 µg/ ml LH + 1 µg/ml estradiol-17ß + 50 µM cysteamine + 0.81 mM sodium pyruvate + 10% buFF + 50 µg/ml gentamicin sulfate) and placed in separate groups of 10–12 oocytes per 50 µl IVM droplets, overlaid with sterile mineral oil in 35 mm petri dishes and incubated at 38.5°C in a humidified CO_2 incubator (5% CO_2 in air, ≥ 95% humidity) for 24 h to achieve maturation. At the end of 24 h of culture, oocytes were subjected to IVF.

The semen used for IVF throughout the study was from the same proven cattle bulls, obtained from Animal Breeding Research Center of the institute. The processing of spermatozoa for IVF was carried out as per the protocol described by Chauhan *et al.* (1998, 1999). Briefly, the frozen straws were thawed at 37°C and centrifuged twice at 1,000 g for 8 min in BO medium (Brackett and Oliphant 1975). *In vitro* matured oocytes and the processed spermatozoa were incubated in droplets of BO medium supplemented with 1% fatty acid free bovine serum albumin (BSA) overlaid with sterile mineral oil. After 18 h, incubation the cumulus cells were washed off the presumptive zygotes by gentle pipetting, followed by 3 washes in RVCL medium supplemented with 1% fatty acid free BSA. The presumed zygotes were cultured in this medium for 8 days post insemination on original beds of granulosa cells with 50% of the media being replaced every 48 h. The developmental potential of the presumed zygotes was evaluated from their respective cleavage rates and progression through 2–, 4-, 8-to 16-cell, morulae up to blastocyst stage. IVM/IVF cleaved oocytes (Fig. 2) successfully developed to the morula (29.41%, Fig. 3) and blastocyst (11.76%, Fig. 4) stage after 9 days of IVC in culture medium. The morphologically normal blastocysts were transferred non-surgically into the ipsilateral uterine horn of sahiwal recipients and a female Sahiwal calf named 'Holi' (Fig. 5) was born on 7 March 2012 through normal delivery after standard gestation period with normal weight 23 kg. Some of the reports (Hammer *et al.* 2001, Holm *et al.* 1996) described that the animals generated via *in vitro* production (IVP) of intact embryos are generally health compromised, regardless of oocyte source (live donor or ovaries post mortem). In particular, the 'large offspring (or foetal oversize) syndrome', an enigmatic and multi-variate phenomenon of unpredictable frequency, indicates developmental sensitivities and aberrations that lower the chances of survival of young ones derived from bovine IVP embryos and under instances of severe dystocia, that of their surrogate dams. In this case, no such problem was observed and the Holi calf was absolutely healthy and has already completed two years, inseminated with AI after coming in estrus and is three months pregnant now.

This is the first report of production of a Sahiwal calf through OPU-IVF in India which identifies and promises an approach for obtaining calves from live cattle and thus propagating their superior herd. This technique also enables us to exploit the genetic merit of elite indigenous cattle, which are otherwise clinically subfertile, infertile or aged. Further study is required for optimization of OPU in our indigenous cattle. The technique could also be extended to other species, notably goat and buffalo.

SUMMARY

In the present study, we report the birth of India's first cattle calf produced through Ovum Pick-up – IVF technology. The oocytes were retrieved from an aged Sahiwal cattle using the technique of transvaginal oocyte retrieval, under ultrasound guidance and subjected to *in vitro* fertilization and *in vitro* culture for 8 days after which blastocysts so formed were transferred into reproductively healthy Sahiwal recipients. A female Sahiwal calf named 'HOLI' was born through normal delivery after standard gestation period. This work proves ovum pick-up as a promising technique to multiply the elite herd having superior germplasm, which are otherwise clinically infertile or aged.

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