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Review on ovum pick up in cattle and buffalo

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Abstract
Among the assisted reproductive technologies Ovum Pick-Up (OPU) and *In vitro* Embryo Production is potential alternative traditional *in vivo* embryo production mainly for the conservation of indigenous cattle breeds, augmentation of productivity and exploitation reproductive potential superior cows. OPU we can collect more oocytes from genetically superior animals and in turn production of a greater number of embryos per donor animal with subsequent fresh transfer of embryos or cryopreservation of embryos for frozen transfer and finally production of superior elite animals. This can be accomplished by selection of donor animals for OPU and donor preparation by stimulation with or without gonadotropins, efficient culture media and fresh transfer embryos. OPU can performed pre pubertal animals, pubertal calves, non-pregnant animals and even pregnant animals up to three months of gestational age. Present review emphasizes mainly on the history of OPU, factors affecting the OPU, donor preparation, OPU procedure and OPU complications.

Keywords: Ovum pick-up, embryo production, FSH, superior animals

Introduction
Most indigenous breeds are genetically degraded as a result of indiscriminate crossbreeding and irregular breeding among breeds in close proximity (Ramesha, 2001) [48]. Indigenous cattle population have declined in their native land, emphasizing the importance of conservation strategies and use of appropriate reproductive technologies to prevent further population reductions. Ovum Pickup (OPU) - Invitro Embryo Production (IVEP) in conjunction with Embryo Transfer (ET) will be a viable option for conservation and faster multiplication of breed as OPU will increase the number of oocytes available from living cows and serve as a potential source for embryo production. The researchers demonstrated that repeated oocyte collection by OPU could be done without endangering one's health or reproductive activity (Hasler *et al*., 1995) [27]. The first OPU in water buffalo was documented by Boni *et al*. (1994) [8]. The initial study involved weekly oocyte aspirations after FSH priming was administered to donors in severe anestrus. The average recovery rate for untreated controls was 31.9%, but for donors who received FSH stimulation, it was 44%. In recent years, some have successfully used exogenous gonadotropins for super-stimulating donor cows with FSH before OPU session in order to increase the population and diameter of follicles (Demissie *et al*., 2021) [17] for aspiration and recovery of oocytes suitable for IVEP (Vieira *et al*., 2016). OPU can be performed on nearly all animals with or without donor hormonal stimulation and aspiration can be done up to twice a week from prepubertal animals of 10-12 months as well as pregnant animals up to 3 months of pregnancy.

History of OPU
Initially oocytes have been aspirated from abattoirs ovaries by puncturing all of the visible (2–5 mm) follicles (Lonergan *et al*., 1992) [33]. Later due to its drawbacks like lack of reproductive status of animal, pedigree and repeatability of oocytes collection etc. other methods have been identified (Bols *et al*., 1995) [6]. Laparoscopy is one of such methods with repeatability but is quite traumatic to the oocyte donor and thus its use is limited due to scarring and adhesions at the operation site (Lambert *et al*., 1983) [32]. Later, Callesen *et al*. (1987) [12] proposed ultrasonically guided aspiration of bovine follicular oocytes wherein through rectal palpation ovaries were positioned against Sacro-sciatic ligaments and follicles punctured with the help of ultrasound to recover 2.3 oocytes per heifer.

The adaptation of human transvaginal ultrasound guided follicular aspiration for ovum pick-up (OPU) in bovines was a breakthrough since it is less traumatic, less invasive and having a higher degree of repeatability (Pieterse *et al*., 1988) [43]. Through the transfer of frozen embryos created *in vitro*, the Avantea team initiated the development of the Ovum Pick Up (OPU) technology in 1997, resulting in the birth of the world's first buffaloes the following year. According to Avantea's prior findings, the Ovum Pick Up is the most successful method of reproduction for preserving the genetic legacy of excellent animals and enhancing a buffalo farm's financial productivity. The technique of OPU in buffaloes is the same in cattle (Hufana-Duran & Duran, 2015) [28]. In India, the first buffalo calf (Saubhagya) was produced through this technique by Prasad *et al*. (2013) [47].

Factors affecting the OPU

Technically the OPU efficiency is influenced by different technical and biological factors. Technical factors that have been investigated included vaccum pressure, needle diameter (Bols, 1997) [7], scanner resolution and needle guidance system (Mullaart *et al*., 1999) [37] and operator experience (Scott *et al*., 1994) [54]. Biological factors investigated include the origin of the oocytes (Karadjole *et al*., 2007) [31] and donor animal herself (Ferret *et al*., 2006 and Merton *et al*., 2008) [19, 35], hormonal pre-stimulation (Getz, 2004 and Chaubal *et al*., 2007) [22, 15], timing and frequency of OPU sessions (Blondin *et al*., 2002 and Petyim *et al*., 2003) [5, 42] follicular wave synchronization and dominant follicle removal (Garcia *et al*., 2000 and Chaubal *et al*., 2006) [21, 15].

Breed of the donor

Bos indicus breeds and their hybrids were able to produce more oocytes and embryos than Bos taurus breeds and also in the pregnancy rate (2-fold) per OPU session (Arreseigor *et al*., 2021) [2] with higher oocyte quality, total number of oocytes recovery (22.5±1.5 vs. 14.6±0.9), higher numbers of viable oocytes (19.3±1.2 vs. 10.8±0.7) and higher *in vitro* embryo yield (3.8±0.4 vs. 0.7±0.1) than *Bos taurus* (Sales *et al*., 2015) [51]

Individual donor

In terms of the follicles aspirated and the number of oocytes recovered per session, there are significant variations between different species. Individual animal variation influenced the oocyte recovery rate and was the most important factor for the results of OPU (Backer *et al*., 1996., Santl *et al*., 1998 and Roschlau *et al*., 2001) [3, 52, 49] and different animals respond differently to the same doses of pFSH in terms of numbers and diameters of follicles (De Roover *et al*., 2005b) [16].

Donor reproduction status

Pieterse *et al*. (1991b) [44] performed OPU and obtained more follicle on day 3 or 4 of oestrous cycle but can be performed at any time of the estrous cycle, including the early growth phase of the first follicular wave (Paul *et al*., 1995) [41]. Pontes *et al*. (2009) [46] performed follicular aspiration procedures on a random stage of the estrous cycle in *Bos indicus* cows with or without the follicular wave synchronization and collected 25.6±15.3 immature oocytes per OPU session. González *et al*. (2018) [24] concluded that the number of recovered oocytes, the rate of cleavage, oocyte maturation and embryonic development through the OPU technique in pregnant dairy cattle were similar to what is obtained from pregnant heifers.

Super stimulation with eCG / FSH

With the main goal of increasing oocyte yield per aspiration session, OPU is now administered to animals that have been super-stimulated as well as animals that have not been super-stimulated. Pre-stimulation with gonadotropins prior to OPU led to the greatest increase in oocyte quantity and quality (Fry *et al*., 1994) [20]. Of the different hormones used viz., GnRH (Bordignon *et al*., 1997) [10], FSH (Gibbons *et al*., 1994., Bungartz *et al*., 1995., Goodhand *et al*., 2000 and Chaubal *et al*., 2006) [23, 11, 22, 15] and eCG (Vos *et al*., 1994., Van de Leemput *et al*., 1999., Sendag *et al*., 2008 and Aller *et al*., 2012) [60, 56, 55, 1], FSH has given the best results in terms of number of follicles aspirated and oocytes retrieved.

Donor preparation and OPU procedure

To perform OPU, the animal is restrained in an adjustable squeeze chute. Rectum is emptied by back racking and is not allowed to aspirate air. Caudal epidural anaesthesia is induced by administering 3-5 mL 2% lignocaine hydrochloride to prevent defecation, abdominal straining and to facilitate easy handling of the ovaries through rectum. The vulva and perineal area are washed with plain water and dried with a sterilized napkin. The tail is held away and tied to the neck of animal with a cotton rope. The vulval lips are mopped with tissue paper soaked in 70% alcohol before insertion of vaginal probe. During OPU the ovaries are manipulated per rectally by positioning each ovary between the fingers for under taking OPU activity. After thorough cleaning and lubrication with sterile paraffin transvaginal Ultrasound probe fitted to OPU handle is advanced into anterior vagina (fornix vagina) and the transducer surface was kept in position either at the left or right side of the external Os of the cervix. Then the ovary is manipulated gently and positioned against the probe head in order to obtain a clear image of the follicles on the ultra-sonographic monitor. The number of follicles per ovary and the diameter of the follicles are recorded after freezing the image on the monitor with the help of an inbuilt electronic calliper. The diameter of follicle is obtained by considering the average of measurements taken in two directions, *i.e*., vertical and horizontal (Nagai *et al*., 2015) [38]. Based on the diameter, the follicles are classified as small (<4 mm), medium (4-8 mm), large (>8 mm). After stabilisation of ovary and targeted follicle the needle fitted with aspiration line is inserted through the OPU handle and advanced to reach the fornix vagina and into the follicle antrum. Follicular fluid of each follicle is aspirated using continuous negative pressure applied with a vacuum pump (Saleem *et al*., 2022) [50] into the centrifuge tube which is placed in pocket tube heater to maintain the temperature of follicular fluid collected until carried to the laboratory.

Post OPU complications

Physical injury

According to Greve and Jacobsen (2001) [26], OPU procedures can result in vaginal and ovarian puncture, frequent epidural anaesthesia, tail pain, and vertebral fusion. Chastant-Maillard *et al*. (2003) [13] performed histological examination of ovaries collected by ovariectomy 4 days after puncture revealed blood filled follicles and haemorrhagic foci on ovarian stroma, but examination after 30 days of OPU revealed only limited fibrosis, indicating that repeated OPU has no negative impact on cow welfare. Jeyakumar (2004) [30] investigated the effect of OPU on donor cows and discovered no pathological changes at the epidural injection site, fornix vagina, or ovary. El-Shawarby *et al*. (2004) [18] investigated the complications

associated with transvaginal oocyte retrieval for *in vitro* fertilization and discovered that the most common complications were haemorrhage, pelvic trauma and injury, and pelvic infection. Other risks include adnexal torsion, endometriotic cyst rupture, anaesthesia, and even vertebral osteomyelitis. Oliveira *et al.* (2019) [40] examined the macroscopic and microscopic changes in the ovaries of zebu donor cows and concluded that follicular puncture promotes gross lesions such as ovarian cysts, fibrosis and scarring side and may reduce fertility. Studies on the ultrastructure revealed a large number of cytoplasmic granules with a notable lipid content (Hufana-Duran, 2008) [29], which most likely makes buffalo oocytes and embryos more vulnerable to oxidative stress.

Ovarian changes after OPU

Although the ultrasound guided transvaginal follicular aspiration method was less traumatic for the vagina and especially for the fornix (Backer *et al*., 1996) [3], it caused more injuries to the ovarian stroma (Pieterse *et al*., 1991a; Backer *et al*., 1996 and Santl *et al*., 1998) [45, 3, 52] particularly during small follicle aspiration (Backer *et al*., 1996) [3] Gibbons *et al*. (1994) [23], Jeyakumar (2004) [30] Intrafollicular haematoma (Van der Schans, 1991; Pieterse *et al*., 1991a; Gibbons *et al*., 1994; Bergfelt *et al*., 1994; Mc Evoy *et al*., 2002 and Chastant Maillard *et al*., 2003) [57, 45, 23, 4, 34, 13], a slight hardening of the ovarian tunica albuginea (Pieterse *et al*., 1991a and Van der Schans, 1991) [45, 57], thickening and hardening of the ovarian tunica albuginea which decreased the number of follicles (Yang *et al*., 2005) [61], fibrous tissue accumulation around the ovaries (Gibbons *et al*., 1994 and Jeyakumar, 2004) [23, 30], scar formation in the ovarian cortex and stroma, reducing oocyte recovery efficiency overtime (Jeyakumar, 2004; Neglia *et al*., 2011; Monteiro *et al*., 2010 and Oliveira *et al*., 2019) [30, 39, 36, 40]. Several studies found that the risk of ovarian damage from follicle aspiration increased with the number of OPU sessions and the total number of COCs recovered per session. They have also stated that the greater the number of follicles on the ovary, the greater the number of needles punctures and thus the high risk of ovarian damage and scar formation (Viana *et al*., 2003 and Oliveira *et al*., 2019) [58, 40], wherein a greater number of follicles will be recruited per follicular wave in *Bos indicus* cattle (Sartori and Barros, 2011). It also evident that repeated OPU can be performed without side effects both in cattle and buffaloes with a minimal stress to the animal (Boni, 2012) [9].

Conclusions

Ovum pick -up can be successfully applied on buffalo cows. The P-FSH priming significantly increased either the number of punctured follicles or the number of recovered oocytes in deep anestrus. Buffaloes. In order to manipulate and improve the health, productivity, and reproductive performance of any livestock species, these emerging techniques should be judiciously supplemented with best practices in animal nutrition, health, and management at the level of stake holders. This will help to produce and disseminate superior germplasm, thereby increasing the overall productivity of livestock species.

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