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Ovum Pick-up in Ruminants

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ABSTRACT

Ovum Pick-up (OPU) associated with IVF has become advancement in ruminant reproduction. Transvaginal OPU guided by ultrasound is a non- invasive technique for cumulating oocytes from Graafian and antral follicles from live donor animals especially from cattle. Laparoscopic OPU in combination with *in vitro* embryo production increases the possibility of obtaining viable offspring in small ruminants. OPU does not impede with the usual reproduction and production performance of the donor animals and can be apply in various physiological states and circumstances of animals like different ages and reproductive disorders. The persistent collection of oocytes through OPU techniques concedes us to acquire the highest possible progeny of animals with merit genetic value, genetic improvement and advancement in animal selection. Here, we are presenting an overview of OPU from ruminants with factors affecting their outcome.

Introduction

In recent years, there has been advancement in the field of reproductive techniques including artificial insemination (AI), embryo transfer (ET), *in vitro* embryo production (IVEP) that have aided in genetic gain, improvement in productivity and performance. The remarkable contribution of the livestock to the economy has shifted the attention towards the use of the advanced techniques. The India, being a developing country, has to rely mainly on agriculture production to meet the increasing food demand for its more than 1.25 billion population. Total population dependent on agriculture is 49.8% and livestock sector employs 8% of total Indian work force. India has the world's largest livestock population, with 535.78 million domesticated animals; in which cattle population is (192.49 million), buffalo (109.85 million), goat (148.88 million) and sheep (74.26 million) as per 20th livestock census, 2019 (https://ruralindiaonline.org/en/library/resource/20th-livestock-census-2019-all-india-report/). Livestock sector contributes about 4.9% of GDP during 2017-18 (DADF, 2019). India stands first in milk and carabeef production, second in goat meat production, 9th in wool production, largest exporter of the sheep and goat meat to the world (APEDA, 2021). The ruminant is an important part of the livestock economy whether it is for milk production or meat production. To deal with the growing demand for increasing the productivity and performance of the animal, the reproductive techniques including the following embryo technologies have been taken into consideration: Multiple

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ovulation and embryo transfer and IVEP regarding ovum collection either from live animals or slaughtered animals.

However, while dealing with the IVEP, few limitations were being encountered, including the collection of competent oocytes from the same animal, as only oocytes present within the follicles involved in the follicular wave dynamics have the proper competence needed for IVEP. All these limitations can be overcome by the collection of oocytes from living animals by the technique termed ovum pick-up (OPU, Galli, 2001). Moreover, OPU does not need the normal reproductive cycle of the donor animal, as compared to IVF and thus has proved its importance in shortening the generation interval and increasing genetic gain. In accordance to the American Embryo Transfer Association, a total number of 1,22,431 OPU has been done, out of which 2 million oocytes have been recovered for the purpose of ET (Demetrio et al., 2020). OPU is first known to be introduced in case of cattle by a Dutch team (Pieterse et al., 1988). Since years the techniques of OPU has undergone through various advancements starting from endoscopy, transvaginal ultrasound, laparoscopy including hormonal interventions. OPU is a highly flexible technique as it can be performed at least twice a week. The optimisation of the OPU depends on the frequency of sampling from the live animals. IVF techniques have been merged along with OPU technique in order to exploit the advantages of the elite animals, leading to overall growth in production. OPU technique has proved to be a low invasive procedure for the retrieval of high quality and quantity of oocyte in terms of elite living animals, thus has been used as an alternative to invasive surgical techniques (Fig.1). The success of the OPU has been demarcated on the basis of the quantity and quality of oocytes recovered, expressed in the percentage of total number of follicles punctured

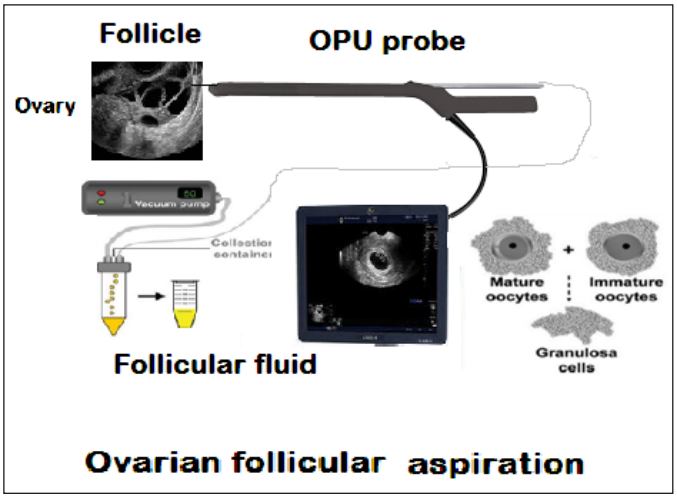

Fig. 1. Sketch diagram of ovarian follicular aspiration

(Ward et al., 2000). Since cow slaughter is not allowed in India for religious reasons, OPU can help to provide developmentally competent oocytes on the laboratory bench for use in various reproductive technologies.

Advantages of ovum pick-up over traditional methods

Increase in the number of embryos harvested, resulting in an increase of 2 -3 times in the number of offspring from elite females (Kruip et al., 1994). Using this technique, ovum could be recovered from both pregnant and non-pregnant female. It has aided in increasing the number of oocytes fertilised using unusual, costly, or sexed semen. The process of OPU in the case of ruminants includes epidural anaesthesia, positioning of the ovary through per rectal palpation, visualisation of the follicle using a transvaginal transducer, aspiration of the oocyte using a needle. The OPU is done mostly twice a week for the retrieval of more oocytes. Decrease in the collection time, minimizing the cost of operation, increasing the efficiency of the collection are the basic objectives for the OPU technique (Boni, 2012).

Ultrasound guided transvaginal ovum pick up

The first ultrasound-guided transvaginal OPU was reported in the year 1988. It is defined as a non-invasive method for recovering oocytes from antral follicles in the case of live animals (Callesen et al., 1987). It uses a scanner with an endovaginal sector probe fitted with a guided needle which is further connected to a test tube and vacuum pump that helps in the aspiration of the follicular fluid. A probe of at least 6 MHz is used to envisage follicles of 2-3 mm in size (Qi et al., 2013). This has been helpful in the collection of oocytes from problematic, pregnant, acyclic cows.

Laparoscopic ovum pick up

Laparoscopic ovum pick up (LOPU) in combination with IVEP increases the possibility of obtaining viable offspring. Snyder and Dukelov originally proposed the notion of LOPU in 1974, when they retrieved 6 oocytes by aspirating 21 follicles from a sheep using laparoscopy (Snyder and Dukelow, 1974). LOPU-IVEP has proved to be useful in the case of pre-pubertal cows of 2–6 months of age. It led to the establishment of genetic marker selection in dairy cattle, which allowed for the prediction of the production

phenotype of dairy females, immediately after birth. An average of 22 viable oocytes and a 20% transferable blastocyst rate were found, with a >50% pregnancy rate following the transfer, making the platform fit for commercial use. The potential of LOPU has been exploited to produce 'calves from calves' (Baldassarre and Bordignon, 2018; Baldassarre, 2021). It is a less invasive procedure, having more repeatability and reliability, with the observation of ovaries with the help of a laparoscope. The advent of laparoscopy and video surgery has been useful in the OPU in the case of small ruminants (Wieczorek et al., 2018).

The procedure followed in LOPU can be described as follows: animal needs to be deprived of hay, grain, and water for at least 36, 24, and 12 h respectively. The procedure is carried out under general anaesthesia. Follicles larger than 2 mm in diameter are aspirated under laparoscopic monitoring using a 20G needle placed on an acrylic pipette and coupled to a collecting tube connected to a vacuum pump. The laparoscopic equipment comprises a 5 mm/0° laparoscope, three trocar/cannula components, an atraumatic gripping forceps, and a cabled light source. The grasping forceps are used to draw the mesosalpinx in different directions, exposing the various surfaces of the ovaries and permitting aspiration of all follicles (2 mm). The vacuum pressure is set at 50–70 mm Hg. Following the aspiration of all follicles, wash the ovarian surface with warm saline solution injected through one of the cannula parts using a pipette (Baldassarre and Bordignon, 2018).

LOPU can be classified as competitive applications (use of non-pregnant, non-lactating, adult females as a donor of oocyte) and non-competitive applications (used in case of repeated luteal regression, failure to fertilise or ovulate, etc) (Ali, 2021).

Effect of hormones on ovum pick-up and collection from a different category of animals

OPU can be either non-stimulatory type or pre stimulatory type. Earlier the OPU was practised with non-stimulated animals. However, in recent years, super stimulation of the animals has been practised that has increased the retrieval of oocytes. Oestrus synchronisation can be used as an accessory protocol in the procedure of OPU. This is carried out with the supplementation of gonadotropins or progesterone. Progestogen is containing intra-vaginal devices such as CIDR; sponge can be applied for 7–11 days in combination with a luteolytic dose of prostaglandin 48 h given before LOPU. Recently a 'one-shot FSH' protocol has also been used as 80 mg of FSH with 300 IU of eCG, administered simultaneously 36–48 h before LOPU for recomposing the lyophilized FSH inside the Folltropin® vial, 10 mL of a 0.5 % hyaluronic acid solution (MAP-5®, Vetoquinol, Lavaltrie, QC, Canada) is used. In the case of goats, a week of GnRH analogue administration increased the number of follicles as well as oocytes (Baldassarre et al., 2002). A large variability has been marked in the case of goats ranging from 4 to 33 follicles and 2 to 12 oocytes recovered in response to the use of estrus synchronisation protocols (Gibbons et al., 2007).

Oocyte collection from post-partum cattle

Oocyte collection from the cattle just after calving has also been possible with the use of FSH hormone. It has been noted that retrieval of more number of oocytes is possible on 25th day and 35th day, of administration of FSH before 18 hrs of collection (De Roover et al., 2005)tailored to the individual donor response, to evaluate its advantages and disadvantages in terms of follicle numbers and diameters, the numbers of retrieved oocytes and day 7 cultured blastocysts. Ten adult non-lactating dairy cows were superstimulated with pFSH and subjected to ovum pick up-in vitro fertilisation (OPU-IVF.

Oocyte collection from pregnant cattle

It has been reported that cows continue to have follicular growth and development during early and mid-gestation. Mostly trans-vaginal ultrasound-guided aspiration is used for the collection of oocytes. Since the cervix and uterus are not penetrated during aspiration, so oocytes can be collected without disturbing the foetus. This technique has proved to be highly effective in getting a jump to the next generation.

Oocyte collection from calves and pre-pubertal cattle

To achieve genetic improvement in the case of cattle, the generation interval plays an important role. It has been found that follicular development occurs in a wave-like manner in calves as early as 2 weeks of age; also the ovaries contain large pools of antral follicles that can yield oocytes suitable for maturation. Recently a modified 5MHz convex-array ultrasound transducer has been used in the case of calves, capable of collecting ovum from calves in

a standing position (10-16 weeks old) and dorsal recumbency (6 weeks, old) (Majerus et al., 1999).

Factors affecting ovum pick-up

To obtain a good number of oocytes, there must be a greater number of follicles, which is influenced by many factors such as breed, nutritional factors, climatic conditions, etc. The type of breed used as a donor plays a significant role for instance *Bos indicus* has more follicular waves and more small follicles as compared to *Bos taurus*. Climatic factors such as heat stress result in changes in follicular growth, suppressing the follicular cycle (Al-Katanani et al., 2002). It has been reported that the number of follicles (about 3-8 mm in diameter per ovary) was higher in winter as compared to summer (Roth et al., 2001). It has been found that oocytes obtained in autumn are of poor quality, while there is an increase in oocyte quality with the approach of winter. In the case of ewes, the season also influences the number and competency of retrieved oocytes, with the cleavage rate being greater during the anestrous season (Gonzalez-Bulnes et al., 2010). Melatonin implanted subcutaneously enhances oocyte developmental competence in ewes during the anestrous season (Gonzalez-Bulnes et al., 2010). In sheep and goats, the developmental competence of follicles improves with size.

The success rate of OPU is also affected by various individual factors such as age (oocyte developmental competence is lower in pre-pubertal heifers than in the case of cows), the individual response of the animal, etc (Ali, 2021). It has been seen that oocyte competence has a relationship with follicular size. With the increase in follicular size, there is an increase in oocyte competence. Oocyte competence is greatly increased in follicle of size having 8mm.The quality and quantity of oocyte retrieved also depends on body score condition of the animal. Several studies marked that oocyte competence is also affected by the nutritional status, for example, small follicles (less than 4mm) and medium-sized follicles (more than 4-8 mm) are found to be sensitive to excess dietary energy and rumen degradable nitrogen respectively (Armstrong et al., 1994).

Advantages of ovum pick-up

OPU has been useful in fulfilling the demands of third generation assisted reproductive techniques, which not usually met by conventional superovulation techniques. It eliminates the variability problem (such as collection of oocytes from pregnant animals, lactating animals, increase in oocyte competence etc.) faced as in the case of superovulation. The OPU techniques can be practised on a sporadic or a regular basis such as twice a week. It has helped obtain a greater number of oocytes per female, being efficient enough for the collection of oocytes from females starting from pre-pubertal age to acyclic cows, pregnant cows etc. For example, collection of about 15-20 oocytes once a week or 5-10 oocytes twice a week from zebu cattle. It is applied on the cows irrespective of their reproductive status, for instance, cows with any genital tract infections, with blocked fallopian tubes, those non-responsive to superovulation treatment. It has helped reduce the generation interval (as female calves of 2-3 months can also be used as donors) and increase the genetic gain over the years.

Future prospective of OPU

Persistent collection of oocytes by ultrasound-guided trans-vaginal OPU unconditionally correlated with IVEP and become effective alternative of superovulation technique which performed in ruminants for embryo production. Simple oocyte collection by follicular puncture or trans-vaginal OPU could be repeatedly performed in animals without any risk to their health and reproductive performances. Conjugation of OPU with IVF facilitates production of large number of embryos outside the animal body and available for implantation in many surrogates' animal. Some of the probable superiority of OPU in contrast to traditional techniques of ET in cooperate the likelihood of enhanced harvested embryos number and ultimately higher offspring's production from elite female animals. The OPU strategies is potentially useful in both pregnant and non-pregnant donor animals as well as recipient animals, can maximize oocyte fertilization and also helps in increasing livestock population and production.

Conclusion

The advent of OPU has been highly helpful in the collection of oocytes from live animals. OPU techniques are also useful for animals of various age groups including 2 months of aged female calf. The only exceptions are for the animals with severe ovarian hypoplasia, animals in the immediate postpartum phase where ovarian activity is not yet restored. Since the collection of an oocyte from a slaughterhouse has its limitation in terms of unknown pregree and their genetics, contamination, quality and recovery rate of the oocyte, OPU can help combat these limitations. Thus, the application of OPU will be invaluable in rapidly multiplying rare genes and provide basis for

more advanced technologies such as in the development of embryo sexing programmes, transgenesis, cloning, etc.

Conflict of interest

Author declare none

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