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Review Article

Advanced assisted reproductive technology in cattle: OPU-IVF review

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Abstract

India ranked 1st in cow and buffalo population in the world with annual production of 187.75 MT of milk and 8.11 MT of meat, livestock contributes 4.1% in of total GDP. Even though, production is inadequate to fulfill the nutritional demands of population of the country. To generate bulk quantity of food products to fulfill the demands required to adopt new technologies or refining of the technologies, which in turn can increase in production and here, assisted reproductive technology (ART) can play a major role including *In vitro* fertilization (IVF) technology. In world, 6,66,000 embryos produced by IVF in 2016 and in Brazil IVP increased by 184.0% between 2005 and 2016. Live offspring of 25 species already been achieved though embryo transfer technology. Initially, *in vitro* embryo production (IVEP), the oocytes were collected from the slaughterhouse ovaries and at present, oocytes are being collected from the live animal by using OPU methodology. To maintain optimum quality of embryo production and production rate clean room preparations is radically important. Polluted air and volatile organic compounds are contributing in poor *in vitro* embryo production. IVF is become a regular tool to produce embryos for different purpose including research throughout the world. This technology needs further refinement and here we reviewed about Ovum pick up - *In vitro* fertilization (OPU- IVF) technique for cattle, clean room methodologies and pointed out the major considerations that can definitely help researchers.

Key words: Assisted reproductive technology, *In vitro* Fertilization, Ovum pick up, Volatile organic compounds

Introduction

India ranked 1st in cow and buffalo population in the world. India produces 187.75 MT of milk and 8.11 MT of meat. Livestock contributes 4.1% of the total GDP in India, per capita availability of milk in India is 394 g/day (BAHS, 2019). Even though, production is inadequate to fulfill the nutritional demands of population of the country. To generate bulk quantity of food products to fulfill the demands required to adopt new technologies or refining of the technologies, which in turn can increase in production. Reproduction is key area to produce livestock with desirable traits and here, assisted reproductive technology (ART) can play a major role including *In vitro* fertilization (IVF) technology. In the year 2016, total 6,66,000 number of embryos produced by IVF worldwide. Alone in Brazil the *in vitro* embryo production increased by 184.0% between 2005 and 2016 while *in vivo* derived embryos decreased by 73.7%. Till now, by using embryo transfer technology in 25 species live offspring already been acquired (Verma *et al*., 2012; Abdullah *et al*., 2018; Reuben *et al*., 2018). It represents there are vast opportunities to do further research to develop highly productive animals in high number in a short time span. Here, we have discussed the historical development of the IVF technology, production

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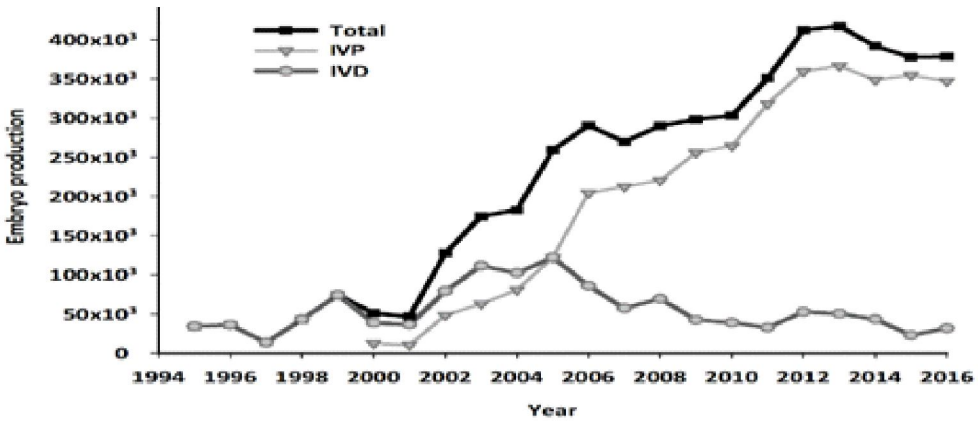
rates constrains, future scope of this IVF and OPU-IVF technology.

Historical background of assisted reproductive technologies

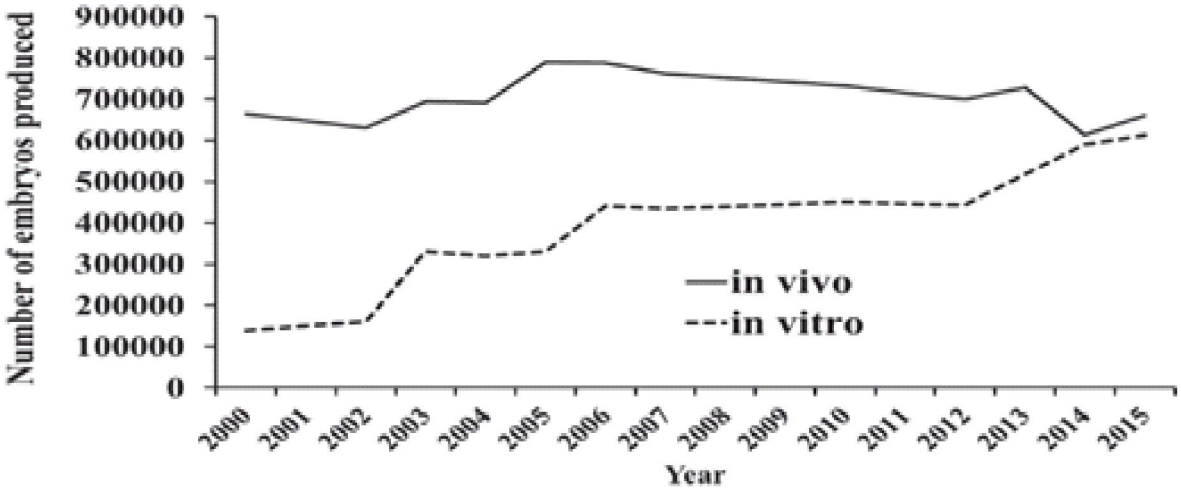
The first generation assisted reproductive tool is artificial insemination. Following that there was further development of ART's namely super ovulation, embryo transfer technology (ETT), *In Vitro* fertilization (IVF), ovum pick up technique (OPU), combination of OPU-IVF, pregnancy diagnosis by ultrasonography. Now, at the present genomic selection by using DNA analysis can be recognized as ART of 21st century (Moore and Hasler, 2017; Purohit, 2018).

In 1976, "assisted fertilization" experiment was done by micro injection of Hamster oocyte with human sperm pioneered by Uehera and Yanagimachi (1976). Around the same time, on 1780 first artificial insemination had done by L. Spallanzani in Beagle dog and produced three pups. Although in India, first artificial insemination was done in cattle by S. Kumaran, 1939 at Mysore cattle palace. Sequentially the first super ovulation technique reported by Casida *et al*., 1943 and first embryo transfer was done by Willett, 1951 and produced calf. In India, first embryo transfer was done at National Institute of Immunology, New Delhi, 1987. The *in vitro* fertilization (IVF) techniques yield first calf, "VIRGIL" by Brackett *et al*., 1982. In India, first buffalo calf "PRATHAM" produced by using this technology in National Dairy Research Institute, Karnal, 1990 (Madan *et al*., 1991; Nain *et al*., 2006) and recently produced first calf of Tharparker breed by Dr. Zawar, Pune on 5th Sept. 2017 (Purohit, 2018). Most advance technique of oocyte collection from the live animal i.e., first ovum pick up technique (OPU) was introduced by Callesen *et al.*1987, performed in Bovine. In India, OPU followed by IVF and development of embryo upto Blastocyst-reported by Verma, 2005, in Bovine. The first OPU- IVF in aged Sahiwal cattle done at National Dairy Research Institute, Karnal, 2012 and produce Sahiwal calf "HOLI" (Saini *et al*., 2015; Moore and Hasler, 2017; Purohit, 2018). Even though, in India this technological development confined within reputed institutes, here can see in Brazil (exemplary country) where they utilised this technology for the development of livestock and getting successful increasing production and productivity.



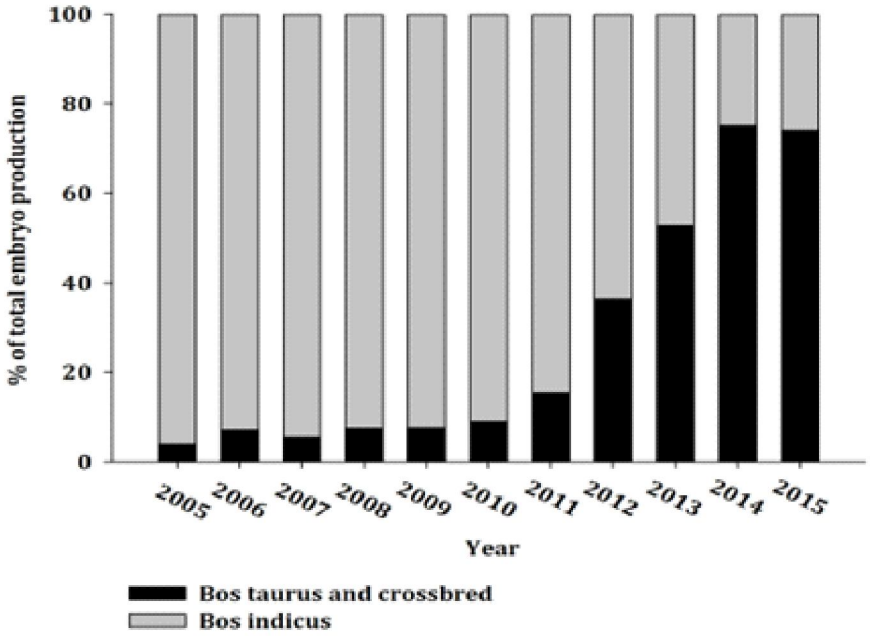
Growth of IVP Technology in Brazil from 1995 to 2016

Fig. 1. The pattern of production of bovine embryos in Brazil during 1995 to 2016. Total indicates combination of *in vivo* and *in vitro* embryos; IVP- *In vitro* production of embryo, OPU/ IVF; IVD- *In vivo* derived embryo produced by using super ovulation followed by embryo collection; observed increases trend of IVP due to modification and development of superior culture medium and increased conception rate followed by IVF (Reuben *et al*., 2018)



Worldwide scenario upon IVD and IVF

Fig. 2. Continuous increasing trend in IVP embryos compared to IVD since year 2000 onwards; IETS (Moore and Hasler, 2017)



Trend (in percentage) of Brazilian embryo production in *Bos taurus* and *Bos indicus*

Fig. 3. Brazilian embryo production (in percentage) in two bovine species from 2005 to 2015, majorly the *B. taurus* animals are using for production of embryos compared to *B. indicus* (Viana *et al*., 2017)

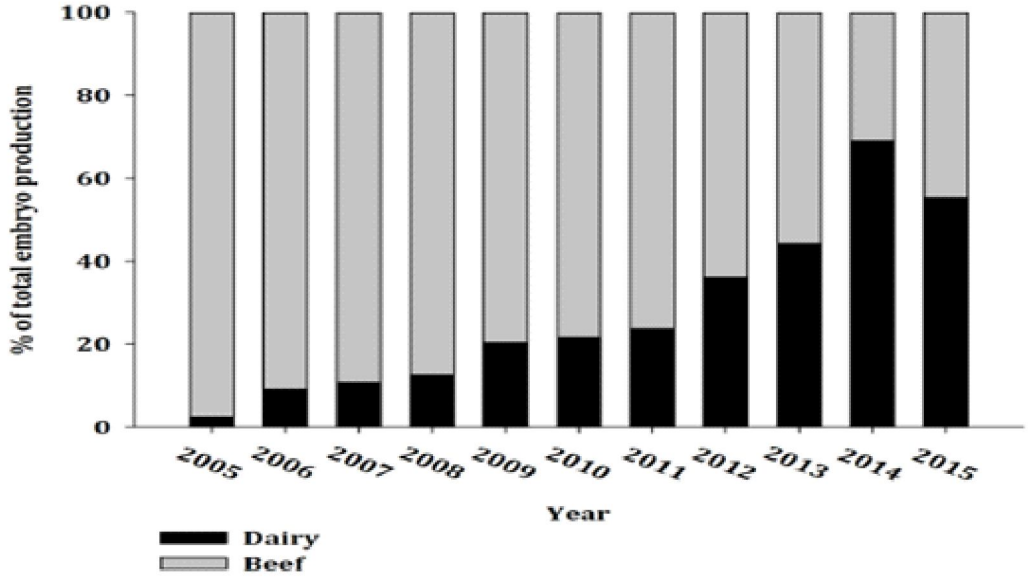


Fig. 4. Brazilian embryo production (in percentage) in dairy and beef breeds from 2005 to 2015 (Viana *et al*., 2017)

In Vitro embryo production

In vitro embryo production (IVEP) is the combination of three major steps: a) *in vitro* maturation (IVM) of collected oocytes either from slaughterhouse ovaries or from live animal by OPU technique from live animals, b) *In vitro* fertilization (IVF), where matured oocytes are fertilized by the spermatozoa from sexed semen or semen from elite bull and c) *In vitro* culture (IVC) procedure, the fertilized oocytes are being cultured in appropriate media where they are nourished to develop up to the blastocyst stages. Generally, the time duration of culture is 7 days for cow. As per species, the culture duration varies. The appropriate timing of transfer of embryo maximizes the pregnancy rate in the recipient animal.

Oocyte collection: Previously, in the *in vitro* embryo production programmes, embryos were developed from the procured ovaries from slaughterhouse (Lu and Polge, 1992; Sinclair *et al*., 1995; Kuwayama *et al*., 1996; Lazzari and Galli, 1996). Currently due to the technological improvement, the oocytes are collected from the ovaries of elite live animal by using OPU method. This enables repeated oocyte recovery from the animal within a gap of very short period and yield more embryos from the elite animal (Galli and Lazzari, 2008). This technique is using in many countries for production of IVEP. This technique is being used for collection of oocytes from endangered species to conserve the genetic resources in much faster way (Hafez, 2015).

Grading of oocyte: Grading of oocytes can be done based on number of cumulus-oocyte complex (COC) layers, expansion of cumulus cell, cytoplasmic appearance in terms-colour, consistency and integrity of zona pellucida (Cavalieria *et al*., 2018).

***In-vitro* maturation** (IVM)**:** IVM process requires certain *in vitro* environment to develop the embryo and these requirements are summarized as 38.5°C temperature, 5% CO_2 in air, 90-95% humidity for 22-24 hours kept in 35 drops of 75 µL of maturation medium. This media contains TCM 199, earls salt, glutamine, sodium bicarbonate, 10% fetal calf serum, 0.5 µg/mL FSH, 22 µg/mL of pyruvate, 50 µg/mL of gentamicin, 50 µg/mL of LH, 1µg/mL estradiol (Chowdhury *et al*., 2019).

Sperm selection: Semen straws are thawing at

38.5°C for 1 min and collected semen in DPBS medium followed by centrifugation at 750-x g for 5 min at room temperature. After the centrifugation, the semen sample should resuspended in the IVF medium containing tyrode lactate solution with bovine serum albumin (BSA) 6 mg/mL, sodium pyruvate 22 mg/mL, penicillin 100 IU/mL, streptomycin 0.1 mg/mL, supplemented with heparin 20 mg/mL. Followed by incubated at 38.5°C in a humidified atmosphere of 5% CO_2 in air for 15 min to facilitate capacitation. Final concentration of semen sample is $1x10^6$ sperm/mL in IVF medium (De *et al*., 2011; Ayman *et al*., 2017; Chowdhury *et al*., 2019).

Sperm selection for sexed embryo: In recent development, scientists are trying to sexing of semen by using the monoclonal antibody. It is reported that sex sorting has a negative effect on sperm quality. It altered the pattern of sperm motility; reduce the period of cell viability (Chowdhury *et al*., 2019). Researcher has approached a new technique to produce preselected embryo by using a monoclonal antibody which been developed against bull sperm epitopes. This antibody separates the X and Y sperm in a simple and easy process and provides preselected embryo production. The mechanism of action of this method involves immobilization of the male spermatozoa and the XX spermatozoa will bind with the oolemma of oocyte followed by fertilization. They found that there was no significant differences ($P>0.05$) in the percentage of presumptive zygotes production between the control and the X-sperm sorted group, but there was a difference in early cleavage embryos with 81.2±1.4, 78.3±1.0 and 66.7±1.1% for the control, X-sperm sorted, and Y-sperm sorted groups, respectively.

Chowdhury *et al*. (2019) also found that there were significant differences ($P<0.05$) in the percentage of embryo development up to the blastocyst stage (Day 7) 34.8±1.0, 32.1±0.8, and 23.7±1.0% in the control, X-sperm sorted, and Y-sp sperm sorted groups, respectively. The author also reported that by analyzing the gene expression (B-SRY F2 and B-SRY R2) of *in vitro* produced embryos that the detection accuracy of female embryos was 81.0% and for the male embryos 72.5%.

Chowdhury *et al*. (2019) concluded that no differences in cleavage and blastocyst developmental rates with use of sperm with the X chromosome and sperm unsorted sperm with X or Y chromosomes (control). This cell sorting technique is less time consuming and produces less stress on sperm cells compared to current method of sperm sexing. This technique can be useful in sperm selection in case of IVEP technique.

Other method of sperm selection is swim-up method. Application of this method yields more male embryos (58%) and compared to female embryos (Wolf *et al.*, 2008). It is due morphological character of Y-chromosome bearing sperm i.e., due to lighter, smaller and faster compared to the X-chromosome bearing sperm (McEvoy, 1992; Johnson, 2000). However, with 67.5% continuous percoll density gradient centrifugation for 10 min yield more amount of female sperm (Wolf *et al.*, 2008), and female embryo following the *in vitro* fertilization. There still a scope to use larger volume of percoll gradient and increasing in centrifugation can induce more sex sorted sperm for production of IVF embryo.

***In-vitro* fertilization:** Matured COCs are kept in 4-well dishes for fertilization. Each well contains 500 mL of IVF medium and spermatozoa. These 4-well dishes are incubated at 5% CO_2 in air 38.5°C for 18-20 hr (Rosenkrans *et al*., 1993; Ayman *et al*., 2017).

***In-vitro* culture:** This process carried out at 38.5°C temp, 5% CO_2 in air and 90-95% humidity. The culture kept in 4-well dishes, which contain 500 mL CR1-aa medium. This media contains 3 mg/mL BSA, 310 mg/mL glutathione for initial 3 days, 44 mg/mL sodium pyruvate, 100 IU/mL penicillin, 14.6 mg/mL glutamine, 0.1 mg/mL streptomycin, 10% fetal calf serum (FCS) after 3 days of culture (Rosenkrans *et al*., 1993; Ayman *et al*., 2017).

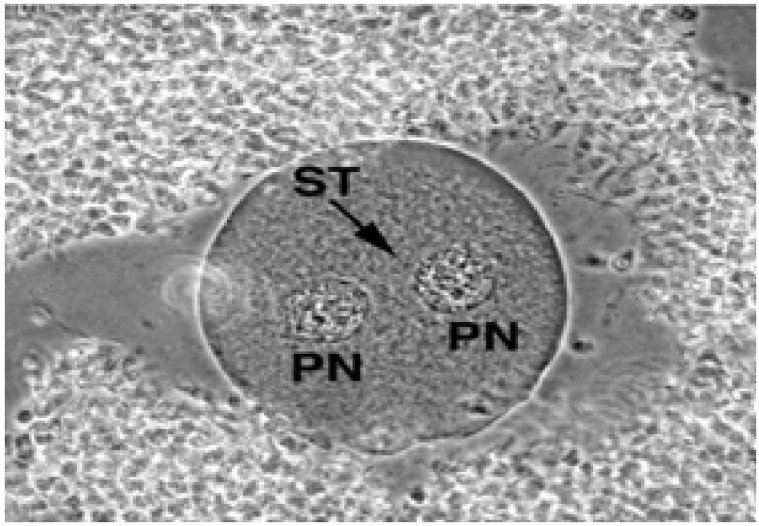


Fig. 5. One of the embryos produced after using heparin for capacitation (Parrish *et al*., 1986)

Effect of light exposure upon embryo during ART

IVF lab procedures, embryos exposed to radiation energy from light. This produces stress upon its biological systems. The blue light (400-500 nm) is harmful than longer wave length visible light due to generation of H_2O_2, hydroxyl radicals production, damaging the amino acids, phospholipids, nucleotides, organic acids and ultimately changes in the respiratory chain leading to impaired mitochondrial function and lastly cellular damage (Aggarwal *et al*., 1978; Hirao *et al*., 1978; Ramadan-Talib and Prebble, 1978; Halliwell *et al*., 1989; Umaoka *et al*., 1992; Squirrell *et al*., 1999; Hockberger *et al*., 1999). The damages can reduce by mounting filters on the inspection microscopes. Therefore, without hampering the visualisation can exclude the radiation energy within the range of the 400–500 nm. On the other hand, reducing the exposure time as well as narrowing the illuminated area by just focusing upon the embryo can reduce the damage (Ottosen and Hindkjaer, 2007).

IVF room

The laboratory room should have smooth, non-porous walls, impervious unbroken surfaces, corners with rounded edges and easy to clean, no sliding doors, sinks and drains. Ducts and pipes can be insulated internally i.e., hidden between wall panels or externally (covered within an alcove) to prevent dust accumulation. As the most of the laboratory located in cities and towns, "clean room technology" can easily controlled the entry of particles and contaminants in significantly manner. Clean room cells (CRC) that are stand-alone prefabricated laboratory can provide instant clean room environment within pre-existing buildings or in locations with poor overall air quality or in the absence of local materials and expertise. In this kind of rooms, hermetically sealed doors and windows are used. Adjacent rooms are interconnected by single door. Positive air pressure achieved by using high efficiency particle air (HEPA) filters which can highly filtrate the air. Also can be use central air handling system that includes activated carbon pre-filters, chemical filters and photo catalytic conversion that damage volatile compounds, bacteria and moulds. Maintenance of HEPA filter is crucial to maintain standard equipment status, safeguards against drop in air quality (Forman *et al*., 2004).

Air in urban areas contains high levels carbon monoxide, nitrous oxide, sulphur

dioxide, heavy metals and in addition, inside the lab contains volatile organic compounds (VOCs), dusts from construction materials, MDF (medium-density fiberboard), PVC flooring, paints, adhesives, chemicals like cleaning fluids, floor waxes, cosmetics and cigarette smoke which produce the phenomenon "sick building syndrome" (Takigawa *et al*., 2010). The poor air quality within an IVF lab has negative effect upon on fertilisation and embryo development (Boone *et al*., 1997; Dean, 2015). VOC levels can be monitored either by electronically or organic chemical sensors. The furniture and workbenches has to make up of stainless to reduce VOC emission. Highest VOC levels were acetone can reach up to a level of 15.5 ppm (Martine and Verheyen, 2016).

Cohen *et al*. (1997) has shown that inside the IVF lab sometimes-high levels of aldehydes, noxious compounds, VOCs are higher than outside air. The air contaminants can settle on work surfaces, dissolve in aqueous or lipophilic oil solutions, embryo culture medium ultimately harming the embryos. Reactive aldehyde present in air which can arrest mouse embryos at the 8-cell stage at concentration of 2.1 parts per million (ppm) and live birth rate was reduced at a concentration of 0.58 ppm (Hall *et al*., 1998; Destaillats *et al*., 2002; Dean, 2015). In a study in Japan upon sick building syndrome they found that chemical like aldehydes are the responsible for the SBS condition among the human (Takigawa, 2010).

In instruments, the copper pipes are prone to oxidation should not be used for transportation of medical grade gasses from cylinder to incubator and best to be replace with inert stainless steel tubes. Compressed gas like medical grade N_2 and CO_2 used for embryo production may contains harmful compounds like benzene, isopropanol and pentane, these should be eliminate before entering the incubators (Hall *et al*., 1998; Mayer *et al*., 1999).

Staff is among one of the biggest contaminants in a clean environment. Exhaled air from smokers contains residual tobacco particles (0.5 and 2 micron in size) that can remain indefinitely in the air or settle on surfaces and walls. Therefore, smokers should be discouraged from smoking within the working day. Beards long than 3-4 mm should be covered. "Air shower" before entering the laboratories acts as physical barrier which can reduce particle contamination. Polyester garments are most suitable for IVF laboratory because it does not shred fibres; carry less residual traces of laundry detergent (Martine and Verheyen, 2016). A moving person sheds 1 million particles ≥0.5 μm per minute, and a walking person sheds over 5 million particles ≥0.5 μm per minute (Martine and Verheyen, 2016).

Ovum pick up

Transvaginal oocyte retrieval (TVOR) technique is the process of collecting immature unfertilized oocyte directly from the ovaries of donor cow or heifer by using a specially designed probed equipped with an ultrasound transducer to visualize the ovary during oocyte aspiration (Pieterse *et al*., 1988). The compatibility of oocyte size ranges from 2-8 mm, (Abdullah *et al*., 2018). OPU sessions averaging 15 oocyte and 6 embryos per session of oocyte collection from live donor (Meiyu *et al*., 2013; Chowdhury *et al*., 2017)

Donor: Donor should be selected carefully. Outstanding donors are found in all the breeds. It is reported that oocytes retrieved through OPU per session is consistent with time but production of oocyte varies with individual animal. *Bos taurus* consistent in oocyte production even after 32 times retrieval (Petyim *et al*., 2003); *Bos indicus* decreases in oocyte retrieval with time (Gimenes *et al*., 2015). Certain factors are closely correlated with the long term production of oocyte through OPU. Anti-Mullerian hormone (AMH) level is directly correlated with number of population of oocyte present in the ovaries as well as correlated with oocyte retrieval through OPU. Higher the

retrieval of oocyte higher the number of *in vitro* embryo production, (Boni *et al*., 1997; Baldrighi *et al*., 2014; Batista *et al*., 2014; Guerreiro *et al*., 2014; Monteiro *et al*., 2017). Oocyte production is repeatable trait for female animals (Boni *et al*., 1997; Ireland *et al*., 2007; Baruselli *et al*., 2015; Monteiro *et al*., 2017; Watanabe *et al*., 2017).

Hormonal protocol OPU

Administration of exogenous hormones leads to appearance of more number of follicles in the ovary at the time of oocyte collection through OPU. Subsequently, more number of oocytes retrieval and embryos production with minimal labour (Meiyu *et al*., 2013). It is reported that in once-a-week OPU procedure, the average number of follicles aspirated, oocytes retrieved and blastocysts produced on day 7 was significantly higher in a FSH-treatment than non-stimulated OPU procedure per cow per session basis (Chaubal *et al*., 2007). By FSH treatment followed by OPU produces more number of Grade A oocytes than Grade C oocytes. In researches, it is concluded that oocytes yield per animal increased ($p<0.05$) in multiple FSH administration compared to single administration (Sendag *et al*., 2008). Also FSH treatment increases the quality of oocyte (Sendag *et al*., 2008). Administration of LH 6h prior to OPU, it is increased the number of blastocysts per OPU session (Chaubal *et al*., 2007; Sendag *et al*., 2008; Meiyu *et al*., 2013). In spite of all still has big window to left to scientists hand to do the improvement in the part of hormone-stimulated OPU.

Factors affecting OPU

1. Breed difference: *Bos indicus* breeds have more follicular waves, large pool of follicular reserve, contain more population of small follicles (<5 mm) at a time (Segerson *et al*.,1984; Figueiredo *et al*., 1997; Viana *et al*., 2000; Meiyu *et al*., 2013).
2. Nutrition and health: Enough amount of nutrition which maintains the proper health as well as it helps in the yield of good number of oocyte through OPU. It also plays role in embryo viability followed by successful pregnancy (Rubin *et al*., 2005; De *et al*., 2011; Watanabe *et al*., 2017).
3. Age: This technique helps to collect oocyte from heifer, adult cow, pregnant cow (De *et al*., 2011).
4. Heat stress: Less quantity and quality of oocyte observed during the period of summer while the number of follicles of 3-8 mm range of diameter per ovary was higher in winter. Recovery of oocyte in winter vs summer is ranges between 7.5 oocytes vs 5.0 oocytes per ovary (Zeron *et al*., 2001; Roth *et al*., 2002; Meiyu *et al*.,

Practical benefits of OPU over ET

OPU	ET
1. Once or twice a week	1. 45-60 days waiting period
2. Super ovulation is not necessary	2. Super ovulation is necessary
3. Use of female at early age, velogenosis, pregnant animal, estrous cycle not disturbed	3. Not possible
4. Pathological conditions of uterus or oviduct cannot hamper the collection of oocyte and capable of producing more than 50 calf a year.	4. Not possible
5. Average oocytes per OPU/ session is 18 to 25	5. Pathological conditions of uterus or oviduct cannot be exploited.
6. Experience plays a vital role compared to ET	6. Super ovulation produce 8-30 oocyte

(Wagtendonk, 2000; Sirard *et al*., 2006; Van Wagtendonk-de, 2006; Rodriguez, 2011; Verma *et al*., 2012; Meiyu *et al*., 2013, Purohit, 2018)

2013). Some author reported that bovine oocyte development was arrested in summer (Roth *et al*., 2002).

5. Operator's experience: To collect enough amount of oocyte need expertise hand and yields ranges from 0- 25 oocytes per session (Meiyu *et al*., 2013).
6. Individual variation: Variation among the donors persists in terms of production of oocyte in the ovary and followed by collection of oocyte by OPU procedure. The variation of response of the exogenous gonadotropin has observed among individuals (Meiyu *et al*., 2013).
7. Absence of dominant follicle (DF): Improved the blastocysts produced on day 7 (Gradela *et al*., 2000; Meiyu *et al*., 2013).
8. Animal: Heifers yield significantly higher number of total oocytes (4.7 vs. 2.8, P<0.001) and Grade A- B oocytes (3.0 vs. 1.8, P<0.05) compared to cow (Rizosa *et al*., 2005). It is recorded that pregnant cows produce good quality blastocyst and freezable embryos than non-pregnant cows (Takuma *et al*., 2010; Meiyu *et al*., 2013). Both the technique can yield more than 50 calf a year.

Increase in demand of OPU 2000- 2015

In US, there is increased demand of OPU (2,099 to 32,636), 29% to 53% while reduction in embryo flushes 39% to 30% (Moore and Hasler, 2017).

Limitation of hormone treatment for OPU

Long-term application of exogenous hormone causes disturbances in the donor endocrine system and having the capability to develop infertility to the animal. The response of the donor animal towards to hormone stimulation is varies with individual to individual leading to variation in the result (Meiyu *et al*., 2013). It is also found that ovarian response, appearance of number of follicles on ovaries and oocyte quality are affected by the type of gonadotropin used (Sendag *et al*., 2008). The best way to use the exogenous hormones in the donor is to apply short period followed by a resting period for normalization and recovery to the endocrine system (Meiyu *et al*., 2013).

Constrains

Oocytes collected from slaughter house ovarian samples shows sub-optimal maturation of COCs after OPU (Merton *et al*., 2003; Lonergan, 2007). OPU is applicable where slaughter house is unavailable. OPU is the one and only way to collect oocyte from live elite animal (Manik *et al*., 2003). Many instances observed low oocyte yield per session after continuous successive oocyte collection session (Machado *et al*., 2006). *In vivo* mature oocyte has greater competency than IVM oocyte. Species differences also a factor like, IVP efficiency in lower in buffalo compare to cattle (Rodriguez *et al*., 2012). Large calf syndrome, heavier birth weights, twining rate over 30% in cow, large ovary syndrome, higher rates abortion, extended gestation periods and increased rates of perinatal mortality observed (Lu *et al*., 1989; Hasler, 2000). Still research and improvement is going on throughout the world to minimize the all flaws of this technology.

Future scope

Greve and Madison (1991) has observed the potentiality of IVF technology and assumption of development this technology with development of OPU technique of oocyte collection. In India till this technology in grass root level. To standardize this technique, OPU-IVF technique initially must be utilized in large farms in Indian sub continent. Once the results are coming at scientifically significant level than can starts dispense this technology to other small-scale farmers. To dispense this technology in the field, need extensive training to the technical people willing to acquire the technical knowledge at the field level followed by adaptation of this technology by the farmers itself. The trained people can select the best females from the population regards of their production and productivity.

The extension work for this biotechnological tool to the enthusiastic farmer can lead to self-advertisement for this technology followed by adaptation of other farmers. The selection farmers and their satisfaction will give the best outcome of the technology. For the implementation of this technology in a wide range need Governmental and institutional help at all the level.

Application of IVF

IVF may perform like genetic 'insurance policy', when the outbreak of lethal diseases or for endangered species. IVP can salvage the irreversible genetic diseases which lead to culling. By this technique can generate embryo with known/fixed genomics, sex, date, donor and recipient and can produce more than 50 calves/ year (Hasler, 2003; Wrathall *et al*., 2004; Meiyu *et al*., 2013). IVF technology increases the genetic gain by reducing the generation interval. It is also contributing to produce embryos for research, purpose, embryonic stem cells (ESCs) (Gordon and Lu, 1990; Mapletoft and Hasler, 2005).

OPU-IVF maximizes the use of sexed semen, cloning, embryo sexing, analysis of pattern of gene expression, analysis of cytogenetic disorders and analysis of rapidly multiplying rare genes (Galli and Lazari, 2008; Verma *et al*., 2012). Increase scope in production of transgenic animal production by nuclear transfer technique and simultaneously production of recombinant medicine for human use (Blash *et al*., 2012). Combination of somatic cell banking and nuclear transfer technology can be used as a tool to conserve endangered breeds. Present days, there are increases in the production of transgenic animal after CRISPR/C as genome editing (Abdullah *et al*., 2018). This technique extensively used for commercial production of calf, especially beef breeds in Japan and Italy (Hamano *et al*., 2006). In total, due to extensive use of this technology for production of experimental embryos leading to submission of more reports upon discoveries, findings about embryos in site like Pub Med. In this well known site there are recorded over 1000 publications related to bovine embryos from the year 2011 - 2014 (Meiyu *et al*., 2013; Parrish, 2014).

Conclusions

The future of IVF and OPU- IVF technology is very bright can generate more employment, can add most valuable animals to the population such as pure indigenous breed of animals, reconstructing the population of endangered animals. Being superior to the ETT, this technology has the power to provide and produce high number of superior sexed embryo with economical traits, disease resistance, thermo-tolerance, higher feed efficiency. Here can develop general recording system for selected animals produced by IVF method, which makes easy for progeny testing and genomic selection of animal. Application of this technology followed by dissemination of new and improved genetics will leads to increase in reproductive performance and it corresponding to increase in economic growth in livestock sector. There is a vast field open for research and application of the IVF and OPU-IVF in cattle as well as other domestic animals. Large ovary syndrome, high rates of abortion, extended gestation periods, large calf syndrome and increased rates of perinatal mortality are required for further studies which put emphasis on reduction in the incidences. The ssuccessful application of IVF and OPU-IVF may become a boon and boom for a country like India with huge population livestock is present.

Conflict of interest: Authors have no conflict of interest in this study.

Author contributions: All authors contributed equally to the manuscript.

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