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Popular Article

Invitro Fertilization- an Art and Science conglomeration

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Abstract

In the domain of reproductive biotechnology, In Vitro fertilization has revolutionized various aspects of reproductive processes. It has re-defined evolution in getting back fertilization from internal to external. This technology is becoming an inevitable solution to major problems of human and animal reproduction. This expounding technology has huge potential in the fields of genomics, transgenics, and embryo physiology. Nevertheless, the cost of embryo production and the outcome is still an area of concern. Henceforth, to avoid exploitation of this technology concerning human and animal welfare, regulations and surveillance are to be incorporated to enhance the efficiency of Invitro fertilization.

Introduction

One of the aspects of biology that fascinated at the same time challenged human intellectual ability is the idea of "ARTIFICAL CREATION" which was thought to be impossible in the past until the birth of "LOUIS BROWN" the world's first baby born out of In vitro fertilization in 1978. IVF technology is a third-generation reproductive technology with immense scope in humans, domestic and wild animals. Though the methodology remains similar there exists a diversified purpose. In humans, it is to address the potential problems of Infertility, while in production animals the prime intention is to propagate elite animals and attain rapid genetic gain. However, in wild animals, it is urgency to conserve and preserve unique endangered species. The first successful birth of IVF in Rabbit was reported by Chang in 1959. Further, it has taken another 23 years not until 1982 for this technology to be successful in domestic animals with the first calf "Virgil" was been born out of IVF technology using In vivo matured oocytes. Followed by the birth of IVF animals was also reported in various species in due course of time.

Annual statistics from the International Embryo Transfer Society Denver, Colorado reported that more than **4lakh** bovine embryos were transferred worldwide in **2020**. In India, IVF technology is still in the budding stage. Department of animal husbandry and dairying, Government of India has taken massive initiation on conservation and propagation of Indigenous breeds under Rashtriya Gokul Mission with special emphasis on establishing IVF labs by **100%**central funding basis. These labs across India are now fully functional and being monitored intensively under the surveillance of the central committee. The prime intention of this **herculean task** is to establish a high-potential genetic herd of various unique breeds within the Indian subcontinent.

Mammalian ovaries are provided with a non-replenishable follicular pool approximately 2,00,000 primordial follicles per ovary constituting ovarian reserve. Nevertheless, a single such follicle finally reaches the preovulatory stage to release oocyte at each estrous cycle remaining undergoing atresia which is the most dominant event occurring all the time. Otherwise, undergo development on gonadotrophin stimulation. This principle is being utilized in IVF technology.

In conventional breeding/AI, a Cow can produce an average of 6-8 calves per lifetime. In contrast through IVF technology, 2-3 calves can be made to born from an opu session which can be done conveniently and frequently without detrimental effect on the animal. Hence 48-72 calves can be made to be born per animal each year. Such is the potential of this technology once the procedure is standardized.

Methodology

IVF is a long and continuous process every step is important and interdependent. The outline of the procedure is as follows.

❖ Oocyte Collection

Oocytes are the starting and crucial material of the whole IVF process. The Source of oocytes can be from slaughter or live animals. Oocytes from slaughterhouses contribute largely to the IVF industry worldwide. Ovaries are procured and cumulus-oocyte complexes are collected by aspiration technique which is often used or by slicing or follicular dissection. Follicles greater than 2mm in diameter are aspirated as these follicles host the developmentally competent oocytes. In our country, a major source of oocytes from the bovine species is donor aspiration. Transvaginal oocyte aspiration is most often used others include surgical flank incision through Laparoscopic procedures via Para lumbar Fossa. No. of oocytes collected depends on Vacuum pressure, gauge needle, length of the bevel, age, frequency, season, and efficiency of collection. An important aspect is aspiration time should coincide with follicular wave emergence. Donors are aspirated with or without stimulation. Porcine follicle stimulating hormone comes under two trades names Follitropin/ Stimufol is used for ovarian stimulation in either single or multiple doses. A coasting period / FSH fasting of 44–68 hours is usually recommended between the last FSH injection and OPU session. Intact cumulus-oocyte complexes are important for further maturation and fertilization. Advantages of stimulation include an increase in no of oocytes, blastocysts, grade 1 oocytes, and conception rate (Oliveira 2016). The cost of hormones is a major setback in stimulation protocols.

❖ *In Vitro* Maturation

Oocytes in follicles are growth arrested at the dictyate stage in prophase- I of meiosis under inhibitory forces of granulosa cells which is resumed upon gonadotrophin surge at ovulation. The cytoplasmic and nuclear changes between diplotene nuclear arrest and metaphase II at ovulation are termed maturation (Pan et al. 2019). The follicles that are in different stages of development at collection have to undergo similar phases of development in *In vitro* maturation step that enables the oocyte to fertilize the male gamete. Oocytes procured are graded those with a greater number of cumulus layers with even cytoplasm are ideal candidates to incubate in the media containing hormones, growth factors, energy resources, etc for a 20-24hour period in IVM media (Fig: 1). The expansion of the cumulus with the release of the polar body is the external sign of maturation.

❖ ***In Vitro* Fertilization**

Involves co-incubation of spermatozoa with matured oocytes in IVF media for 16-18hours to yield embryo. Capacitation changes are the obligatory maturational changes that spermatozoa have to undergo before they could fertilize the oocyte. These are characterized by elevated intracellular calcium and cholesterol efflux. The process of capacitation usually happens in the female reproductive tract to be specific in the isthmus region of the oviduct. The primary capacitating agent added to IVF media is heparin. Others are caffeine, adenosine, etc.

***In Vitro* CULTURE**

Newly fertilized eggs are transferred to an IVC medium and require six days of incubation under favorable conditions to transform into an embryo. There is significant evidence that bovine embryos develop under5% CO2, 5% O2, and 90% N2 gaseous mixture. These embryos are cultured in a CO2 / benchtop incubator. Embryo handling for longer times outside media leads to contamination and decreased blastocyst rate. All that comes in contact with the embryo must be tested and maintained at 37 ^{o} c including glassware and media to minimize stress on the developing embryo.

❖ **Embryo Transfer**

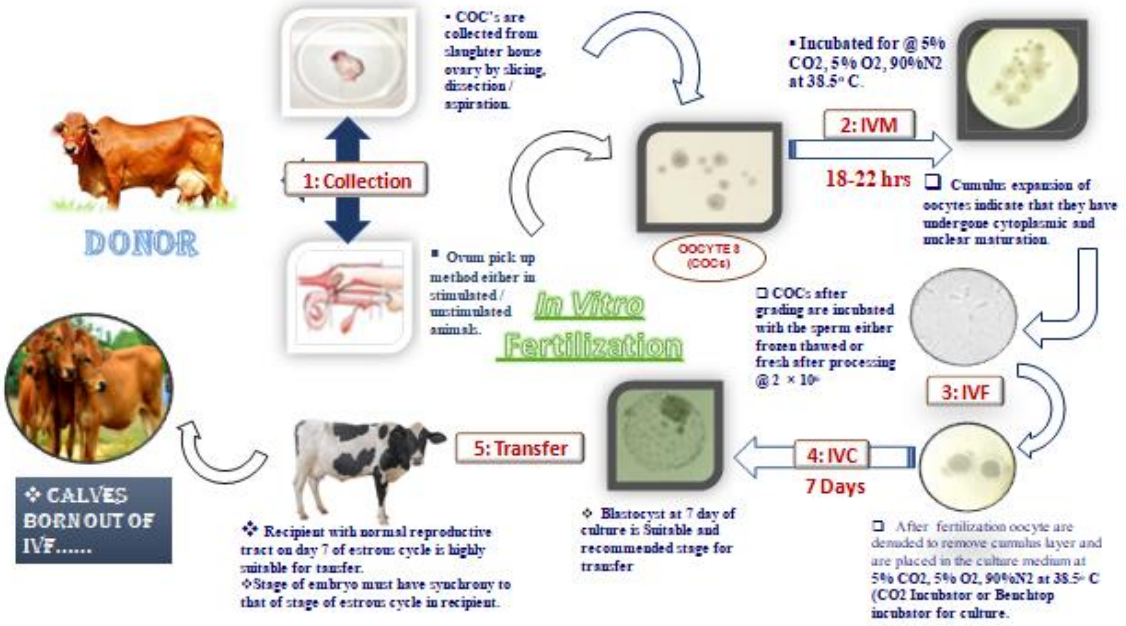

Fig 1: Illustrates outline of the methodology of *In Vitro* Fertilization.

On day 7 of culture, blastocysts are graded to segregate. This is very crucial as it not only enables us to evaluate the quality of an embryo that increases the likelihood of Positive Pregnancy but also an indirect indication reflecting the operating skills of the IVF lab. The diameter of the bovine embryo measures 170 to 190µm and it is a 3D structure hence stereomicroscope is required to evaluate its quality. IETS Standardized coding system to describe the stage and grade of an embryo. Grade A and B are selected for transfer while Grade A is reserved for cryopreservation. Recipient animals with regular reproductive cycles and free from anatomical abnormalities are estrous synchronized naturally or through hormones. The day of the estrous cycle on the day of transfer should be similar to that of the embryo developmental stage. In bovine species transfer is done on day 7 under epidural anesthesia (Fig: 1). Precautions have to be taken for utmost hygienic maintenance.

❖ **Applications Of IVF**

- IVF and Embryo transfer together revolutionized genetic improvement protocols that enable us to carry out conservation programs most efficiently to propagate and preserve endangered and rare species.
- Production of progeny is possible in dead animals and animals with reproductive tract abnormalities.
- Production of progeny from pregnant animals till the first trimester.
- IVF with sexed semen yielded double-fold results.
- Genomics, facilitating blastocyst testing to interpret bioinformatic analysis made it possible to determine the breeding value which is widely in practice.
- Transport of valuable genoplasm in the form of oocytes and embryos across continents.
- Interdisciplinary research for deeper understanding is possible with the studies on bovine species as they match in similarity with human physiology.

❖ **Challenges of IVF**

- The cost of production in terms of hormones, media, equipment, embryo transportation, and herd maintenance are high.
- Need for skilled personnel
- The lower blastocyst development rate is around 35-40% and conception rate of 25-35% is still an area of concern.
- Availability of recipient animals.
- Lack of enthusiasm from the farmers.

Apart from the challenges the IVF industry is rapidly developing in India with improved results across the country.

Conclusion

In vitro fertilization has the potential to establish a high genetic merit herd of indigenous bovine species such as Sahiwal, Gir, Red Sindhi, Tharparker, Ongole, Punganur, etc. IVF demands herculean efforts where Inter-institutional collaboration between universities, state livestock boards, national institutes, and most importantly farmers are very crucial for this technology to flourish and get established in India.

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