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Popular Article

New insights on Assisted Reproductive Technologies

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The livestock industry is one of the fastest-growing sectors of the economy, accounting in agricultural GDP and providing a significant portion of the nation's dairy farmers' income. In order to provide farmers with the highest possible profits, dairy animals need to be robust and healthy throughout their lives in order for this industry to flourish.

Advancement in farm animal reproduction has been increasingly dependent on advance scientific research besides to an understanding of the physiological processes involved in reproduction. Reproductive physiology has come a long way in recent years, but infertility brought on by low conception rates and high embryonic mortality rates still poses a serious challenge. The use of assisted reproductive techniques (ART) has helped owners to produce offspring from valuable farm animals that were considered infertile using standard breeding techniques. However, rather than growing the number of animal herds in order to achieve high quality and quantity output, there is a growing need to develop animals with high genetic potential in order to boost the productivity of animal units.

In order to solve reproductive issues, enhance the number of children from chosen females, and shorten generation intervals in farm animals, assisted reproductive methods such as artificial insemination, superovulation, in vitro fertilization, and embryo transfer have been adopted. Utilizing all new technologies, particularly contemporary reproductive biotechnologies, is necessary to fulfil future needs and be able to sustain agricultural production, research, and its uses.

In present article effort has been made to enrich the knowledge about various recent assisted reproductive techniques which may be helpful for improving the current status of livestock reproduction.

Oesturs Synchronization

One method of controlling the detection of oestrus signals is synchronization of oestrus, which is achieved by coordinating follicular waves and/or luteal regression. Most animals that undergo oestrus modification of the bovine oestrus cycle eventually reach standing oestrus. Increasing the percentage of animals that are bred at the start of the breeding season is a very effective strategy. Increasing the use of artificial insemination by using sires with better germplasms is made possible by this method.

In India, the following techniques are frequently employed.

1) Using PGF2a Injection: In this case, cyclic females should have two PGF2a injections spaced 11 days apart.
2) Oestrus Synchronization by GnRH-PGF2a-GnRH Protocol: In this approach, cyclic animals were treated to the GnRH-PGF2a-GnRH protocol of oestrus synchronization on the first, eighth, and eleventh day, respectively, regardless of which day of the oestrus cycle they were in.
3) Oestrus Synchronization with CIDR: The basic technique entails putting the CIDR (Controlled Internal Drug Release -Progestin impregnated plastic devices) into the vagina for seven days and delivering an injection of PGF2a on day 6 of implantation and noting the presence of oestrus on day 8.

Artificial Insemination (AI)

The act of physically inserting sperm cells into a female animal's reproductive canal from a genetically superior male animal is known as artificial insemination, or AI. In both developed and developing nations, AI technology is widely used in commercial dairy cattle operations. It is more than fifty years old and has been used all around the world.

By introducing new genetic material through the import of semen rather than live animals and thereby reducing international transport costs, AI technology maximizes the use of exceptional males, disseminates superior genetic material, improves the rate and efficiency of genetic selection, allows for the use of frozen semen even after the donor has passed away, and lowers the risk of sexually transmitted diseases. AI will become more effective only when farmers will have access to considerably better technical and organizational facilities.

Multiple Ovulation and Embryo Transfer (MOET)

Smith presented the idea of MOET and gave an example of how carefully thought-out MOET programs could result in shorter generation intervals and more intense selection, which would improve genetic gains. The rate at which genetic progress is occurring is revolutionized by recent developments in embryo transplantation procedures.

The following are the crucial phases:

- Multiple ovulation (MO) is the process by which donor cows of animals with a high pedigree release more eggs during ovulation by administering hormone treatments (FSH and LH).
- Afterwards, semen from a reliable bull is used to artificially inseminate the cows.
- A catheter inserted into the uterus is used to non-surgically drain out the embryos after 6-7 days. This is conceivable since embryos in cattle take longer to implant in the uterine wall. Four to seven embryos are typically collected.
- After that, the embryos may be implanted into recipient cows whose oestrus cycles are appropriately receptive—typically as a result of hormone therapy. Embryos can also be frozen and stored (though exact control of the regime is somewhat more necessary).

Invitro Fertilization (IVF)

The technology for In Vitro Embryo Production (IVEP) of embryos in farm animals has advanced at a rate never seen in the previous few decades, and this progress has accelerated in the recent ten years. Technologies for in vitro production aid in the creation of animals with superior genetic qualities and serve as a reliable source of embryos for cutting-edge biotechnologies such as nuclear transfer, transgenesis, cloning, and embryo sexing.

Crucial steps of *in vitro* embryo production technique include:

1. Oocyte collection (oocyte pick up from living animal or post-mortem from slaughter houses).
2. Selecting and cleaning oocytes and placing oocytes in maturation medium for 18-24 h.
3. Sperm purification using percoll gradient.
4. Inseminate matured oocytes with purified sperm cells for 8-24h.
5. Removal of Cumulus Cells Complexes [can be done by mechanical (vortexing, pipeting) or by enzymatic digestion].

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6. Placing putative zygotes in culture medium for 7-9 days.
7. Obtaining early bovine embryos that is ready to be transfer to surrogate mothers.

The capacity to recover oocytes has limited the initial deployment of in vitro embryo generation in both cattle and buffaloes, despite the technology's many benefits. Nonetheless, these challenges have mostly been eliminated with the advent of low invasive ultra sound guided transvaginal oocyte retrieval (TVOR) and oocyte pick up (OPU). In a laboratory, unfertilized eggs are fertilized and then cultivated for a few days until they develop into early embryos.

Cloning

Cloning is a potent procedure that has the ability to reduce genetic variation in experimental animals and multiply elite animals. The method of reproducing an entire organism from a single, genetically identical cell extracted from the parent organism is known as animal cloning. This indicates that the cloned animal has the exact same DNA as its parent, making it a perfect replica in every manner.

Chromosomes from mature oocytes are removed during the cloning procedure and replaced with a cell from the donor animal. After the donor cell and enulected oocyte are united, the donor cell is stimulated chemically or electrically to cause the somatic cell genome to become reprogrammed to resemble an embryonic genome. Following the reconstruction of cloned embryos, healthy embryos are transplanted to recipients that are synchronized and bear the cloned offspring till parturition.Cloning offers the potential to produce thousands of exact replicas of genetically altered animals without using traditional breeding methods.

Intra cytoplasmic sperm injection (ICSI)

The successful treatment of male infertility of a different origin provided by the development of the intracytoplasmic sperm injection technique has reignited interest in the technology's possible application in farm animal reproduction. ICSI is important not only in clinical settings but also in the creation of transgenic animals and the investigation of fertilization mechanisms.A single spermatozoa or sperm head is directly injected into ooplasm using the micro-fertilization procedure known as ICSI. The procedure is performed by laparoscopy. Crucial steps of ICSI technique includes: (1)Sperm aspiration for ICSI. (2) Sperm uptake and microinjection into mature oocyte.This technique can further be used to extend the sperm vector system for transgenic animal production. The main indication for ICSI is severe male infertility due to various abnormalities of ejaculated spermatozoa, epididymal spermatozoa or testicular spermatozoa.

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Sexing of semen and embryos

Predicting the sex of offspring would result in a higher proportion of males or females, which would aid in the selection of people with the best genetic make-up for the following generation. Elements that are often found on the Y chromosome either present or absent determines the sexual differentiation of an embryo.Some of the techniques employed commercially for the embryo sexing are: (i) chromosomal analysis of demi embryos (ii) immunological detection of embryonic H- Y antigen (iii) use of Y-specific probes (iv) Fluorescence in situ hybridization (v) rapid sexing method for pre implantation embryos of bovine using Loop-Mediated Isothermal Amplification (LAMP) reaction (vi) Standard flow cytometry equipment

Oocyte/embryo cryopreservation

Significant advancements in the cryopreservation of mammalian oocytes and embryos have been made in recent decades. Oocyte preservation lowers the dangers of disease transmission, lowers the cost and risk of transporting live animals, and offers protection from natural calamities. The introduction of vitrification of germplasm coincided with advances in conventional cryopreservation techniques. Compared to slow freezing, vitrification is a simpler, quicker, and less expensive process.The novel vitrification techniques have been nearly solely responsible for the recent development in the cryopreservation of mammalian oocytes and embryos, and they may present new opportunities for routine alternatives to commercial embryology and breeding programs in the majority of domestic animal species.

Embryo transfer

Using the method of embryo transfer, cattle can be improved more quickly and the genetic contributions of both male and female animals can be used simultaneously.

Embryo freezing is a crucial part of commercial embryo transfer (ET) programs since, after 12 hours in holding media, embryo viability begins to diminish, making interim storage of the embryos necessary until they are transferred. The main goals of the well-established commercial practice of freezing embryos are to prevent the creation of ice crystals, which could injure the embryo when it thaws, and to maintain the embryo in a viable state from which it may be revived to continue a normal development. Embryo transfer and other associated reproductive technologies facilitate genetic improvement and have been successively used for rapidly multiplying the population of elite breeds of domestic animals.

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Zygote Intra-Fallopian Transfer (ZIFT) and Gamete Intra-Fallopian Transfer (GIFT)

Zygote Intra-Fallopian Transfer **(**ZIFT), also referred to as Tubal Embryo Transfer (TET), is an ART technique in which embryos are transferred into the fallopian tubes for purposes of achieving pregnancy. Meanwhile, Gamete Intra-Fallopian Transfer (GIFT) allows the transfer of gamete into the fallopian tubes. The obvious advantage of ZIFT over GIFT is that as in IVF, it is possible to document fertilization.

Transgenesis

Breeding and biomedicine can both benefit from the use of transgenic farm animals. Transgenic people created through breeding have better quantitative and qualitative features as well as illness resistance.Currently, a number of biotechnological methods are being used to create transgenic animals, including sperm-mediated gene transfer of lentivectors, cytoplasmic micro-injection, pro-nuclear micro-injection, retrovirus-based vectors, DNA transfer to embryos or embryonic stem cells via retroviral vectors, and RNA interference.

Nanotechnology

A recent development in cellular and molecular biotechnology is nanotechnology. Apart from its potential uses in genetics, biotechnology, therapeutic medicine, and cellular biology, this technique could also prove beneficial for farm animal breeding and reproduction. Newer technologies called microfluidic and nanofluidic have made in vitro fertilization (IVF) and in vitro embryo generation easier. Recent studies have shown how effective microfluidics can be for isolating motile sperm without the need for centrifugation. When breeding farm animals, heat sensing can be achieved by subcutaneously implanting a nanotube to monitor variations in the blood's estradiol concentration. In order to activate breeding, the signal from this sensor will be integrated into a central monitoring and control system.

Laser technology

A number of laser applications have been examined as ART-assisted methods to improve sperm or oocyte competence for fertilization by utilizing the energy of various laser types. A review has been conducted on the impact of lasers on parameters related to sperm motility, oocyte maturation, and semen characterization in livestock. The paucity of published works in this emerging discipline diminishes the knowledge and application of such techniques in everyday field work.

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