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An overview of Assisted reproductive technologies and its applications

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Farm animals' reproductive performance determines the profitability and the intensity of achieved genetic improvement. The reproductive performance of either accretion type or secretion type animals is affected by such genetic improvement in the male side and/or the female side. Assisted Reproductive Technologies (ART) refers collectively to the various procedures and techniques involving the laboratory handling of human or animals' sperm, oocytes and/or embryos such as IVF, GIFT, ZIFT/TET, cryopreservation/vitrification, ICSI, … etc

Artificial Insemination

This technology has now become a practical technology in commercial dairy cattle programs in both developed and developmental countries. Artificial insemination (AI) is the process of collecting sperm cells from a genetically superior male animal and manually depositing them into the reproductive tract of a female. Artificial insemination, using the semen of highly selected males, has been the most powerful tool for livestock improvement ever available to breeder. The successful use of artificial insemination (AI) as a means of animal breeding relies upon three major premises: first, that spermatozoa can survive outside the body; second, that they can be reintroduced into the female genital tract in a way that results in an acceptable conception rate; and third, that the fertile period of the female can be identified.

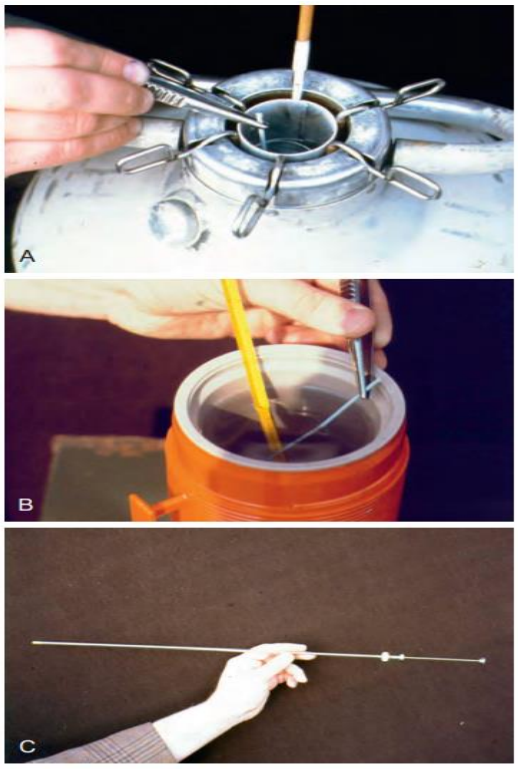
Advantages of artificial insemination

1. 1.Artificial insemination greatly increases the utilization of proven sires. The services of outstanding, superior sires are made available to many more owners. Milk production has doubled since artificial insemination was introduced through better genetics, feeding and management.
2. Bulls used in artificial insemination are more carefully and scientifically selected from outstanding cows and proven sires than is possible in individual dairy herds. Computers scan the records of all the top tested cows and matings are made with the best sires available. About 90 percent of the possible genetic improvement in a commercial herd depends on the genetic improvement through artificial insemination. Artificial insemination permits earlier and more rapid proving of young bulls on a greater number of cows under varied conditions. This gives a more accurate proof of a male's transmitting ability than can the testing of a bull on only one small group of females in a herd under similar conditions of management and feeding. This mode of testing also distributes the risk so that inferior bulls cause little loss to individual owners
3. The danger, work, and expense of keeping and handling, what in many cases proved to be, an inferior male is eliminated for the average dairyman. This added saving by the use of artificial insemination can be spent on maintaining an additional cow, which, may defray part or all of the yearly cost of breeding a herd artificially.
4. If the males are handled carefully, examined closely from a health standpoint to make certain that they are free from disease, if regular semen examinations are made and accurate breeding records maintained, artificial insemination lessens or eliminates the occurance of veneral/bacterial/viral diseases.
5. With the advent of the widespread use of frozen semen and the classification of the physical characteristics of a proven sire's daughters, careful selection of bulls for type and line breeding is possible for the breeder who so desires.

6. The intensive selection and record keeping has reduced the incidence of lethals and other genetic defects.
7. Miscellaneous advantages of artificial insemination include the following:
    a) It makes possible the mating of animals with a great difference in size without injury to either animal.
    b) It may extend the usefulness of sires which for some physical reason are unable to copulate normally.
    c) It may be used to increase the usefulness of males of monogamous species such as the fox. It is of value in experiments on hybridization where natural mating cannot take place
    d) Artificial insemination usually stimulates greater interest in livestock breeding and in better management practices.
    e) Artificial insemination has proved to be of value for male dogs that are timid or have premature erection, as complete erection of the canine penis does not ordinarily occur until after entry into the female's vagina. In toy breeds of dogs it may be used to prevent exhaustion due to repeated attempts to copulate during hot weather. It may also be of value in relatively impotent male dogs.
    f) It may be helpful when used in females which are in true estrum and ovulate but refuse to stand or accept the male, An example of this is in zoo animals where pairs refuse to mate. Semen can be collected by electroejaculation,and used when the female is in estrus.

Disadvantages of artificial insemination

1. A well trained operators are required to supervise the collection, examination, extension, freezing, shipping, and the insemination of the females in order to ensure that infectious diseases such as brucellosis, vibriosis, trichomoniasis and others are not spread. All major A.I. organizations in the U.S. maintain studs free of these diseases. If semen is not collected, extended and handled properly, poor breeding efficiency results. Careful observations for estrum and insemination at the proper time is important. Careful, complete records must be kept. Inseminators, if they are not careful, could be means of spreading diseases from one farm to other.
2. There could be a possibility of spreading genetic abnormalities in cattle, such as: cystic ovaries, spastic syndrome, poor conformation, especially of feet and limbs, and lack of libido. However, large artificial insemination organizations have systems for routinely evaluating udder, legs, feet and other type traits. Only those sires passing this evaluation are used to produce sons for further testing. The increase in cystic ovaries in dairy cattle may be due partly to the wide use of certain bulls by means of artificial insemination. Improved feeding practices and successful treatment of cows with cystic ovaries also may have increased the incidence of cystic ovaries.



• **Fig. 43.3** Semen handling for bovine artificial insemination. (A) Withdrawing a straw of frozen semen from the liquid nitrogen flask. The canister containing the semen should not be lifted higher than the level of the top of the neck of the flask. (B) Thawing. After checking the identity of the sire, the straw is thawed. Water temperature is not really critical but placing the straw in water at 37°C for 10 seconds is a typical thawing regime. (C) The straw is placed in an insemination catheter, which is then covered with a plastic sheath. The catheter is then ready for use, but care must be exercised not to allow the semen to become chilled again before it is inseminated.

3. Miscellaneous disadvantages:
    a. Intrauterine insemination of a pregnant female may result in abortion.
    b. Uncontrolled or unscrupulous operators or owners could substitute sperm of less valuable animals unless blood typing is routinely employed. However, few errors have been found, so this seems to be a minor problem.

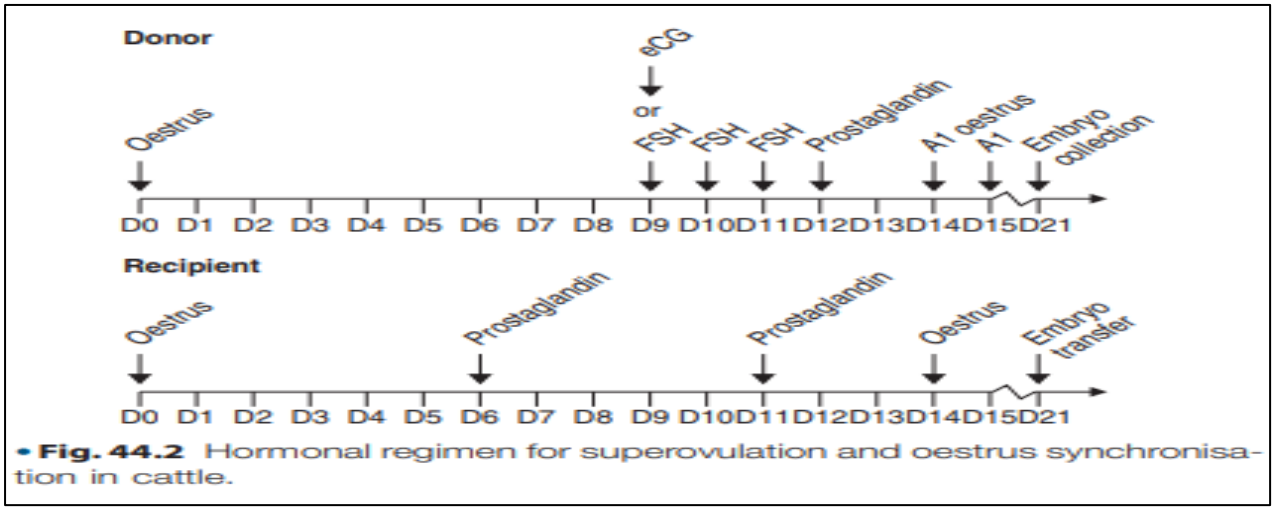
c. Service is not always available. Many dairymen take training to do their own insemination. Artificial insemination cannot be used conveniently on all species or breeds of animals. In some species much more work must be done before artificial insemination becomes practical.

Multiple Ovulation and embryo transfer (MOET)

Multiple ovulation and embryo transfer (MOET) is often referred as the ART that is "to the female, while AI is to the male", i.e. a method of producing more offspring from a genetically valuable female than would be possible by natural breeding. Meanwhile, MOET has not yet become a widespread tool for genetic improvement for a variety of reasons including its costs, technical demands, and variable and unpredictable efficiency (Cognié et al., 2003). An early review by Callesen et al. (1996) showed the significance of MOET in dairy cattle. Meanwhile, the use of MOET procedures remains affected by a high variability in the ovulatory response to hormonal treatment and by a low and variable number of transferable embryos and offspring obtained. This variability has been classically identified with both extrinsic (source, purity of gonadotrophins and protocol of administration) and intrinsic factors (breed, age, nutrition and reproductive status) (González-Bulnes et al., 2004). In a more recent review, Menchaca et al. (2010) summarized that the application of recently acquired knowledge has resulted in relevant improvements in MOET programs in small ruminants.

Superovulation

Superovulation, also called superstimulation, is a treatment intended to increase the ovulation rate and thus the number of available oocytes in the donor animal without disrupting the physiological and endocrinological processes associated with oocyte maturation, ovulation, and fertilisation, as well as subsequent embryonic and fetal development. Superovulation is a prerequisite for the successful application of embryo transfer in species with a physiologically low ovulation rate (cattle, sheep/goats, and horses), and the current description of superovulation will focus on these species. A close synchrony of oestrus between the donor and the recipient is crucial for obtaining optimal pregnancy rates and is an important part of the entire embryo transfer planning process.
undertaken to increase the likelihood of success.



• **Fig. 44.2** Hormonal regimen for superovulation and oestrus synchronisation in cattle.

The crucial steps of MOET were reviewed by Chakravarthi and Sri Balaji (2010) as follow:

1. Donor cows of good pedigree animals are treated with hormones (FSH and LH) to increase the number of eggs released at ovulation - multiple ovulation (MO).
2. Then the cows are artificially inseminated using semen from a proven bull or sexed semen.
3. After 6-7 days, the embryos are flushed out non-surgically using a catheter placed into the uterus. This is possible because, in cattle, there is a delay in embryos becoming implanted in the uterine wall. On average, 4-7 embryos are collected.
4. The embryos may then be implanted into recipient cows whose oestrous cycle is at correct receptive stage-usually as the result of hormonal manipulation.
5. Embryos may be frozen and stored, using techniques similar to those applied to semen, (though precise control of the regime is somewhat more critical).

Thus, embryo transfer has the potential to bring about genetic improvement twice as fast as AI alone. Moreover, the use of MOET technique could lead to increased selection intensity and reduced generation intervals, resulting in improving genetic gains.

Intra-cytoplasmic sperm injection (ICSI)

Intra-cytoplasmic Sperm Injection is a procedure in which a single sperm is injected directly into an egg, thus sperm and egg interactions involved with normal fertilization are by-passed. This technique is used to overcome severe male infertility. The procedure is performed by laparoscopy. Development of ICSI technique furnished a successful treatment for male infertility of different origin and has led to a resurgence of interest in its potential use in farm animal reproduction. In addition to its clinical usefulness, ICSI can be applied for the production of transgenic animal and to study the mechanism of fertilization. Since the first report of ICSI success in hamster (Uehera and Yanagimachi, 1976), the transfer of embryo produced by ICSI has given rise to live young in rabbits, mice, sheep, humans, horses, cattle and pigs. The ICSI is the micro-fertilization technique of the direct injection of a single spermatozoa or sperm head into cytoplasm.

Crucial steps of Intracytoplasmic sperm injection (ICSI) technique includes:

1. Sperm aspiration for ICSI.
2. Sperm uptake and microinjection into mature oocyte.

In vitro embryo production IVP

The potential for commercial production of genetically superior embryos by in vitro ferti1ization is apparent. In the past few decades there have been unprecedented evolution of technology for in vitro embryo production of farm animals, with the rate of progress getting intensified in the last decade with the characterization of effectively defined and semi defined medium for different species. The first succeeded IVF was achieved in rabbits in 1959 (Chang, 1959), next success was with mice in 1968. The first IVF production in human was in 1978 (Steptoe, 1980) and the first born calf produced with IVF was in 1981 (Brackett et al., 1982). Kane (2003) wrote an excellent review about in vitro gamete maturation and embryo culture. In vitro production technologies not only help in production of high genetic merit animals, but also provide an excellent source of embryos for emerging biotechnologies like embryo sexing, cloning, nuclear transfer, transgenesis, etc. Furthermore, it allows analyzing developmental potential of embryos, including the pattern of gene expression, epigenetic modifications and cytogenetic disorders during the development (Galli and Lazzari, 2008). Early stages of bovine embryo development show many similarities with human embryos. Crucial steps includes;

1. Oocyte collection (oocyte pick up from living animal or post-motrem 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].
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.

Oocyte/embryo cryopreservation and vitrification

Continuous availability of viable, developmentally competent oocytes and/or embryos has been critical to recent progress in IVP because of the relatively short fertile life span of mammalian oocytes and/or embryos. Hence, storage of unfertilized oocytes would generate a readily available source, which allow the experiments to be carried out at convenient time and could therefore be of practical importance for establishment of gamete bank, from which particular genetic combinations could be derived. During the past few decades, significant progress in cryopreservation of mammalian oocytes and embryos has been achieved. Live offspring of at least 25 species resulted from transfer of cryopreserved embryos or oocytes have been successfully produced (Gajda and Smorg, 2009).

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 transfered 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. On the other hand, both ZIFT and GIFT procedures require the female to have at least one functioning fallopian tube which is a disadvantage when compared with IVF. These different techniques are used to achieve pregnancy in high genetically merit animal with reproduction problems or to achieve implantation of produced embryos in surrogate mothers.

Cloning

Cloning is a powerful technique and potentially it could be used for multiplication of elite animals and minimize the genetic variation in experimental animals. It can be used for the conservation as

well as tool for the production of stem cells for therapeutic purposes, as therapeutic cloning. Cloning using somatic cells offers opportunities to select and multiply animals of specific merits (Das et al., 2003). Numerous types of somatic cells are used as donors in somatic cloning; foetal fibroblasts, adult fibroblasts, granulosa cells, hepatocytes, lymphocytes, etc. (Campbell et al., 2007).

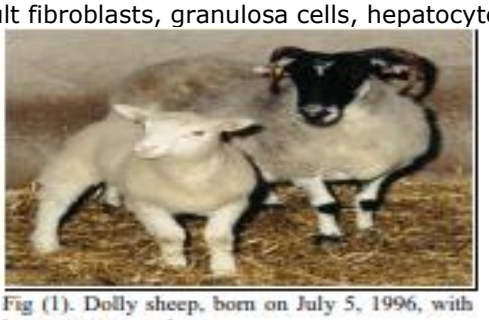


Fig (1). Dolly sheep, born on July 5, 1996, with her surrogate mother

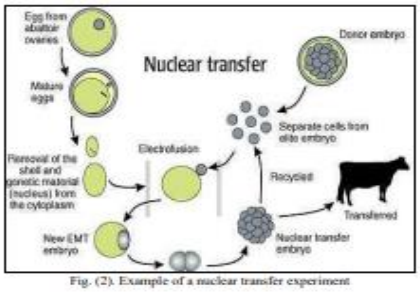


Fig. (2). Example of a nuclear transfer experiment

Transgenesis

Several biotechnological techniques such as pro-nuclear micro-injection, cytoplasmic micro-injection, retrovirus based vectors, transferring DNA to embryos or embryonic stem cells via retroviral vectors, sperm mediated gene transfer of lentivectors and RNA interference, are presently being used to produce transgenic animals. Transgenic farm animals can be used both in breeding and biomedicine (Robet al., 2007; Wells, 2010). In breeding, transgenic individuals produced are equipped with disease resistance and improved qualitative traits. For example transgenic cows producing milk of increased β and κ-caseine content, pigs with high body weight gain or fat to muscle tissue, expressing human growth hormone and human haemoglobin (Niemann et al., 2005), sheep with integrated keratin-IGF-I gene and higher production of wool (Kues and Niemann, 2011), sheep and goat with antithrombin III and alpha anti trypsin on milk. An important achievement was production of transgenic cows resistant to mastitis (Wall et al., 2005). There is a high interest in using transgenic farm animals as bioreactors producing human recombinant proteins in mammary gland (Kues and Niemann, 2004; Redwan, 2009). Transgenic domestic pigs are used in studies on xenotransplants, i.e. transplantation of animal body parts into humans (Niemann et al., 2005). Researches are going on for production of environment-friendly transgenic individuals or using such animals in basic studies as a model to understand various physiological processes in farm animals and humans (Niemann et al., 2005). The technique of sperm mediated gene transfer (SMGT) is to produce transgenic animals using spermatozoa as a natural carrier to transfer an exogenous gene into oocyte during the fertilization process. Lavitrano et al. (1989) demonstrated for the first time that

a) the epididymal sperm of the mouse can spontaneously incorporated with exogenous plasmid DNA molecules
b) genetically modified offspring can be generated by using sperm cells containing plasmid by in vitro fertilization procedures; exogenous DNA sequences are expressed in the progenitors, and that the sperm-carried exogenous DNA incorporated in the fertilized ovum. Louis-Marie (2002) showed that sperm incubated in the presence of DNA could carry it to the oocyte during fertilization, leading to the generation of transgenic mice

Nanotechnology

Nanotechnology is recent advancement in cellular and molecular biotechnology. It has an enormous potential to revolutionize agriculture and livestock sector. This technology allows researchers to handle biological materials and media in minute quantities usually nanoliters or picoliters. In addition to its applications in cellular biology, biotechnology, theraputic medicine and genetics, it might be useful technique in farm animal breeding and reproduction. Microfluidic and nanofluidic (Schuster et al., 2003; Eijkel and Berg, 2005) are recent tools to simplify traditional procedures of in vitro fertilization (IVF) and in vitro embryo production (Suh et al.,2006). More recent reports have demonstrated the utility of microfluidics in isolation of motile sperm without centrifugation (Schuster et al., 2003). Genetic diseases. In farm animal breeding heat detection can be done by implanting a nanotube (O'Connell et al., 2002) under the skin to detect the changes in the level of estradiol in the blood. The signal from this sensor will be incorporated as a part of a central monitoring and control system to actuate breeding.

Laser technology

Laser effects on sperm motility parameters (Fig. 6), improvement of oocyte maturation and characterization of semen in livestock have been reviewed (AbdelSalam and Harith, 2014). Several application of laser had been reviewed as assisted techniques of ART to benefit the energy from

different types of laser to excite either sperm of oocyte to improve their competence toward fertilization. Also, they mentioned the promising types of laser and wave length in the applications of improving semen parameters either pre- or post- thawing, oocyte maturation for IVF and avoid the bacterial contamination shorter wavelengths, e.g. λ= 532 nm and λ= 405 nm is promising and more reasonable than longer laser wavelength for biostimulative purposes because they will be better absorbed by the cellular chromophores. The limited publication in this new field reduce the awareness and the speared of such technique in routine field work.

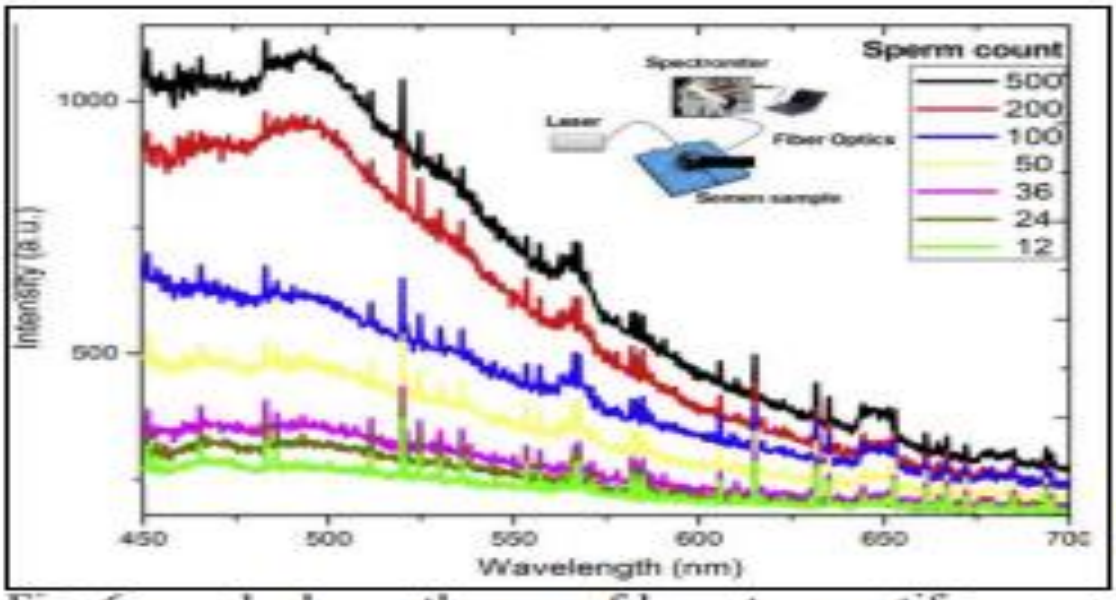


Fig. 6. graph shows the use of laser to quantify sperm number adapted from (Abdel-Salam and Harith, 2014).

Twinning

In certain beef cattle breeds, twinning may be an attractive prospect. With the transfer of two in vivo derived or in vitro produced embryos (IVP) to each recipient, it is possible to obtain a calving rate for each embryo that is greater than 100%. Embryos should be placed in both horns, as this will improve the number of fetuses that survive (Sreenan & Beehan 1976). It was speculated that the technology would gain a broader application with the use of IVP embryos, which can be produced at a lower price (Lu & Polge 1992), but it has not been used widely in practice, as it requires expensive equipment and advanced training; in some countries, it is even prohibited by law.

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