

## Improving Livestock Production Using Auxiliary Reproductive Biotechnology

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### Abstract

From the beginning, many reproductive techniques have been used in domestic animals to achieve the desired goals. These goals include: Increasing reproductive and productive efficiency, improved genetic makeup of farm animals, reducing the risk of diseases and injury, producing resistance against climate and infectious agents, and research. Major Assisted Reproductive Techniques (ART) discussed in this brief review are, Artificial Insemination (AI), Estrus Synchronization, Superovulation, Semen Sexing, Cryopreservation, and Embryo Transfer (ET). Artificial insemination is the most basic and widely used technique for improving genetics and increasing conception rate. Estrus synchronization is the technique in which the selected females are targeted to come in heat (estrus) simultaneously in a brief period using different protocols. Super-ovulation is producing multiple ova at a single time using many combinations of hormones. Semen Sexing, as the name implies, is a method in which sperms are sorted out, using different techniques, to gain animal of desirable sex. In Cryopreservation, long-term preservation of living cells and tissues is done at a very low temperature (conventionally -196°C) without compromising the structural and functional integrity of the preserved specimen. Embryo transfer involves producing and collecting embryos in donor animals and then implanting them in recipients. Reproduction is the backbone of any livestock enterprise, and to achieve remarkable results, the use of Assisted Reproductive Techniques (ART) is inevitable. This mini review article will provide a brief comparison of such great reproductive biotechnological methods which are using in livestock farming.

**Keywords:** Artificial Insemination; Estrous Synchronization; Super Ovulation; Semen Sexing; Cryopreservation; Embryo Transfer

### Introduction

Assisted breeding techniques are key to improving animal production efficiency in livestock businesses. Unfortunately, these techniques are being used in only a few farms to maintain production levels [1]. These technologies aim to eradicate common reproductive problems in the dairy industry like infertility, low conception rate, less production, the poor genetic makeup of animals, and

early embryonic mortality [2]. The livestock industry is suffering from a huge economic loss because of little use of these beneficial techniques [3]. This article targets to cover an overview of major and easily applicable assisted reproductive techniques (ART) and why there is a need for using all these technologies in today's world [10,11,14].

### Artificial insemination

Artificial insemination (AI) is the first and basic assisted reproductive technique that is now being practiced all over the world, including developed and under-developed countries [9,10]. Artificial insemination (AI) is the unnatural depositing of semen in the female reproductive tract and was first practiced in bitch by Spallanzin in the year 1784. Later on, Ivanoff (1922) also used this technique in domestic animals including horses, poultry, cattle, and sheep. Nowadays, almost all corporate and commercial dairy farms are using AI in a routine. Every year almost 100 million cattle, 40 million pigs, 33 million sheep, and 0.5 million goats are artificially inseminated [12]. AI is practiced less in small ruminants, especially goats, as compared with large ruminants (cattle and buffalo). AI involves a collection of semen from the male by any method (most commonly artificial vagina and electro-ejaculation methods), examining its vigor and motility, processing it, and then loading it into the straws. Either freshly chilled or frozen-thawed semen can be used to inseminate the animal [15]. Although Freezing and long-term storage require the use of cryoprotectants. It is also noted that the conception rate is low if the frozen-thawed semen is used. AI is an easy way to improve the genetic makeup of progeny from the side of a male. Also, there is an improved conception rate, less chance of injury, and a low risk of spreading sexually transmitted diseases (STD) [4].

### Estrus synchronization

The sole purpose of estrus synchronization is to induce estrus in a desired group of females at a predestined date and time. While synchronizing the time of induction of heat, we are ultimately scheduling the time of parturition. Detection of heat is a crucial part of the good management of animals and this is even harder when we have to deal with a large herd of hundreds and thousands of animals. Using the Estrus synchronization technique, relieve from this difficulty by bypassing the estrus detection phase. The most commonly used approaches for synchronizing the ovulation time are Prostaglandin Treatment, Controlled internal drug-releasing regimen, and GnRH-PGF2 $\alpha$ -GnRH protocols. During Prostaglandin treatment we are using a luteolytic agent (PGF2 $\alpha$ ) 11 days apart in cyclic females regardless of the stage of estrus in animals and then after the appearance of estrus signs, timely AI is performed. CIDR (controlled internal drug-releasing) device maintains the blood progesterone concentration in the targeted animals and is inserted into the vagina for 7 days. One injection of PGF2 $\alpha$  is used on day 6 and estrus is seen on day eight. GnRH-PGF2 $\alpha$ -GnRH protocol is a good regimen to induce estrus in non-cyclic animals. Usually on day 1 GnRH is injected, and then 7 days later, PGF2 $\alpha$  injection is ad-

ministered. Another injection of GnRH is given on the 10th day and AI is performed on day 11. The use of estrus synchronization technique eliminates the possibility of human error in the detection of estrus. It is also very beneficial for animals, which exhibit low external heat symptoms (silent heat) like sheep and buffalo [2,3,7].

### Superovulation or multiple ovulation

As the name superovulation or multiple ovulations indicates, it is the induction of ovulation of multiple ova from the ovary of an animal using different injectable hormones [8]. Any cyclic animal can be made to ovulate multiple ova at a single time using synthetic follicle-stimulating hormone (a gonadotropin which is naturally released from the anterior lobe of the pituitary gland) [11,17]. Multiple ovulation techniques combined with embryo transfer can bring revolution in the livestock industry. Although superovulation can be induced in any cyclic animal it is usually applied in animals of superior genetic. As Artificial insemination can be used for the improvement of genetic from the male side, multiple ovulations is being used as an improvement of genetics from the female side [18].

Usually, a follicle-stimulating hormone is injected at the end of the luteal phase or before 1-2 day of completion of the estrus synchronization regimen [14,15]. Superovulation is a crucial technique for embryo biotechnology. Several factors should be considered before inducing superovulation in an animal. Most important of these are breeds, reproductive health status of the animal and nutrition. Also, repeated super ovulations have a significant effect on the next one [16,17].

### Semen sexing

For producing progeny of desired gender, either male or female, the technique of semen sexing is used. There is a difference in the DNA content of X chromosome bearing sperm and Y-Chromosome bearing spermatozoa [5-18]. Semen sexing is done by flow cytometric separation of fluorescent-labeled X-Chromosome possessing spermatozoa and Y-Chromosome possessing spermatozoa [31]. In recent times, this technology was capable of sorting 15 million spermatozoa into X and Y chromosome bearing chromosomes [5,19]. The accuracy of predicting the sex of the resulting progeny is 85-95 percent. Although new generation flow cytometers with the high efficiency of sorting of sperms are also being devised and utilized nowadays. There are some flaws in the sexing of semen like low sperm concentration, reduced motility, viability, decreased life span of spermatozoa, and some structural defects. Also, sexed semen needs deep intra-uterine insemination [20]. But still, the benefits of this technique outnumber the drawbacks. Major advantages include a high ratio of progeny testing programs, enhanced male

production for this purpose, low risk of sex-limited diseases, and the last but not least, conservation of superior and rare genetics of animals [5,21].

### Cryopreservation

It is a technique in which living cells and tissues are preserved in a state of suspended animation at a very low temperature (-196 °C) for a long period while maintaining the structural integrity of the specimen [6,22]. At a very low temperature, the processes going on in a living cell are dramatically reduced [13]. To alter these processes different cryoprotective agents (CPA) are used like Glycerol, Dimethyl sulfoxide (DSMO), and some polymers. Liquid Nitrogen is used as a freezing agent [1,8]. Some difficulties like ice crystal formation at a fast cooling rate and osmotic changes due to the high concentration of intracellular and extracellular solutions at a low cooling rate were faced in conventional methods of freezing [23]. These problems can lead to cryo-injury. But now, vitrification has reduced the risk of cryo-injury very much. Cryopreservation of sperms, oocytes, and embryos has brought a revolution in breeding programs and research systems. Other benefits of cryopreservation are cryosurgery, preservation of organs and tissue for transplantation, and application in food sciences [5,29].

### Embryo transfer

Embryo Transfer is a technique which can improve the genetic worth of a herd at a faster rate than any other techniques discussed above. Embryo Transfer is a process that involves many steps. Mainly, it involves generating and obtaining embryos from females of superior genetics called as the donor and then implanting these embryos after processing and grading, into the uterus of different females known as recipients [25,26]. ET is an expensive technique and is only practiced in animals which can justify its cost. While producing embryos in the donor females, estrus synchronization and superovulation methods are applied to gain better results [26]. By using this technique, genetic combinations of both males and females can be used to enhance genetic gain in the offspring. Surgical, as well as non-surgical embryo transfer techniques, are being practiced in elite cattle. Although, non-surgical embryo transfer is cost-effective, safe, and shows better results [19,30]. This technique is not much common as other assisted reproductive techniques. It requires highly skilled technicians and embryologists. The International Embryo Transfer society provides technicians for this purpose. This is the best way devised yet for the rapid expansion of animals of elite genetic makeup [27,28].

### Conclusion

The use of auxiliary breeding techniques in farm animals has increased over the past few decades. With time assisted reproduc-

tive techniques are drawing attention and becoming popular in the livestock industry due to astonishing results. Yet many technical refinements and public awareness are needed in this field. Artificial Insemination, superovulation, semen sexing, cryopreservation and embryo transfer have proved to be valuable in improving the genetic merit of animals of low genetic worth. Estrus synchronization has eliminated many managerial problems in the reproduction of farm animals. New technologies like stem cell culture technique, Intra-cytoplasmic sperm injection and nano-technology are also being trialed. Therefore, in order to deal with the future crisis, there is an urgent need to expand reproductive biotechnology with large and basic support at all levels around the world.

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