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Review on Major Assisted Reproductive Technologies

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Abstract- Since early period, several reproductive technologies practiced as a prime concern for researchers, employed for genetic improvement of farm animals. This review deals with the assisted reproductive technologies (ARTs) among the known approaches for genetic improvements. This review paper focused on artificial insemination (AI), estrus synchronization, multiple ovulation and embryo transfer (MOET), cryopreservation (freezing) of gametes or embryos and in vitro embryo production (IVEP). Briefly to see, AI is the manual placement of semen in the reproductive tract of the female by a method other than natural mating. AI is the most effective method being used for the genetic improvement of animals. Estrous synchronization is another process of targeting female mammals to come to heat within a short time frame (36 to 96 hours) that is to have a number of females in estrus during a very short period.

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Review on Major Assisted Reproductive Technologies

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I. Introduction

Genetic improvement of farm animals is a prime concern over the years for researchers. Several reproductive technologies have been employed to achieve this (Vikrama, 2010). Among them, the application of assisted reproductive technologies (ARTs) plays a great role, which enables the rate of genetic progress to be increased (Nicholas, 1996; Vivanco-Mackie, 2001). Major assisted reproductive technologies, which play paramount importance in livestock utilization, include artificial insemination (AI), estrus synchronization, multiple ovulation and embryo transfer (MOET), cryopreservation (freezing) of gametes or embryos and *in vitro embryo* production (IVEP). To take full advantage of the benefits of assisted reproductive technologies, one must understand the basic physiology of the female and male reproductive systems as well as various methods of reproductive cycles (Paterson *et al*., 2003).Therefore; this review is aimed to achieve the major assisted reproductive technologies (ARTs).

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II. Assisted Reproductive Technologies (ARTs)

a) *Artificial Insemination (AI)*

Artificial insemination (AI) is the manual placement of semen in the reproductive tract of the female by a method other than natural mating. It is one of a group of technologies commonly known as "assisted reproduction technologies" (ART), whereby offspring are generated by facilitating the mating of gametes (spermatozoa and oocytes). AI is the most effective method being used for the genetic improvement of animals. According to Durrant (2009) and Vishwanath (2003), AI is the ART that is less complex, invasive and costly and is therefore the first logical choice for companion animals or non-domestic endangered animal species. In the present scenario a large number of AI are performed globally, more than 100 million cattle, 40 million pigs, 3.3 million sheep and 0.5 million goats are artificially inseminated every year (Boa- Amponsem and Minozzi, 2006).

Moreover, by the 1960s, significant improvements in cryopreservation and storage of semen made AI even more accessible to livestock producers (Vishwanath, 2003). In the modern dairy industry, where a large number of dairy cows are managed intensely, AI is widely used. Semen from bulls is especially amenable to freezing and long-term storage. In contrast, for reasons not yet well understood, semen from other livestock species such as horses, pigs, and poultry are more difficult to freeze and store.

b) *Synchronization of Estrus*

Estrous synchronization is the process of targeting female mammals to come to heat within a short time frame (36 to 96 hours) that is to have a number of females in estrus during a very short period of time. This is achieved through the use of one or more hormones (http://en.wikipedia.org/wiki/Estrous_synchr-

onization). Oestrus is synchronized by using PGF2a, GnRH and controlled Intravaginal Drug (Progesterone) Releasing device (CIDR) (Vikrama and Balaji, 2010). It can be also achieved through the use of prostaglandin F2a and progesterone, repeated progesterone injections for 16-17 days, intravaginal sponge [30-40 mg of fluorogestone acetate (FGA) for 11-18 days or 50-60 mg of medroxy progesterone acetate (MAP) for 15-18 days], or by using subcutaneous ear implants with a dose rate of 2-6 mg of progesterone for 9-17 days (Ishwar and Pandey, 1990; Stenbak *et al*., 2001, 2003). Moreover, it is possible to synchronize estrus through luteolysis. Prostaglandin F2a and its analogues have luteolytic action and two injections administered 11 days apart in cycling females' gives satisfactory results (Trounson, 1976; Ishwar and Memon, 1996). According to Vikrama and Balaji (2010), synchronization of oestrus is one of the ways to regulate the oestrus signs detection. It is a very effective method to increase the proportion of animals that are bred at the beginning of the breeding season.

c) Multiple/Superovulation

Superovulation is when an animal is induced (usually through use of injectable hormones) to ovulate multiple ova (the hormonal treatment for harvesting increased number of oocytes from the ovary than normal). This is usually done on animals with superior g enetics, but any cycling female can be superovulated (ht tps://uk.answers.yahoo.com/question/index?qid=20090 306063423AANtpZw). Principles of inducing superovulat ion in sheep are the same as in cattle. A follicle stimulating gonadotropin is administered either near the end of the luteal phase of the cycle (Days 11-13) or around 1 or 2 days before the end of the synchronizing treatments (Stenbak *et al*., 2001, Grazul-Bilska *et al*., 2003). Multiple ovulations are an effective means of increasing the contribution of superior females to breeding programs and it is also an essential procedure of embryo biotechnology. Despite its application in many domestic species, there are still many problems. For instance, rates of ovulation are still unpredictable. This causes problems not only in animal production but also in the application of embryo biotechnology. It is known that factors such as breeds (Terawaki *et al*., 2002), ovarian status (Gonzalez- Bulnes *et al*., 2000; 2002), gonadotrophin preparation (Lopes da, 2001; Gonzalez-Bulnes, 2000), nutrition (O'Callaghan *et al*., 2000; Armstrong *et al*., 2001), season (Mitchell *et al*., 2002; Chagas *et al*., 2003), photoperiod (Mutiga *et al*., 1984) and repeated superovulation (Cognie, 1999; Magarey *et al.,* 2003) affect superovulation as well as the quality of embryos produced.

d) Cryopreservation

Cryopreservation is a long-term storage technique with very low temperatures to preserve the structurally intact living cells and tissues for extended period of time at a relatively low cost (Tsai and Lin, 2012). It is the freezing of cells or tissues to subzero temperatures, typically -196 ^{o} C. This temperature is the boiling point of liquid nitrogen, a common agent using in the freezing and storage process. At this temperature, all biological activity is stopped or paused until it is thawed. The freezing of sperm needs vitrification agents that minimize damage to the cells during the freezing and thawing process (Wikipedia, 2015). With the development of ARTs the necessity of developing successful cryopreservation methods for reproductive cells and embryos became quickly evident. The freezing of sperm was initiated over 50 years ago. This was the first successful cryopreservation of spermatozoa. Cryopreserved sperm, oocytes and embryos are used for artificial insemination and embryo transfer in the livestock industry. Frozen semen can be used during AI and during *in vitro* embryo production (IVEP) schemes. In the 1950s, with the use of glycerol as cryoprotective, frozen bull semen methods allowed a great increase in the use of AI in the dairy industry (Polge, 1949; Woods, 2004). Bull semen has the best cell recovery percentage after thawing (50-70%) (Hiemstra, 2005; Vishwanath, 2003) compared to other livestock species.

Cryopreserved oocytes and embryos provide the opportunity to overcome the difficulties of donor recipient synchronization during super/multiple ovulation and embryo transfer. Maturation, fertilization and embryo development of cryopreserved oocytes has been achieved in a number of species (Hiemstra, 2005). The feasibility of the technique has been demonstrated by the birth of live animals using cryopreserved oocytes (Maclellan, 2002; Otoi, 1996; Stachecki, 2002). Moreover, cryopreservation of embryos of many mammals has achieved acceptable rates of success. The birth of live offspring from cryopreserved embryos is possible for many species (Hasler, 2001; Squires, 2003). In bovine, cryopreservation of embryos is highly successful with both slow freezing and vitrification protocols (Woods, 2004). However, pregnancy rates with fresh embryos are still significantly higher than after cryopreservation (Hasler, 2001).

e) Embryo Transfer (ET)

Embryo transfer is a multi-step process that involves the production and collection of preimplantation embryos from genetically superior females (called donors) and the subsequent transfer of the harvested embryos into reproductively healthy females (called recipients) for the purpose of establishing pregnancies and producing live offspring that is genetic ally unrelated. Embryo transfer is carried out on a variety of agricultural animals, to a greater or lesser extent depending on the species. It is used extensively in the beef cattle industry (http://hsc.csu.edu.au/agriculture/ electives/ 21st/2409/ embryo_ transfer.htm).

The first successful embryo transfer in mammals was performed with rabbits in 1890 (Heape, 1891), but it was more than 70 years later that the first successful embryo transfer in cattle was reported (Willett et al., 1951). Today, more than ¾ million bovine preimplantation embryos are transferred each year throughout the world (Thibier, 2006). Embryo transfer technology is an important tool to improve livestock at faster rate as well as gives an opportunity to utilize the genetic contribution of both male and female at the same time. With the help of ET (embryo transfer) or MOET (multiple ovulation embryo transfer) techniques faster improve of livestock, rapid expansion of elite animals, genetic gain, accelerated herd development and conservation of rare genetic stocks could be achieved (Nicholas and Smith, 1983).

Surgical embryo transfer is in principle possible in all mammalian livestock species. In contrast, non-surgical embryo transfer is only possible in cattle (routinely performed), horses and also pigs, although still not as efficient as in cattle and horses. For embryo transfer purposes, embryos can either be flushed from donors or can be produced *in vitro*. Depending on the species, embryos can be recovered from donor females of superior genetic merit by surgical or non-surgical techniques. In cattle and horses, efficient techniques recover fertilized embryos without surgery, but only one or sometimes two embryos are produced during each normal reproductive cycle. The recovered embryos are then transferred to recipient females of lesser genetic merit. They are transferred to the uterus or oviduct of recipients by laparotomy or using a laparoscopic technique. Comparison of the laparoscopic and surgical transfer of embryos showed that the laparoscopic method can achieve high pregnancy rates (Stefani *et al*., 1990). It appears, that laparoscopic transfer is a safe, minimally invasive surgical procedure and it should be recommended for transfer of embryos in small ruminants.

f) In vitro Embryo Production (IVEP)

IVEP includes three major steps: *in vitro* maturation (IVM), *in vitro* fertilization (IVF) and *in vitro* development (IVD) of the resulting embryos. However, primary oocytes collection should be added upstream of these major steps and embryo management (freezing, transfer) should be added downstream to give a complete overview of the whole process. Once the immature oocytes have been removed from the ovary, they are matured, fertilized, and cultured *in vitro* for up to seven days until they develop to a stage that is suitable for transfer or freezing (http://www.nifa.usda.gov/nea/animals/infocus/reproduction_if_assisted.html). Since the birth of the first IVEP calf in 1982, thanks to intensive research programs worldwide, cattle IVEP has done significant progress (Mermillod *et al*., 2006).

Although each ovary contains hundreds of thousands of oocytes (eggs) at birth, many thousands undergo atresia and are lost, starting before birth. This tremendous loss of genetic material could be salvaged by harvesting oocytes from the ovary and using IVEP techniques (Hasler *et al*., 1995). Bovine IVEP is now a reasonably efficient procedure; trans vaginal ultrasound-guided oocyte aspiration at frequent intervals, in combination with in-vitro fertilization (IVF) has proved its worth in improving the yield of embryos from designated donors, salvaging irreplaceable genetics following slaughter in the face of infectious disease control or in culling for other reasons (Hasler, 2003).

III. Conclusion

Animal biotechnologies related to reproduction have contributed too many improvements in agriculturally important traits in livestock. Reproduction lies at the heart of any livestock breeding enterprise and is vital to maintain or increase the number of animals required for production. Among the reproductive technologies, animal breeders have made widespread use of ARTs to accelerate genetic improvement programs aimed at obtaining more, better and cheaper food products. ARTs like AI, estrus synchronization, MOET cryopreservation (freezing) of gametes or embryos and IVEP have contributed to animal breeding programs faster transmission of desirable traits/genetic improvement by increasing offspring of selected males and females and the reduction of the generation interval in livestock populations in a shorter period of time compared to classical approaches as well as they provide a number of advantages. Therefore, ARTs require technical improvements and refinements but this will not be sufficient to provide benefits if the public opinion is not correctly informed and made aware of the advantages and the risks associated with the progress of science.

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