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REPRODUCTIVE BIOTECHNOLOGIES IN DAIRY INDUSTRY IN PAKISTAN

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ABSTRACT

Biotechnology is founded upon an ever-increasing understanding of the mechanisms that maintains living organisms and allows them to reproduce from generation to generation. We are living in an age of biotechnology, which is developing rapidly to expand further in the next few decades. It is a modern science that deals with the biological process through which technological innovation can be achieved and subjected to deliver goods and services for the benefit of human being. This "august" journey, which started with the production of insulin by the bacteria *E. Coli,* experienced a phenomenal growth since than. Reproductive biotechnologies include, semen processing, cryo-preservation, vitrification, sexing of sperm and embryos, artificial insemination, embryo transfer, *in vitro* fertilization, cloning, transgensis, juvenile *in vitro* embryo transfer, chimera production, multiple ovulation and embryo transfer, aspiration of oocytes from the live animals and zygote intra-fallopian tube transfer. In Pakistan these modern technologies have yet to play their due role in different areas especially in the fields of agriculture, medicine, dairy industry and environment. In the present post-flood scenario, the dairy supply chain recognized the need to work together in order to address this significant challenge. Especially in Pakistan, this future intra-disciplinary cooperation will also be needed among the industries, consumers and research institutes. Failure to achieve a high level of cooperation can potentially lead to a delay in reproductive biotechnologies application in development and will result in serious long lasting economic losses. This review is an attempt to analyze the current situation of the reproductive biotechnologies in dairy industry in the country and propose means for research and development interventions in the dairy sector through coordinated efforts of academia, government departments, development agencies and private sector organizations to achieve the highest possible benefits.

Key words: Milk, cow, buffalo, dairy production, artificial insemination, biotechnology.

INTRODUCTION

Biotechnology has the potential to enhance quality of life in many ways, while helping the environment by reducing our dependence on non-renewable resources. But that's just the beginning. We have to understand the importance of biotechnology and its role and influence on our future growth, health and environment. Reproductive biotechnologies are a combination of assisted reproduction, cellular and molecular biology and genomic techniques. Their classical use in animal breeding has been to increase the number of superior genotypes but with advancement in biotechnology and genomics they have become a tool for transgenesis and genotyping (Lopez *et al.,* 2004). Multiple ovulation and embryo transfer has been well established for many years and still accounts for the majority of the embryos produced worldwide (Dargatz *et al.,* 2004). Somatic cell cloning is a rapidly developing area and a very valuable technique to copy superior genotypes and to produce or copy transgenic animals. More knowledge in oocyte and embryo biology is expected to shed new light on the early developmental events, including epigenetic changes and their long lasting effect on the newborn (Van Winkle, 2001). Embryo technologies are here to stay and their use will increase as advances in the understanding of the mechanisms governing basic biological processes are made (Li *et al.,* 2004).

Artificial Insemination (AI): A.I. is the means whereby elite genes can be more efficiently distributed compared with natural mating (Cavalieri *et al.,* 2004). Pivotal to this means is the ability to handle semen, freeze it and to transport it both nationally and internationally. Generally, semen compared with other cells of most of the body is challenge to handle – it is very prone to temperature shock, its pH (acidity) can change quickly and it is susceptible to the presence of contaminants e.g. dust (Van Soom *et al.,* 1997). The use of frozen semen presents its own set of challenges (Verberckmoes *et al.,* 2004). Despite advances in the freezing technology, frozen-thawed semen is compromised in terms of viability and motility compared with fresh semen.

Multiple ovulation and embryo transfer (MOET): MOET is a well-established technology and is used to obtain over 80% of the embryos produced for commercial purposes (Neglia *et al.,* 2003). Most of the pituitary extracts available on the market have varying ratios of FSH and LH. They are administered in the mid-luteal phase of the estrus cycle of the donor over 4 – 5 days period and are combined with induced luteolysis. At the estrus donor is inseminated, usually with at least two straws of semen 12h apart, and 7 days later the uterus is flushed to recover the embryos (Sinclair *et al.,* 2000). On average four to six transferable quality embryos are recovered (Dawuda *et al.,* 2004). This is the main drawback of MOET because breeding companies usually require a few particular sire – dam combinations (Silva *et al.,* 2004).

Ovum pick up (OPU): The most flexible and repeatable technique to produce embryos from any given live donor is offered by the technique of ovum pick up (OPU) or ultrasound guided follicular aspiration (Techakumphu *et al.,* 2004). A scanner with an adequate endovaginal (or adapted for vaginal use) sector probe with a guided needle is required to perform this procedure. The needle is connected to a test tube and to a vacuum pump to aspirate the follicular fluid and the oocyte contained in it. Virtually any female starting from 6 months of age up to third month of pregnancy and also soon after calving (2 – 3 weeks) is a suitable donor.

Sexing sperm and embryos: For millennia, mankind has sought to control the sex of offspring of domestic animals (Smeaton *et al.,* 2003). The desire is deeply ingrained in those who work directly with breeding animals of monotocous species, especially cattle (Vishwanath, 2003). As a potential reproductive technology, sexed sperm is an attractive option for many farm producers, due to the following reasons:

- ♦ The use of AI is a readily understood technology
- ♦ Farmers can have their desired sex of calf
- ♦ There are few ethical problems or animal welfare issues with the use of AI
- ♦ There is no new reproductive management requirement with AI such as the need for synchronization.

In some report the success of sexed semen, whether the separation of X and Y spermatozoa passing through x-rays ionization may lead to genetic damage.

Sexing embryos can be a useful tool; sexing fetuses via transvaginal ultrasound is used very widely in several species including cattle; and cloning, which results in automatic sex selection, may have an important role in the future (Tominaga and Hamada, 2004). Sexing embryos is usage an antibody to H-Y antigen, a protein found on the cell membrane of male, but not on female, mammalian cells. For sexing, the antibodies to H-Y antigens are usually made in rodents, although monoclonal antibodies are also used.

Cryopreservation of ova and embryos: The reliable cryopreservation of mammalian sperm was first demonstrated by Polge et al. in 1947. The use of frozen/thawed bovine semen successfully achieved for the first time in allowed progeny testing and subsequent intensive use of valuable sires and has significantly improved productive traits (Gali et al., 2003). Significant progress has been made in freezing of livestock embryos with main emphasis on the bovine. This has led to practical application of freezing and thawing procedures for bovine/ovine morulae and blastocysts that are nonsurgically collected and transferred.

Chimera formation: Chimeras are composite animals in which the different cell populations are derived from more than one zygote. Chimera can be produced experimentally by mixing two or more cell populations at a very early stage of development or by combining tissues from two or more individuals after the period of organogenesis (Boediono and Suzuki, 1994). Chimera can be produced by two ways i.e., Morula aggregation and Blastocyst injection.

Juvenile *in vitro* embryo transfer (JIVET): JIVET is an emerging tool or technique that is appealing because (a) it generates a reduction in the generation interval and (b) it has the potential to be more successful than convention ET. The technology is based on an ability to harvest large numbers of eggs from 6-8 week old lamb or calf and to mature and fertilize those eggs in the laboratory. Embryos that are produced are transferred to recipients. Current expected success rates are between 10-20 offspring per donor although, as with adult ET, responses can be variable (Presicce *et al.,* 2002).

Mature in vitro embryo transfer (MIVET): As one can harvest immature eggs from juveniles, so it is possible to harvest immature eggs from adults and to have them matured and fertilized in the laboratory. MIVET is being proposed an alternative to ET i.e., it has the potential to generate more offspring per animal per year than conventional ET. This technology remains to be fully evaluated but it does offer considerable potential as a means of increasing the production of offspring from elite animals.

Cloning: Cloning is a potentially useful breeding tool because it is a means of producing "carbon copies" of elite animals that would not otherwise be available to commercial farmers (Da Silva *et al.,* 2002). This direct availability of elite bulls/rams provides an immediate but "one off" jump in the rate of genetic progress equivalent to about 10-12 years of conventional genetic selection (Dean *et al.,* 1998).

Scope of biotechnological applications in Pakistan: Biotechnology is making it possible for researchers and developers to deliver products that help farmers protect their crops and livestock; and improve the economy and environment while grow grains, develop dairy products that improve the quality of the food we eat. Biotechnology will enhance quality of life in many ways, while helping the environment by reducing our dependence on non-renewable resources. But that's just the beginning. We have to understand the importance and its proper role and influence on our future growth, health and environment. Embryo biotechnologies applied to animal breeding have the important role of increasing the impact of superior genotypes in the population. However, a more widespread and competent use of the available techniques is required in order to gain benefit from their applications.

Future developments, linked to the newest area of research such as somatic cloning and embryo genotyping, are expected to find a role in advanced animal breeding. Together with the requirement for continuous scientific progress there is also a need to address public concern over the new biotechnologies. In this respect, more knowledge is needed to demonstrate the safety of embryo biotechnologies and the suitability of the derived products to enter the food chain.

It is clear that the next 10 years will see many exciting developments in domestic animal reproductive biotechnologies. Of the techniques described in this paper, just how many will become commercially available in Pakistan are not yet clear. Nevertheless, it is instructive to remember that fewer than 15 percent of cows in Pakistan conceive by artificial insemination, and this is a proven technology that is inexpensive and easy to apply compared with most genetic engineering techniques. Thus, because a technology is available does not necessarily mean that it will widely be applied. Previous experience suggests that few will have widespread impact on Pakistan's domestic animal production. Nevertheless, the challenge has been, and will continue to be, for researchers to find ways of manipulating reproduction and genetic change. Similarly industry has to consider and evaluate each new development for its application.

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