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Review

Reproductive Biotechnologies and Their Future in Pakistan

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ABSTRACT

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 has yet to play their due role in different areas especially in the fields of agriculture, medicine, industry and environment. Particularly every developed and developing country is engaged in molecular biological activities. To obtain maximum benefits from biotechnology, some important points need due consideration. In order to be successful and competitive, an appropriate infrastructure is required. It is essential that the people involved have the necessary knowledge and training in the various aspects of this multi-faced discipline. Intradisciplinary 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 biotechnology application in development and will result in serious long lasting economic losses. In this paper, reproductive biotechnologies and their future in Pakistan have been discussed.

Key Words: Reproduction; Biotechnology; Pakistan

INTRODUCTION

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 transgensis and genotyping (Boland *et al.*, 2001; Smeaton *et al.*, 2003; Gali *et al.*, 2003; Techakumphu *et al.*, 2004; Silva *et al.*, 2004; 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 (Buhi, 2002; Walker *et al.*, 1992; Hoshi, 2003; Merton *et al.*, 2003; Vishwanath, 2003; Dargatz *et al.*, 2004). *In vitro* embryo production is a newer and more flexible approach, although it is technically more demanding and requires specific laboratory expertise and equipment that are most important for the quality of the embryos produced (Walker *et al.*, 1992; Bavister, 1995; Smeaton *et al.*, 2003; Lucci *et al.*, 2004; Tominaga & Hamada, 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 (Wilson *et al.*, 1995; Telfer *et al.*, 1999; Wrenzycki *et al.*, 2000; Squirrell *et al.*, 2001). 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 (Gandolfi *et al.*, 1992; Suzuki *et al.*, 1993; Farin & Farin, 1995; Steeves & Gardner, 1999; Kakar *et al.*, 2001; Kleemann *et al.*, 2001; 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 ((Merton *et al.*, 2003; Mtango *et al.*, 2003; Schafer-Somi, 2003; Li *et al.*, 2004).

Artificial insemination (AI). AI is a method of breeding in which semen is obtained from the male and introduced into the female reproductive tract by means of instruments. Direct contact of male and female is avoided. AI is the means whereby elite genes can be more efficiently distributed compared with natural mating (Wilmut *et al.*, 1985; Guillomot *et al.*, 1993; Merton *et al.*, 2003; Dargatz *et al.*, 2004; 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 (Ashworth, & Bazer, 1989; Van Soom *et al.*, 1997). The use of frozen semen presents its own set of challenges (Vishwanath, 2003; 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. Consequently, the results obtained with frozen semen seldom match those obtained with fresh semen (Anderson, 1977; Stegner *et al.*, 2004; Anderson *et al.*, 2004). Advantages of AI include, i) Increased rate of genetic gain, 2) Easy transport of genetic material, 3) Long-term storage of semen, 4) Increased efficiency of breeding, 5) Reduction or elimination of the need to maintain males on the farm, 6) Prevention and control of diseases (venereal diseases), 7) Use of

incapacitated males, 8) Accurate record keeping, 9) Use of synchronized or out-of-season breeding, 10) Use of other technology

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 (Walker *et al.,* 1989; Wilmut *et al.,* 1986; Adams, 1994; Assay *et al.,* 1994; Merton *et al.,* 2003; Smeaton *et al.,* 2003; 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 (Scaramuzzi *et al.,* 1993; Webb *et al.,* 1999). At the estrus donor is inseminated, usually with atleast two straws of semen 12h apart, and 7 days later the uterus is flushed to recover the embryos (Sirard *et al.,* 1998; Yoshida *et al.,* 1997; Sinclair *et al.,* 2000). On average four to six transferable quality embryos are recovered (Dawuda *et al.,* 2002; Kakar *et al.,* 2003; Neglia *et al.,* 2003; Preis *et al.,* 2004; Dawuda *et al.,* 2004). The outcome of the treatment is very variable: one third of the donors treated do not respond to superovulation, another third produces an average of one to three embryos and only one third actually superovulates giving a large number of embryos (Baker, 1982; Baird, 1987; Downing & Scaramuzzi, 1997; Li *et al.,* 2004). This is the main drawback of MOET because breeding companies usually require a few particular sire–dam combinations (Hunter, 1994; Yaakub *et al.,* 1997; Kaye and Gardner, 1999; Silva *et al.,* 2004). Another limitation is the requirement of a donor in perfect gynecological condition and this is best achieved with donors that have passed their lactation peak since the superovulatory treatment induces a drop in milk production (Barnes, 2000; Presicce *et al.,* 2004). In many cases donors are dried off to achieve better results.

Repeated superovulatory treatment of heifers or cows has side effects on fertility (development of cystic syndromes, difficulties in getting them pregnant) and on udder ligaments that can relax and downgrade the morphology in show animals (Bazer *et al.,* 1987; Fahey *et al.,* 2001; Edwards & McMillen, 2002). In young heifers, the superovulatory treatment can cause excessive premature udder development. For long time several researchers have attempt to improve embryo yield with little success (Walker *et al.,* 1996; Wathes *et al.,* 1998). However, these research efforts have resulted in the simplification and rationalization of the procedures involved in MOET (Jorritsma *et al.,* 2004). For example, dominant follicle ablation and estrogen administration can synchronize follicular wave emergence and the superovulatory treatment can be scheduled at the most convenient time (Fortune, 1994; Driancourt & Thuel, 1998; Merton *et al.,* 2003).

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 (Hasler *et al.,* 1995; Techakumphu *et al.,* 2000; Gali *et al.,* 2003; 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. A scanner with good resolution and with a probe of at least 6 MHz is used to envisage follicles down to 2–3 mm in size and also to view the needle during follicle aspiration. This procedure can be performed either on-farm or in an IVP residential center (Lonergan *et al.,* 1994; Merton *et al.,* 2003; Berlinguer *et al.,* 2004). The donor is confined to a crush, mildly sedated and given an epidural anaesthesia just before collection. OPU has virtually no drawbacks for the donor and can even have a therapeutic effect in some infertile donors affected by ovarian cystic syndrome or similar pathologies that compromise reproductive function (Techakumphu *et al.,* 2000; Hoshi, 2003). 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. This makes OPU a very flexible technique that, unlike MOET, does not interfere with the normal reproduction and production cycles of the donor (Hammon *et al.,* 2000; House, 2000).

OPU can be performed sporadically or on a regular basis such as two times a week for many weeks or months (Gali *et al.,* 2001; Merton *et al.,* 2003; Smeaton *et al.,* 2003). The twice a week protocol is the one that yield the maximum number of competent oocytes in a given period of time. Another advantage of OPU is that it is not necessary to treat the donor with gonadotropins (some laboratories however do this, often because of a poor scanner not suitable for small follicles) with the inevitable side effects (Manik *et al.,* 2003). This is a very important advantage especially for young heifer in which gonadotropin-stimulation can cause mammary oedema and ovarian cystic syndrome. A final advantage is the possibility of using over a short time, or even on the same collection (when many oocyte are produced), more sires to achieve in a short time several different dam–sire combinations (Vishwanath, 2003). The drawbacks of OPU are higher cost compared to MOET and the requirement of specialized laboratory equipment to perform all the steps of embryo production. By this procedure around 2.5 freezable/ transferable embryos per cow and about 1.4 for heifers can achieved.

The OPU technique tends to be more consistent than MOET and it allows repeatable and safe embryo production without interfering with the reproductive cycle or milk production of dairy donors (Hasler *et al.,* 1995). However, only a minority of the collected oocytes develops into viable embryos. Several factors have a role to play in this context Manik *et al.,* 2003). The nutritional status of the donor together with intraovarian factor such as the stage of the oocyte within the follicular wave, are likely to substantially affect embryo development (Techakumphu *et al.,* 2004).

Parthenogenesis. The phenomenon of virgin birth, or parthenogenesis, occurs in at least one species of every vertebrate class except mammals. There are parthenogenetic birds, reptiles, fish and amphibians. Although the predominant strategy for reproduction in each of these vertebrate classes involves sexual recombination and mating, the existence of parthenogenetic species is conclusive evidence that development can occur in the absence of a paternal contribution to the genome (Boediono & Suzuki, 1994). In other words, maternal and paternal genomes can be functionally equivalent in every significant way except in mammals. Despite claims of successful development of mice both from normal and from parthenogenetic nuclei transferred to normally fertilized eggs cytoplasm, it is now clear that the parthenogenomic genome is incapable of supporting development to term of the individual mammalian embryo (Mclaren, 1980; Watson *et al.,* 1994; McEvoy *et al.,* 2001; Sakaguchi *et al.,* 2002).

Developmental failure of parthenotes does not generally occur at preimplantation stages. Spontaneous parthenogenesis occurs in ovarian oocytes of the mice. Experimental parthenogenesis can be induced with a variety of treatments, all of which yield apparently normal activation, cleavage, and blastocyst formation in the diploid individuals that occur as a result of suppression of second polar body formation (Boediono, 1996). These diploid parthenogenetic individuals develop until postimplantation stages, with a rare few developing into small 25 somite embryos by day 10 of gestation (Boediono & Suzuki, 1994). What makes this delayed death of parthenotes even more intriguing is that parthenogenetic embryos can contribute viable cells to chimeras made by aggregation or by injection of the parthenogenetic inner cell mass into a normal blastocyst.

Sexing Sperm and Embryos

Sperm sexing. For millennia, mankind has sought to control the sex of offspring of domestic animals (Seidel Jr., 2003; 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: i) The use of AI is a readily understood technology, ii) Farmers can have their desired sex of calf, iii) There are few ethical problems or animal welfare issues with the use of AI, iv) There is no new reproductive management requirement with AI such as the need for synchronization.

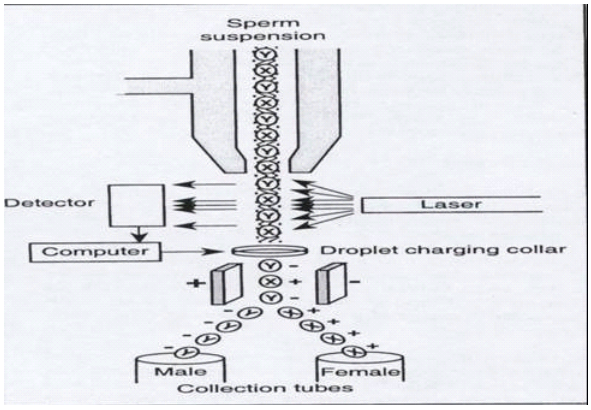
Unfortunately, at present sexed sperm are not available as a commercial product in Pakistan. However, since technical progress is continuing in this field, the use of sexed sperm may become more viable in future.

Sperm sexing procedures. More than a dozen approaches for the sexing of sperm have been proposed, but to date only one procedure has proven efficacious in any practical sense (Erickson, 1997). That procedure measures DNA content of individual sperm via fluorescence of the DNA-binding dye, Hoechst, while sperm are processed through a flow cytometer/cell sorter. Bovine X-sperm have 3.8% more DNA than Y-sperm. Accuracy of sexing is about 90% is achieved routinely, but this can be set by the operator to be lower or as high as >95% (Hohenboken, 1999; Doyle, 2000).

Sorting speed is the primary limitation to the technology. To some extent, speed is a function of accuracy, but having to evaluate sperm one at a time is the inherent drawback of cell sorting. Sorting speed also depends on characteristics of the individual ejaculate, for example, slower with more dead sperm and on the species. It is unlikely that additional major advances in sorter technology will occur in the immediate future (Vishwanath, 2003).

To utilize these sperm efficiently, fewer sperm are used per insemination dose than normally, usually around 2 million sexed, frozen sperm/straw (Patrat *et al.,* 2002; Gali *et al.,* 2003). A major consequence is somewhat reduced fertility, which is exacerbated by some damage to sperm during the sexing process. With excellent management of cattle, fertility with low doses of sexed sperm has been around 70–80% of normal doses of unsexed sperm; while in average to marginal management, fertility can fall in between 50–60% than of controls (Vishwanath, 2003). Importantly, calves are normal. The population of calves resulting from sexed sperm is no different from control calves in any of the trait studied, including gestation length, birth weight, calf mortality, and weaning weight. Recent improvements in sorting procedures, especially lowering system pressure, result in considerably less damage to sperm than procedures used for sexed sperm prior to 2002 (Fig. 1).

Fig. 1. Note: Sperm are oriented to follow each other in a single line. A laser beam then causes fluorescence of the DNA-specific fluorochrome used to stain the sperm heads. Based on the amount of fluorescence, sperm "charged" with either +ve or –ve polarity and are then sorted by passing through an electric field.



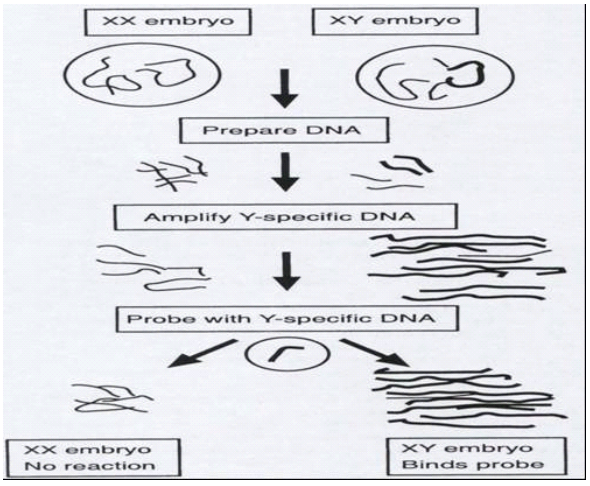
Sexing embryos. Sexing embryos also can be a useful tool; sexing fetuses via transvaginal ultrasound is used very

widely in several species including cattle; and cloning, which results in automatic sex selection, may have an important role in the future (Seidel Jr., 2003; Vishwanath, 2003; Tominaga & Hamada, 2004).

The first approach to sexing embryos was conducted on the basis of sex chromatin in biopsied trophoblast cells. Since then, numerous investigators have successfully sexed mammalian embryos on the basis of the karyotype of biopsied cells (Moore & Kemler, 1997; Hoshi, 2003). Under ideal circumstances, about two thirds of the embryos can be karyotyped accurately for sex chromosomes while suitable metaphase chromosome preparation are not obtained from the other one third. The net result is a 1:1:1 sex ratio of males, females and unknown. The biopsy and karyotyping procedures are tedious and time consuming, making them impractical for routine commercial use.

A second method of 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 (Fig. 2). Detectable H-Y antigen is present at the eight-cell stage if the ovum was fertilized by a Y-bearing sperm, which contains the genetic information for producing this protein. For sexing, the antibodies to H-Y antigens are usually made in rodents, although monoclonal antibodies are also used. The embryos are exposed to anti-H-Y antibodies, which bind to the male embryos (Seidel Jr. 2003; Smeaton *et al.,* 2003). Two methods are commonly used to detect the bound antibodies. The first method involves adding complement, which results in death of the male embryos. The more practical method is the anti-antibody method. This consists of producing a fluorescent-labeled antibody to the first antibody so that the male embryos will fluoresce in the appropriate light. This procedure is about 80 percent accurate for sexing mouse embryos and is being developed by several companies for use with bovine embryos (Smeaton *et al.,* 2003). A major concern is that the procedure might damage embryos (Erickson, 1997). One way around this might be to detect sex-specific secretion products after-term culture of embryos. Developing sexing procedure is expensive, and the commercial companies will have to charge high rates for sexing embryos to recover these costs (Brackett, 1981). However, with continuous research and development, costs are likely to drop quickly (Eppig, 1994).

Fig. 2. Embryo sexing procedure for domestic animals

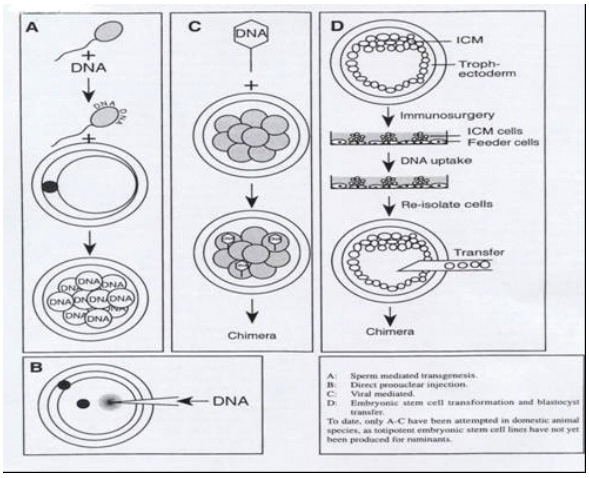


Cryopreservation of ova and embryos. Over the last two decades major progress has been made in techniques for cryopreservation of different cell types, more complex tissues and even organs (Whittingham, 1971; Lucci *et al.,* 2004). This rapid development pertains also cryopreservation of mammalian gametes. Later Chang and Hunter (1975) reported normal development of fertilized rabbit ova stored at low temperature for several days. The reliable cryopreservation of mammalian sperm was first demonstrated by Polge *et al.* (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). The first successful freezing and thawing of mouse embryo was reported by Whittingham (1971), followed two years later by the birth of the first calf following surgical transfer of frozen thawed embryos. Since then, significant progress has been made in freezing of livestock embryos with main emphasis on the bovine (Arceci *et al.,* 1992; Bavister, 1995; Anderson *et al.,* 2004). This has led to practical application of freezing and thawing procedures for bovine/ovine morulae and blastocysts that are nonsurgically collected and transferred. Based on cryobiological principles originally studied in cells like lymphocytes or fibroblasts, a variety of procedures has been developed that allow almost similar pregnancy rates as following nonsurgical transfer of fresh embryos (Tominaga & Hamada, 2004). Thus, successful cryopreservation of mammalian embryos can be achieved by either controlled freezing and thawing procedures, the one-step procedure, vitrification or ultrarapid freezing (Suzuki *et al.,* 1993; Jousan *et al.,* 2004; Berlinguer *et al.,* 2004).

Chimera formation. Chimeras are composite animals in which the different cell populations are derived from more than one zygote (Fig. 3). 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 & Suzuki, 1994). Chimera can be produced by two ways.

Morula aggregation. It is possible to aggregate two or more cleavage stage embryos together; making composite morula that will develop into a chimeric animal when transplanted to a foster mother (Tarkowski, 1961). Methods for the aggregation of cleaving mouse embryos were first described. Aggregating two or more, eight to sixteen cell stage mouse embryos after removing their zona pellucida

Fig. 3. Chimera production procedures



produced the chimeras (Boediono, 1996). In mammals, an important stage in the development is the time when cell compaction occurs. Bovine embryo will begin to compact and form tight junction from 16 to 32-cell stage. Once the compaction begin to occur, aggregation of morula becomes progressively more difficult (Shim *et al.,* 2004). In bovine, the use of *in vivo* embryos for producing chimera by morula aggregation method is inconvenient, as developed embryos in this stage (precompaction) normally located in the oviduct. However, by using the *in vitro* produced embryos, these problems did not occur. For analytical purposes, the embryos obtained from strains that differ in coat or eye colour, chromosome morphology or biochemical traits can be studied (Boediono & Suzuki, 1994).

Blastocyst injection. Once mammalian embryos have reached the blastocyst stage, two distinct tissues are present, the inner cell mass (ICM) and the trophectoderm (TE), both of which have restricted potential and different fate in development. At this stage it is possible to introduce cells into the cavity of the blastocyst to obtain chimeras. Injection of individual cells into blastocyst let to a new generation of chimera. In this way it is possible to assess the fate and potency of cells at later stage with greater resolution than chimera produced by aggregation method (Gali *et al.,* 2003; Shim *et al.,* 2004).

The blastocyst injection method has certain advantages over morula aggregation despite technically being more difficult. First of all, the zona pellucida does not need to be removed, allowing adaptation of this method to species such as the rabbit in which the zona pellucida is necessary for subsequent development (Tarkowski, 1961). It also makes possible interspecific combinations that would otherwise fail to implant, since the trophoblast will be entirely composed of the host blastocyst type, unlike aggregation chimeras where the trophoblast will be a mosaic of cells from both components. In these studies scientist generally used the biochemical markers developed for aggregation chimeras and analyzed the progeny either at mid gestation or after birth (Kwong *et al.,* 2000).

The aggregation technique using whole embryos may prove to be more successful means of producing mammalian chimeras. The aggregation of demi-embryos or the microinjection of the ICM has the disadvantages of being a complex procedure and of requiring costly equipment.

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 (Gali *et al.,* 2003; Hoshi, 2003). 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 (Merton *et al.,* 2003). As a result, lambs or calf themselves can have their lambs or calves "on the ground" between 6-7 months of age (given a gestation period of five months in sheep). Current expected success rates are between 10-20 offspring per donor although, as with adult ET, responses can be variable (Goodman, 1988; Pushpakumara *et al.,* 2002; Presicce *et al.,* 2002).

Mature in vitro embryo transfer (MIVET)

1. 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.
2. 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.
3. The salient features of the technology are: a) Donors are treated with a progestagen pessary and FSH is administered in much the same way as it is for ewes prepared for ET, b) The follicles do not ovulate – rather the eggs are harvested from the follicles at a precise time before ovulation, c) The eggs may be harvested by either laparoscopy or by midventral laparotomy. The laparoscope is less invasive and suitable for multiple collections although bleeding from the ovary surface (and hence adhesions) is a possible problem, d) After maturation and fertilization, embryos are grown in the laboratory for about 5–6 days before being transferred or frozen, e) It should be possible to do repeat collections throughout the year and the accumulated embryos would be transferred to recipients at preferred times (e.g., in sheep to give a Spring and Autumn lambing).
4. The MIVET ewes themselves need not to be necessarily become pregnant but act as suppliers of eggs.
5. 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" (Figs. 4 and 5) of elite animals that would not otherwise be available to commercial farmers (Bachvarova, 1985; Handyside *et al.,* 1987; Willadson *et al.,* 1991; Price *et al.,* 1992; Wilson *et al.,* 1995 Cross, 2001; Da Silva *et al.,* 2002). This direct

Fig. 4. Note: Cloning from somatic cells would replace the nuclear donor embryo cells with ones derived from a cultured cell line

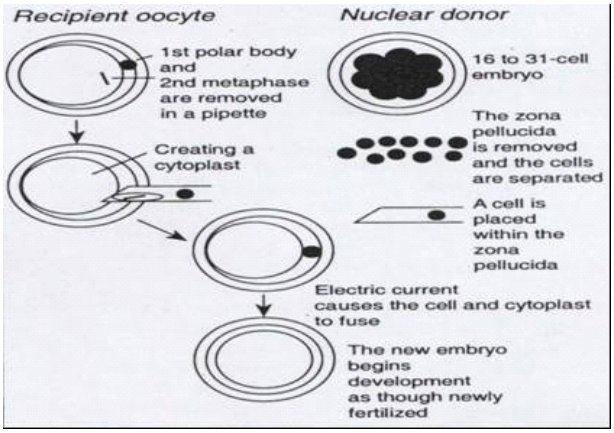


Fig. 5. The MATILDA, the first Australian sheep cloned from somatic cells by Turretfield Research Station Rosedale SARDI, University of Adelaide South Australia

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 (Du *et al.*, 1995; Dean *et al.*, 1998).

Preparation of donor cells

1. Cloning involves removing the maternal DNA from the egg and replacing it with DNA of a somatic cell (e.g. skin cell). The latter contains both maternal and paternal DNA and so fertilization is not necessary.
2. Somatic cells (often sourced from ear tissue) can be grown in millions in the laboratory after which they are frozen ready to use.
3. Each somatic cell, after it has been transferred to the egg, has the potential to lead to the production of an animal that is a clone of the animal from which the somatic cells was obtained. Given that there are millions of these cells frozen, it is therefore possible to generate many copies of the donor animal.

Collection and enucleation of eggs

1. Eggs can be produced from donor animals or from ovaries collected at the abattoir (using *in vitro* maturation procedures).
2. The maternal DNA is removed from the egg (enucleation) under magnification and a somatic cell is fused with the remaining yolk (cytoplasm).
3. The reconstructed egg is then "activated" using an electric pulse. Activation mimics the events of fertilization and ultimately leads to nuclear reprogramming i.e., changing the DNA from acting like that of a skin cell to that of a newly formed embryo.
4. Reconstructed embryos are cultured in the laboratory for about six days and potentially viable embryos are transferred to recipient animals.

Production of cloned sheep and their subsequent performance

1. Many cloned lams are lost during pregnancy and of those that do survive to term, approximately half are lost during or shortly after birth.
2. Cloned that do survive appear to be able to reproduce normally (Dolly has given 5 normal births) and their offspring also appear to be normal.
3. One particular problem with clone pregnancies is the inability of the ewe/fetus to always initiate parturition– consequently, many cloned lambs are delivered by caesarian section.
4. This technology is in its genesis and so there are many problems. The two main problems are:
   - A lack of knowledge of nuclear reprogramming which is ultimately responsible for the correct development of the embryo and fetus, and
   - The lack of a natural onset of parturition although this problem can be side-stepped to some extent by administering corticosteroids to the ewes just prior to the due lambing time.
5. Even if technical advances are made in the laboratory, field evaluations of cloned animals throughout their lifetimes will need to be conducted before cloning can be considered to be a useful breeding tool.

CONCLUSIONS

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.

The challenge is out there for the biological scientists in Pakistan to achieve the above demanding requirements, coupled with the ethical and human factors involved in the introduction of any new technology. 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 AI, 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. One research area, genetic engineering, is likely to have enormous significance on agriculture as a whole, including livestock production. This will result in unimaginable changes to our traditional farming practices and encourage the application of other emerging and new technologies. Whatever pathways are followed, ethical and animal welfare considerations will have to be kept in mind if commercial exploitation of new technology is to become a reality. Nevertheless, we need to be aware of new genetic engineering tools and remain prepared to develop, refine and apply them to serve the needs of mankind.

Livestock sector in Pakistan is generally characterized by low per animal productivity. The average productivity of ruminants is far below than its potentially achievable levels. Though improved feed, control of parasites, and most importantly through better breeding strategies and introduction of modern biotechnologies productivity gap can be narrowed. However, so far no serious attempts have been made to take the new knowledge on the farm level nor have these new research findings been adequately disseminated to those concerned in the country. The public sector infrastructure and institutional base needs to be strengthened and reorganized to meet the emerging needs of the growing animal population. So far, emphasis has been mainly on the animal health side, on prevention of livestock diseases and their control measures. Time has now come to devote equal attention towards the livestock production and management through modern biotechnologies and their application. But we are still away from the starting line of advancement especially in animal production.

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