ASSISTED REPRODUCTIVE TECHNOLOGY IN MAMMALS

A.A. Mohammed

Department of Animal and Poultry Production, Faculty of Agriculture, University of Assiut, Egypt

SUMMARY

This review describes the use of modern reproductive biotechnologies or assisted reproductive techniques (ART) including artificial insemination, embryo transfer, in vitro fertilization, gamete/embryo micromanipulation, semen sexing, genome resource banking, and somatic cell nuclear transfer (cloning) for mammalian species. Such biotechnologies allow more offspring to be obtained from selected parents to increase productivity of milk and meat, reduce the interval between generations, therapy of diseases and conservation of endangered mammalian species. Practically, current reproductive biotechnologies are species-specific because of differences in estrous cycle, seasonality, structural anatomy, gamete physiology and site for semen deposition or method of embryo transfer.

Keywords: In vitro, embryo production, embryo transfer, micromanipulation

INTRODUCTION

Assisted reproductive technology (ART) is the application of laboratory or field technology to gametes and/or embryos for the purposes of reproduction (Mohammed *et al.,* 2005; Mohammed, 2006a; Mohammed, 2006b; Mohammed, 2008; Mohammed *et al.,* 2008; Mohammed, 2009a, and b; Mohammed, 2010; Mohammed *et al.,* 2010; Wani and Skidmore, 2010; and Wani *et al.,* 2010). Reproductive technology on mammals has made significant strides over the past fifty years. This is a continuum which began with artificial insemination (AI). The utilization of AI was greatly enhanced with cryopreservation of semen and the ability to synchronize estrus. Embryo transfer is now commonly used to produce artificial insemination (AI) males from highly proven females and males. Embryo transfer offered a means by which their numbers could be multiplied rapidly. The animal production industry has focused on developing elite males and selection through pregnancy testing. Genetic progress was further enhanced via embryo transfer technology. Improved methods of synchronization and superovulation, surgical and non-surgical collection and transfer of embryos and cryopreservation of embryos have improved efficiency, decreased costs, and increased the utilization of embryo transfer by animal producers. The continuum of reproductive technology continues with techniques such as in vitro fertilization, semen sexing, and nuclear transfer or cloning. Each of these areas will be discussed in greater depth in this article.

Assisted reproductive technology in males:

The application of assisted reproductive technologies in the field of male reproductive physiology has advanced greatly during the past couple of decades. Since, the advent of frozen semen and artificial insemination, reproductive

physiologists have attempted to manipulate male reproductive rates of mammals. Availability of frozen semen has resulted in improvement fertilization to livestock and medicine. Fertilization occurs using either sperm or germ cells through the techniques of in vitro fertilization, intracytoplasmic sperm injection (ICSI), subzonal sperm injection (SUZI) and intracytoplasmic cell injection (ICCI). Currently, the sex has been predetermined with 85-95% accuracy by sexing spermatozoa.

Artificial insemination:

The first ART developed was artificial insemination (AI), which in its simplest form involves the collection of semen from males and its subsequent human-assisted introduction into a physiologically receptive female. It is an important technique for the genetic improvement of animals, as a few select males can produce sufficient sperm to inseminate thousands of females per year, while natural service would provide for the insemination of only a fraction of those animals.

Although there are several methods for collecting semen, most involve training males to ejaculate into an artificial vagina. Semen is then diluted to maximize the number of services that one male can provide. A normal ejaculate from a dairy bull usually contains between 5 and 10 billion sperm; good conception rates generally require about 12-20 million sperm to be introduced. The diluting solution contains factors that help to stabilize and preserve the sperm, as well as antibiotics to inhibit bacterial growth and reduce the danger of spreading any potential disease or contamination. Most collected semen is stored in plastic straws and kept in liquid nitrogen (-196˚C). To date, there appears to be no limit on the amount of time that bovine sperm can remain frozen and regain viability upon appropriate thawing.

AI of the female is usually performed either by trained technicians employed by breeding companies or large farms or by the producers themselves. The most common technique employed today for dairy cows involves the use of sterile, disposable catheters that are inserted vaginally and extended through the cervix into the body of the uterus of the recipient cow (whose estrous cycle has been documented). Thawed semen is warmed to the appropriate temperature, and sperm are deposited in the uterine/cervical regions.

The primary advantages of AI to farmers include the ability to use semen from bulls anywhere in the world rather than those that are more geographically proximate, and thus to have desirable genetics available for propagation. It also allows the farmer to use multiple sires in a herd without the attendant costs of maintaining animals that are often difficult to handle and in multiple breeding pastures. AI tends to be less expensive than natural service (a straw of semen generally costs less than transporting a female to the sire and the stud fee) and avoids the potential physical risks to either sire or dam as part of the mating process. The disadvantages of AI include the need to train personnel engaged in the breeding operations on how to detect estrus in females and training or retaining individuals to perform the insemination. Further, care needs to be taken not to rely excessively on a few apparently superior sires so as not to reduce the genetic diversity of the resulting herds.

Sperm collection and AI were further improved by the advent of sperm sexing, or selection of sperm carrying an X (female) or Y (male) chromosome. Development of an effective and simple method for producing animals of the desired sex is economically desirable for livestock producers; sperm sexing is currently being used

when available and economically feasible (Wheeler *et al*., 2006; Palma *et al*., 2008; and Blondin *et al*., 2009). For example, in the dairy industry, females are desired because males do not produce milk; and excess males often become veal. In the beef industry, however, males are desired because they grow faster. The question arises, is it possible to increase milk and meat production in Egypt through AI of superior males?

In Vitro Fertilization:

The term "in vitro fertilization" (IVF) refers to fertilization of the oocyte outside of the body; IVF typically is used to refer to standard methods (incubation of sperm and egg together in media). The overall technique for IVF is similar among species, and involves significant manipulations *in vitro*, or outside the body of animals for gametes; sperm and oocytes. Either fresh or frozen-thawed semen can be used for fertilization. Sperm need to be capacitated *in vitro* in order to penetrate the zona pellucida and fuse with the ovum or to undergo the same maturation process that they would normally undergo in the female reproductive tract. Capacitation involves a series of cellular changes to the sperm including increased motility, calcium uptake and protein binding (binding to proteins produced by the female reproductive tract). *In vitro* capacitation is accomplished by creating a medium designed to simulate the female reproductive tract and allowing the sperm to incubate in it for a period of time. Sperm are then added to ova, incubated in culture medium for approximately 8-22 hours, and the resulting fertilized ova, called zygotes, are washed, examined for appropriate development, and allowed to continue to divide for up to seven days, again in culture. At that time, if embryos appear normal, they may either be frozen for future use or inserted into the uterus of a reproductively competent female.

IVF can be applied for oocytes collected from slaughterhouse materials or live females through transvaginal ovum pick-up (OPU) guided by ultrasonography (Mohammed *et al.,* 2005; and Aller *et al.,* 2010). The initial purpose of commercial IVF was to obtain viable embryos from females that may not be able to produce progeny through conventional techniques. At present, IVF is a complement to an embryo transfer (ET) program. Its application could be for females that will not respond to super stimulatory treatments, fail to produce transferable embryos, or possess abnormalities in their reproductive tracts (i.e. ovarian adhesions or blocked fallopian tubes). IVF is also used for females that are terminal (age, accident, disease, etc.), or that are pregnant during the first trimester of gestation, and for animals with and without offspring during the first one, two or three months after parturition (post-partum period). It also has applications for normal cyclic animals, and prepuberal animals. IVF allows an improvement in efficiency of utilization of sperm. IVF still provides opportunities to use relatively low numbers of sperm to produce viable embryos. This allows for the utilization of high value semen and may provide significant opportunities when coupled with gender separated semen. Commercial and research centers have used OPU-IVF in diverse categories of females (pre-puberal calves, heifers, cows), age (pre-puberal, post-puberal, aged cows), breeds, reproductive status (cyclic, pregnant, post-partum), aspiration frequency (twice per month, once weekly & twice weekly), use of hormones (FSH, rBST) and IVF protocols (co-culture BRL cells, chemically defined media, serum) with different degree of success (Guyader-Joly *et al*., 2000; Blondin *et al.,* 2002; Ferré *et al.,* 2002 and Reis *et al.,* 2002; Mohammed *et al.,* 2005;and Aller *et al.,* 2010).

Assisted in vitro fertilization:

Laser-assisted in vitro fertilization:

Given the fact that eggs, sperms and embryos are all microscopic, it is hardly surprising that one of the successful advances in recent times has been the introduction of lasers in the IVF lab (Obruca *et al*., 1994), since lasers allow the embryologist to deliver a controlled amount of energy at a specified spot with exquisite precision. After a considerable amount of experience, lasers have been successfully integrated into routine IVF practice, and are now found in most advanced and well-equipped IVF labs. The advantages of a laser are multiple – it is precise; a traumatic, and non-contact, which means there is no need to touch the egg or embryo when using the laser.

The initial concerns regarding the safety of the laser have now been put to rest after years of extensive experience with lasers in IVF labs (Hammoud *et al*., 2010). Lasers now allow to safely perform cellular microsurgery in the IVF lab, and are routinely used for assisted hatching in many labs. Assisted hatching involves using the laser to create a precise opening in the shell (zona) of the embryo prior to transferring it. Such an opening helps the embryo to hatch more easily, thus increasing the chances of it implanting successfully in the uterus.

The laser are used routinely in labs for performing embryo biopsies for preimplantation genetic diagnosis (Taylor *et al*., 2010). The laser allows to create a well-defined opening through the zona, through which it is possible to safely and atraumatically remove a single blastomere from the embryo.

Researchers have also used the laser for ICSI (intracytoplasmic sperm injection). The trickiest part of ICSI is the technical difficulty involved in capturing a single motile sperm and crushing its tail in order to immobilize it prior to injecting it into the egg. Using a laser beam, this can be performed with much greater ease and speed, thus dramatically reducing the time taken to perform ICSI. Moreover, Tyrode solution is used to create one hole in the zona pellucida of each oocyte providing a means for less motile or less progressive sperm to penetrate the oocyte and induce fertilization (Mohammed 2009 a).

Intracytoplasmic and subzonal sperm injection and intracytoplasmic cell injection:

Techniques of intracytoplasmic (ICSI) or subzonal (SUZI) sperm injection and intracytoplasmic cell injection (ICCI) are used to fertilize metaphase II and/or immature oocytes (Tsirigotis *et al*., 1994; Kimura and Yanagimachi, 1995 and Tarín, 1995). Transfer of male germ or embryonic or somatic nuclei into MII or GV cytoplasts might enable creating the new type of oocytes carrying the haploid introduced nuclear genome of male through meiotic maturation. Cell transplantation can be accomplished with a high degree of efficiency without compromising the maturation. Kimura and Yanagimachi (1995) injected nuclei of mouse secondary spermatocytes (1N and 2C) into MII oocytes, whereas nuclei of primary spermatocytes (2N/4C) transferred into GV oocytes in other studies (Kimura *et al.,* 1998; Ogura *et al.,* 1998; Sasagawa *et al.,* 1998; and Nan *et al.,* 2007), all generating artificially haploidized ‘‘spermatocytes’’ (1N/1C) in vitro that were capable of fertilizing an oocyte in vitro and resulting in full-term development. In reproduction of mammals, may this indicate that males before puberty or males without sperm could be used for fertilization of oocytes and offspring could be obtained? Moreover,

nuclei of growing oocytes were transferred into mature germinal vesicle cytoplasts in mice (Mohammed *et al*., unpublished data) and cow (Bao *et al*., 2003). The resulting reconstructed oocytes were matured in vitro into MII oocytes. When they were fertilized and transferred into recipient animals, the fetuses were aborted during the last stage of pregnancy in cow. Furthermore, birth of healthy offspring was through the transfer of embryos produced from young animals.

***Semen sexing*:**

The possibility of sex pre-selection always had sparked great interest among livestock producers. Sexed semen could contribute to increasing the profitability desired by the animal industries through desired sex offspring production, thus taking advantage of specific marketing or commercial production demands (like herd replacement, herd expansion, or increasing the male sales to slaughter). The clearest examples could be the production of females for dairy or replacement and males for meat production. Other applications would be for cattle breeders and AI semen companies to test elite bulls on a small number of females. Thus, methods are needed to determine the sex of sperm or embryos so producers can control the sex of the offspring of their livestock. Using a specific dye that binds to DNA (the Hoechst 33342 stain) and a flow cytometer/cell sorter, the DNA content of individual sperm is measured (Johnson and Clarke, 1988; Morrell *et al*., 1988; and Johnson *et al*., 1989). In cattle, the X-bearing sperm contain 3.8 percent more DNA than the Y-bearing sperm. In mammals, the presence of a Y chromosome (and one X chromosome) determines that the individual will be a male. Female mammals contain 2 X chromosomes. Although the process to sort the X and Y bearing sperm is slow (approximately 10 million live sperm of each sex can be obtained per hour—this is about the number of live sperm required for one conventional dose of frozen semen for artificial insemination), this procedure determines the sex with higher than 95 percent accuracy (Wheeler *et al*., 2006; Palma *et al*., 2008; and Blondin *et al*., 2009).

Genome resource banking:

There have been a number of approaches proposed to slow or halt the rate of species decline. One suggestion is to undertake a program aimed at preserving genetic material or ex situ cryoconservation of germplasm, specifically spermatozoa, oocytes or embryos, and other cells/tissues or DNA from species.

Semen banks:

Systematic cryopreservation and storage of semen from species can facilitate maintenance of genetic heterozygosity, while minimizing movement of living animals between research centers or countries (Johnston and Lacy, 1995). Using frozen-thawed spermatozoa would facilitate the infusion of new genetic material across populations by AI. The use of frozen sperm from semen banks increases the generation interval indefinitely and allows fewer males to be held in captivity because some of the genetic diversity is maintained strictly as frozen spermatozoa.

Semen banks are currently more developed for rare domestic breeds (bovine, ovine, caprine and porcine) than for non-domestic species, but the concept of using them to facilitate the management and conservation of endangered species is being promoted extensively (Wildt *et al.,* 1997). In order to maximize genetic diversity, a rare animal from the family bovidae could be saved with 1000 sperm doses collected from 25 different males (Comizzoli *et al.,* 2000).

Assisted Reproductive technology in females:

Multiple ovulation and embryo transfer:

Development of embryo transfer technology allows producers to obtain multiple progeny from genetically superior females. Depending on the species, fertilized embryos can be recovered from females (also called embryo donors) of superior genetic merit by surgical or nonsurgical techniques. The genetically superior embryos are then transferred to females (also called embryo recipients) of lesser genetic merit. 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. In swine and sheep, embryos must be recovered by surgical techniques. To increase the number of embryos that can be recovered from genetically superior females, the embryo donor is treated with a hormone regimen to induce multiple ovulations, or superovulation.

Embryo micromanipulation:

Micromanipulation of embryos by treatments such as zona drilling or partial zona dissection could raise their chances of implantation (Edwards and Brody, 1995). Drilling holes on the zona pellucida aims to facilitate earlier hatching of embryos from the zona pellucida when it has been hardened by ovarian stimulation and/or embryo culture. Results obtained by Loskutoff *et al*. (1999) indicate that partial zona dissection improves the hatching frequencies of bovine blastocysts produced in vitro and co-culture conditions can affect survival after thawing.

Production of identical offspring:

Work at Cambridge by Steen Willadsen in the late 1970s was the first to show that each blastomere of a two-cell sheep embryo possessed the potential to develop into a normally organized blastocyst; a technique was developed which resulted in the production of identical twins in cattle and other farm mammals. By the early 1980s, the technology had moved to splitting ruminant embryos at a later stage of development, which attracted commercial interest. For cattle, it was now possible to flush embryos from the superovulated donor animal and split them at the late morula/early blastocyst stage; this permitted the number of pregnancies obtained from a given collection of embryos to be markedly increased. A great many reports appeared on cattle embryo splitting in the late 1980s and early 1990s; occasionally some have appeared in more recent times, usually with reference to embryo sexing (Bredbacka *et al.,* 1994; and Wang *et al.,* 1995). Pregnancy rates of 50% or more per demi-embryo have been reported, resulting in a net pregnancy rate of more than 100% per original bovine embryo (Gray *et al.,* 1991).

Production of transgenic offspring:

Although microinjection of pronuclear stage embryos became the conventional route to the creation of transgenic animals, workers in Poland found that development to the morula/blastocyst stage was about 20% greater for bovine embryos obtained from microinjected two-cell embryos compared with zygotes (Jura *et al.,* 1994). Other work examined the production of transgenics by injecting one- to four-cell in vivo derived embryos (Echelard *et al.,* 2000); their results indicated that transgenic cattle could be created by injecting two-cell embryos. Other methods were used to achieve genetic modification in species. Transfected cells for nuclear transfer

might be used to achieve genetic modification in species. Some researchers have been able to isolate, culture and genetically transform primordial germ cells (PGC) derived from cattle embryos (Lee, *et al.,* 2000). Different transfected cells were used in the production of transgenic embryos. Furthermore, researchers have been able to modify endogenous genes in the mouse by manipulating ES cells; they have been able to generate specific mutations and alter specific gene sequences in cultured ES cells. Such modified cells retained their developmental potential and when inserted into a developing embryo could contribute to all its tissues, including sperm and oocytes (Robl *et al.,* 2003). Genes of transgenic animals are altered, so the animals can secrete rare and expensive drugs in their milk (i.e., transgenic sheep which produced human antihemophylic factor IX in milk; Niemann *et al*., 1999; and McCreath *et al*., 2000). Sheep-derived protein has now entered clinical trials for cystic fibrosis (UK and USA) and congenital emphysema (UK), and the utilization of cloning and transgenic technology is making inroads into more traditional ways of making biopharmaceuticals (Colman, 1999).

Somatic nuclear transfer:

Somatic cell nuclear transfer (SCNT) is a process by which the nucleus (DNA) is moved from a donor cell to an enucleated recipient cell to create an exact genetic match of the donor (Fig 1). If this happens to be a viable embryo that proceeds to term, the resulting offspring has the same genetic complement as the original donor, except for the mitochondrial DNA, which is derived from the recipient (Wolf *et al.,* 2001; Yang *et al.,* 2004).

The figure illustrates using of germinal vesicle (GV), metaphase II (MII) and zygote cytoplasts as recipient cytoplasts. The G0/G1 donor nuclei could be introduced into metaphase II and zygote cytoplasts whereas G0/G1 and G2/M donor nuclei could be introduced into germinal vesicle cytoplasts. Second meiotic division occurs in GV and MII cytoplasts necessitating activation whereas second meiotic division does not occur in zygote cytoplasts and activation is not required upon nuclear transfer for further developmental competence.

Recipient cytoplasts:

The production of cytoplast requires the removal of the nuclear material from oocytes or zygotes. This procedure is essential for the maintenance of the normal number of chromosomes of a given species. The survival of obtaining cytoplasts using enucleation technique is an impotant step of reconstruction new oocytes in assisted reproductive tecnology (ART). Germinal vesicle and metaphase II oocytes and zygotes were used as recipient cytoplasts (Greda *et al.,* 2006; Mohammed *et al.,* 2008; Mohammed *et al.,* 2010; and Wani *et al.*, 2010).

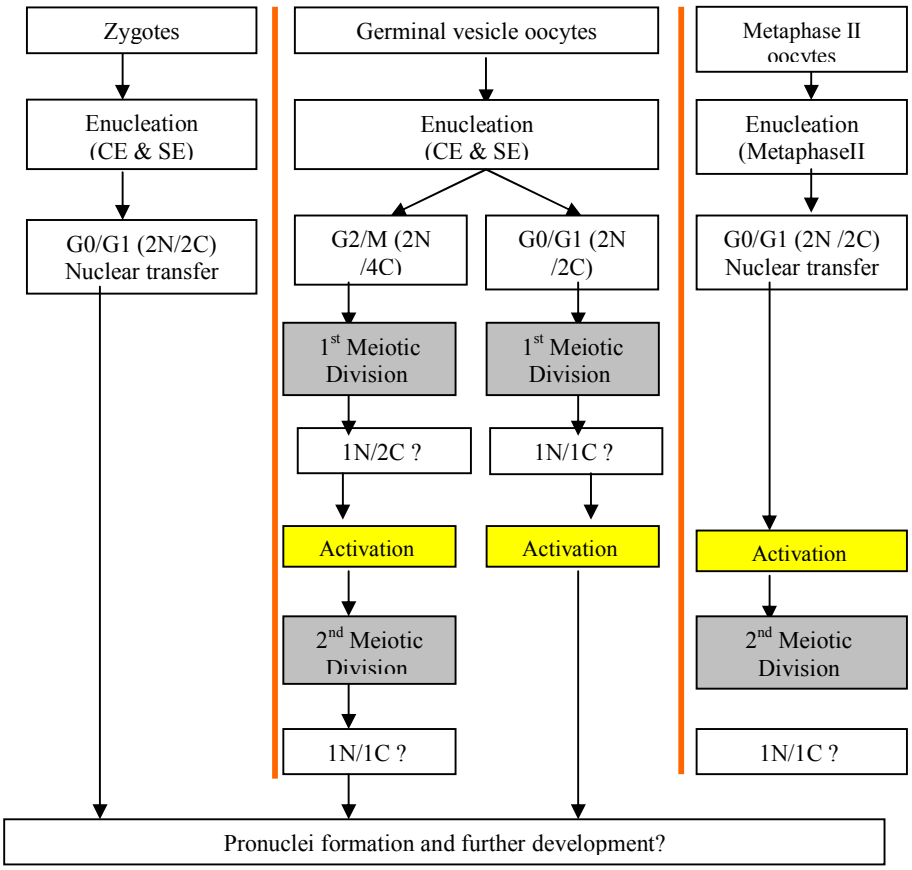

Figure 1. Strategies of reduction divisions of the introduced nuclei (G0/G1 & G2/M) and their further developmental competence into recipient cytoplasts. CE; complete enucleation where the whole germinal vesicle nucleus or pronuclei removed. SE; selective enucleation where the nuclear or pronuclear envelope and chromatin removed only

Donor nuclei:

Transfer of germ, embryonic or somatic nuclei into recipient cytoplasts might enable creating the new type of oocytes carrying the introduced nuclear genome through maturation. Such artificial gametes could subsequently be fertilized by spermatozoa or artificially activated (Mohammed *et al.,* 2008; Mohammed, 2009a &b, Mohammed, 2010; and Mohammed *et al.,* 2010). The effect of the donor cell on cloning success was also studied. Lagutina *et al*. (2005) obtained 24.3% pregnancy rates when adult fibroblasts were used for nuclear transfer. The same authors describe that when fetal cells were used the rate of success was only 5.6%.

Cloning:

Cloning is the production of genetically identical individuals by non-sexual means (Seidel, 1983). The first results for mammalian cloning were obtained by Willadsen (1986), when cloned sheep were born after the split of 8 to 16 cell embryos. At the end of the 20th century, Wilmut *et al*. (1997) surprised the world by producing the first clone obtained from a differentiated cell. Since then, the production of clones by nuclear transfer has been a success in many mammalian species.

Many studies have revealed that cloned mammals suffer from gestational and neonatal developmental abnormalities (Eckardt and McLaughlin, 2004; and Piedrahita *et al.,* 2004). It is relevant to mention that many of the problems associated with SCNT embryos particularly large offspring syndrome have also been found with conventional IVF and ET procedures (Young *et al.,* 1998), however the frequency and severity of the syndrome appears to be much higher with cloning (Wells, 2003). Hence, for the present, it has been suggested that SCNT should be only considered as a useful tool for basic research for the investigation of cell biology and reprogramming (Van Heyman, 2005). In present circumstances, where rapid advances in cloning technology are being made, perhaps it is more appropriate to focus on developing realistic strategies for using these methods in wildlife conservation and ensuring that scarce resources are deployed where they will be most effective (Holt *et al.,* 2004). Interaction between the donor nuclei and the recipient cytoplasts is considered as one of the fundamental questions in the field of assisted reproductive technology. The resulting artificial oocytes might be used for embryonic/somatic cloning or treatment of infertility.

Embryo Sexing:

Although there have been promising developments in semen sorting, it will take time for such procedures to reach the farm in an economically viable form; for that reason, sexing of embryos is likely to remain a major route to gender preselection. Several methods have been used to reach this objective. The result and accuracy of most of these techniques are satisfactory. The only method used routinely on a commercial scale is to biopsy embryos and amplify Y-chromosome -specific DNA using polymerase chain reaction (Martinhago *et al*., 2010; Zoheir and Allam, 2010). This method is effective for more than 90% of embryos and is > 95% accurate (Seidel, 1999). However, determination of embryo sex by PCR is inefficient. All embryos are biopsied, tested, and then approximately 50% of the undesired sexes are discarded. Costs of donor board, superovulation and collection have to be carried by a small number of embryos.

Genome resource banking:

Oocytes and embryos:

Embryo cryopreservation and storage allows conservation of the full genetic complement of the sire and dam and thus has enormous potential for protecting and managing species and population integrity and heterozygosity. The differences among embryos in cryosensitivity are substantial, as demonstrated by the variance between the freezable bovine compared with the difficult to freeze swine embryos (Nieman and Rath, 2001). Conventional freezing and thawing procedures for embryos are time-consuming and require the use of biological freezers and a

microscope. Complicated embryos freezing procedures may soon be replaced by a relatively simple procedure called vitrification. The greatest advantage of vitrification is its simplicity. The situation for oocyte cryopreservation, there has been significant progress in the cryopreservation of oocytes from laboratory animals (Stachecki and Cohen, 2004; Albarracin *et al.,* 2005; Cetin and Bastan, 2006). These studies have made substantial progress in using ultra rapid freezing protocols for retaining the stability of oocyte cytoskeleton. Finally for making the embryo or oocytes banks a practical reality, there is a need to understand the fundamental cryobiological factors that determine embryo and oocyte viability and functionality before and after cryopreservation for virtually every individual endangered species.

Tissue graft banks:

The cryopreservation and subsequent use of gonadal tissue offers fascinating opportunities. This has particularly been the case since the news of a live birth following transplantation of cryopreserved ovarian tissue in humans). Recent developments in the autografting and xenografting of ovaries and testes clearly demonstrate the potential value of cryopreserving gonadal tissue (Oktay and Yih, 2002; and Tibary *et al.,* 2005). Thawed ovarian tissue has been transplanted into conspecific recipients in the mouse, sheep (Demirci *et al.,* 2003), and more recently humans (Donnez *et al.,* 2004), resulting in the birth of normal young.

Research is required now to identify suitable sources of cells which could be exploited for banking and future cloning-based conservation programs. Therefore, the establishment of worldwide tissue graft banks, to store reproductive/somatic tissue and cells collected opportunistically from threatened species, could be a milestone in conservation planning. This is particularly the case in situations where population numbers are critically low, other options have failed and conservationists are faced with the need to rescue all extant genetic diversity, including from dying neonates.

CONCLUSIONS

Progress in assisted reproductive technology for species will continue at a fast pace due to the necessity. In practice, current reproductive biotechnologies are species-specific and this is because of differences on basic reproduction like estrous cycle, seasonality, structural anatomy, gamete physiology and site for semen deposition or embryo transfer in species.

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التكنولوجيا المساعدة للتناسل في الثدييات

عبد الناصر أحمد محمد

قسم الإنتاج الحيوانى والدواجن، كلية الزراعة، جامعة أسيوط، أسيوط

يصف هذا المقال استخدام التقنيات الحيوية الحديثة في التناسل أو التقنيات المساعدة للتناسل مشتملة التلقيح الصناعي ، نقل الأجنة ، الإخصاب المعملي ، المعالجة الدقيقة للجاميطات والأجنة ، تجنيس السائل المنوي ، مصادر الجينوم البنكية ، نقل الخلايا الجسدية للبويضات (الاستنساخ) لأنواع الثدييات. تتيح هذه التقنيات الحصول علي نسل أكثر من الآباء المختارة مؤدية إلى زيادة الإنتاجية من اللبن واللحم ، نقص الفترة بين الأجيال ، معالجة الأمراض ، والمحافظة علي أنواع الثدييات من الانقراض. التقنيات الحيوية من الناحية العملية تخصصية لأنواع الحيوانات بسبب الاختلافات في دورة الشبق ، التركيب التشريحي للجهاز التناسلي ، فسيولوجيا الجاميطات، مكان وضع السائل المنوي في التلقيح الصناعي ، طريقة نقل الأجنة.