

# Advances in Embryo Transfer

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## 1. Introduction

Embryo transfer refers to a step in the process of assisted reproduction in which one or several embryos are placed into the uterus of a female with the intent to establish a pregnancy. Currently this biotechnology has become one of the prominent high businesses worldwide. This technique, which is often used in connection with *in vitro* fertilization (IVF), has widely been used in animals or human, in which situations the goals may vary. In animal husbandry, embryo transfer has become the most powerful tool for animal scientists and breeders to improve genetic construction of their animal herds and increase quickly elite animal numbers which have recently gained considerable popularity with seedstock dairy and beef producers. In human, embryo transfer technique has mainly been used for the treatment of infertile couples to realize their dream to have their children. The history of the embryo transfer procedure goes back considerably farther, but the most modern applicable embryo transfer technology was developed in the 1970s (Steptoe and Edwards, 1978). In the last three decades, embryo transfer has developed into a specific advanced biotechnology which has gone through three major changes, "three generations"---the first with embryo derived from donors (*in vivo*) by superovulation, non-surgical recovery and transfer, especially in cattle embryos, the second with *in vitro* embryo production by ovum pick up with *in vitro* fertilization (OPU-IVF) and the third including further *in vitro* developed techniques, especially innovated embryo micromanipulation technique, which can promote us to perform embryo cloning involved somatic cells and embryonic stem cells, preimplantation genetic diagnosis (PGD), transgenic animal production etc. At the same time, commercial animal embryo transfer has become a large international business (Betteridge, 2006), while human embryo transfer has spread all over the world for infertility treatment. Just only in the United States of America there are 442 assisted reproductive technology (ART) centers with IVF programs in 2009 report of the Centers for Disease Control and Prevention. Embryo transfer, besides male sperm, involves entire all process from female ovarian stimulation (start) to uterine receptivity (end). During this entire process, many new bio-techniques have been developed (Figure 1). These techniques include optimal ovarian stimulation scheme, oocyte picking up (OPU) or oocyte retrieval, *in vitro* maturation (IVM) of immature oocytes, *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI), preimplantation genetic diagnosis (PGD), blastocyst embryo culture technology, identification of optimal uterine environment etc. Here, we will review some key newly developed biotechnologies on human embryo transfer.

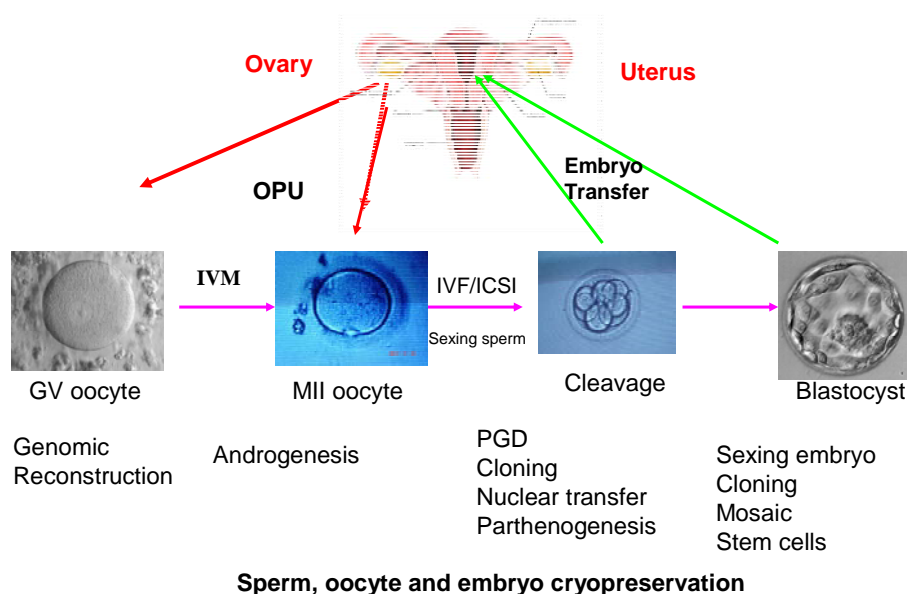


Fig. 1. Schematic representation of main embryo biotechnologies which can involve from ovary to uterus.

## 2. Ovarian stimulation technique

So far we have known that female ovary at birth has about 2 million primordial follicles with primary oocytes and at puberty ovary has 300,000 to 400,000 oocytes. From puberty, oocytes start frequently to grow, mature and ovulate from ovaries under endocrine hormone stimulation, such as follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol, but normal fertile woman usually ovulate only an oocyte per menstrual cycle. This will ensure women to be able to have a normal single baby pregnancy. However, in an attempt to compensate for inefficiencies in IVF procedures, patients need to undergo ovarian stimulation using high doses of exogenous gonadotrophins to allow retrieval of multiple oocytes in a single cycle. Current IVF stimulation protocols in the United State generally involve the use of 3 types of drugs: 1) a medication to suppress the LH surge and ovulation until the developing eggs are ready, GnRH-agonist (gonadotropin releasing hormone agonist) such as Lupron and GnRH-antagonist such as Ganirelix or Cetrotide; 2) FSH product (follicle stimulating hormone) to stimulate development of multiple eggs such as Gonal-F, Follistim, Bravelle, Menopur; 3) HCG (human chorionic gonadotropin) to cause final maturation of the eggs. The use of such ovarian stimulation protocols enables the selection of one or more embryos for transfer, while supernumerary embryos can be cryopreserved for transfer in a later cycle (Macklon et al. 2006). Currently the standard regimen procedures for ovarian stimulation have been set up in all IVF centers (Santos, et al., 2010) in which almost centers use exogenous gonadotrophins as routine procedure to stimulate patients' ovaries to obtain multiple eggs. This technique works very well in most patients. Thus, this leads to setting up many drug companies to produce all kind of

stimulation drugs for human assisted reproductive technology (ART). However, evidences of recent several year studies have showed that ovarian stimulation may itself have detrimental effects on oogenesis, embryo quality, uterus endometrial receptivity and perhaps perinatal outcomes. The retrieval oocyte number is also considered to be an important prognostic variable in our routine IVF practice (see Chapter 3 in this book). Also, if this stimulation scheme produces too many eggs, it often results in hyperstimulation syndrome. Standard IVF requires the administration of higher dosages of injectable medications to stimulate the growth of multiple eggs. These medications are expensive and are associated with certain potential health risks. Careful monitoring must be performed to ensure safety and efficacy. While these factors lead to increased costs and time commitment for the patient, the result is an increased number of embryos available for transfer. Additionally, in standard or traditional IVF, cryopreservation of surplus embryos for transfer in a non-stimulated cycle may be available. The overall expected take-home baby rate with standard IVF varies considerably, based primarily upon the patient's age. Recently some IVF centers have begun to use natural or minimal stimulation on IVF and obtained good results.

The recent popularity of Mini-IVF (minimal stimulation IVF), Micro-IVF, natural cycle for IVF, oocyte *in vitro* maturation (IVM) has attested to the changes taking place in the practice of advanced reproductive technologies (Edward, 2007). This technique has some advantage and has good future use. A common feature all of these procedures share is the use of less infertility medications. The reduction in medication use compared to a normal IVF cycle ranges from a 50% to a 100% reduction. If the less medication is used, the less monitoring is required (blood test and ultrasounds). The amount of reduction in monitoring depends on the procedure being done and the philosophy of the practice. For most of these approaches, fewer eggs are involved, which may mean there is less work for the laboratory to do. Some programs will discount their routine laboratory charges compared to regular IVF and patients may pay less expense (see Chapter 2). Also, the most significant risk of routine IVF, severe ovarian hyperstimulation syndrome, could be decreased in all of these procedures or completely eliminated in some pure natural IVF cycles and programmed IVM cycles (Tang-Pedersen et al., 2012). This can be very important for some women with severe polycystic ovary syndrome (PCOS) who are at increased risk for significant discomfort or even (rarely) hospitalization with routine IVF approaches. However, this technique needs more times of oocyte retrieval and results in a lower pregnancy rate per egg retrieval cycle because only one or a very few eggs may be retrieved per cycle. Thus, this technique may be used in some specific woman populations, such as younger women less than 30 years old or aged women over 40 years old. In this book, the impact of ovarian stimulation and underlying mechanisms will be reviewed and some strategies for reducing the impact of ovarian stimulation on IVF outcomes are also addressed. (Please further read Chapter 2 in this book).

As describing above, ovarian hyperstimulation syndrome (OHSS) usually occurs as a result of taking hormonal medications that stimulate oocyte development in woman's ovaries. In OHSS, the ovaries become swollen and painful and its symptoms can range from mild to severe. About one-fourth of women who take injectable fertility drugs get a mild OHSS form, which goes away after about a week. If woman becomes pregnant after taking one of these fertility drugs, her OHSS may last several weeks. A small proportion of women taking fertility drugs develop a more severe OHSS form, which can cause rapid weight gain, abdominal pain, vomiting and shortness of breath. In order to prevent OHSS occur, some

effective steps should be taken. If the OHSS has happened, specific treatment should be guided by the severity of OHSS. The aim of the treatment is to help relieving symptoms and prevent complications. Chapter 4 of this book has given a detail review about OHSS diagnosis, prevention and treatment.

### 3. Advances in insemination and *in vitro* fertilization technology

Embryo development begins with fertilization. Prior to fertilization, both the oocytes and the sperm must undergo a series of maturational events to acquire their capacity to achieve fertilization. Much research in this area has been geared toward improving reproductive efficiencies of farm animals and preserving endangered species. An important milestone of embryo transfer is *in vitro* fertilization (IVF). In animals, IVF has offered a very valuable tool to study mammalian fertilization and early embryo development. *in vitro* fertilization is a process by which retrieval oocytes fertilized by sperm outside the body, *in vitro*. IVF is a major treatment in infertility when other methods of assisted reproductive technology have failed. This technique has become a routine procedure and widely been used in human infertile treatment all over the world. IVF was initially created to help those women whose fallopian tubes were blocked not allowing for fertilization to occur. Over the years through, this technique has become very efficient in achieving pregnancies for several other situations including unexplained infertility. So far many infertile couples may obtain their dreamed children by IVF technique. However, IVF isn't just for issues relating to women. Male sperm amount and quality has a determined effect on egg fertilization. Sperm dysfunction is associated with the inability of sperm to bind and penetrate the oocyte zona pellucida. During last two decades, micromanipulation techniques have undergone some major developments which include partial zona dissection (PZD), subzonal sperm injection (SUZI) and intracytoplasmic sperm injection (ICSI). These methods greatly improve oocyte fertilization rate. More importance is that ICSI technique can solve sever male infertility problem including 1) complete absence of sperm (azoospermia); 2) low sperm count (oligozoospermia); 3) abnormal sperm shape (teratozoospermia); 4) problems with sperm movement (asthenozoospermia); 5) completely immobile sperm (necrozoospermia). To further review the development of these technologies, four chapters about sperm treatment have been listed in this book.

Firstly, some basic knowledge of sperm physiology has been described and evaluation of male infertility has been discussed (Chapter 5). Secondly, the sperm chromosomal abnormality has a significant effect on embryo quality and pregnancy rate. Even so it may result in a lot of miscarriage after pregnancy. Currently there are many researches about sperm abnormality including sperm chromosomal examination, sperm DNA fragmentation analysis. Thus, a chapter about meiotic chromosome abnormalities and spermatid FISH in infertile patients with normal karyotype has listed (Chapter 6). This chapter indicates that the incidence of spermatid aneuploid in the infertile population is as three times as in the fertile population and using FISH technique may diagnosis testicular sperm meiosis. This is a very interesting result. Thirdly, in the recent years, ICSI technique has experienced a great development to intracytoplasmic morphologically-selected sperm injection (IMSI) and a method for selection of hyaluronan bound sperm for use in ICSI (PICSI) (Parmegiani et al., 2010a,b; Said & Land 2011; Berger et al., 2011). These innovations have significantly increased egg fertilization and pregnancy rates in many IVF clinics. The major aim of these

techniques is to select a normal good spermatozoon without any dysfunction for ICSI to obtain a good quality embryo for transfer. Oligozoospermic men often carry seminal populations demonstrating increased chromosomal aberrations and compromised DNA integrity. Therefore, the *in vitro* selection of sperm for ICSI is critical and directly influences the paternal contribution to preimplantation embryogenesis. Hyaluronan (H), a major constituent of the cumulus matrix, may play a critical role in the selection of functionally competent sperm during *in vivo* fertilization (Parmegiani et al., 2010a). Hyaluronan bound sperm (HBS) exhibit decreased levels of cytoplasmic inclusions and residual histones, an increased expression of the HspA2 chaperone protein and a marked reduction in the incidence of chromosomal aneuploidy. The relationship between HBS and enhanced levels of developmental competence led to the current clinical trial (Worrilow et al., 2010). Thus, as a HBS test, a PCISI technique, a method for selection of hyaluronan bound sperm for use in ICSI, has been developed to treat oligozoospermic and asthenozoospermic man infertility problem (Parmegiani et al., 2010b). Additionally, recent advanced intracytoplasmic morphologically-selected sperm injection (IMSI) technique has been used to treat sperm morphology problem (teratozoospermia). These techniques have begun to be used in some IVF centers. Thus, some detail technologies for sperm selection have been reviewed in chapter 7.

Finally, the most sever cases of male infertility are those presenting with no sperm in the ejaculate (azoospermia). Some men have a condition where their reproductive ducts may be absent or blocked (obstructive azoospermia or OA), where others may have no sperm production with normal reproductive anatomy (non-obstructive azoospermia or NOA). Azoospermia is found in 10% of male infertility cases. Patients with OA due to congenital bilateral absence of the vas deferens or those in whom reconstructive surgery fails have historically been considered infertile. Men who can not produce sperm in their testes with apparent absence of spermatogenesis diagnosed by testicle biopsy are classified as NOA. Once testicular and epididymal function can be verified, surgery is justified to correct or remove the blockage. Current optimal method for treatment of azoospermic men is to acquire sperm from testicles or epididymides by means of surgery or non-surgery (Wu et al., 2005). However, in some situations, no any mature spermatozoon can be obtained from either semen or surgical testicular biopsy tissues. Thus immature haploid spermatids or diploid spermatocytes or spermatogonia, or even somatic cells like Sertoli cell nuclei or Leydig cells may also be considered as a sperm to transfer paternal DNA into maternal oocyte to form embryo for transfer. In the chapter of advances in fertility options of azoospermic men (Chapter 8), the optimal applications of testicular biopsy sperm, round or elongated spermatids from azoospermic men to human IVF have been discussed and some new technologies to produce artificial sperm from stem cells and somatic cells as well as sperm cloning have been designed. Application of these technologies will make no sperm men realize their dream to have a child.

#### 4. Procedure for embryo transfer

The procedure of embryo transfer is very crucial and great attention and time should be given to this step. The embryo transfer procedure is the last one of the *in vitro* fertilization process and it is a critically important procedure. No matter how good the IVF laboratory culture environment is, the physician can ruin everything with a carelessly performed embryo transfer. The entire IVF cycle depends on delicate placement of the embryos at the

proper location near the middle of the endometrial cavity with minimal trauma and manipulation. The ultimate goal of a successful embryo transfer is to deliver the embryos atraumatically to the uterine fundus in a location where implantation is maximized.

The transfer of embryos can be accomplished in several different fashions including transfallopian (ZIFT), transmyometrial and transcervical ways. Today the majority of embryo transfers are performed via the cervical canal into the uterine cavity by a specific catheter. In order to optimize the embryo transfer technique, although Mansour and Aboulghar (2002) had a good review paper about embryo transfer procedure, two chapters of this book indicated that several precautions should be taken (Chapter 9 and 10). The first and most important is to avoid the initiation of uterine contractility. This can be achieved by the use of soft catheters, gentle manipulation and by avoiding touching the fundus. Secondly, proper evaluation of the uterine cavity and utero-cervical angulation is very important, and this can be achieved by performing dummy embryo transfer and by ultrasound evaluation of the utero-cervical angulation and uterine cavity length. Another important step is the removal of cervical mucus so that it does not stick to the catheter and inadvertently remove the embryo during catheter withdrawal. Finally, one has to be absolutely sure that the embryo transfer catheter has passed the internal cervical os and that the embryos are delivered gently inside the uterine cavity.

Embryo stage for transfer also has an important influence on IVF pregnancy outcome. As we know, the time and number of transfer embryos have an obvious effect on pregnancy. Current IVF technique may make many infertility couple to realize their dream to have children, but many treated patients by IVF program have multiple pregnancy problems which present a serious perinatal risk for mother and child. This is mainly due to the transfer of three or four early cleavage stage embryos. In order to reduce multiple pregnancies, the best way is to transfer single embryo. However, this will greatly decrease pregnancy rate. Many studies have showed that good quality embryo on morphology will have a high chance for implantation, especially good blastocyst stage embryo for transfer. Thus, prolonged cultivation of embryos to the blastocyst stage has become a routine practice in the human *in vitro* fertilization program (IVF) since the first commercial sequential media were developed in 1999. The advantage of blastocyst culture is able to select the activated genome embryos (Braude et al., 1988) which have higher predictive values for implantation on the basis of their morphological appearance as compared with earlier embryos (Gardner and Schoolcraft, 1999; Kovačič et al., 2004) and in a reduction in the number of transferred embryos without compromising pregnancy rate (Gardner et al., 2000). Also transfer of blastocyst stage embryos is matching better synchronized with endometrial receptivity for embryo implantation. Interestingly, Kovačič et al's studies (see chapter 11) have showed that single or double blastocyst transfer results in similar pregnancy rates in young patient groups, but the twin rate remains unacceptably high after the transfer of two blastocysts, especially if at least one of them is morphologically optimal. Thus, based on evaluation of blastocyst embryo morphology, single embryo transfer is feasible for young couple patients so as to prevent multiple pregnancies in IVF program.

Also, frozen/thawed embryo transfer (FET) has become a routine procedure in all IVF centers throughout the world. This treatment involves implanting embryos that were retrieved from the patient during a previous IVF cycle and held safely in a frozen state. However, FET often results in lower pregnancy rate than fresh embryo transfers. This is

because freezing and thawing may damage the morphological characteristics of embryos and survival rate of embryo blastomeres resulting into lower implantation rates. Thus, the evaluation after embryo thawing and transferring one or several real alive embryos will greatly improve pregnancy rate. In chapter 12, a simple research report has showed a pregnancy comparison following transfer of cultured versus non cultured frozen thawed human embryos. This study provides a feasible method to determine embryo alive after embryo thawing by overnight embryo culture to select the embryos with blastomere cleavage for transfer. Transferring cleaving embryos after embryo frozen and thawing will significantly increase pregnancy rate (Joshi et al., 2010).

So far, there is still a contradictory whether intercourse is encouraged or not after embryo transfer. A large of randomized control trials suggest that intercourse around the time of embryo transfer improve embryo implantation rates and increase pregnancy rate, but some studies showed no significant difference. Chapter 13 examines the available evidences suggesting why intercourse is beneficial or harmful to assisted reproductive technique outcome.

## 5. Embryo implantation and endometrial receptivity

After transfer procedure, embryo will continue growing and finally hatching out from zona pellucida to start implantation in the uterus. Thus, implantation is the final frontier to embryogenesis and successful pregnancy. Over the past three decades, tremendous advances have made in the understanding of human embryo development and its implantation in the uterus. Implantation is a process requiring the delicate interaction between the embryo and a receptive endometrium. This interactive process is a complex series of events that can be divided into three distinct steps: apposition, attachment and invasion (Chapter 15, Norwitz et al., 2001). This intricate interaction requires a harmonized dialogue between embryonic and maternal tissue. Thus, implantation represents the remarkable synchronization between the development of the embryo and the differentiation of the endometrium. As long as these events remain unexplained, it is very difficult to improve the success of IVF treatment. In last few years, many researches have focused on both enhancing the quality of the embryos and understanding the highly dynamic tissue of the endometrial wall (Horne et al., 2000) because there is a close relationship between endometrial receptivity and embryo implantation. Not only does woman pregnancy depend on embryo quality, but also it depends on uterine receptivity because uterine endometrium must undergo a serious changes leading to a short time for embryo implantation called the "implantation window". Outside of this time the uterus is resistant to embryo attachment. How to determine this window time is very important for obtaining a high pregnancy rate. Determining molecular mechanisms of human embryo implantation is an extremely challenging task due to the limitation of materials and significant differences underlying this process among mammalian species. Recently some papers have reviewed some adhesion molecules in endometrial epithelium during tissue integrity and embryo implantation (Singh and Aplin, 2009) and the trophinin has been identified as a unique apical cell adhesion molecule potentially involved in the initial adhesion of trophoctoderm of the human blastocyst to endometrial surface epithelia (Fukuda, 2008). In the mouse, the binding between ErbB4 on the blastocyst and heparin-binding epidermal growth factor-like growth factor on the endometrial surface enables the initial step of the blastocyst implantation. L-selectin and its ligand carbohydrate have been proposed as a system that mediates initial

adhesion of human blastocysts to the uterine epithelia. The evidence suggests that L-selectin and trophinin are included in human embryo implantation and their relevant to the functions and these cell adhesion mechanisms in human embryo implantation have been described (Fukuda, 2008). Interestingly, some important biomarkers including essential expression of proteins, cytokines and peptides can be detected in the uterine endometrium during embryo implantation (Aghajanova et al., 2008). Also, human cumulus cells may be used as biomarkers for embryo and pregnancy outcomes (Assou et al., 2010). Thus, this book selected two very interesting papers about mechanism of embryo implantation (Chapter 14, and 15). These two articles have explored the mystery of the mechanisms controlling the receptivity of the human endometrium. About 20 biomarkers have been described and studied to distinguish embryo implantation window time as days 20-24 of menstrual cycle. This is very interesting to determine embryo transfer time and to improve pregnancy rate. Additionally, screening for receptivity markers and testing patients accordingly may allow for increasing use a single embryo transfer.

From a clinical point of view, the repeated implantation failure is one the least understood causes of failure of IVF. The causes for repeated implantation failure may be because of reduced endometrial receptivity, embryonic defects or multifactorial causes. Various uterine pathologies, such as thin endometrium, altered expression of adhesive molecules and immunological factors, may decrease endometrial receptivity, whereas genetic abnormalities of the male or female, sperm defects, embryonic aneuploidy or zona hardening are among the embryonic reasons for failure of implantation. Endometriosis and hydrosalpinges may adversely influence both. Recent advances into the molecular processes have delineated possible explanations why the embryos fail to implant. Our selected chapters also have a detail description about embryo implantation failure and some feasible treatment methods have been recommended.

## 6. Fertility cryopreservation

Fertility cryopreservation is a vital branch of reproductive science and involves the preservation of gametes (sperm and oocytes), embryos, and reproductive tissues (ovarian and testicular tissues) for use in assisted reproduction techniques. The cryopreservation of reproductive cells is the process of freezing, storage, and thawing of spermatozoa or oocytes. It involves an initial exposure to cryoprotectants, cooling to subzero temperature, storage, thawing, and finally, dilution and removal of the cryoprotectants, when used, with a return to a physiological environment that will allow subsequent development. Proper management of the osmotic pressure to avoid damage due to intracellular ice formation is crucial for successful freezing and thawing procedure. So far there are two major techniques for reproductive cell or tissue cryopreservation: slow program frozen-thawing processes and vitrification method. Slow program has widely used in many IVF programs for a long time and it has been proved to be a feasible practice for human and other animal sperm and embryo freezing. In the last decade, many scientists and embryologists are more interested in vitrification method because this technique may freeze oocytes and embryos with an ultra-fast speed to avoid ice formation within cell during cryopreservation. Thus, it may save freezing time and obtain a higher survival rate. In order to understand and apply these two methods to human and other animal IVF program, a detail review on reproductive cell cryopreservation including sperm, oocyte, embryo and testicular/ovarian biopsy tissues has been included in this book (Chapter 16).



## 7. Future use of newly developed embryo transfer technologies

As our description in introduction section, embryo transfer has experienced three major changes, "three generations." In the human, major application of these techniques focus on the second stage where *in vitro* embryo production is performed by ovum pick up with *in vitro* fertilization (OPU-IVF) for infertile couple treatment. However, the third stage including further developed techniques, especially innovated embryo micromanipulation techniques, can promote us to perform various embryo manipulation including cloning involved somatic cells and embryonic stem cells, preimplantation genetic diagnosis (PGD), transgenic animal production etc. As Figure 1 showed, early oocytes may be obtained by current *in vitro* culture of ovarian tissue and primordial germ cells (PGCs), because female PGCs may become oogonia, which are mitotically divided several times in the ovaries and enter the prophase of first meiosis (Eppig et al., 1989). In the germinal vesicle stage (GV), oocyte reconstruction may be conducted by the nuclear transfer technique (Takeuchi et al., 1999). In higher organisms including humans, both nucleus and mitochondria contain DNA. Mitochondrion is located outside the nucleus in the cytoplasm and is an organelle responsible for energy synthesis. Oocyte contains rich mitochondria in a large amount of cytoplasm (Spikings et al., 2006). In normal sexual reproduction, offspring inherit their mitochondrial DNA from the mother. This type of inheritance pattern is generally known as maternal inheritance. When the mother passes defective mitochondria to the child, fatal heart, liver, brain or muscular disorders can result. In order to prevent this genetic disease, getting rid of mother defective mitochondrial DNA, mother nuclear DNA may be transferred into a normal enucleated ovum provided a third donor (Figure 2). The purpose of the donation of an enucleated cell is to provide the child with non-defective mitochondria, from a woman other than the mother. This results in a three-parent embryo. Its nucleus is formed by the fusion of sperm and mother's oocyte nucleus, and its cytoplasm is provided by the enucleated donor cell. Thus, this child has the inheritance of DNA from three different sources, the nuclear DNA is from his father and mother, and his mitochondrial DNA is mainly through the donor (Zhang et al., 1999).

Also, some aged women can not produce normal fertilization eggs and well-development embryos. The major problem is that the aged egg lacks synthesizing some components of maturation promoter factors, such as cyclin B, *c-Mos* proto-onco protein, cytostatic factor (Wu et al., 1997a, b). Thus, the new developed technique of oocyte (egg) reconstruction including nuclear transfer and cytoplasm replace may increase age woman pregnancy opportunity. Nuclear transfer is to transfer an age woman nucleus into young woman enucleated egg so that aged woman nucleus may complete a normal meiosis (Figure 2). The cytoplasm nuclear transfer (Figure 3) may replace partly aged oocyte cytoplasm with younger oocyte cytoplasm by transferring part of one woman's egg into another's (Cohen, 1998). In this case, the healthy portion of a donor egg (the cytoplasm) may supplement the defective portion of the infertile recipient's egg and to help it survive, hence making one good egg. Thus, the infertile woman's genetic legacy is preserved because the nucleus of this egg is made available from the infertile woman and the donor cytoplasm (which simply contains mitochondrial DNA that gives the egg energy to survive) contributes only one percent of the embryo's genetic makeup. Once the egg is fertilized, the embryo is implanted in the infertile woman's uterus. Unfortunately, after many babies were born in the U.S. using human cytoplasmic transfer (HCT), ethical and medical complications spurred the

U.S. government to curtail the procedure in 2001. Today, fertility scientists must file an investigational clinical trial application to continue research in this area, and the New Hope Fertility Center in New York intends to obtain approvals and continue our research. It is likely that with continued research this technique may prove its efficacy and safety in the future. It should be understood that the methods of the present invention are applicable to non-human species and, where the law permits, to humans.

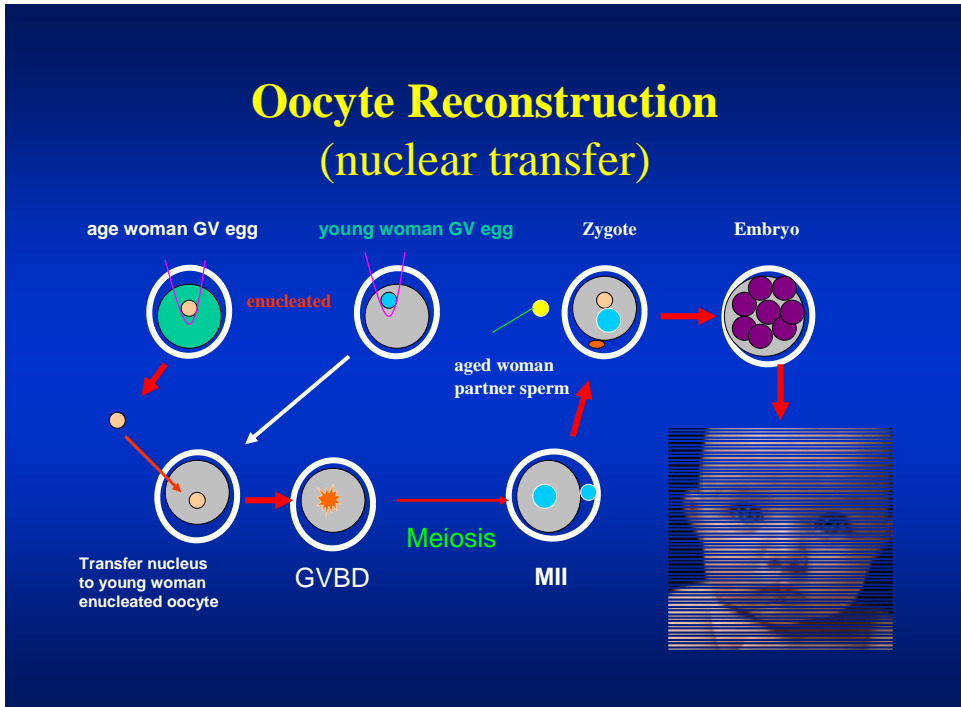


Fig. 2. Scenario for oocyte reconstruction by nuclear transfer technique. An aged woman oocyte nucleus is transferred into a young woman enucleated oocyte so that young woman immature oocyte will induce aged woman nucleus to complete meiosis during oocyte maturation. Then the aged woman husband sperm will be injected into this reconstructed oocyte to form a normal embryo for transfer.

Further, during oocyte *in vitro* maturation (IVM) and *in vitro* fertilization (IVF), some techniques such as sperm sexing, oocyte activation, parthenogenesis have been developed and applied in animal researches and human infertility treatment. Sex selection is the attempt to control the sex of the offspring to achieve a desired sex animal. It can be accomplished in several ways, including sperm sex selection and preimplantation embryo sex selection. A number of reviews have addressed the use of sexed semen in cattle (Seidel and Garner, 2002; Seidel, 2003). The current successful method for separating semen into X- or Y-bearing chromosome sperm is to use flow cytometry to sort sperm for artificial insemination or IVF (DeJarnette et al. 2007, Blondin et al., 2009). However, in human treatment, sex selection seems to have ethical problem. Thus, sperm sexing may be used in

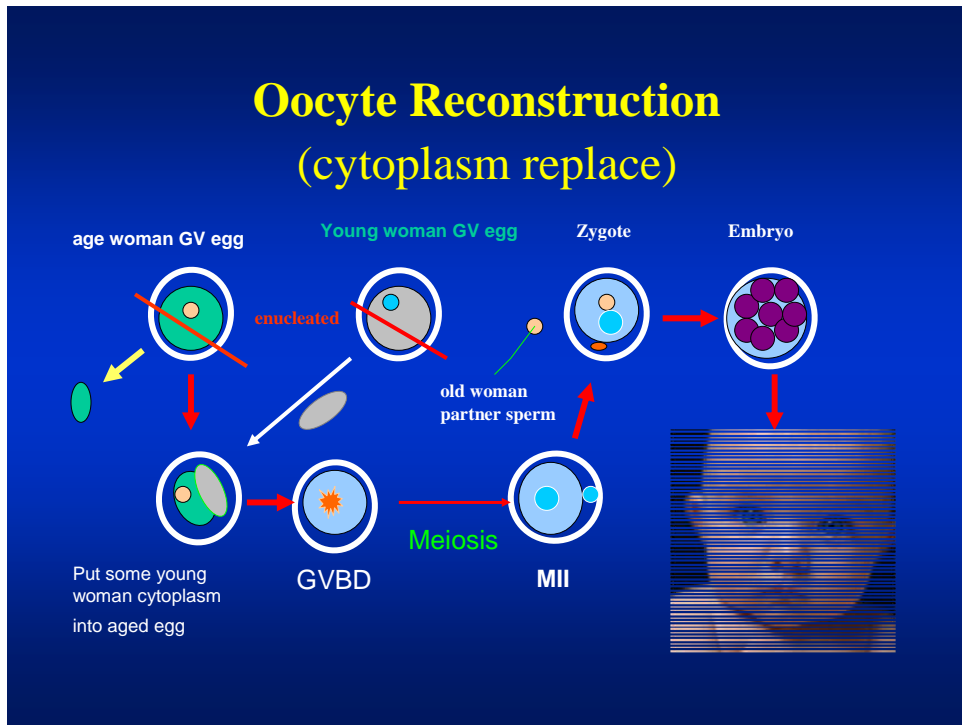


Fig. 3. Scenario for oocyte reconstruction by partly cytoplasm replace technique. Firstly small amount cytoplasm of aged woman oocyte is removed out and partly young woman oocyte cytoplasm will be transferred into this oocyte so that young woman oocyte cytoplasm will induce aged woman nucleus to complete meiosis during oocyte maturation. Then the aged woman husband sperm will be injected into this reconstructed oocyte to form a normal embryo for transfer.

related x-chromosome disease inherit treatment. One major limitation of sperm sexing is low efficient for low sperm motility. Oocyte activation may increase oocyte fertilization or result in parthenogenesis. Combining with reliable nuclear transfer method, this oocyte activation may produce pathenogenetic bimaternal embryos (Kawahara et al., 2008) or androgenetic bipaternal embryos (Wu and Zan, 2011, Tesarik 2002). In the chapter 9 of this book, the scenario for using immature oocyte to induce male somatic cell complete meiosis has been described. The nucleus of immature oocyte is removed and a male diploid cell was injected to this enucleated oocyte. After completing meiotic division, the induced haploid nucleus was transferred into normal female mature oocyte to form a biparental embryo for transfer. Also, a scenario for sperm genome cloning technique is displayed in this chapter. A single sperm is injected into enucleated oocyte and this oocyte goes through a parthenogenesis process to become a 4-8 cell haploid embryo. A single blastomere is transferred into a normal mature oocyte to form a zygote. The developed embryos are transferred to recipient mice to deliver offspring. Also, nuclear transfer studies have shown that nuclei from not growing oocytes have already been competent to mature into MII stage

when transferred into fully grown germinal vesicle-stage oocytes. However, the resultant oocytes lack developmental competence, and nuclei from oocytes more than 65  $\mu\text{m}$  in diameter first become competent to support term development after fertilization *in vitro* (Niwa et al., 2004).

After the fertilization, the zygote will be formed and two obvious pronuclei could be observed at this stage. The genetic manipulation of the pronuclear stage embryo has resulted in two fundamental discoveries in reproductive biology (Wilmut et al., 1991). By pronuclear removal and exchanges, the principle of genetic imprinting has been convincingly demonstrated. By injecting foreign DNA into one of the two pronuclei of the zygote, the resulting offspring may contain a functional foreign gene in the genome, known as transgenesis. Production of transgenic animals has great application in agriculture and medicine (Niemann and Kues 2003). In agricultural animals, the transgenic technology may be applied to develop lines of animals for faster growth, higher quality beef products or disease resistance (Greger 2010). Transgenic practices of last decade have proved that by inserting a single growth regulating gene into an animal of agricultural value, animal growth rate and feed efficiency could be greatly increased and fat deposition could be obviously reduced. This technique has been transforming the entire meat animal industry (Wheeler, 2007). In human, it is possible to target genetic sequences into predetermined sites in the host DNA, to transfer a given gene for some genetic disease therapy.

Animal cloning may involve embryo cloning and adult somatic cell cloning. During early development before 8-cell stage, embryonic cells may be dis-aggregated into individual blastomeres. Each blastomere has a totipotency which is able to potentially to develop into a viable embryo following nuclear transfer and to regenerate whole new individuals, this is, cloning. Also, embryo division is a kind of cloning and it may produce identical twin. In the human IVF, one blastomere often is removed from embryo for genetic diagnosis to examine some genetic disease and sex determination by fluorescent *in situ* hybridization (FISH) technique or polymerase chain reaction (PCR). The biopsied embryo could develop normal fetus and deliver health babies. Also, the process of freezing and thawing can be fairly harsh on the embryos and often not all of the cells or embryos survive. After freezing and thawing, one or several lysed blastomeres often occur in some embryos and these damaged cells are thought to either disrupt the development of the embryo or produce negative factors as they degenerate to affect survival blastomere growth. Recently new technique attempts to remove these lysed cells from embryo by making a small hole in the zona pellucida with acid or laser. The removal of lysed cells will restore the embryo's developmental potential. Cell number and morphology was also significantly improved compared with embryos without lysed cell removal (Elliott et al., 2007). This method has been shown to dramatically increase the implantation potential of human embryos and pregnancy rate (Nagy et al., 2005).

Also, many data showed a significant negative correlation between the degree of embryo fragmentation and rate of blastocyst development (Eftekhari-Yazdi et al., 2006). As above method, this fragmentation of embryo also may be removed. Some studies have indicated that the removal of fragmentation from fresh embryo on day 3 may increase the rate of blastocyst development (Alikani et al., 1999; Eftekhari-Yazdi et 2006).

At the blastocyst stage, two distinct cell lines in the embryos may be observed, the inner cell mass (ICM) and the trophectoderm (TE) cells. Inner cell mass cells are totipotent stem cells

which will give rise to all different tissues in the fetus. By *in vitro* culturing ICM cells, the lines of embryonic stem (ES) cells have been developed. ES cells have the ability to remain undifferentiated and proliferate indefinitely *in vitro* while maintaining the potential to differentiate into derivatives of all three embryonic germ layers. Thus, combining cloning and nuclear transfer technique, the specific stem cells may be produced from ICM of embryos. Recent achievement showed that completely differentiated cells (both fetal and adult) may be reprogrammed to return to multipotential embryonic cells, that is the induced pluripotent stem cells (iPSCs) with qualities remarkably similar to embryonic stem cells-like state by being forced to express genes and factors important for maintaining the defining properties of embryonic stem cells (Takahashi and Yamanaka 2006; Yu et al., 2007). This discovery has created a valuable new source of pluripotent cells for drug discovery, cell therapy, and basic research.

For more than a decade, preimplantation genetic screening (PGS) and PGD have been used to assist in the identification of aneuploid embryos on Day 3. However, current strategies, based upon cell biopsy followed by FISH, allow less than half of the chromosomes to be screened. Currently, the FISH technique has gradually been replaced by the competitive genomic hybridization (CGH) or microarray analysis (Wells, et al., 2008). This analysis can evaluate all chromosomes by the trophoctoderm biopsies of blastocyst embryos, which may significantly reduce embryo harm than Day 3 embryo. Trophoctoderm biopsy involves removing some cells from the trophoctoderm component of an IVF blastocyst embryo. The removed cells can be tested for chromosome normality, or for a specific gene defect using PGD or preimplantation genetic screening test. Some microarray platforms also offer the advantage of embryo fingerprinting and the potential for combined aneuploidy and single gene disorder diagnosis. However, more data concerning accuracy and further reductions in the price of tests will be necessary before microarrays can be widely applied.

## 8. Conclusions

Not only has embryo transfer already been one of the prominent high businesses worldwide for animal breed genetic improvement and creating new animal breeds, but also it has become a major tool for the treatment of infertile couples to realize their dream to have their children. The new developed embryo biotechnology has been able to make no sperm (azoospermia) men realize their dream to have a child. In the meantime, the innovation of various technologies, such as ovarian optimal stimulation scheme, new developed ICSI techniques, ultra-sound guide embryo transfer, embryo selection, seeking uterus biomarkers, have greatly improved transfer embryo pregnancy rates. As new embryo culture method improved and PGD, PGS, CGH and microarray analysis techniques developed, a single good quality embryo may be chosen for transfer and multiply pregnancies may significantly be reduced. Also, embryo cryopreservation technique, especially vitrification, has greatly increased embryo survival after thawing and made a single egg retrieval have more opportunity for pregnancy. Newly developed technologies such as embryo cloning, nuclear transfer, transgenic animals, stem cells etc. have demonstrated great promises for application in agricultural and biomedical sciences. Currently, these technologies have been being or will be used in human infertility treatment. In the next decade, these technologies will not only greatly promote animal genetic improvement and create new animal breeds, but also significantly improve human reproductive health.

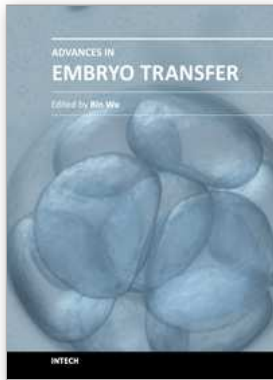
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Embryo transfer has become one of the prominent high businesses worldwide. This book updates and reviews some new developed theories and technologies in the human embryo transfer and mainly focus on discussing some encountered problems during embryo transfer, which gives some examples how to improve pregnancy rate by innovated techniques so that readers, especially embryologists and physicians for human IVF programs, may acquire some new and usable information as well as some key practice techniques. Major contents include the optimal stimulation scheme for ovaries, advance in insemination technology, improved embryo transfer technology and endometrial receptivity and embryo implantation mechanism. Thus, this book will greatly add new information for readers to improve human embryo transfer pregnancy rate.

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