Review Article

Roles of Assisted Reproductive Techniques in Mammals: Developmental Competence of Oocytes and Embryos

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Abstract | The assisted reproductive technologies or techniques (ARTs) have advanced greatly during the past couple of decades. The ARTs include several techniques including artificial insemination, *in vitro* embryo production, oestrous synchronization, superovulation, embryo transfer, germ cells or embryos' micromanipulation, sperm sexing, cryopreservation of ovarian tissues and germ cells and embryos, and genome resource banking for mammalian species. Such biotechnologies allow more offspring to be obtained from selected parents to increase milk and meat productivity, 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. Therefore, this review was conducted to collect and consolidate the current knowledge of assisted reproductive techniques of males and females in addition to their importance for oocyte maturation, artificial oocyte reprogramming, embryo development and their resulting newborns.

Keywords | *In vitro*, Oocyte, Embryo production, Embryo transfer, Micromanipulation

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INTRODUCTION

Assisted reproductive techniques (ART) is the application of field or laboratory techniques to gonads, germ cells and embryos for the purposes of increasing reproductive performance or treatment of reproductive dysfunctions (Wani and Skidmore, 2010; Wani et al., 2010; Mohammed and Attaai, 2011; Al-Zeidi et al., 2022a, b; Mohammed et al., 2022). Several millions of humans are treated of reproductive dysfunctions annually in the world through assisted reproductive techniques (Dyer et al., 2016; Kushnir et al., 2017).

Significant strides of development have made in assisted reproductive techniques during the past seventy years (Al-Zeidi et al., 2022a, b). This is a continuum, which started with artificial insemination. Artificial insemination application was greatly maximized with the advent of semen cryopreservation and synchronize estrus procedures in different species. Then, embryo transfer technique was arisen to produce offspring from females and males that were proven genetically superior (Seidel Jr and Seidel, 1991). Embryo transfer program enabled to shorten the interval between generations and multiply the number of species rapidly. Oestrous synchronization and superovulation protocols are necessitated for successful embryo transfer (Gordon, 2003; Mohammed et al., 2011). In addition, the procedures of *in vitro* embryo production including oocyte maturation, fertilization and culture were improved in addition to cryopreservation of oocytes and

embryos (Mohammed et al., 2005; Arav, 2014). Several techniques for fertilizing oocytes were developed as *in vitro* fertilization, subzonal sperm injection (SUZI), and intracytoplasmic sperm injection (ICSI) (Gordon, 2003). Semen and embryos' sexing enable getting either males or females offspring for meat and milk production, respectively, and avoiding the genetic diseases (Mohammed and Al-Hozab, 2016). Moreover, the progress of creating artificial oocytes through transfer of germ, embryonic and somatic cells enabled to solve the severe reproductive problems in different species (Mohammed et al., 2019a, 2022). The continuum progress of assisted reproductive techniques is helpful to livestock sectors and human medicine (Al-Zeidi et al., 2022a, b). Therefore, this review was designed to collect and consolidate the current knowledge of assisted reproductive techniques and their importance for oocyte maturation, artificial oocyte reprogramming, embryo development and their resulting newborns.

Assisted reproductive technology in males

Assisted reproductive techniques in males include semen collection, *in vitro* fertilization of oocytes in addition to intracytoplasmic sperm injection (ICSI), subzonal sperm injection (SUZI). Furthermore, genome resource banking is established to cryopreserve semen for hundreds of years (Ugur et al., 2019) (Figure 1).

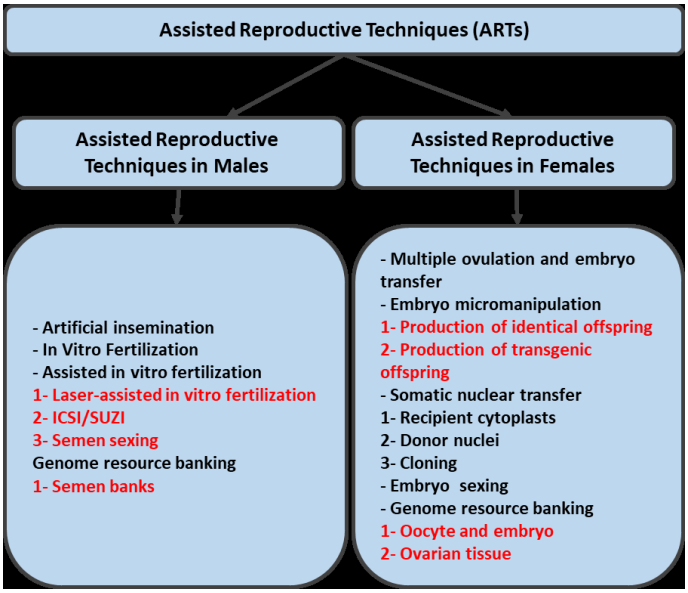

Figure 1: Assisted reproductive techniques in males and females. Intracytoplasmic sperm injection (ICSI), subzonal sperm injection (SUZI).

Artificial insemination

The first assisted reproductive technique primarily emerged was artificial insemination (AI), which simply includes semen collection from males and subsequently followed by introduction of the collected semen into physiologically receptive females of domestic and wild mammalian species (Manafi, 2011; Waberski, 2018). Artificial insemination in different mammalian species is an important technique for the genetic improvement, as the selected proven male can produce sufficient spermatozoa to inseminate hundreds or thousands of females annually compared to natural service, which provides insemination for tenth of females annually (Dalton et al., 2021).

Although there are several techniques for semen collection, most of them include artificial vagina and electro-ejaculation methods (Jiménez-Rabadán et al., 2012). The collected semen is then diluted according to species to maximize the number of services per collection or male. The dilution solution contains components or factors that help preserving and stabilizing the sperm in addition to antibiotics to inhibit microorganism growth and reduce the spreading of contamination (Jiménez-Rabadán et al., 2012). The collected semen is packaged in plastic straws and might be stored either cooled (5°C) or frozen in liquid nitrogen (-196°C). It appears that the frozen semen can remain frozen for hundred years and regain viability upon appropriate thawing. The most common technique employs for inseminating receptive females involving the use of disposable and sterile catheter that inserts vaginally and might be extended through the cervix into the body of uterus depending on the species (Manafi, 2011). The frozen semen with desirable genetic characters could be exported or imported to any place in the world. Furthermore, the advent of sperm sexing technique of collected semen enables to get male or female offspring after parturition (Wheeler et al., 2006; Palma et al., 2008; Blondin et al., 2009; Mohammed and Al-Hozab, 2016).

In vitro fertilization

In vitro fertilization refers to fertilizing oocytes using fresh or frozen-thawed spermatozoa through their incubation in fertilization medium (Mohammed et al., 2005; Chang et al., 2014). Spermatozoa need to be capacitated *in vitro* through swim-up or percoll-gradient techniques in order to penetrate the zona pellucida and fuse with the oocyte (Gordon, 2003; Mohammed et al., 2005; Volpes et al., 2016). Capacitation procedure results in a series of sperm changes including increased motility, calcium uptake and protein binding (Donà et al., 2020). The capacitated sperm are incubated with oocytes in fertilizing medium for approximately 8-22 hours, and the resulting fertilized oocytes are called zygotes (Greda et al., 2006). *In vitro* fertilization can be applied for matured oocytes, which collected from either slaughterhouse ovaries and matured *in vitro* or live females through ovum pick-up (Mohammed et al., 2005; Aller et al., 2010). The purposes of *in vitro* fertilization are to obtain embryos from terminal females (age, accident, disease, prepuberal, pregnant females) or commercial purposes. Commercial and research centers have used OPU-IVF in several categories including females age, species, reproductive status, aspiration frequency,

culture protocols (co-culture BRL cells, chemically defined media, serum) with different degree of successes (Blondin et al., 2002; Aller et al., 2010) (Tables 1 and 2). Several techniques could be used for assisted *in vitro* fertilization including laser-assisted *in vitro* fertilization (Hammoud et al., 2010; Taylor et al., 2010), cumulus cells removal (Mohammed, 2008; Mohammed et al., 2008, 2010), zona thinning or opening through Tyrode solution (Mohammed 2006, 2009a, b), subzonal sperm injection, intracytoplasmic sperm injection, and intracytoplasmic cell injection (Kimura and Yanagimachi, 1995; Gordon, 2003) (Figure 2). Semen sexing procedure is used for sex pre-selection and is determined the sex with accuracy more than 95% (Wheeler et al., 2006; Palma et al., 2008; Blondin et al., 2009; Mohammed and Al-Hozab, 2016). The sexed semen could be used for AI or IVF programs. There are several approaches proposed to preserve male genetic materials including germ cells, spermatozoa, cells and tissues (Johnston and Lacy, 1995). Semen banks are currently more developed for domestic and non-domestic species to facilitate the management and conservation of endangered species (Wildt et al., 1997; Comizzoli et al., 2000). Finally, *in vitro* fertilization of oocytes is considered a complement of an embryo transfer program (Zhao et al., 2019a, b).

Assisted reproductive technology in females

The mammalian male and female reproductive systems is in partnership to produce offspring (Figure 3). The abnormal functions in either male or female reproductive systems result in infertility, which necessitates the urgent need for assisted reproductive technologies (Gordon, 2003). Several techniques were applied concerning oestrous or menstrual manipulation, oocytes and embryos manipulation in addition to ovarian cryopreservation and transplantation (Mohammed et al., 2011, 2019, 2012a, b; Mohammed and Farghaly, 2018).

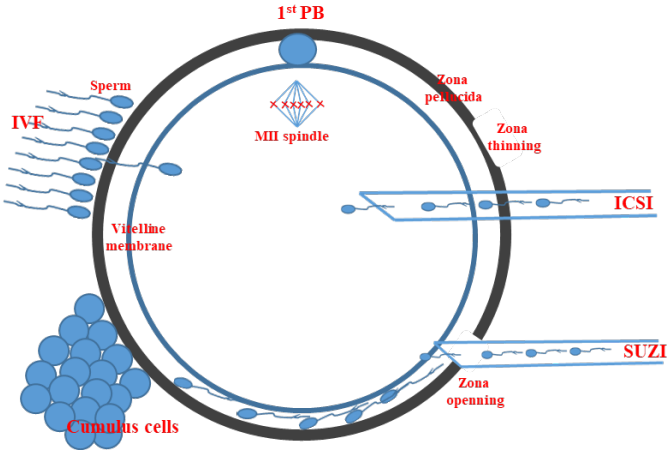

Figure 2: Techniques of assisted *in vitro* fertilization: IVF, *In vitro* fertilization; ICSI, intracytoplasmic sperm injection; subzonal sperm injection, SUZI; PB, polar body; MII, metaphase II.

Table 1: Factors affecting developmental competence *in vitro* of aspirated oocytes from slaughterhouse ovaries or live organisms ovaries.

Ovaries	Treatments	Effects	References
Live organisms	Follicular and luteal stages	↑ development of oocyte to embryos	Gordon, 2003
	Follicle sizes	↑ development of oocyte to embryos	Maylinda *et al.*, 2018; Hasler 1998; Raj *et al.*, 2018
	Follicular waves	↑ development of oocyte to embryos	Gordon, 2003; Cavalieri *et al.*, 2018; Baby and Bartlewski, 2011
	Feed additives	↑ reproductive performance	Mohammed and Al-Hozab, 2020; Senosy *et al.*, 2017, 2018
	Nutrition	Variable effects	Gordon, 2003
Slaughter-house ovaries	Follicle size	↑ development of oocyte to embryos	Gordon, 2003; Shabankareh *et al.*, 2014; Maylinda *et al.*, 2018
	Defined and undefined media	Variable effects ↑ development of oocyte to embryos	Mohammed *et al.*, 2005; Madkour *et al.*, 2018; Spacek and Carnevale, 2018; Abdoon *et al.*, 2018
	Co-culture cumulus and oviduct cells	↑ development of oocyte to embryos	Lee *et al.*, 2018
	Cumulus cells surrounding the oocytes	↑ development of oocyte to embryos	Mohammed *et al.*, 2005; Al Zeidi *et al.*, 2022a, b; Mohammed, 2006; Mohammed *et al.*, 2008, 2010
	Follicular fluid	variable effects due to follicle size and added %	Mohammed *et al.*, 2005; Sinclair *et al.*, 2008
	Fetal calf serum	↑ development of oocyte to embryos	Mohammed *et al.*, 2005; Moulavi *et al.*, 2019
	IGF-1 and FF	Variable effects	Oberlender *et al.*, 2013
	Hormone	↑ development of oocyte to embryos	Moulavi and Hosseini, 2019
	Storage the ovaries at 30 °C for 3-4 hours	↑ development of oocyte to embryos	Luu *et al.*, 2011

Table 2: Timing (hr.) of oocyte maturation from germinal vesicle to metaphase II stage *in vitro* due to different treatments in different animal species and human.

Species	Duration, h	Treatments	References
Sheep	24	Oxygen tension C-type natriuretic peptide	Sánchez-Ajofrín *et al.*, 2020 Zhang *et al.*, 2018
		Resveratrol	Zabihi *et al.*, 2021
		Sericin and growth factors	Tian *et al.*, 2021
Goat	24	Cysteamine, leukemia inhibitory factor, and Y27632 Physiological oocyte maturation Leptin	An *et al.*, 2018; Suresh *et al.*, 2021; de Senna Costa *et al.*, 2022
Cattle	24	FF and FCS Retinoic acid Lycopene	Mohammed *et al.*, 2005 Borges *et al.*, 2021 Residiwati *et al.*, 2021
Buffalo	24	- Brain-derived neurotrophic factor Retinoic acid	Marin *et al.*, 2019 Zhao *et al.*, 2019b Gad *et al.*, 2018
Camel	42-48	- Macromolecule Roscovitine	Wani and Hong 2018, Wani *et al.*, 2018 Moulavi and Hosseini 2019 Wani and Hong 2020
Pig	48	Dihydroartemisinin Zearalenone CRH and ACTH Allicin	Luo *et al.*, 2018 Wang *et al.*, 2022 Gong *et al.*, 2022 Li *et al.*, 2022
Rabbit	24	Gonadotropin-releasing hormone -	Yoshimura *et al.*, 1991 Arias-Álvarez *et al.*, 2017
Rodents	17	Cumulus cells Cumulus cells Cumulus cells Perfluorooctane CRH and ACTH	Grabarek *et al.*, 2004; Mohammed 2006, 2008, 2009, 2011; Mohammed *et al.*, 2008, 2010, 2019; Wei *et al.*, 2021; Gong *et al.*, 2022
Human	24	- - Coenzyme Q10 A fertilin-derived peptide	Chian *et al.*, 2004 Baruffi *et al.*, 2004 Ma *et al.*, 2020 Sallem *et al.*, 2022

Multiple ovulation and embryo transfer

Multiple ovulation or superovulation protocol is a procedure meant that more than one oocyte is released by an ovary of different species and human as well (Mohammed et al., 2011; Macmillan et al., 2018). Embryo transfer program allows animals' producers to obtain multiple progeny from superior genetic females (Gordon, 2003; Mohammed et al., 2011) (Figure 4). The embryos can be recovered from fallopian tube or uterus of females through surgical or nonsurgical techniques depending on species. Thereafter, genetically superior collected embryos are transferred to recipient females of lesser genetic merit or cryopreserved (Neto et al., 2005; Mohammed et al., 2011).

The collected embryos could be manipulated as partial zona dissection or zona drilling to raise their chances of hatching and implantation (Edwards and Brody, 1995). Partial zona dissection improves hatching rate of bovine blastocysts *in vitro* (Loskutoff et al., 1999). In addition, the embryo could be split into two embryonic parts. Fifty percent pregnancy rate of splitting embryos compared to hundred percent of intact embryos has been reported (Gray et al., 1991).

Meiotic maturation of germinal vesicle oocytes *in vivo* and *in vitro*

The germinal vesicle oocyte resumes meiotic maturation after removing from ovarian antral follicle or after LH surge (Dieleman et al., 1983; Grynberg et al., 2022). Meiosis resumption of fully-grown germinal vesicle oocyte is initiated *in vivo* after LH surge (Mehlmann et al., 2006). Several layers of compact cumulus-enclosed cells are surrounding the oocytes before LH surge. Numerous projections of the surrounding cells penetrate the zona pellucida through gap junctions and end on the oocyte oolemma (Gordon, 2003). Gap junctions disruption occur shortly after LH surge. Several changes occur in the GV nucleus and cytoplast of the fully grown germinal vesicle oocyte of pre-ovulatory follicle known as oocyte maturation.

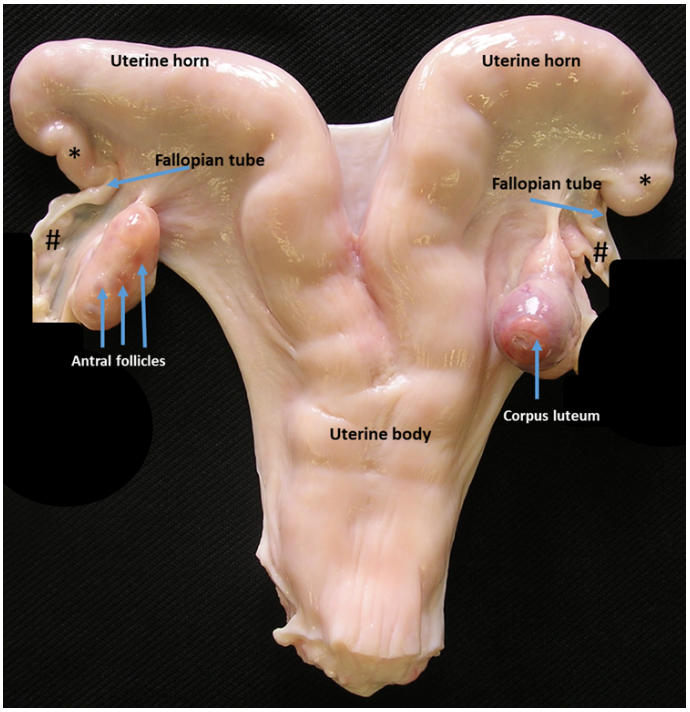

Figure 3: Caprine reproductive system; cumulus-enclosed germinal vesicle oocytes aspirate from antral follicles for embryo production; (#) the place in fallopian tube where the 1-8 cell-stage developed embryos are collected or transferred; (*) the place in the uterine tube where the morula-blastocyst embryos are collected or transferred (Mohammed and Kassab, 2015).

Figure 4: Rabbit offspring obtained after transferred embryos to uterus of surrogate mothers (Mohammed *et al.*, 2011).

The first sign of meiotic maturation is germinal vesicle breakdown (GVBD), which occurred approximately 2-3h of starting maturation in rodent oocytes (Gao et al., 2002; Mohammed et al., 2019a, 2022; Al-Zeidi et al., 2022a, b) and is continued to reach the MII stage (Liu et al., 2006). The oocyte remains arrested at the MII stage until activation or fertilization, which makes the oocytes complete the second meiotic division and follow the embryonic developmental competence. Simultaneously, cytoplasmic maturation of oocytes involves morphological and ultrastructural changes that support fertilization and further embryo development (Chang et al., 2005). Mitochondrial migration and organelles redistribution in addition to changes in mitogen activated protein kinase and maturation promoting factor. Factors affecting developmental competence (Mohammed and Attaai, 2011; Mohammed and Al-Hozab, 2020; Mohammed et al., 2020) of aspirated oocytes *in vitro* are indicated in Table 1. In addition, duration of oocyte maturation *in vitro* from germinal vesicle to metaphase II stage using different treatments in different species is indicated in Table 2.

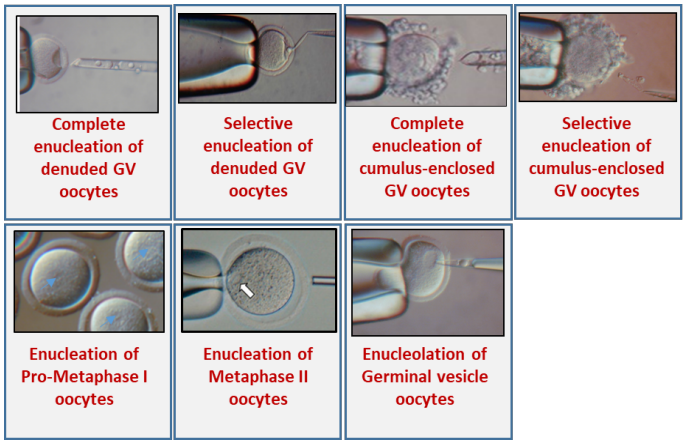

Figure 5: Micromanipulation of germinal oocytes, Pro-Metaphase I and Metaphase II oocytes in addition to enucleolation of germinal oocytes (Mohammed 2006; Mohammed *et al.*, 2008, 2010; 2019; 2022).

Nuclear transfer

Germ, embryonic, and somatic nuclear transfer were used for treatment of infertility and production of cloned and transgenic animals (Wilmut et al., 1997; Wani and Hong, 2018). Cytoplasmic and nuclear maturation of intact and reconstructed oocytes control the developmental competence of embryos (Mohammed et al., 2019a, 2022). The nuclear and cytoplasmic maturation are essential for remodeling of the penetrated sperm or the introduced germ or embryonic or somatic nuclei in addition to further embryonic developmental competence (Mohammed et al., 2008, 2010, 2022). The roles of germinal vesicle, nuclear sap and nucleoli, and surrounding cumulus cells on oocyte maturation and embryonic developmental competence were investigated through micromanipulation of germinal vesicle or metaphase II oocyte (Al-Zeidi et al., 2022a, b). The micromanipulation techniques applied to GV or MII oocytes help to explore cellular reprogramming of the introduced nuclei. Such assisted reproductive techniques of GV or MII oocytes are hypothesized to treat infertility, enhance meat and milk production, and save endangered species. Further studies are still required on gene expression of the manipulated oocytes.

Different techniques were used to produce transgenic embryos and offspring including microinjection of nuclear of one-cell, two-cells, four-cells embryos (Jura et al., 1994; Echelard et al., 2000). Furthermore, transfected somatic cells, embryonic cells, primordial germ cells after nuclear transfer might be used to achieve genetic modification in several species (Lee et al., 2000). Moreover, the transfected cells retained their developmental competence and when inserted into a developing embryo could contribute to all its tissues (McCreath et al., 2000; Robl et al., 2003).

Recipient cytoplasts

Different stages of oocytes were enucleated including germinal vesicle, Pro-Metaphase I, Metaphase I and Metaphase II oocytes (Figure 5). The germinal vesicle or MII cytoplasts reconstructed with germ, germinal vesicle, embryonic and somatic nuclear transfer were used for meiotic maturation and further embryonic development till obtaining fetus (Wilmut et al., 1997; Mohammed, 2006; Mohammed et al., 2008, 2010, 2019a, 2022). The resulting embryos and offspring have the same genetic materials as the original donors, except for the mitochondrial DNA, which are derived from the recipient oocyte cytoplasts (Wolf et al., 2001; Yang et al., 2004). The cytoplasts of oocytes (Mohammed, 2006; Mohammed et al., 2008, 2010, 2019a; Wani et al., 2010; Al-Zeidi et al., 2022a, b) or zygotes (Greda et al., 2006) were used over nuclear transfer for studying meiotic maturation, cellular reprogramming, pre- and post-implantation embryonic development.

Donor nuclei

Different types of donor nuclei (germ, germinal vesicle, cumulus, muscle, skin, fibroblast etc.) with different cell cycle stage (G0/G1, S-phase and G2/M stage) were used for introducing into enucleated oocytes (Mohammed, 2006; Mohammed et al., 2008, 2010, 2019a, 2022; Al-Zeidi et al., 2022a, b). The donor nuclei of G0/G1 stage could be introduced into germinal vesicle cytoplasts whereas donor nuclei of G2/M stage could be introduced into MII cytoplasts (Grabarek et al., 2004; Mohammed 2014a, b). On the other hand, donor nuclei of interphase stage (S-phase) resulted in abnormalities in meiotic maturation and further developmental competence upon introducing into GV cytoplasts (Mohammed, 2006). The resulting artificial gametes could be subsequently activated or fertilized by spermatozoa (Polanski et al., 2005; Mohammed, 2006). The effects of the donor nuclei types and their cycle stages on the success of cloning has been confirmed (Lagutina et al., 2005).

Animal cloning

Animal cloning could be applied through embryo splitting or embryonic/somatic cloning (Willadsen, 1986; Wilmut et al., 1997). The resulting cloned animals were confirmed in several studies to be suffered from neonatal developmental and gestational abnormalities (Eckardt and McLaughlin, 2004; Piedrahita et al., 2004) including abnormal placenta and large offspring syndromes (Wells, 2003). Hence, it has been suggested that nuclear transfer could be considered a useful strategy for cellular reprogramming basic research (Van Heyman, 2005). The interaction between the recipient cytoplasts and the donor nuclei is considered one of the fundamental questions in the field of assisted reproductive technology (Mohammed et al., 2022).

Embryo sexing

Embryo sexing could be controlled through oocytes' fertilization using sorted semen as well as insemination of cycled mammalian females using sorted semen (Mohammed and Al-Hozab, 2016). The semen sorting is still an uncommercial viable form made embryo sexing a major route of gender preselection. There are several methods for embryo sexing. 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; Mohammed and Al-Hozab, 2016).

Oocytes, embryo and tissues resource banking

Embryo storage and cryopreservation are allowing conservation of the full genetic complement of the dam and sire and thus has an enormous potential for managing and protecting species. The differences among mammalian embryos are substantial in cryo-sensitivity as demonstrated by the variance between the freezable bovine embryos versus the difficulties to freeze swine embryos (Nieman and Rath, 2001). Two embryo-freezing procedures were adapted including conventional freezing and vitrification protocols (Albarracin et al., 2005; Nicacio et al., 2012; Varago et al., 2014; Araújo-Lemos et al., 2015). The conventional freezing of embryos requires biological freezers or incubators and it is time-consuming, which replaced by a fast vitrification procedure (Araújo-Lemos et al., 2015). The procedures of freezing and vitrification were adapted to immature and mature oocytes of different species (Bautista and Kanagawa, 1998; Cetin and Bastan, 2006). The studies in freezing and vitrification procedures for oocyte and embryos have made substantial progress in retaining the stability of cytoplast cytoskeleton (Ledda et al., 2019).

Gonadal tissues' cryopreservation and transplantation offer fascinating opportunities for both animals and Humans as well (Mohammed, 2019). The recent developments in the auto-grafting and xeno-grafting of testes and ovaries demonstrate the potential values of cryopreserving gonadal tissues (Oktay and Yih, 2002; Tibary et al., 2005; Mohammed, 2019). Thawed ovarian tissues has been

transplanted into animals and humans as well resulting in the birth of normal young (Demirci et al., 2003; Donnez et al., 2004). Such helpful techniques give the females who suffer from caner the elixir of life through ovariectomy and cryopreservation before chemotherapy followed by re-transplantation after recovery. Furthermore, germ, embryonic, and somatic cells could be exploited for genome resource banking and used in the future for cloning conservation program (Wilmut et al., 1997).

CONCLUSIONS AND RECOMMENDATIONS

The continuous progresses in assisted reproductive techniques are necessitated for both animals and human as well. There are differences among mammalian species to apply assisted reproductive techniques due to differences in anatomy of reproductive systems, duration of oestrous cycle, and gamete physiology, which require studies of the specific species.

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NOVELTY STATEMENT

The continuous development of assisted reproductive techniques is necessitated to overcome the problems of reproduction.

AUTHOR'S CONTRIBUTION

Authors contribute equally in Conceptualization, writing and editing manuscript. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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