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Do the Mammalian Artificial Oocytes Repair Reproductive Dysfunctions in Mammalian Species?: A Review

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ABSTRACT

Artificial oocytes were used of different mammalian species for repairing reproductive dysfunctions. Artificial oocytes are those created *in vitro* using enucleated germinal vesicle, metaphase I and metaphase II oocytes followed by embryonic cells, somatic cells, pluripotent stem cells and germ cells nuclear transfer. Artificial oocytes are under development, they have the potential to revolutionize reproduction in mammalian species. The potential applications of artificial oocytes include 1) treatment of infertility as premature ovarian failure, ovarian cancer, or other conditions that damage the ovaries, 2) preservation of fertility, 3) creating cloned and transgenic animals. While artificial oocytes have the potential to revolutionize reproductive medicine, there are still a number of challenges that need to be addressed before they can be effectively and safely used in humans including the adapting efficient and safe enucleation techniques. Furthermore, cellular reprogramming is the biggest obstacle for creating artificial oocytes in addition to activation procedures to ensure that artificial oocytes are genetically and epigenetically normal and producing healthy offspring. Several studies are designed to overcome these challenges and there is significant progresses being made. Offspring are obtained in several animals species whereas developed blastocysts were obtained in humans. This review is developed and implemented to discuss the progress in creation of "artificial oocytes" in addition to the factors affecting the developmental competence of reconstructed oocytes.

Key words: Artificial, Blastocyst, Embryonic, Enucleation, Oocytes, Somatic.

Recent potential interest has arisen in the use of enucleated GV, MI and MII oocytes as recipientscytoplasts for GV nucleus, embryonic and somatic nuclei in addition to germ cells in the last couple of decades for different purposes (Mohammed *et al.,* 2019; Melo *et al*., 2022). The stages of enucleated oocyte include germinal vesicle, metaphase I and metaphase II stage oocytes. In addition, the types of introduced cells or nuclei include embryonic cells, somatic cells, pluripotent stem cells and germ cells. Furthermore, the cell cycle stages of donor nuclei include G0/G1, S-phase and G2/M stages (Mohammed 2008, 2014a,b).

Several techniques were adapted to increase the efficiency of creation artificial oocytes and their further embryo developmental competence and the resulting produced offspring (Al Jubran *et al*., 2023; Al Zeidi *et al.,* 2022a,b). The adapted techniques include the recipient cytoplast (Mohammed *et al*., 2006a,b, Mohammed *et al*., 2008, 2010), the technique of enucleation (Mohammed *et al*., 2019), activation method (Montgomery *et al.,* 2022), nucleolus transfer (Benc *et al.,* 2019). The reconstructed "artificial oocytes" seems to be an interesting model for studying meiotic progression of the introduced nuclei or cells, reproductive disorder treatments or for embryonic/ somatic cloning. Therefore, the recipient GV, MI and MII cytoplasts and donor GV, embryonic and somatic nuclei at G0/G1, S-phase and G2/M stages affect the maturation or activation efficiency in addition to the developmental competence of the resulting embryos. Furthermore, the factors that increase the quality of recipient cytoplasts were presumably increase the quality of reconstructed oocytes and their developmental competence as *in vivo* cytoplast transfer and culture conditions (Mohammed *et al.,* 2005). Therefore, this review is designed to collect and discuss the progress in creation of "artificial oocytes" in addition to factors affecting the developmental competence of reconstructed oocytes.

The current study was carried out according to the procedure approved by Deanship of Scientific Research, King Faisal University, Saudi Arabi from August to February 2024. Ethical approval for this study was not required and the data were obtained from science direct databases.

Artificial oocytes are required for infertility treatment of people around the world and the number increasing every year (Oqani *et al.,* 2022). In recent years, assisted reproductive technologies have been widely developed (Pavlović *et al.,* 2024; Desy and Marre 2024).

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Differences among mammalian oocytes

Mammalian oocytes are similar in some ways, but there are also some key differences between them (Albertini 2015). These differences are important to consider when using these models to investigate reconstruction of oocytes. The differences will be concentrated on mouse, bovine in addition to human oocytes. The diameter of mouse oocytes is about 70 micrometers, bovine oocytes is ~120 micrometers while human oocytes is ~100 micrometers. In addition, mouse oocytes have a spherical shape, while bovine and human oocytes have a more oval shape (Mohammed *et al.,* 2022). Bovine oocytes also have a unique feature of containing lipid droplets making the germinal vesicle (GV) invisible in the cytoplasm versus mouse and human oocytes, which contain visible GV nucleus. The timing of maturation for bovine and human oocytes lasts 24 h whereas that of mouse oocytes lasts 17 h.

Protein synthetic patterns in immature and mature mammalian oocytes were recorded on cytoplasmic lattices (Schultz and Wassarman 1977; Goff *et al.,* 2001; Jentoft *et al.,* 2023) to store proteins for the early embryo. Protein synthesis is required for germinal vesicle breakdown in bovine oocytes (Li *et al.,* 2021), human oocytes (Cha and Chian 1998), but not in mice (Li *et al.,* 2022). In addition, the cumulus cells is tightly attached to bovine and human oocytes compared to those loosely attached to mouse human oocytes (Mohammed *et al*., 2019).

Cumulus-enclosed germinal vesicle (GV) oocyte aspiration or collection

Cumulus-enclosed GV oocyte could be aspirated or collected from slaughterhouse materials or live females (Mohammed *et al*., 2005; Kagawa *et al.,* 2022). There are three methods for collecting oocytes from slaughterhouse materials include aspiration, dissection and slicing (Gordon 2003). There are two main methods of cumulus germinal vesicle (GV) oocyte collection include transvaginal ultrasound-guided oocyte retrieval and laparoscopic oocyte retrieval. The oocyte could be collected form pre-pubertal (Currin *et al*., 2021), pubertal and pregnant animals (Pongsuthirak and Vutyavanich, 2018).

Exposure to compounds in relation to reproductive dysfunctions

Endocrine-disrupting chemicals can interfere with the normal functioning of the endocrine system, which is responsible for synthesizing and regulating hormone production (Santos *et al*., 2014; Chen *et al.,* 2023). These compounds can be found in a wide range of products, including pesticides, herbicides, plastics, personal care products and food additives. Exposure of animals and human as well can occur through variety of routes, including ingestion, inhalation and skin contact. The symptoms of reproductive dysfunctions might include interfere with the production and release of estrogen hormone, estrous or menstrual cycle irregularities, endometriosis, polycystic ovary syndrome (Fig 1). In addition, the compounds have also been linked to an increased risk of miscarriage, stillbirth and premature birth. The effects of toxic compounds on reproductive health are depending on the type of compound, the dose and the timing of exposure (Santos *et al*., 2014).

Artificial oocytes for dysfunctions' repair

Abnormalities of mammalian oocytes could be nucleus or cytoplasmic in natures due to different factors as chromosome misalignment (Cui *et al*., 2005; Tatíèková *et al*., 2023) or ultrastructure dysfunctions including mitochondrial dysfunction, spindle disorganization (Jiang *et al*., 2021; Xing *et al*., 2021) and mitochondrial and glucose metabolic dysfunctions in granulosa cells (Zhang *et al*., 2022) and nucleolus dysfunction (Fulka *et al*., 2004). Various trials were applied to repair dysfunction oocytes (Jiang *et al*., 2021; Xing *et al*., 2021; Zhang *et al*., 2022). The trials include the recipient cytoplasts (GV, MI and MII stages) (Mohammed *et al*., 2019, Al Jubran *et al*., 2023),

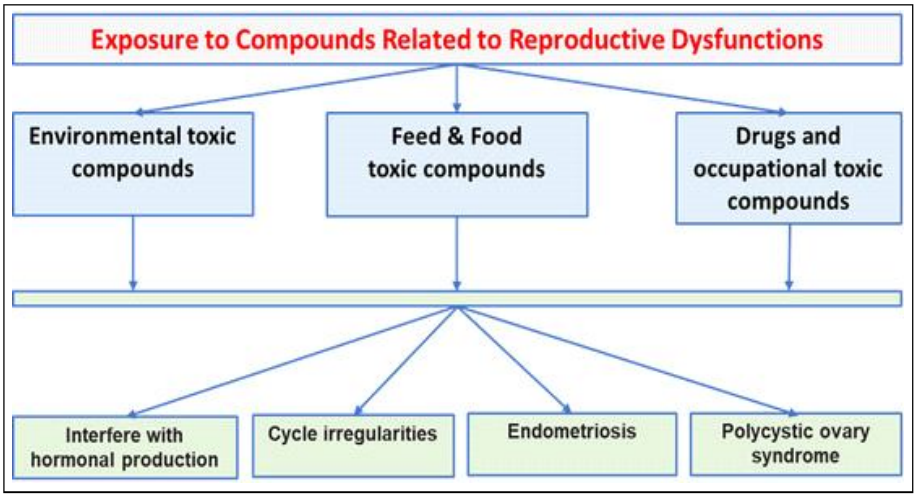


Fig 1: Effects of exposure to toxic compounds relation to reproductive dysfunctions.

the donor nuclei and their cell cycle stage (G1, S-phase and G2/M stages) in addition to the modification of *in vitro* culture media and conditions (Mohammed *et al.*, 2005; Sharma *et al.,* 2024).

The question arose, is it possible for the reconstructed oocytes to repair dysfunction oocytes through different combinations of recipient cytoplasts and donor nuclei in addition to the culture conditions. The low efficiency of success in cloning of different mammalian species with MII cytoplasts as recipient of embryonic/somatic nuclei gave reason for 1) new micromanipulation techniques 2) modified culture systems of oocytes or 3) new combination between the recipient cytoplasts and donor nuclei (Mohammed *et al.*, 2019; Al Jubran *et al.*, 2023).

Thus, for better understanding the background of difficulties in co-operation between foreign nucleus and cytoplasm in reconstructed oocytes, the development of new micromanipulation techniques and/or new culture systems of oocytes are required which might also help to overcome the existing problems and to increase the developmental competence of resulting embryos (Mohammed *et al.*, 2008, 2010, 2019).

Obtaining recipient enucleated cytoplasts

The efficiency of obtaining recipient enucleated cytoplasts is dependent on the oocyte stage and the technique used (Mohammed *et al.*, 2008, 2010, 2019; Al Zeidi *et al.*, 2022a,b; Al Jubran *et al.*, 2023). There are two main methods for obtaining recipient enucleated cytoplasts either the manual enucleation or the chemical enucleation. The oocytes are enucleated at germinal vesicle (GV) (Fig 2), metaphase I (MI) and metaphase II (MII) stage (Fig 3).

To the best of our knowledge, the success in assisted reproduction has been achieved using MII cytoplasts (Wilmut *et al.*, 1997; Son *et al.*, 2021). Therefore, the idea is to introduce the donor nuclei at earlier stage of development "GV or MI oocytes". Hence, techniques were adapted for enucleation of those oocytes. The MI cytoplasts gave success with germ cell donor nuclei (Ogura *et al.,* 1997; Tao *et al.*, 2022). On the other hand, removal the whole GV from oocyte upon enucleated resulted in lack of cytoplasm from nuclear materials, nuclear sap and nucleolus. Upon reconstruction with somatic nuclei, the development has been blocked at one cell-stage and the nucleus without nucleoli. Therefore, our adapted techniques "selective enucleation of GV oocytes" is targeted to leave in the GV cytoplast the nuclear sap. In addition, we were able to enucleated GV oocytes cumulus-enclosed, which promote developmental competence upon reconstruction with embryonic/somatic cells. The developmental competence exceeded one cell-stage embryos to hatching/hatched blastocyst (Mohammed *et al.*, 2008, 2010). In my point of view, such technique needs further studies to apply in other species with different donor nuclei.

Type of donor cells and the cycle stage for reconstruction oocytes

The types of donor cells and their cell cycle-stage for reconstruction oocytes are important factors that affect the success of either maturation or further embryonic development (Al Jubran *et al.*, 2023). The most common types of donor cells used for oocyte reconstruction of oocytes are 1) embryonic cells such as blastomeres or inner cell mass cells, 2) somatic cells such as skin or blood cells, granulosa cells (Nguyen *et al.*, 2021) in addition to polar body for generation of functional human oocytes (Ma *et al.,* 2017).

Regarding to the cell cycle stage of donor nuclei, the synchronization between the donor cell and recipient cytoplast is necessitated for success in maturation and embryonic development (Wani *et al.,* 2018). The donor cells should be in the G0/G1 phase of the cell cycle when they are introduced into the enucleated MII oocyte (Mohammed *et al.,* 2019). This is the resting phase of the cell cycle and it is the time when the DNA is most stable and reduced the risk of chromosomal abnormalities. In addition, the G0/G1 phase is the longest phase of the cell cycle, which gives the donor cell more time to reprogram to the embryonic state. With the emergence of GV and MI cytoplast, S-phase and G2/M phase were investigated for maturation and further embryonic development. The s-phase donor nuclei showed the matured oocytes with chromosomal abnormalities in addition to blocked embryos at one-cell stage. Overall, the the type of donor cells and the cell cycle stage are important factors that affect the success of oocyte reconstruction. G0/G1 phase donor cells are the preferred choice, but S -phase and G2/M phase donor cells may be used in some cases.

Developmental potential of artificial oocyte after activation or fertilization

Oocyte activation of matured oocytes is a fascinating and crucial process for further embryonic development. Out of sperm-mediated oocyte activation, the methods of oocyte activation used in assisted reproductive technologies include electrical or chemical stimulation (Mohammed *et al.,* 2019; AlJubran *et al.,* 2023). Human oocyte activation over round spermatid injection is resulting healthy babies (Tao *et al.,* 2022).

The development of artificial oocytes to embryos and offspring is a rapidly evolving field and there is significant potential for this technology to have a major impact on reproductive medicine. Development of artificial oocyte after activation or fertilization is a complex process include a rise in intracellular calcium levels, completion of meiosis II and completion of meiosis II, decondensation of the chromosomes and formation of the female and/or male pronuclei and proceed to embryo cleavages in definite time (Mohammed *et al.*, 2005, 2008, 2010). To the best of our knowledge, cytoplasts of MI reconstructed with germ cells

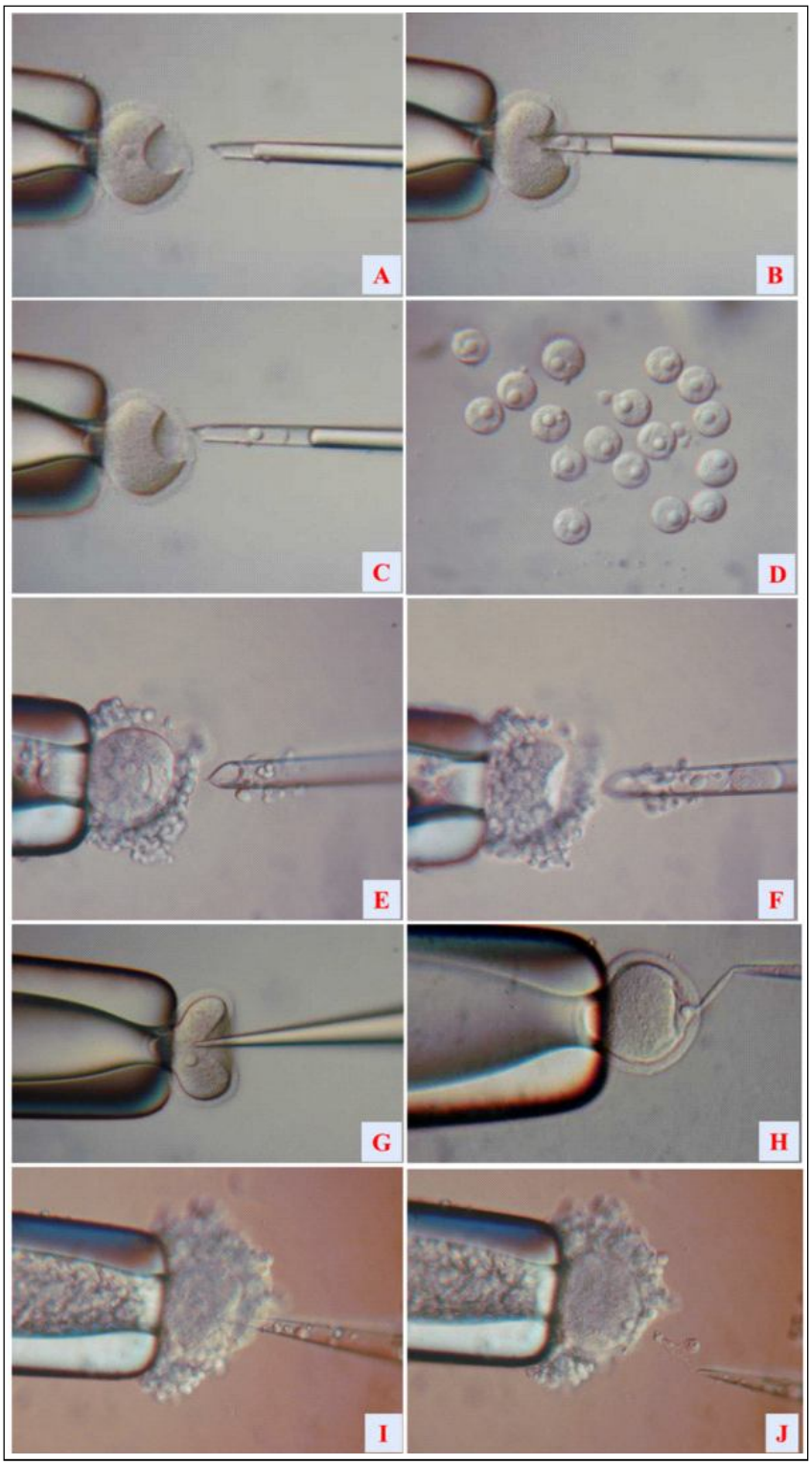


Fig 2: Obtaining recipient enucleated high quality GV cytoplasts for further reconstruction with germinal vesicle nucleus, germ cells or embryonic somatic nuclei at G2/M cell cycle stage; complete enucleation of denuded GV oocyte (A-D), complete enucleation of cumulus-enclosed GV oocyte (E-F), selective enucleation of denuded GV oocyte (G-H) and selective enucleation of cumulus-enclosed GV oocyte (I-J).

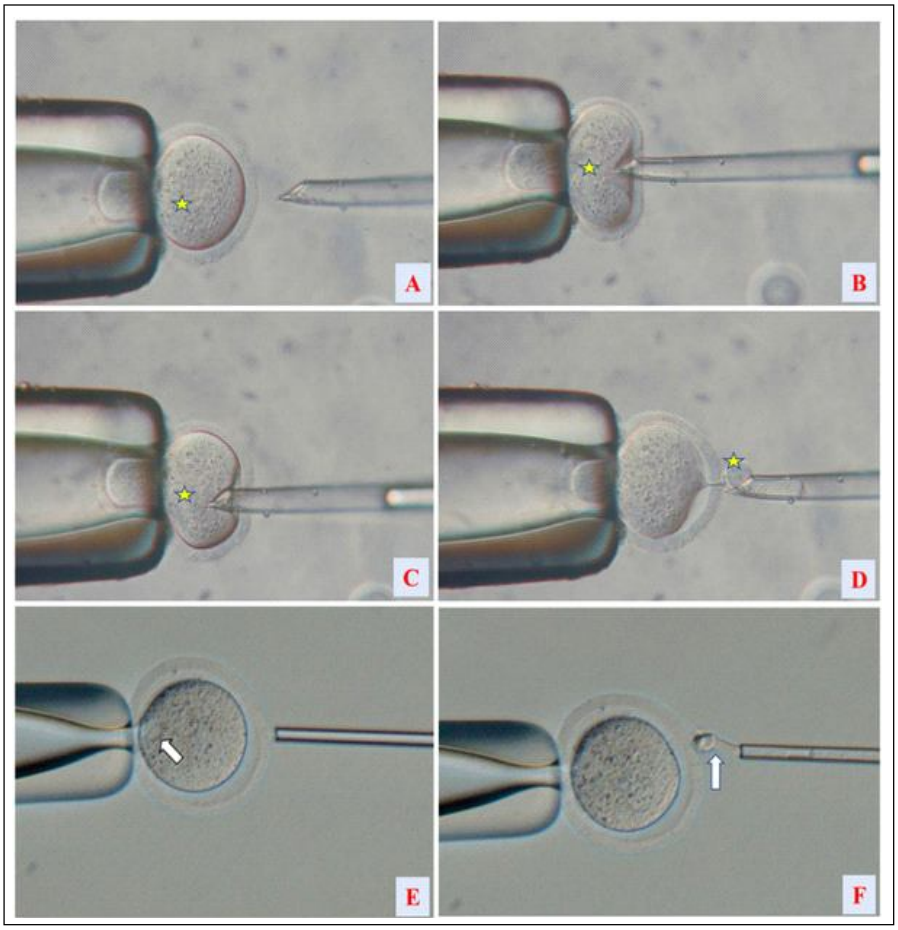


Fig 3: Obtaining recipient enucleated high quality ProMI/MI and MII cytoplasts for further reconstruction with ProMI/MI and MII spindle, germ cells or embryonic somatic nuclei at G0/G1 cell stage; enucleation of ProMI/MI oocyte (A-D), enucleation of MII oocyte (E-F).

and MII cytoplasts reconstructed embryonic/somatic cells were developed to full term offspring whereas GV cytoplasts reconstructed embryonic/somatic cells were developed only to hatching/hatched blastocysts (Mohammed *et al.*, 2019).

CONCLUSION

The artificial oocytes are generated using the recipient cytoplasts and donor nuclei to solve the problems concerning the recipient cytoplasts and donor nuclei. The types of the recipient cytoplasts include GV, ProMI and MII cytoplasts and the types of donor nuclei include GV, embryonic and somatic nuclei. The synchronization between the recipient cytoplasts and donor nuclei is required in addition to the protocols of activation and *in vitro* cultures. The potential applications of artificial oocytes are required for treatment of infertility and stem cell research. Further studies are still required in such hot spot area to overcome the implications and to maximize the subsequent pre-implantation and post implantation embryonic development.

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Conflicts of interest

There is no conflict of interest for authors to declare.

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