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Application of parthenogenetic embryonic stem cells: A review

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INTRODUCTION

Parthenogenesis (from the Greek *parthenos*, "virgin", + *genesis*, "creation") is an asexual form of reproduction found in females where growth and development of embryos occurs without fertilization by a male. Parthenogenesis occurs naturally in some invertebrate animal species (e.g. water fleas, aphids, some bees, some Phasmida, some scorpion species, and parasitic wasps), and vertebrates (e.g. some reptiles, fish, and, very rarely, birds and sharks) and this type of reproduction has been induced artificially in other species. Although such embryos lack the potential to develop to full term, they can be used to establish parthenogenetic embryonic stem (pES) cells for autologous cell therapy in females without needing to destroy normally competent embryos.Stem cells are one of the most fascinating areas of biology today. Stem cells are cells found in all multi-cellular organisms. They retain the ability to renew themselves through mitotic cell division and can differentiate into a diverse range of specialized cell types. Research in the stem cell field grew out of findings by Canadian scientists Ernest A. McCulloch and James E. Till in the 1960s. Research on stem cells is advancing knowledge about how an organism develops from a single cell and how healthy cells replace damaged cells in adult organisms. This promising area of science is also leading scientists to investigate the possibility of cell-based therapies to treat disease, which is often referred to as regenerative or reparative medicine**.** It has been hypothesized by scientists that stem cells may, at some point in the future, become the basis for treating diseases such as Parkinson's disease, diabetes, and heart disease. Embryonic stem cells (ES cells) are stem cells derived from the inner cell mass of an early stage embryo known as a blastocyst. Human embryos reach the blastocyst stage 4–5 days post fertilization, at which time they consist of 50–150 cells.Embryonic Stem (ES) cells are pluripotent. This means they are able to differentiate into all derivatives of the three primary germ layers: ectoderm, endoderm, and mesoderm. These include each of the more than 220 cell types in the adult body. Pluripotency distinguishes ES cells from multipotent progenitor cells found in the adult; these only form a limited number of cell types. When given no stimuli for differentiation, (i.e. when grown *in vitro*), ES cells maintain pluripotency through multiple cell divisions. The presence of pluripotent adult stem cells remains a subject of scientific debate; however, research has demonstrated that pluripotent stem cells can be directly generated from adult fibroblast cultures.

The two broad types of mammalian stem cells are embryonic stem cells (ES)

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that are found in blastocyst, and adult stem cells that are are found in adult tissues (like endothelial stem cells, etc.).

Embryonic stem cells can become all cell types of the body because they are pluripotant. Adult stem cells are generally limited to differentiating into different cell types of their tissue of origin. Therfore, the regenerative or reparative medicine with embryonic stem cells has got the prime importance. However, some evidence suggests that adult stem cell plasticity may exist, increasing the number of cell types a given adult stem cell can become.

The use of adult stem cells in research and therapy is not a problematic or controversial. But the use of embryonic stem cells encounters several common problems like-

i) It requires the formation and destruction of an embryo.

ii) Both male and female germ cells are required, which may not be available at the time when it is needed.

iii) The use of human embryos to derive embryonic stem cells ethically is not permissible.

iv) These process may not offer a way for creating stem cells that are genetically matched to a particular individual for the treatment of degenerative diseases.

v) The stem cells derived from embryos may not immunologically match to the individual to whom it is going to transfer.

1) A new source of deriving pluripotent stem cells is coming up recently from the oocyte that undergone a process known as parthenogenesis.

By using this technique, ES cells were derived without the need to create or destroy a viable embryo. Therefore, this artificial parthenogenesis seems to offer a way to sidestep these ethical pitfalls and various immunological problems arise in regenerative or reparative medicine, especially for female patients..

INCIDENCE OF PARTHENOGENESIS:

Parthenogenesis is a common mode of reproduction in lower organisms in nature. It has been reported in about 70 lower species of invertebrates. Flies, ants, lizards, snakes, fish, birds, reptiles, amphibians, honeybees, and crayfish routinely reproduce in this manner.

Dec 21(2006), zoologist Kevin Buley for the first time documented about a female Komodo dragon at Chester Zoo in England, who fertilized her own eggs.

A small hammerhead shark (bonnethead), was thought to have produced a pup, born live on the 14th December 2001 at Henry Doorly Zoo in Nebraska, in a tank containing three female hammerheads, but no males. The testing showed that the pup's DNA matched only one female who lived in the tank, and that no male DNA was present in the pup. This investigation revealed that the birth was through parthenogenic means. In 2002, two white-spotted bamboo sharks were born at the Belle Isle Aquarium in Detroit, where the mother shared an aquarium with only one other female shark.

In the nature the mammalian parthenotes fails to produce a successful pregnancy and are not capable of this form of reproduction. But eutherian oocytes can undergo parthenogenesis *in vitro* with variable success. When mammalian oocytes are activated (emulating the fertilization process) and transferred to a surrogate mother, they are capable of surviving to day 10 of development in the mouse, day 21 for sheep, day 29 in pigs, and day 11.5 in rabbit (Vrana *et al*., 2003)

The birth of live parthenogenetic rabbits has been reported by Pincus during

1940s. Beaty (1957) expected that out of 200 mammalian parthenogenetic embryos, one would reach the full term of pregnancy.Kaufman (1977) reported normal postimplantation development of mouse parthenogenic embryos to the forelimb bud stage.

However, chimeras of parthenogenetic cells coupled with biparentally derived embryonic tissues have generated apparently normal offspring, and the parthenogenetic origin of several tissues has been confirmed in such chimeric animals (Boediono *et al.*, 1999). In a reported case of a human parthenogenetic chimera, contribution to several tissues has been demonstrated, including blood where 100% of the leukocytes were found to be of parthenogenetic origin (Strain *et al.*, 1995).

Attempts at artificial parthenogenesis in humans have not yet been successful. There is some evidence, however, that natural parthenogenesis does occasionally occur in humans. There are many instances in which impregnation has allegedly taken place in women without there being any possibility of the semen entering the female genital passage. In some cases it was found either in the course of pregnancy or at the time of childbirth that the female passages were obstructed. In 1956 the medical journal *Lancet* published a report concerning 19 alleged cases of virgin birth among women in England, who were studied by members of the British Medical Association. The six-month study convinced the investigators that human parthenogenesis was physiologically possible and had actually occurred in some of the women studied.

It has been known for many years that human eggs occasionally undergo spontaneous cell divisions. These dividing eggs lead to dermoid cysts and to benign tumors known as teratomas that contain several cell types including skin and hair. If eggs could be routinely stimulated to undergo cell division in the laboratory, this could be an especially valuable source of stem cells.

SOME FACTS ABOUT PARTHENOGENIC STEM CELLS RESEARCH:

Jacques Loeb (1859–1924) considered as the founding father of artificial parthenogenesis and published his research in 1899.

Hwang Woo-Suk (2004 and 2005), a South Korean biomedical scientist, reported to have succeeded in creating human embryonic stem cells by cloning. On August 2, 2007, after much independent investigation, it was revealed that Hwang's team succeded in extracting cells from eggs that had undergone parthenogenesis, the same indicators of parthenogenesis in those extracted stem cells as are found in the mice created by Tokyo scientists in 2004.

Cibelli *et al*. (2002) produced the first embryonic stem cells by parthenogenesis in primates. Vrana (2002) from Wake Forest University School of Medicine and Advanced Cell Technology (ACT) of Worcester, Mass, reported that they had generated a pluripotent stem cell line from primate that they called cyno-1 from the blastocyst that results from parthenogenesis which could not become a viable fetus. The cell line has grown continuously for 10 months. Vrana said, "Parthenogenesis offers an important new

therapeutic strategy for a host of medical conditions."

A group of researchers leaded by Nancy L. Jones (2003) from the Stemron Corporation and Reproductive Biology Association have reported on their creation of human embryos by parthenogenesis for the purpose of obtaining stem cells. Dr. Ann Kiessling (2005) at the Bedford Stem Cell Research Foundation, United States, experimented a series taking the first look at Human Parthenogenetic Stem Cells. She prepared to activate three human eggs.

Jin Young Ju *et al*.(2006) reported Establishment of stem cell lines from parthenogenetically activated mouse oocytes for therapeutic cloning. The embryonic stem cell line establishment rate was higher from parthenogenetically activated oocytes (15.7%) than nuclear transferred (4.3%) or sham-manipulated oocytes (12.5%). Cell colonies displayed typical morphology of mice embryonic stem cells and could be maintained successfully with undifferentiated morphology after continuous proliferation for more than 120 passages maintaining normal karyotype.

Steps for development of parthenogenetic stem cells.

1. Follicular Stimulation and Oocyte Collection:

Oocytes are collected either surgically or by flushing. In animals oocytes are collected after super ovulation to get more numbers of oocytes and even slaughterhouse ovary can also be used to collect oocytes. Cumulus-oocyte complexes (COC) with impact multiplayer of cumulus cells are chosen for culture. If the oocytes are collected by aspiration of antral follicles, they are to hold in maturation medium to reach the metaphase of second meiotic division (MII). Then the cumulus cells are stripes off and denuded oocytes with a spherical shape, a visible polar body and an evenly granulated ooplasm are selected for activation.

2. Oocyte Activation:

In vertebrates, mature oocytes are arrested at metaphase of second meiotic division (MII) with elevated maturation promoting factor (MPF) activity maintained by cytostatic factor (CSF). CSF prevents ubiquitin-dependant degradation of cyclin B and thus, inactivation of MPF. During fertilization, sperm entry triggers a series of intra cellular calcium oscillations critical to oocyte activation. MPF and mitogen activated protein (MAP) kinase are the most likely targets of calcium-stimulated events. Because, inactivation of these kinases is a prerequisite to the resumption and completion of meiosis, subsequent formation of pronuclei and DNA synthesis. Intracellular Ca^{2+} oscillations down regulate CSF activity and release the cyclin degradation mechinary. Proteolytic degradation of cyclin B and subsequent MPF inactivation release oocyte from metaphase arrest and allow the beginning or resumption of mitotic cycle.

3. Parthenogenetic Oocyte Activation:

A wide variety of chemical and physical treatments can mimic sperm triggered events and induce parthenogenic development in MII oocyte. For example, ethanol electroporation, calcium ionophore, ionomycin or inositol 1,4,5-triphoshphate induces calcium elevation and release meiotic arrest. In animal the parthenogenetic oocyte activation has been reported long time back by many

scientists. For instances, Kaufman (1977) and Collas *et al.* (1989) reported parthenogenetic activation of mouse oocytes.

In bovine, parthenogenetic activation of oocytes has been reported by Land and Hajkova (1989), Kono *et al.* (1989), Collas *et al.* (1993), Yang X, *et al.* (1994), Landa and Kopecny (1995), Eva Soloy *et al.* (1997), Lin Liu *et al.* (1998), Chung, J.T. *et al.* (1999).Likewise in pig it was reported by Jolliff *et al.* (1997), Kure-bayashi *et al.* (1999), in ovine by Loi *et al.* (1997) and in rabbit Ozil, J.P. (1990), Mitalipov *et al.* (1999) are mentionable for their success. In our laboratory we have also developed activation of parthenogenetic embryos (Kumar et al., 2013, Pankaj et al., 2012)

4. Assesment of oocytes for nuclear activation:

Activation of oocytes was defined as resumption of the second meiotic division; i.e. oocyte that has progressed to anaphase II, telophase II metaphase III, or pronuclear formation was considered as activated. When a stage of pronuclear development was evaluated, only a pronucleus with an evenly granulated nucleoplasm surrounded by a complete nuclear envelope was considered as fully developed.

Telomerase activity is often correlated with replicative immortality and is typically expressed in germ cells, cancer cells, and a variety of stem cells, including ES cells, but absent in most somatic cell types (Kim *et al.*'1994, Armstrong *et al.*'2000, Amit *et al.*'2000). Vrana *et al.*, 2003 also found high levels of telomerase activity in undifferentiated Cyno-1 cells.

Determination of DNA synthsis in activated oocytes also dictates the resumption of MII. It is argued that only fully developed pronucleus can synthesis DNA. . DNA synthesis in activated oocyte can be monitored by ^{3}H-thymidine incorporation into newly synthesized DNA. Incorporated thymidine is then visualized by autoradiography performed on spread oocytes or semithin section of oocyte (Eva Soloy *et al.*'1997). The activated oocytes are cultured either *in vivo* or *in vitro* to blastocyst stage.

5. Seperation of stem cells:

Cibelli (2002) isolated inner cell mass (ICM) form parthenogenetic blastocyst by immunosurgery and cell were cultured and propagated extensively for 10 months in the laboratory. After two weeks of differentiation telomerase activity was lost, which is indicative of not being the tumour cells.

SIGNIFICANCE OF GENETIC COMPLEMENTS IN PARTHENOGENETIC STEM CELLS:

The offspring of parthenogenesis will be all female if two like chromosomes determine the female gender (such as the XY sex-determination system), but male if two like chromosomes determine the male gender (such as the ZW sex-determination system).

Parthenogenic offspring are all genetically identical to its mother and her offspring will be identical to each other, as a parthenogen is homozygous. Each sibling of the first generation of parthenogens will, therefore, create a linage that carries only her unique genes when reproducing via parthenogenesis.

When an egg is parthenogenically stimulated, the cells that result are either monoploid or diploid. But the ultimate goal of parthenogenetic activation of

oocyte is to produce an embryo with a full genetic complement (diploid). Jones (2003) described that this could be achieved either by stimulating eggs that are still diploid to divide or by inducing an egg with haploid chromosomes to replicate its genetic material. Eggs halve their genetic complement relatively late in their maturation cycle, so if early activation is done, a full set of genes is retained. Alternatively, it is possible to stimulate a haploid egg to replicate its genetic material, resulting in a full genetic complement. Unfertilized oocyte has two complete sets of chromosomes. One set is expelled during fertilization, but an electric or chemical shock can make the oocyte develop as if fertilized and retain the extra set. The stem cells can be collected form the resulting embryo.

However, Wise Young (2007) has shown that parthenogentic monoploid cells not only behave like stem cells and are pluripotent both *in vivo* and *in vitro*, but also can transform into cell types that may be therapeutically relevant.

Parthenogenetic stem cell differentiation:

A large variety of specialised cell types could be generated *in vitro*, such as spontaneously beating cardiomyocyte-like cells and cilliated epithelium, smooth muscle cells and cytokeratin-positive cells as well as neural cells. Cyno-1 cells of primates were observed to differentiate to all three germ layers including cartilage, neurons, skin, and hair follicles (ectoderm), intestinal epithelia (endoderm), and muscle and bone (mesoderm) (Vrana *et al.*, 2003).

Advantages of Parthenogenic stem cells:

This could be an especially valuable source of stem cells that could bypass the moral, ethical, and some of the tissue rejection problems associated with fetal and embryonic stem cells, particularly for the woman donating the egg. For example, a woman with diabetes or spinal cord injury could donate her own eggs for her own stem cells.

These cells may provide a novel tool for assessing the effects of genomic imprinting on cell differentiation and function during development in primates and human being.

Their striking differentiation capabilities (electro physiologically active, dopamine-secreting neurons) indicate their therapeutic potential for the treatment of Parkinson's disease and Huntington's disease and suggest a valid alternative to biparentally derived ES cells.

Cells made by parthenogenesis have two identical sets of chromosomes, rather than one set each from the father and the mother, they have less variation in the surface proteins on cells that can trigger immune reactions. So complicated immunocompetability that arouse after cell therapy is said to be nil or negligible in parthenogenetic stem cell therapy.

. Genetically matched pluripotent embryonic stem (ES) cells generated via nuclear transfer or parthenogenesis is a potential source of histocompatible cells and tissues for transplantation. Isolation of parthenogenetic stem cells from murine oocytes that carried the full complement of major histocompatibility complex (MHC) antigens of the oocyte donor engrafted in immunocompetent MHC-matched mouse recipients, demonstrating that selected parthenogenetic stem cells can serve as a source of histocompatible tissues for transplantation. (Kitai Kim, 2007).

Disadvantages of Parthenogenic stem cells

Since eggs are needed to make parthenogenetic stem cells, one potential problem is that the technique could not be used to make matching stem cells for men or for women after menopause. Therapeutic cloning, by contrast, could provide matching stem cells for any individual.

Parthenogenetic stem cell seems to be similar in all respects to traditional ES cells, but it is reasonable to question their viability and utility. They are, after all, exclusively derived from maternal DNA.

Parthenogenetic embryos do not develop to term because of a high frequency of errors in X chromosome inactivation that occurs in extraembryonic tissues when both X chromosomes are derived only from the female.Requires a long time and constant efforts to get a single parthenogentic embryos and tests used to confirm their stemness is also time taking and expensive. Requires expensive instruments, chemicals and highly trained technical support and high technological bank to store the stem cells.

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