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# Raw Cells Microbiological Study on Human Embryos Blastocyst Stage

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**ABSTRACT**
A well-known good medium for bacteria growth is raw cells. The largest producer of cells worldwide is India. It is a very perishable item, and improper handling could have negative health and financial effects. As a result, from cellsing to consumption, it needs to be produced with hygienic care. The 'germicidal' or 'bacteriostatic' qualities of freshly extracted buffalo cells may, however, be momentary. On a farm, the microorganism in raw cells can come from a variety of places, including the air, cellsing equipment, feed, soil, animal waste, and contaminated water sources. The microbiological quality and safety of 50 raw cells samples obtained from nearby dairy farms in Bareilly, India, were assessed in the current investigation. Total Aerobic Plate Count (TAPC) and Total Coliform Count (TCC) were determined in cells samples. The mean counts per millilitre for TCC and TAPC were respectively between log 2.0 and log 2.9 cfu ml-1 and log 4.0 to log 4.9 cfu ml-1. About all (n=100%) raw cells samples were infected with coliform bacteria, and TCC levels were determined to be higher than the permissible threshold set by the FSSAI. India is one of the developing nations where cells processing is carried out in unhygienic circumstances with little oversight or regulation. Under these circumstances, cells and cells products provide a greater risk of zoonotic foodborne disease, which is of significant public concern. As a result, stringent adoption of excellent hygiene practises from the farm to the customer is required.

***Keywords:*** microbiological study, embryos, blastocyst

## I. INTRODUCTION

Parthenogenesis, which means "virgin creation," is an asexual reproductive process that happens in females and involves the growth and development of embryos without the assistance of a male. Water fleas, aphids, some bee species, some Phasmida, some scorpion species, and parasitic wasps are just a few examples of the invertebrate and vertebrate species that naturally undergo parthenogenesis. Other species have also had this type of reproduction artificially induced. The establishment of parthenogenetic embryonic stem (pES) cells for autologous cell therapy in females without the need to kill ordinarily competent embryos is possible even if such embryos lack the capacity to develop to full term. One of the most intriguing areas in biology nowadays is stem cells. In all multicellular animals, stem cells are cells. They can differentiate into a wide variety of specialised cell types and still replenish themselves through mitotic cell division. Scientists Ernest A. McCulloch and James E. Till from Canada made discoveries in the 1960s that led to the development of stem cell research. The understanding of how an organism grows from a single cell and how healthy cells replace damaged ones in adult creatures is being advanced by research on stem cells. Scientists are also looking at the idea of using cell-based therapies, often known as regenerative or reparative medicine, to treat disease as a result of this promising field of research. Scientists have proposed that stem cells could one day serve as the foundation for treating conditions including Parkinson's disease, diabetes, and heart disease. Embryonic stem cells, also referred to as ES cells, are stem cells that are derived from the inner cell mass of a blastocyst, an embryo in its early stages. Four to five days after fertilisation, human embryos reach the blastocyst stage, consisting of 50 to 150 cells. Pluripotent embryonic stem (ES) cells exist. This indicates that they can differentiate into all ectoderm, endoderm, and mesoderm descendants, the three main germ layers. These comprise all 220+ cell types found in the adult body. ES cells' pluripotency sets them apart from adult multipotent progenitor cells, which can only give rise to a small variety of cell types. ES cells maintain pluripotency during numerous cell divisions when they are cultured in vitro in the absence of any differentiation-stimulating factors. Although the existence of pluripotent adult stem cells is still up in the air, studies have shown that pluripotent stem cells can be produced directly from adult fibroblast cultures.

Mammalian stem cells come in two general categories: embryonic stem cells (ES), which are found in blastocysts, and adult stem cells, which are present in adult tissues, particularly those of female patients (such as endothelial stem cells, etc.).

Since embryonic stem cells are pluripotent, they can differentiate into any form of body cell. Adult stem cells can typically only differentiate into the various cell types of the tissue from which they originated. Therefore, the use of embryonic stem cells in regenerative or reparative medicine is of utmost importance. However, some data point to the possibility of adult stem cell plasticity, which would increase the range of cell types that an adult stem cell may differentiate into.

Adult stem cell research and therapy are neither contentious nor troublesome when using them. However, using embryonic stem cells often results in issues like these.

- An embryo must be created and then destroyed.
- Both male and female germ cells are necessary, but they might not be present when they are.
- It is unethical to exploit human embryos to create embryonic stem cells.
- These techniques might not be able to produce stem cells that are genetically compatible with a specific patient in order to treat degenerative disorders.
- It's possible that the stem cells taken from embryos won't be immunologically compatible with the recipient.
- An oocyte that has experienced parthenogenesis, a procedure known for producing pluripotent stem cells, is a new source that has recently come to light.

This method allowed for the creation of ES cells without the requirement to produce or dispose of a viable embryo. As a result, this synthetic parthenogenesis appears to provide a means of avoiding these moral dilemmas, and various immunological issues arise in

## II. PARTHENOGENESIS INCIDENCE

In nature, parthenogenesis is a typical method of reproduction for lesser creatures. Approximately 70 lower species of invertebrates have been documented to have it. This method of reproduction is frequently used by flies, ants, lizards, snakes, fish, birds, reptiles, amphibians, honeybees, and crayfish.

Kevin Buley, a naturalist, initially noted on December 21, 2006, that a female Komodo dragon at Chester Zoo in England fertilised her own eggs.

On December 14, 2001, at the Henry Doorly Zoo in Nebraska, a small hammerhead shark (bonnethead) was believed to have given birth to a live pup in a tank with three female hammerheads but no males. Testing revealed that the pup had no male DNA and only one female match among the inhabitants of the tank. This inquiry established that parthenogenic methods were used to give birth. At the Belle Isle Aquarium in Detroit, where the mother and just one other female shark shared an aquarium, two white-spotted bamboo sharks were born in 2002.

In the natural world, mammals are incapable of this type of reproduction and are unable to create a viable pregnancy. Eutherian oocytes can, nevertheless, partially succeed in parthenogenesis in vitro. Mammalian oocytes can develop to day 10 in the mouse, day 21 in sheep, day 29 in pigs, and day 11.5 in the rabbit when activated (simulating the fertilisation process) and transferred to a surrogate mother (Vrana et al., 2003).

Pincus reported on the birth of live parthenogenetic rabbits in the 1940s. Out of 200 mammalian parthenogenetic embryos, Beaty (1957) predicted that one would progress to full-term pregnancy. According to Kaufman (1977), mouse parthenogenic embryos developed normally after implantation all the way to the forelimb bud stage. The parthenogenetic origin of various tissues in such chimeric animals has been proven (Boediono et al., 1999).

However, hybrids created from parthenogenetic cells and embryonic tissues acquired from biparental sources have produced offspring that appear to be normal. Contribution to multiple tissues, including blood, where 100% of the leukocytes were confirmed to be of parthenogenetic origin, has been shown in a reported case of a human parthenogenetic chimaera (Strain et al., 1995).

Human parthenogenesis induced artificially has not yet proved successful. However, there is some proof that natural parthenogenesis does occasionally take place in people. There are numerous reports of impregnation occurring in females without the semen ever having a chance to reach the female vaginal tract. In rare instances, it was discovered that the female passageways were blocked during labour or throughout pregnancy. A report on 19 reported occurrences of virgin birth among women in England that British Medical Association members had looked into was published in the medical journal Lancet in 1956. Researchers were persuaded by the six-month study that human parthenogenesis was physiologically feasible and had actually taken place in some of the women who were subjected to it.

Human eggs occasionally experience spontaneous cell division, as has long been recognised. These proliferating eggs produce teratomas, which are benign tumours that include skin and hair cells among other cell types, and dermoid cysts. Eggs could be a particularly useful source of stem cells if they could be regularly encouraged in the lab to go through cell division.

## III. PARTHENOGENIC STEM CELL RESEARCH FACTS

The research of Jacques Loeb (1859–1924), who is regarded as the father of artificial parthenogenesis, was first published in 1899.

A South Korean biomedical researcher named Hwang Woo-Suk claimed to have succeeded in cloning human embryonic stem cells in 2004 and 2005. After extensive independent research, it was discovered on August 2, 2007, that Hwang's team had successfully retrieved parthenogenic stem cells from parthenogenesis-affected eggs. These stem cells had the same parthenogenic markers as the mice developed by Tokyo scientists in 2004.

Cibelli et al. created the first embryonic stem cells in primates through parthenogenesis in 2002. Parthenogenesis produces a blastocyst that cannot develop into a viable foetus. Vrana (2002) from Wake Forest University School of Medicine and Advanced Cell Technology (ACT) of Worcester, Massachusetts, reported that they had created a pluripotent stem cell line from a primate that they called Cyno-1 from this blastocyst. For ten months, the cell line has grown without interruption. Parthenogenesis "offers an important new therapeutic approach for a variety of medical conditions," according to Vrana.

Nancy L. Jones led a group of researchers from the Stemron Corporation and the Reproductive Biology Association who reported on their parthenogenesis-based human embryo production in 2003. In 2005, Dr. Ann Kiessling conducted a series of experiments at the Bedford Stem Cell Research Foundation in the United States to examine human parthenogenetic stem cells for the first time. She got ready to turn on three human eggs.

Jin Young Ju et al. described the establishment of stem cell lines from parthenogenetically activated mouse oocytes for therapeutic cloning in 2006. Nuclear transfer (4.3%) and sham manipulation (12.5%) had lower rates of embryonic stem cell line creation than parthenogenetically activated oocytes (15.7%). After sustained proliferation for more than 120 passes while preserving a proper karyotype, cell colonies successfully maintained an undifferentiated morphology and demonstrated the typical appearance of mouse embryonic stem cells.

## IV. PARTHENOGENETIC STEM CELL FORMATION

### 1. Oocyte Collection and Follicular Stimulation

Oocytes can be removed surgically or by flushing. Animals' oocytes are retrieved after superovulation to obtain more of them, and even ovarian tissue from slaughterhouses can be used to gather oocytes. For cultivation, cumulus-oocyte complexes (COC) with the impact multiplication of cumulus cells are used. If the oocytes are extracted from the antral follicles, they must be kept in maturation media until the second meiotic division (MII) metaphase. Following the stripping off of the cumulus cells, denuded oocytes with a spherical form, a visible polar body, and an equally granulated ooplasm are chosen for activation.

### 2. Oocyte Activation

At the metaphase of the second meiotic division (MII) in vertebrates, mature oocytes are stopped due to heightened maturation-promoting factor (MPF) activity that is kept in check by cytostatic factor (CSF).CSF prevents cyclin B's ubiquitin-dependent degradation from inactivating MPF. Sperm entrance during fertilisation sets off a sequence of intracellular calcium oscillations that are essential for oocyte activation. The two proteins that calcium-stimulated events are most likely to target are MPF and mitogen-activated protein (MAP) kinase. Because the restart and completion of meiosis, the ensuing production of pronuclei, and DNA synthesis all depend on the inactivation of these kinases, The cyclin degradation mechanism is released, and CSF activity is downregulated by intracellular $Ca^{2+}$ oscillations. Cyclin B proteolytic breakdown and subsequent MPF inactivation free the oocyte from metaphase arrest, allowing the mitotic cycle to start or resume.

### 3. Activation of Parthenogenetic Oocytes

Numerous physical and chemical interventions can stimulate parthenogenic development in MII oocytes and imitate sperm-triggered processes. For instance, ethanol electroporation, calcium ionophore, ionomycin, or inositol 1,4,5-triphoshphate are the causes of calcium elevation and release from meiotic arrest. Many scientists have long described parthenogenetic oocyte activation in animals. For instance, parthenogenetic stimulation of mouse oocytes was described by Kaufman (1977) and Collas et al. (1989). 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), and Chung, J.T. et al. (1999) have all demonstrated parthenogenetic activation of oocytes in cattle. Similar results were reported for pigs by Jolliff et al. (1997), ovines by Loi et al. (1997), and rabbits by Ozil, J.P. (1990), Mitalipov et al. (1999), who are notable for their success. In addition, we have created parthenogenetic embryo activation in our lab (Kumar et al. 2013, Pankaj et al. 2012).

**4. Oocyte Nuclear Activation Assessment**

Oocyte activation was defined as the restart of the second meiotic division; therefore, an oocyte was deemed to be active if it had reached anaphase II, telophase II, metaphase III, or pronuclear formation. Only a pronucleus with a uniformly granulated nucleoplasm encircled by a full nuclear envelope was judged to be fully formed when a stage of pronuclear development was evaluated.

Since most somatic cell types lack telomerase activity, telomerase activity is frequently associated with replicative immortality (Kim et al., 1994; Armstrong et al., 2000; Amit et al., 2000). Telomerase activity is commonly expressed in germ cells, cancer cells, and a variety of stem cells, including ES cells. In 2003, Vrana et al. also discovered high levels of telomerase activity in undifferentiated Cyno-1 cells.

The amount of DNA synthesis in activated oocytes also affects the restart of MII. Some claim that only completely formed pronuclei are capable of synthesising DNA. The inclusion of 3H-thymidine into freshly synthesised DNA allows for the monitoring of DNA synthesis in an active oocyte. Then, using autoradiography on dispersed oocytes or a semithin segment of an oocyte, the incorporated thymidine is seen (Eva Soloy et al., 1997). The activated oocytes are grown to the blastocyst stage either in vivo or in vitro.

**5. Stem Cell Separation**

Immunosurgery was used by Cibelli (2002) to isolate the inner cell mass (ICM) from parthenogenetic blastocysts, and cells were subsequently extensively grown and propagated for ten months in the lab. Telomerase activity declined after two weeks of differentiation, indicating that the cells weren't cancer cells.

## V. GENETIC COMPLEMENTS IN PARTHENOGENETIC STEM CELLS

If the gender of the female children is determined by two identical chromosomes (such as the XY sex-determination system) and the gender of the male offspring by two identical chromosomes (such as the ZW sex-determination system), the kids of parthenogenesis will all be male.

Given that parthenogens are homozygous, all of their children will be genetically identical to their mothers and to each other as well. As a result, when parthenogenesis is used to reproduce, each sibling of the first generation of parthenogens will produce a linage that has solely her particular genes.

The cells produced after parthenogenically stimulating an egg are either monoploid or diploid. However, the end result of parthenogenetic activation of the oocyte is to create a diploid embryo with a complete genetic makeup. According to Jones (2003), this can be accomplished by either encouraging diploid eggs to divide or by inducing haploid eggs to copy their genetic material. A full set of genes is kept if early activation is carried out, since eggs only retain half of their genetic complement towards the end of their maturation cycle. A haploid egg can also be induced to reproduce its genetic material, producing an entire genetic complement. There are two complete sets of chromosomes in an unfertilized egg. An electric or chemical shock might cause the oocyte to develop as though fertilised and maintain the additional set even when one set is ejected during fertilisation. The resultant embryo can be used to harvest stem cells.

However, Wise Young (2007) has demonstrated that parthenogentic monoploid cells can differentiate into cell types that might be useful for therapeutic purposes in addition to behaving like stem cells and being pluripotent both in vivo and in vitro.

## VI. CONCLUSION

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

Given that parthenogens are homozygous, all of their children will be genetically identical to their mothers and to each other as well. As a result, when parthenogenesis is used to reproduce, each sibling of the first generation of parthenogens will produce a linage that has solely her particular genes.

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**Differentiating Parthenogenetic Stem Cells**

In vitro production of a wide range of specialised cell types was possible, including brain cells, smooth muscle cells, cytokeratin-positive cells, and cells that beat spontaneously, such as cardiomyocyte-like cells and cilliated epithelium. In primates, Cyno-1 cells have been seen to differentiate into all three germ layers, including the ectoderm, which contains cartilage, neurons, skin, and hair follicles; the endoderm, which contains intestinal epithelia; and the mesoderm, which contains muscle and bone (Vrana et al., 2003). Parthenogenic stem cells have several advantages.

This could be a particularly advantageous source of stem cells that avoids the moral, ethical, and some tissue rejection issues related to foetal and embryonic stem cells. This is especially true for the woman donating the egg. An individual with diabetes or spinal cord damage, for instance, could give her own eggs to obtain her own stem cells.

These cells might offer a cutting-edge method for examining how genomic imprinting affects cell differentiation and function in primates and humans during development.

Their remarkable differentiation skills (electrophysiologically active, dopamine-secreting neurons) point to their therapeutic promise for treating Parkinson's and Huntington's diseases and suggest a viable alternative to ES cells produced from two parents.

Parthenogenetic cells have less variety in their surface proteins that can elicit immunological responses because they contain two identical sets of chromosomes rather than one set from each parent. Parthenogenetic stem cell therapy is claimed to have negligible or no effect on the complex immunocompetability that develops after cell therapy. A potential source of histocompatible cells and tissues for transplantation is genetically matched pluripotent embryonic stem (ES) cells produced through nuclear transfer or parthenogenesis. A source of histocompatible tissues for transplantation can be found in parthenogenetic stem cells that have been isolated from murine oocytes and carry all of the donor's major histocompatibility complex (MHC) antigens. These cells were grafted into immunocompetent, MHC-matched mouse recipients. In 2007 (Kitai Kim).

**Parthenogenic Stem Cells' Drawbacks**

Parthenogenetic stem cells cannot be created in either men or women after menopause because eggs are required for parthenogenetic stem cell production. In contrast, therapeutic cloning might provide each person with the exact same stem cells.

Although parthenogenetic stem cells appear to be identical to conventional ES cells in every way, it is legitimate to doubt their survival and usefulness. After all, they are only descended from maternal DNA.

Due to the high frequency of X chromosome inactivation mistakes that take place in extra embryonic tissues when both X chromosomes are derived exclusively from the female, parthenogenetic embryos do not grow to term. It takes a lot of time and effort to produce just one parthenogenetic embryo, and the tests needed to verify their stemness are both expensive and time-consuming. Costly equipment, chemicals, highly qualified technical support, and a bank with cutting-edge technology are needed to store the stem cells.

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