INTRODUCTION

Since 1988 when human umbilical cord blood (UCB)-derived stem cells were transplanted for the first time as an alternative to marrow or circulating blood-derived progenitor cells ***(Gluckman et al., 1989).*** UCB is increasingly used as an alternative source of hematopoietic stem cells in related and unrelated allogeneic transplantation in children and adolescents ***(Reboredo, 2000)***, it has been successfully used to treat a variety of genetic, hematological, and oncological disorders in children and adults.

Umbilical CB transplantation has been performed in more than 4000 patients worldwide ***(Ballen, 2005)***. In fact, CB has become a real alternative source of hematopoietic stem cells for bone marrow reconstitution in a variety of genetic, hematological and oncological disorders. As a response to this increasing activity, CB banks have been developed to guarantee the quality of processed CB units ***(Stanworth et al., 2001)***.

One of the major problems with long-term CB banking is the required storage space. Red blood cell (RBC) depletion of CB not only maximizes storage space, but also has other advantages like the reduction of the potential side-effects of dimethylsulfoxide (DMSO) and the reduction of side-effects of ABO-mismatched or haemolysed red blood cells in the unfractionated infusates ***(Oliveira et al., 2004)***.

Volume reduction is essential for cord blood banks to be economical and efficient. Several different methods have been employed to reduce the volume prior to cryopreservation without loss of progenitor cells: density gradient separation ***(Harris et al., 1994)***, sedimentation of red cells by gelatin ***(Nagler et al., 1993)***, rouleaux formation induced by hydroxyethyl starch (HES) and centrifugation ***(Rubinstien et al., 1995 & Sousa et al., 1997)***, and differential centrifugation with expression of RCB and plasma ***(Ademokun et al., 1997)***.

AIM OF THE WORK

This study is purposed to clarify the impact of maternal and neonatal factors on total nucleated cell count of collected umbilical cord blood of full term delivery > 36 weeks completed gestation.

HISTORICAL BACKGROUND OF STEM CELL RESEARCHES

- **1960s:** ***Joseph Altman and Gopal Das*** presented an evidence of adult neurogenesis, ongoing stem cell activity in the brain; their reports altered ***Cajal's*** "no new neurons" concept ***(Goldman et al., 2006)***.

- **1963:** ***McCulloch and Till*** illustrated the presence of self-renewing cells in mouse bone marrow ***(Goldman et al., 2006)***.

- **1978:** Haematopoietic stem cells were discovered in human cord blood ***(Friedenstein et al., 2000)***.

- **1997:** Leukemia was shown to originate from a haematopoietic stem cell, the first direct evidence for cancer stem cells ***(Goldman et al., 2006)***.

- **2003: Dr. Songtao Shi** of National Institute of Health (NIH) has discovered new source of adult stem cells in children's primary teeth ***(Shostak et al., 2006)***.

- **2007:** Scientists at Wake Forest University led by *Dr. Anthony Atala* and Harvard University report discovery of a new type of stem cell in amniotic fluid ***(French et al., 2008)***.

- **2008:** Embryonic-like stem cells were derived from a single human hair ***(French et al., 2008)***.

- **2009:** ***Andras et al.*** discovered a way to produce embryonic-like stem cells from normal adult cells by using a novel "wrapping" procedure to deliver specific genes to adult cells to reprogram them into stem cells without the risks of using a virus to make the change. The use of electroporation is said to allow for the temporary insertion of genes into the cell.

- **2010:** Transplanted cells that contain their new host's nuclear DNA could still be rejected by the invidual's immune system due to foreign mitochondrial DNA. Tissues made from a person's stem cells could therefore be rejected, because mitochondrial genomes tend to accumulate mutations ***(Ishikawa et al., 2010)***.

- **2011:** ***Friedrich*** led a team which produced the first stem cells from species exposed to danger; this could save animals in danger of extinction.

- **2012:** ***Katsuhiko Hayashi et al. (2012)*** reported that they used mouse skin cells to create stem cells and then used these stem cells to create mouse eggs. These eggs were then fertilized and produced healthy baby offspring. These latter mice were able to have their own babies.

CLASSIFICATION OF STEM CELLS

The four broad categories of mammalian stem cells are:

1- ***Embryonic Stem (ES) cells***, derived from blastocysts (a structure formed in the early development of vertebrates).

2- ***Fetal stem cells***

3- ***Adult stem cells***

4- ***Umbilical cord blood stem cells.***

(Tuch, 2006)

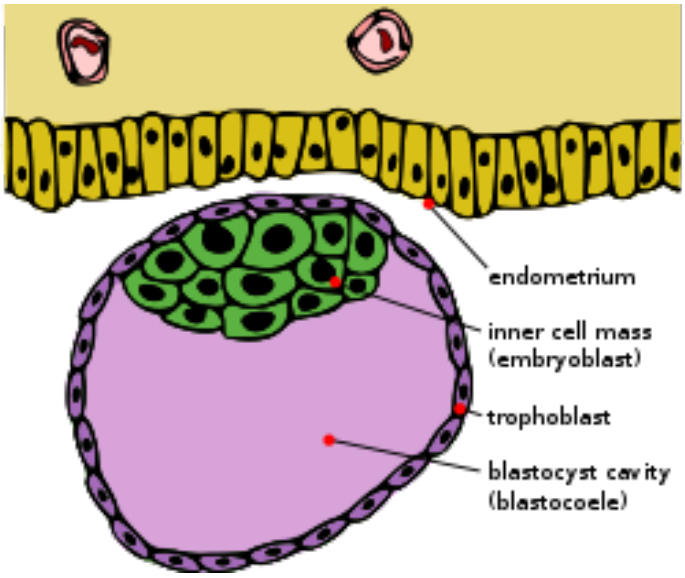

Figure (1): Human blastocyst showing inner cell mass "embryoblast" ***(Reubinoff et al., 2000).***

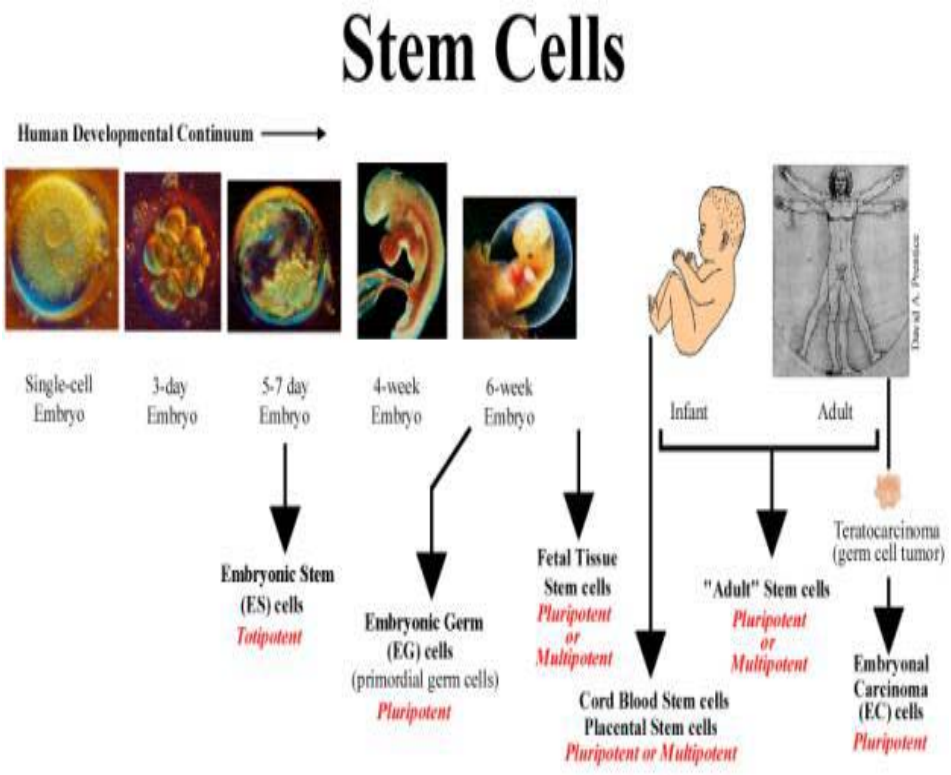

Figure (2): Similarities and differences between embryonic and adult stem cells ***(French, 2010)***

(I) Embryonic stem cells:

Human Embryonic Stem (ES) cells have an almost unlimited developmental potential after many months of growth in culture dishes. The proliferative and developmental potential of human ES cells promises an essentially unlimited supply of specific cell types for basic research and for transplantation therapies for diseases ranging from heart disease, Parkinson's disease to leukemia ***(Trounson et al., 2003)***.

Defining properties of an embryonic stem cell (figure 4):

- The Inner Cell Mass (ICM) cells have the potential to generate any cell type of the body, but after implantation, they are quickly depleted as they differentiate to other cell types with more limited developmental potential ***(Martin et al., 2003)***.
- 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 (a biological cell that, like a stem cell, has a tendency to differentiate into a specific type of cell, but is already more specific than a stem cell and is pushed to differentiate into its "target" cell ***(Andrews et al., 2005)***.
- The most important difference between stem cells and progenitor cells is that stem cells can replicate indefinitely, whereas progenitor cells can divide only a limited number of times. The terms "progenitor cell" and "stem cell" are sometimes equated 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 ***(Andrews et al., 2005)***.
- The first study that describes successful separation of human Inner Cell Mass (ICM) cells and their continued culture for at least two passages in vitro was in 1994. Separated ICM cells either differentiate or produce cells with typical Human

Embryonic Stem (HES)-cell-like morphology positive for alkaline phosphatase staining and with normal karyotype ***(Bongso et al., 2004)***.

- Capable of integrating into all fetal tissues during development. (Mouse ES cells maintained in culture for long periods can still generate any tissue when they are reintroduced into an embryo to generate a chimeric imaginary animal ***(Bongso et al., 2004)***.
- Capable of colonizing the germ line and giving rise to egg or sperm cells ***(Odorico et al., 2001)***.
- Clonogenic (that is a single ES cell can give rise to a colony of genetically identical cells, or clones, which have the same properties as the original cell) ***(Odorico et al., 2001)***.

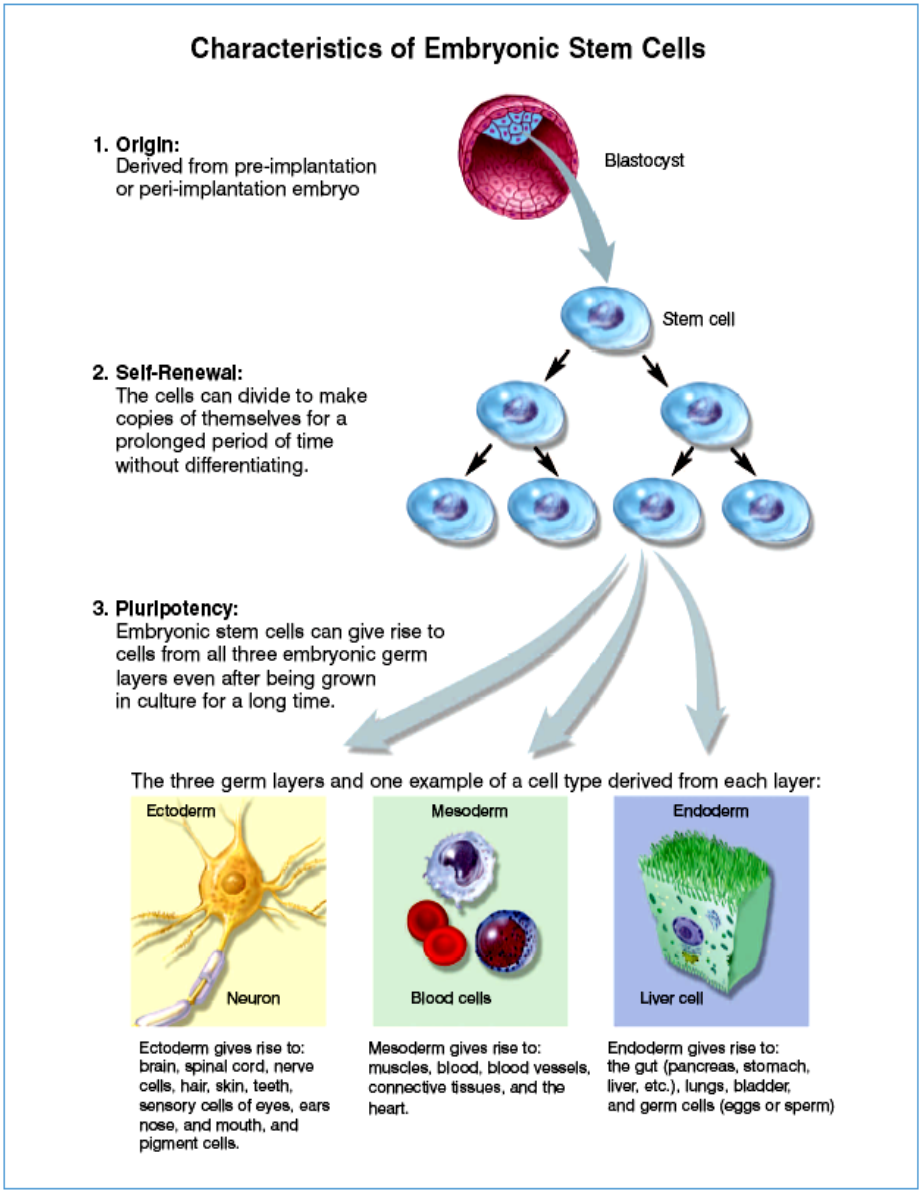

Figure (3): Characteristics of Embryonic stem cells ***(Odorico et al., 2001).***

Derivation of human Embryonic Stem cells (HES) (figure 5):

Nearly all described HES cell lines have been efficiently derived using the immunosurgery procedure, however mechanical isolation is advantageous since there is no contact of blastocysts with animal antibodies. On the other hand, there is a risk that not all of the Trophectoderm (TE) cells may be removed during mechanical isolation and these may subsequently overgrow and inhibit the growth of ICM cells ***(Pickering et al., 2003)***. Success rate in deriving HES cell lines is highly dependant on the quality of recovered blastocysts, isolation conditions and experience of the group ***(Mitalipova et al., 2003)***.

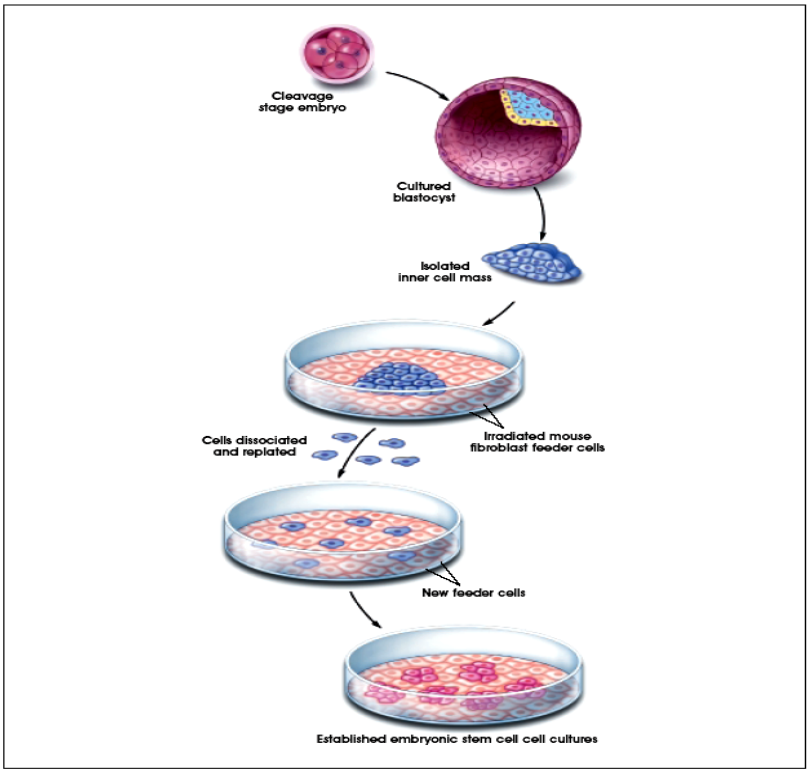

Figure (4): Derivation of embryonic stem cells ***(Hwang et al., 2004).***

Differentiation potential of Human Embryonic Stem Cell (HESC) into functional tissues (figure 6):

Despite the well-recognized pluripotency of HESC, it has proven rather challenging to direct the differentiation of HESC into specific lineages. However, an ESC based strategy could permit the generation of an unlimited supply of desirable, fully functional cell types from an abundant, renewable, and readily accessible source ***(Sudhanshu et al., 2006)***.

When embryonic stem cells in culture are grown under certain conditions, they remain undifferentiated, or in other words, unspecialized. However, if cells are permitted to clump together to form embryoid bodies, they start to spontaneously differentiate into muscle cells, nerve cells, and many other cell types. Though the spontaneous differentiation is a good sign that a culture of embryonic stem cells is healthy, it is not an efficient way to generate cultures of specific cell types ***(Zubair, 2010).***

To generate cultures of specific types of specialized cells such as heart muscle cells or nerve cells, scientists try to control the differentiation of embryonic stem cells. They change the surface of the culture dish, alter the chemical composition of the culture medium, or modify cells by inserting specific genes. Years of experimentation have produced basic protocols for the directed differentiation of embryonic stem cells into specific cell types ***(Schuldiner et al., 2001)***.

Scientists may be able to use differentiated cells to treat certain diseases at some point in the future if they learn to reliably direct the differentiation of embryonic stem cells into specific cell types. Diseases that may be treatable by transplanting cells generated from human embryonic stem cells include diabetes, Parkinson's disease, heart disease, traumatic spinal cord injury, Duchenne's muscular dystrophy, Purkinje cell degeneration, and vision and hearing loss ***(Hwang et al., 2004).***

Current methods for obtaining human embryonic stem cells require destroying the embryo. Many Americans oppose such embryo destruction because they believe that a human life has dignity and should not be violated from fertilization to natural death. Many others, however, believe that the benefits of advances in biomedical science outweigh these moral concerns by the altered nuclear transfer ***(Barbara et al., 2008).***

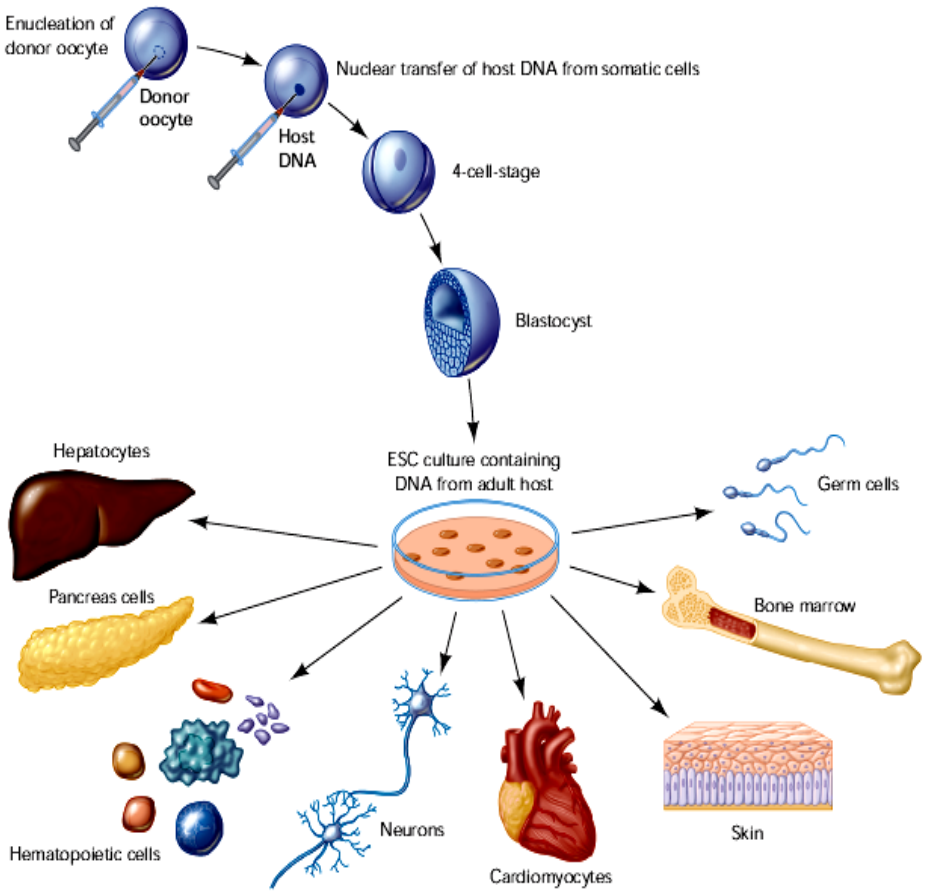

Figure (5): Nuclear transfer and directed differentiation
(Sudhanshu et al., 2006)

Use of human embryonic stem cells as models for human genetic disorders:

Verlinsky (2012), who specialized in embryo and cellular genetics (genetic cytology), developed prenatal diagnosis testing methods to determine genetic and chromosomal disorders a month and a half earlier than standard amniocentesis. The techniques are now used by many pregnant women and prospective parents,

especially those couples with a history of genetic abnormalities or where the woman is over the age of 35, when the risk of genetically-related disorders is higher. In addition, by allowing parents to select an embryo without genetic disorders, they have the potential of saving the lives of siblings that already had similar disorders and diseases using cells from the disease free offspring.

Table (1): Embryonic stem cells

Stem cell type	Current research uses	Ready for the clinic?	Advantages	What we don't know yet: Limitations and question
Embryonic stem cells	Understanding how our bodies develop from a fertilized egg; Investigating how to produce different types of specialized cell	The first clinical trials are now beginning, focused on treating eye disorders; these are early stage safety trials.	Can produce all the different types of cells in the body Can self-renew (copy themselves) almost forever, so large supplies can be made	Still learning how to fully control differentiation of these cells Some groups have concerns on ethical or religious grounds

(Euro Stem Cell, 2013)

(II) Fetal stem cell:

Although ES cells seem the most flexible type of stem cell, the ethical dispute and safety issues surrounding their potential clinical use have encouraged the search for other sources of stem cell ***(Tuch, 2006).***

Gotherstrone et al. (2004) suggested that fetal stem cells are more pleuripotent than adult stem cells, and hence have greater therapeutic potential. In addition, there is evidence that they have important differences in gene expression. Some fetal stem cells