The Role of Mesenchymal Stem cells in Treatment of Endometriosis

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Abstract

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key word:

Endometriosis Mesenchymal Obstetrics

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List of Abbreviations

T A	Transient amplifying cells
MSCs	Mesenchimal stem cells
UCB	Umbilical cord blood
BMSCs	Bone marrow stem cells
non-ESC	Non-embryonic stem cells
ESC	Embryonic stem cells
hESC	Human embryonic stem cells
HSC	Hematopoietic stem cells
BM	Bone marrow
GnRHa	Gonadotropin releasing hormone agonist
GnRH	Gonadotropin-releasing hormone
OA	Osteoarthritis
HRT	Hormone replacement therapy
VEGF	vascular endothelial growth factor
IVF	In-Vitro Fertilization
IP	Intra-peritoneal
MMP-3	Matrix metalloproteinase
LH	Luteinizing hormone
MCP-1	monocyte chemotactic protein 1
TIMP-2	Tissue inhibitor of MMP
mRNA	messenger ribonucleic acid
Q-RT-PCR	**quantitative reverse transcription polymerase chain reaction**
VCAM-1	vascular cell adhesion molecule 1

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Introduction

INTRODUCTION

Endometriosis is a common gynecological disorder that is defined by the presence of endometrial tissue outside the uterine cavity. This disease often results in extensive morbidity, including chronic pelvic pain and infertility. The pathogenesis of endometriosis is multi factorial, and extensive investigations have explored the role of genetics, environmental factors, and the immune system in predisposing patients to developing endometriosis. The incidence of endometriosis is between 6% and 10% of all women. (Kuohung W. et al., 2002)

The clinical manifestations of endometriosis and the presence of endometrial tissue outside the uterine cavity is probably the end point of a combination of several aberrant biological processes. These include retrograde menstruation in a woman with an improper immune response and a genetic predisposition to developing endometriotic lesions, possibly in the setting of an uncharacterized environmental exposure. (Giudice L.C.2006)

The origin of endometriotic implants and the pathogenesis of endometriosis has long been an area of active investigations. Multiple hypotheses have been explored including retrograde menstruation, coelomic metaplasia, embryonic rest theory and the lymphovascular metastasis theory. (Taylor R.N. et al 2002.)

Stem cells are undifferentiated cells that have the ability to self renew as well as to produce more differentiated daughter cells (Gargett C.E et al., 2007). Broadly, stem cells can be divided into two categories: embryonic and adult. Embryonic are derived from blastocysts. Adult derived from postembryonic cell lineages.

Totipotent stem cells are fully undifferentiated and able to generate all embryonic germ layers as well as the extra embryonic tissue (trophoblast, placenta).

Stem cells asymmetrically divided to produce daughter cells known as progenitor or transient amplifying (T A) cells that begin the differentiation process (Bongso A et al., 2004).

T A cells undergo repetitive cycles of cell divisions to increase in number while progressively acquiring markers of the differentiated cell type; consequently, they lose the ability to self renewal. The processes of differentiation, proliferation and migration are often linked in multiple biological systems (Chan R et al., 2004).

The use of stem cell therapy is not new, in that haematopoiotic stem cells (HSC) from bone marrow and cord blood have been used for transplantation for many years.

Degenerative disease that are likely to benefit from stem cell therapy including the brain (Alzheimer's ,Parkinson's) liver (Hepatitis) pancreas (Diabetes) and joints (Arthritis) stem cells could help to overcome the shortage of organs for transplantation not only are they readily expandable.(Jeong YH et al., 2006)

Isolation and culture of UCB-MSCs:

The low-density mononuclear cells were isolated using Ficoll-Plaque Plus (Amersham Biosciences,Sweden).
Then, the cells were cultured in growth medium [Dulbeco 's Modified Eagle media-low glucose with the addition of 10%fetal bovine serum (Gibco-BRL,USA)with 2 mmol/l L-glutamine (Gibco- BRL,USA)] and 0.3%penicillin-streptomycin (Gibco- BRL,USA)at 37 ^{o}C and 5%CO 2 concentration

[1] .The UCB-MSCs were cultured and the mononucleated cells that proliferated from the cord blood were characterized by FACS analysis [2].The cells were prepared as 1 $\times 10^{6}$ in 150 µl of saline solution for the injection.

Key words:

Stem cell, Endometriosis, Esterus cycle, Infertility.

Aim of the work

The aim of this thesis is to assess the results of stem cell therapy in cases with endometriosis and compare it with a non-treated group in experimental animal model.

1. The Stem cells

Introduction:

Stem cells are tissue precursor cells with two contrasting abilities; they self renew and they can differentiate into more specialized cell types. They are found at all stages of development and in all tissues. (Reuhinoff BE, Pera MF 2000)

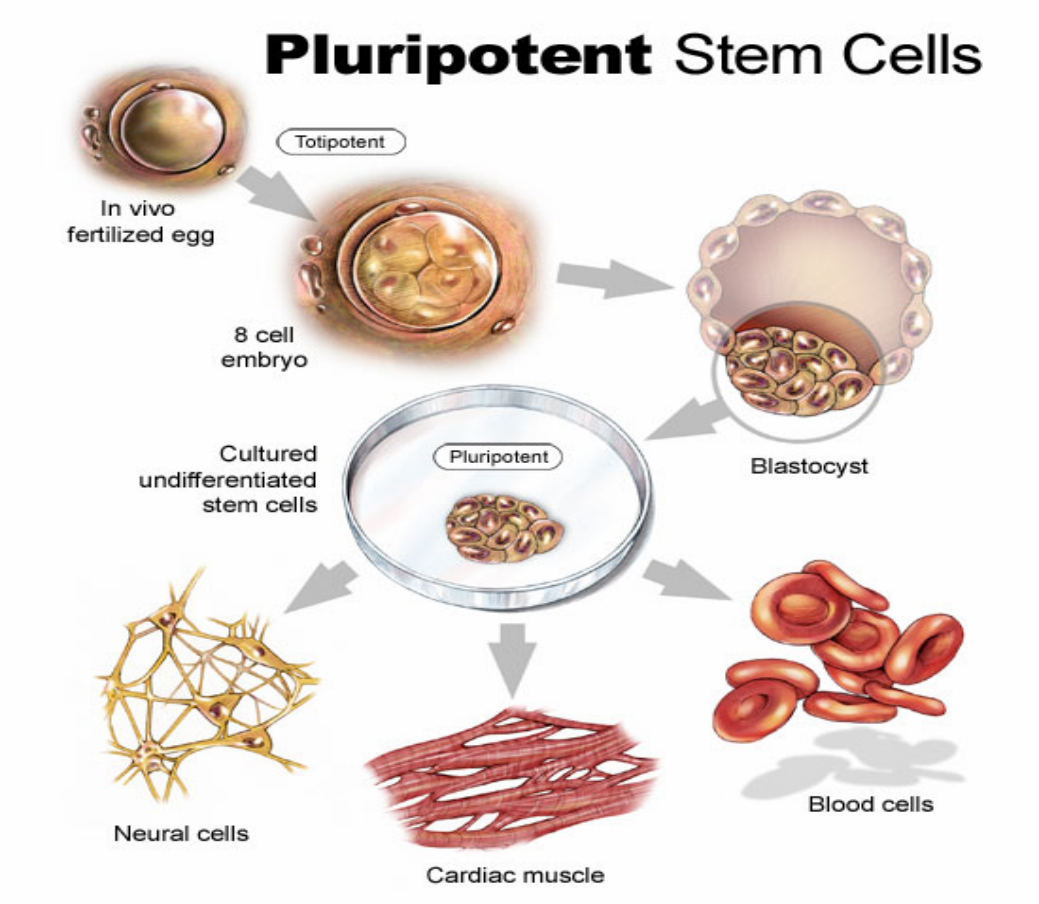

Figure (I)

An orderly chain of highly regulated processes involving cell proliferation, migration, differentiation, and maturation leads to the production and sustenance of most cell lineages in adult organisms. The earliest cell type on this chain has been called a stem cell (**Minguell** et **al., 2001**).

Stem cells are undeveloped cells capable of proliferation, self renewal, conversion to differentiated cells, and regenerating tissues. When a stem cell divides, each new cell has the potential to either remain a stem cell or become another type of cell such as muscle cell, red blood cell, brain cell, …….etc. They are unspecialized, for a long period through cell division. Unspecialized means that the stem cell does not have any tissue-specific structure that allows it to perform specialized function. When unspecialized cell gives rise to specialized cell this is differentiation.

There are two main types of stem cells, embryonic and non-embryonic stem cells (**Amit and Itskovitz-Eldor, 2009)** (Table1). Besides embryonic stem cells, bone marrow has been predominately considered the only significant source of stem cells. However, recent findings have revealed that adult stem cells can reside in most if not every tissue. The marrow and non-marrow stem cells display different characteristics and properties.

Stem cells are classified according to their differentiation potential as totipotent, pluripotent, or multipotent. Totipotent stem cells are capable of forming any tissue in the body, similarly to a fertilized egg which, following cleavage produces cells which differentiate into all types of tissues. Pluripotency is the capability of the cell to create almost any type of cells in the organism, but not the entirety. Multipotent stem cells, finally, are those that can only give rise to cells of the tissue that they were isolated from. Cells in a developing embryo are totipotent at the

beginning, then lose this feature after several cell cycles as a completely developed organism and become pluripotent. Therefore, based on the criteria of differentiation potential, embryonic stem cells are the least differentiated when compared with bone marrow stem cells (BMSCs), tissue-specific stem cells, lineage-specific precursors, and terminally differentiated cells. Non-embryonic stem cells (non-ESC) are multipotent because their potential to differentiate into cell types is more limited. Embryonic stem cells are more prevalent than non- ESC and have a greater potential to spontaneously differentiate than non-ESC **(Preston et al., 2003).**

Table (1): Human Stem Cells

Stem cell	**Location (source)**	**Cells produced**	References
Hematopoietic	Bone marrow	Blood, endothelial, hepatic (oval), and muscle cells	**Gussoni et al., 1999**
Neural	Brain	Neurons, astrocytes, oligodendrocytes, and blood cells	**Armstrong & Svendsen, 2000**
Epithelial	Gut, epidermis	All cells in epithelium crypts; all cells in epidermal layers	**Wright , 2000**
Mesenchymal	Bone marrow	Bone, cartilage, tendon, adipose, muscle, marrow stroma, and neural cells	**Kopen et al., 1999**
Embryonic	Blastocyst inner cell; mass primordial germ cells	All cells	**Thomson et al., 1998**

2. The Embryonic Stem Cell

Embryonic stem cells have the ability to remain undifferentiated and proliferate indefinitely in vitro while maintaining the potential to differentiate into derivatives of all three embryonic germ layers (**Wobus & Boheler , 2005) .**

Embryonic stem cells are derived from the inner cell mass of a blastocyst, which forms several days after an egg is fertilized. If the blastocyst implants into the uterus, the inner cell mass will develop into a fetus, with the surrounding trophoblast developing into the placenta. The first human embryonic stem cells (ESC) line was established in 1998 from the inner cell mass of an embryo (**Pruksananonda et al., 2009**). Since then, at least 225 human ESC lines have been created; five of these being produced in Australia. An ESC line is created by taking the ESC and placing the cells on a feeder layer of fibroblasts. The feeder layer assists in maintaining the ESC in an undifferentiated state.

Human ESC lines can theoretically also be derived in a none physiological manner by a process called 'nuclear transfer' – also known as therapeutic cloning. This requires the transfer of the nucleus of a donor somatic cell, for example, a skin cell, into the cytoplasm of an enucleated egg. There are factors in the cytoplasm of this hybrid cell which then allow its differentiation into a blastocyst. The ESC line, which is then derived from the inner cell mass of the blastocyst, has the same nuclear