Verification of Mesenchymal Stem Cells in Normal Human Endometrium

Thesis

Submitted for fulfillment of Msc. Degree
In Obstetrics and Gynecology

By

Mohamed Ahmed Younes
MB.BCh.
Cairo University

Under Supervision by

Prof. Dr. Mohammad Abd Elhameed Nasr Ad Deen
Professor of Obstetrics and Gynecology
Faculty of Medicine – Ain Shams University

Prof. Dr. Hanaa Ahmed Amer
Professor of Clinical Pathology
Faculty of Medicine – Ain Shams University

Prof. Dr. Gehane Mahmoud Hamed
Professor of Physiology
Faculty of Medicine – Ain Shams University

Faculty of Medicine
Ain Shams University
2014

First thanks to **ALLAH** *to whom I relate any success in achieving any work in my life.*

I wish to express my deepest thanks, gratitude and appreciation to Prof. Dr. Mohammad Abd Elhameed Nasr Ad Deen, *Professor of Obstetrics and Gynecology for his meticulous supervision, kind guidance, valuable instructions and generous help.*

Special thanks are due to Prof. Dr. Hanaa Ahmed Amer, *Professor of Clinical Pathology for her sincere efforts, fruitful encouragement.*

I am deeply thankful to Prof. Dr. Gehane Mahmoud Hamed, *Professor of Physiology for her great help, outstanding support, active participation and guidance.*

I would like to express my deep appreciation and sincere gratitude and express my sincere gratefulness, for her precious assistance and invaluable practical guidance and remarks to Dr. Fatma Abu Zahra; *fellow of clinical pathology, Medical research center, Faculty of medicine, Ain shams University.*

Finally, I'm expressing my gratefulness, heartfelt respect and renewed thanks to my family who helped and encouraged me a lot during the accomplishment of this work.

Mohamed Ahmed Younes

List of Contents

List of Tables

List of Figures

List of Abbreviations

Abb.	Full term
CA125:	Cancer antigen 125
CD:	Cluster differentiation
CFU:	Colony forming unite
DMEM:	Dulbecco's modified eagle's medium
hESCs:	Human embryonic stem cells
hUCB-MSCs:	Human umbilical cord blood-derived mesenchymal stem cells
iPS:	Induced pluripotent stem cells
IUA:	Intrauterine adhesion
mESCs:	Mouse embryonic stem cells
MSC:	Mesenchymal stem cells
PBS:	Phosphate Buffer Saline

Introduction

During the menstrual cycle, the human endometrium, which consists of the functionalis and basalis layers, undergoes proliferation, differentiation, tissue breakdown and shedding (menstruation) under the influence of ovarian steroid hormones. These menstruation associated cyclical changes can repeat throughout a woman's reproductive life ***(Maruyama and Yoshimura, 2008).***

Adult stem cell system(s) has long been believed to be critical for the regeneration and remodeling properties of the female reproductive tract ***(Gargett et al., 2007; Ono et al., 2008 and Maruyama et al., 2010).***

The human endometrium is a cyclically regenerating mucosal tissue comprising glands and an extensive vascularized Stroma. Many findings show that rare individual endometrial cells with colony forming activity (large CFU) display adult stem cell properties of self renewal, differentiation, and high proliferative potential in vitro. This suggests that they are responsible for monthly endometrial tissue regeneration, preparing the endometrium for steroid hormone-initiated differentiation into a receptive environment for embryo implantation ***(Gargett et al., 2009)***.

Aim of the Work

To determine presence of mesenchymal stem cells in normal endometrium by isolation and in vitro culture.

Overview of stem cells

Stem cells are those cells that have the capability of self-renewal and differentiation ***(Morrison et al., 2006).***

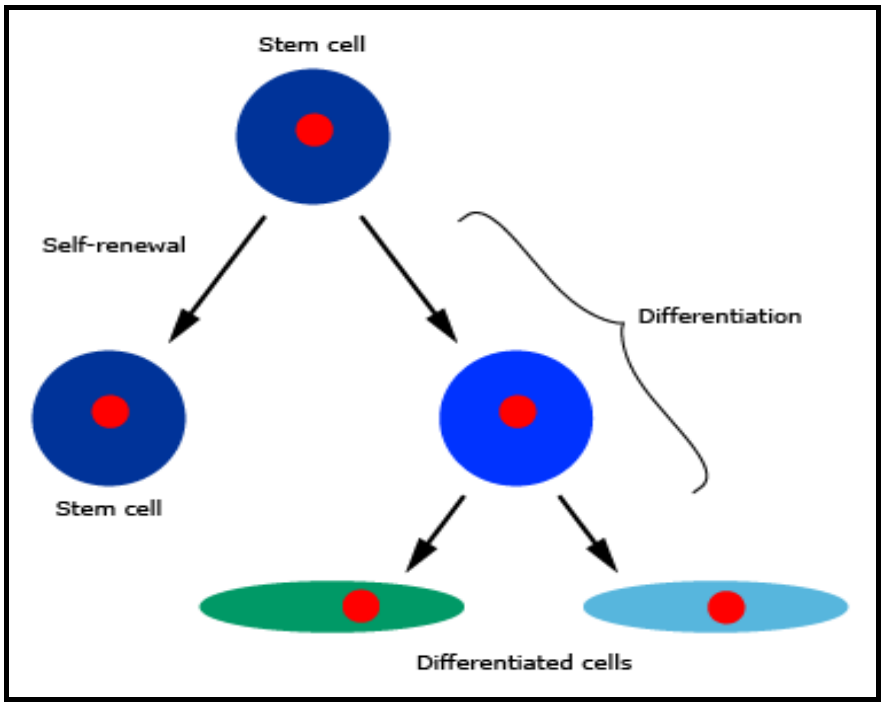

Figure (1): Showing stem cell self renewal and differentiation

The evolving role of stem cells in clinical medicine is developing along at least three lines:

- Stem cells as therapy (either to replace cell lines that have been lost or destroyed, or to modify the behavior of other cells).
- Stem cells as targets of drug therapy.
- Stem cells to generate differentiated tissue for in vitro study of disease models for drug development.

Types of Stem Cells

They are classified according to potency into:

Totipotent cells:

They have the capability to produce all cell types of the developing organism, including both embryonic and extraembryonic (e.g., placenta) tissues.

Pluripotent cells:

They can only make cells of the embryo proper, including germ cells and cells from any of the germ layers. Therefore, they can make any cell of the body.

Multipotent cells:

They can only make cells within a given germ layer. For example, multipotent stem cells from a mesodermal tissue like the blood can make all the cells of the blood, but cannot make cells of a different germ layer such as neural cells (ectoderm) or liver cells (endoderm).

Unipotent cells:

They can make cells of a single cell type. An example is a germ cell stem cell that makes the cells that mature to become egg or sperm, but not other cell types.

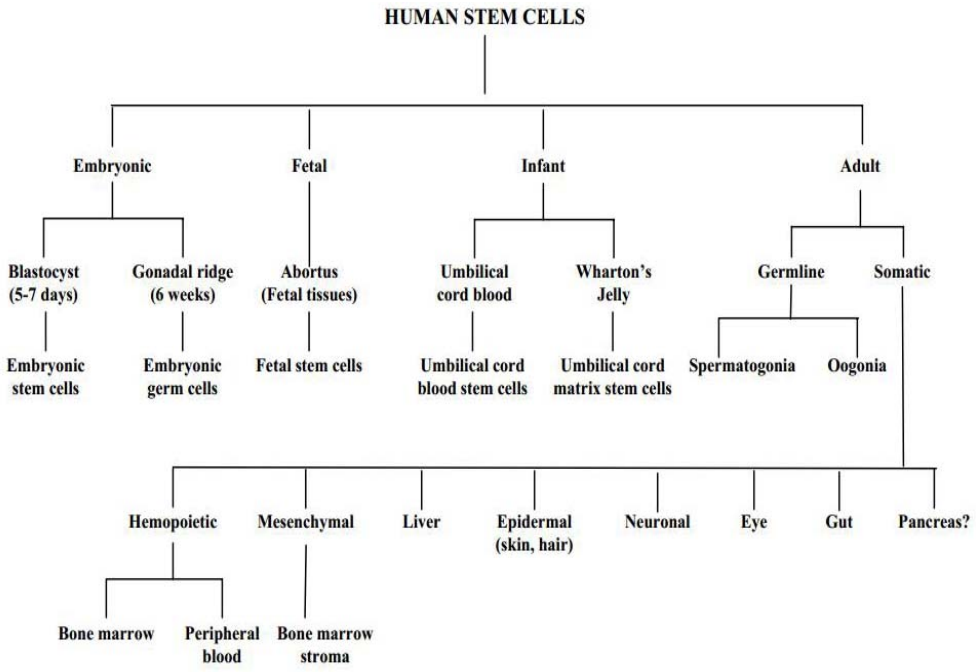

Figure (2): Classification of stem cells

Induced pluripotent stem cells (iPS):

The concept of the close relationship between stem cell potential and stage of development dramatically changed in 2006.In a remarkable set of experiments, Shinya Yamanaka and his colleagues took genes that were expressed in pluripotent ES cells, but not generally in mature cells, and introduced them into mature cells. They did so in a manner such that the genes would now be "ectopically" expressed, i.e., expressed in a cell type where the gene is normally not expressed. A small number of the mature cells reverted back to a highly immature cell state that resembled an ES cell. This process, now called reprogramming, induced a pluripotent state in a previously differentiated cell type ***(Takahashi et al., 2006).***

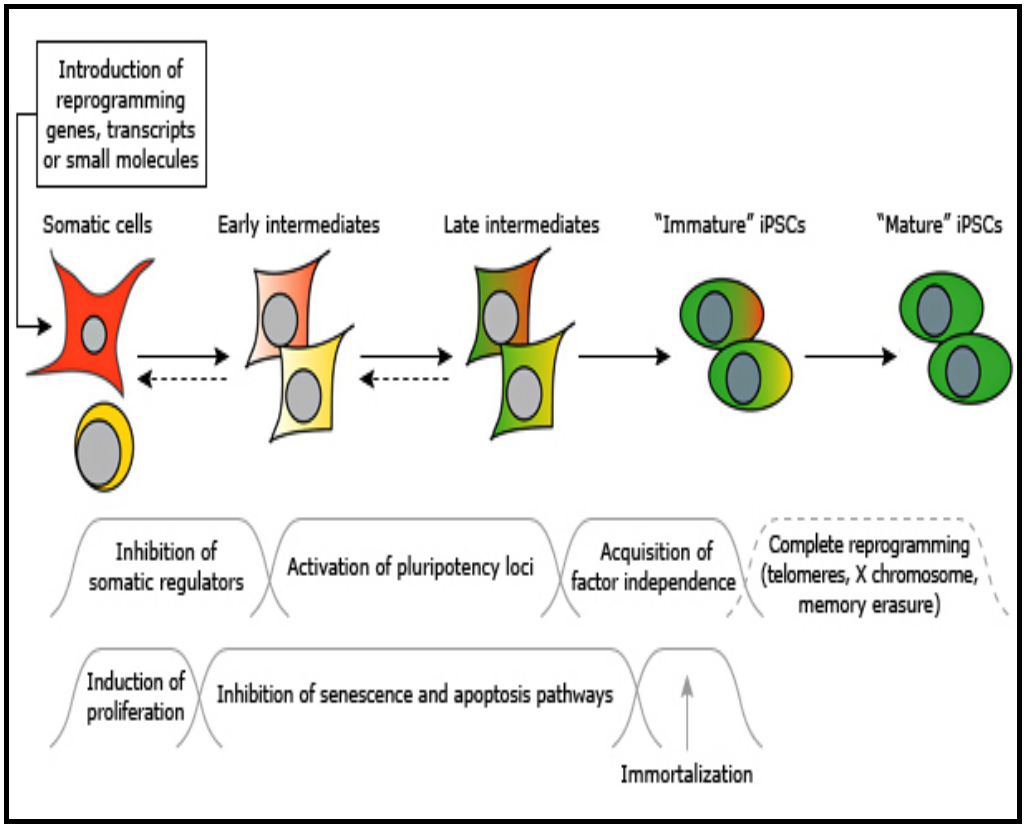

Figure (3): Reprogramming adult cells to become pluripotent cells

N.B: Potency of a stem cell is defined by the types of more differentiated cells that the stem cell can make.

Sources of Stem Cells

A) Emberyonic stem cells:

- Totipotent cells arise from the first few cell divisions following fertilization of the egg.
- Pluripotent cells were thought to be limited to cells derived from either the inner cell mass of the blastocyst (a pre-implantation stage of development occurring approximately 7 to 10 days after fertilization in the human) or nascent germ cells in the embryo.

N.B: Cells cultured and cell lines established from these structures are called embryonic stem (ES) cells and embryonic germ cells respectively ***(Oottamasathien et al., 2007).***

B) Adult stem cells:

Once the primitive streak forms in embryonic development (day 10 to 14 post fertilization in the human), it is thought that most stem cells are restricted to be either multipotent or unipotent. These have often been called 'adult' stem cells. Adult stem cells are thought to be present in most, but not all, tissues and to persist throughout life. This is particularly true for tissues where there is high cell turnover, such as the blood, skin, and intestine ***(Barker et al., 2007).***

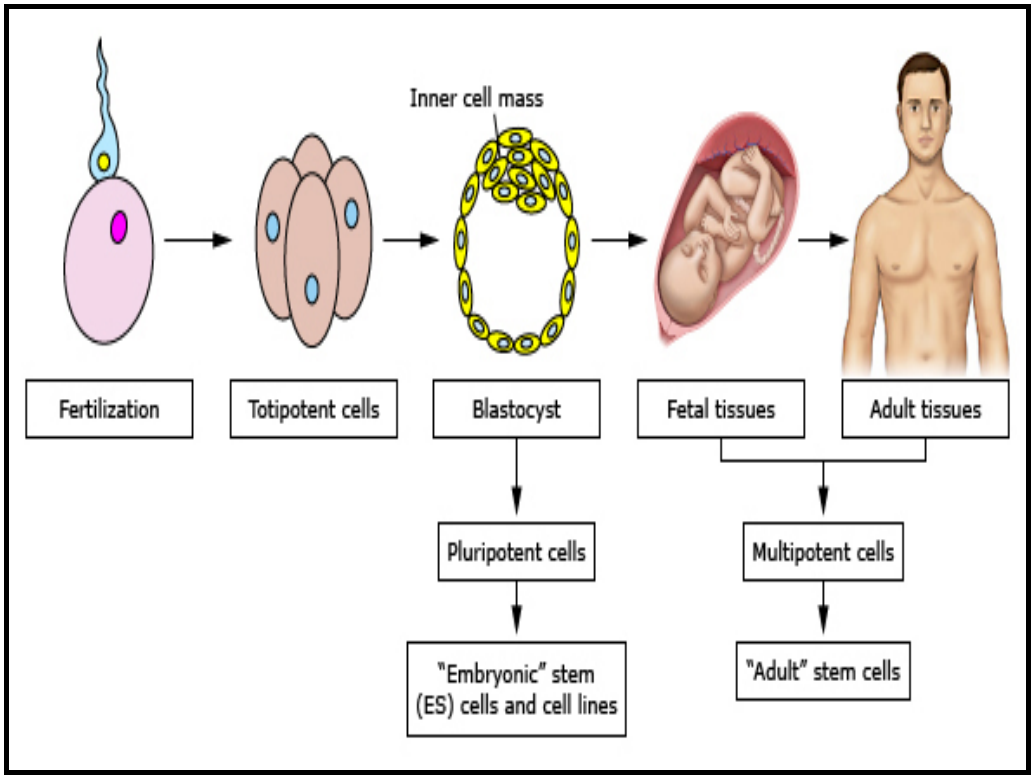

Figure (4): Relationship of development to stem cell type

C) Amniotic fluid stem cells:

They are Multipotent stem cells found in amniotic fluid. These stem cells are very active, expand extensively without feeders and are not tumorigenic. They can differentiate in cells of adipogenic, osteogenic, myogenic, endothelial, hepatic and also neuronal lines ***(Siddiqui et al., 2007).***

Amniotic fluid-derived mesenchymal stem cells (AFMSCs) can be isolated from second-trimester amniocentesis ***(Bieback et al., 2010).***

D) Umbilical Cord Stem Cells:

Human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) are regarded as an alternative source of bone marrow-derived mesenchymal stem cells because collection of cord blood is less invasive than that of bone marrow. hUCB-MSCs have recently been studied for evaluation of their potential as a source of cell therapy ***(Kim et al., 2010).***

Several reports indicate that cells of Wharton's jelly (WJ), the main component of umbilical cord extracellular matrix, are multipotent stem cells ***(Anzalone et al., 2010).***

E) Placental derived stem cells:

The human placenta, besides supporting fetal development, may also represent a reservoir of stem/progenitor cells ***(Brinkmann et al., 2010).***

Placenta, as a temporary organ keeping substance exchange between mother and fetus, thus contain both embryonic and adult stem cells. As a castoff after parturition, along with the ease of accessibility, lack of ethical concerns, placenta may be an attractive source of mesenchymal stem/progenitor cells for basic and clinical application ***(Wu et al., 2005).***