The Role of Human Amniotic Fluid Mesenchymal Stem Cells in the Ovarian Damage Induced by Cyclophosphamide in Adult Albino Rat: Histological and Immunohistochemical Study

Thesis

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List of Abbreviations

Abb.	Full term
AF	Amniotic fluid
AFSCs	Amniotic fluid stem cells
AMF1	Anti-Mullerian hormone
AMH	Antimullerian factor one
Bcl-2	B-cell lymphoma
BLIMP1	B lymphocyte induced maturation protein 1
BMP	Bone morphogenetic protein
BMSCs	Bone marrow stem cells
DAB	Diaminobenzidine tetrahydrochloride
DEMSO	Dimethyl sulfoximide
ECM	Extra cellular matrix
EMT	Epithelial mesenchymal transition
FSH	Follicular stimulating hormone
GFP	Green fluorescent protein
GnRHa	Gonadotropin-releasing hormone agonist
GREL	Gonadal ridge epithelial like cells
GSC	Germ line stem cells
HAFSCs	Human amniotic fluid stem cells
HCG	Human chorionic gonadotropin
HRP	Horse-radish peroxidase
HUMSCs	Human umbilical cord mesenchymal stem cells
IL2	Interleukin two
INFγ	Interferon gamma
LH	Luteinizing hormone
МНС	Major histocompitability antigen
MSCs	Mesenchymal stem cells

List of Abbreviations

Abb.	Full term
MVH	Mouse vasa homologous
NGF	Nerve growth factor
Oct4	Octamer binding transcription factor 4
OSE	Ovarian surface epithelium
PBS	Phosphate-buffered saline
PCNA	Proliferating cell nuclear antigen
PCR	Polymerase chain reaction
PGCs	Primordial germ cells
PMF	Primordial follicle
POF	Premature ovarian failure
POI	Premature ovarian insufficiency
PSCs	Putative stem cells
SCF	Stem cell factor
SCP3	Synaptonemal complex protein
SSA-4	Stage specific antigen 4
TGF-B	Transforming growth factor beta
VEGF	Vascular endothelial growth factor
VSELS	Very small embryonic like stem cells
Wnt4	Wingless family protein member 4
ZPP	zona pellucida protein

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Amniotic Fluid Stem Cells Transplantation Generates Oocytes in Rat Model of Cyclophosphamide-Induced Premature Ovarian Failure. Histological and Immunohistochemical Study

Abstract

Background: Early menopause occurs in female cancer patient after chemotherapy. Amniotic fluid stem cells transplantation has been linked to the return of ovarian function.

Aim of the present work: was to investigate whether intravenously delivered human amniotic fluid stem cells (HAFSCs) could restore the structure and function of ovaries in a model of premature ovarian failure (POF) in rat.

Material and methods:

Forty five adult albino rats weighting 300-350 grams were used in this study. The animals were divided into the following groups group I (control group), group II this group was divided into 2 subgroups in which the animals received single intraperitoneal injection of 70 mg/kg cyclophosphamide, the animals were sacrificed after one week (subgroup IIA), and after two weeks (subgroup IIB). In group III the rats received the same dose of cyclophosphamide and one week later they received single dose of HAFSCs, two weeks later the animals were sacrificed. The ovaries were processed for histological and immunohistochemical study. Moreover hormonal assay, morphometric and statistical study were done

Results:

In subgroup IIA significant increase in the number of ovarian cysts was detected while the number of growing follicle and corpora lutea showed significant decrease. In subgroup IIB nearly all ovarian follicles were atretic with absence of corpora lutea. Sever inflammatory cellular infiltration of ovarian parenchyma with ovarian fibrosis could be seen. These results were associated with significant decrease in estradiol level compared to group I & III. Ovarian surface epithelium of this group revealed negative immunoreaction for CD 105

In group III the ovaries regained their normal histological structure with presence of growing follicles, mature Graafian follicle and recent corpus luteum. These results were associated with positive CD105 immunoreactivity in ovarian surface epithelium (OSE), epithelial cyst and interstitium with significant increase in estradiol level.

Conclusion: Human amniotic fluid mesenchymal stem cells injection (HAFSCs) could improve ovarian damage induced by cyclophosphamide treatment in rats. They could create good microenvironment for transformation of reserve stem cells into ovarian follicles.

Keywords: Premature ovarian failure, Cyclophosphamide, Human amniotic fluid stem cells.

Introduction

Premature ovarian failure (POF) is an ovarian disorder that involves amenorrhea, sex steroid deficiency and elevated (menopausal) levels of serum gonadotropin before the age of 40 years (**Conway**, **2000**).

Santoro (2003) defined (POF) as a primary ovarian defect characterized by premature depletion of ovarian follicles before the age of 40 years.

Most cases of POF are idiopathic, with no identifiable etiology. However, **Goswami et al.** (2003) recorded that genetic aberrations, autoimmune ovarian damage, iatrogenic factors, infectious agents, toxins and environmental factors are associated with POF.

Saoji (2008) stated that 60–80% of women who were treated with cyclophosphamide, methotrexate and 5-fluouracil would develop POF.

Rees and Purdie (2006) declared that when ovarian function were disrupted by cyclophosphamide treatment, the effects can be devastating. Clinically, patients may suffer from complete ovarian failure resulting in amenorrhea and climacteric symptoms.

Introduction

Amniotic fluid is a clear watery fluid that surrounds the growing fetus within amniotic cavity. Recently, amniotic fluid is considered as an attractive source of stem cells of mesenchymal origin for therapeutic applications and with low risk of tumorigenicity (**Klemmt et al., 2011**).

Guan-Yu et al. (2014) declared that injection of amniotic fluid stem cells significantly decreased the number of atretic follicles in mice with POF six weeks after treatment with cyclophosphamide.

Aim of the Work

This study will be conducted to clarify the impact of human amniotic fluid stem cells (HAFSCs) injection on premature ovarian damage induced by cyclophosphamide using adult albino rat.

Review of Literature Ovarian Development

The ovary develops at the mesonephric surface epithelium which forms the future gonadal ridge. Some mesonephric surface epithelial cells change into gonadal ridge epithelial like cells (GREL). Proliferation of GREL occurs with breakdown of basal lamina allowing stromal cells to penetrate into gonadal ridge. Migration and proliferation of primordial germ cells (PGCs) from the yolk sac into the gonadal ridge occur. Proliferation of the stromal cells with presence of blood vessels causes expansion of the ovary and formation of cortex and medulla. Stromal cells penetrate towards ovarian cortex resulting in division of the ovary into ovigerous cords consisting of oogonia and GREL.

The cortex is formed of ovigerous cords and stroma. The medulla is formed of stromal cells, blood vessels and tubules originate from mesonephros (rete ovarii). The GREL cells at the surface differentiate into surface epithelium while ovigerous cords are partitioned into smaller cords then follicles. The follicles contain GREL that form granulosa cells and the oogonia will form primary

Review of Literature

oocyte. The first primordial follicle appears in cortico - medullary junction (Fig. I) (Hummitzsch et al., 2013).

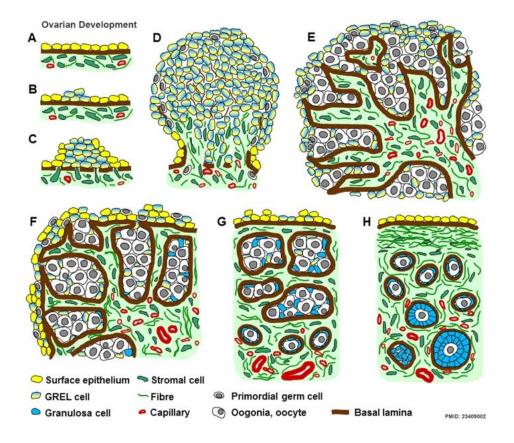


Fig. (I): Ovarian Development Model (Hummitzsch et al., 2013).

A- Represents surface epithelium of mesonephros. **B**- Change of some mesonephros into GREL C- Proliferation of GREL occurs. **D**- Breakdown of basal lamina allows invasion of stromal cells. **E**- Proliferation and migration of PGCs into gonadal ridge **F**- Stromal cells divide the ovary into ovigerous cords with formation of cortex and medulla. **G**- Ovigerous cords become follicles. **H**- GERL cells change into surface epithelium and granulosa cells.