Warm Ischemia Type 1 in Human Uterine Myometrium –Basic Study Toward Uterine Transplantation

Thesis

Submitted for Partial Fulfillment of the Master Degree in Obstetrics and Gynecology

BY

Hany Salah Mohamed Elnemr
M.B.,B.Ch.(2003)-Cairo University
Resident at El-Monira Hospital

Under the Supervision of

Prof./ Magdy Mohammed Mahmoud Abd El-Gawad
Professor of Obstetrics and Gynecology
Faculty of Medicine-Ain Shams University

Prof./ Magda Hassan Abd AL-Hamid
Professor of Pathology
Faculty of Medicine-Ain Shams University

Dr./ Dina Yahia Mansour
Lecturer of Obstetrics and Gynecology
Faculty of Medicine-Ain Shams University

Faculty of Medicine
Ain Shams University
2017

Abstract

Introduction: It is well known that infertility can interfere substantially with psychological health and that most subtypes of uterine infertility still remain untreatable. Thus, patients lacking a uterus due to congenital uterine agenesis or previous hysterectomy (secondary to malignancy, large/inoperable myoma or life-threatening obstetric bleeding), or with untreatable uterine malformations or severe intrauterine adhesions still do not have a real possibility of achieving motherhood.

Aims: The aim of this study is to evaluate the tolerance of human uterine myometrial tissue to warm ischemia type I.

Methodology: Prospective Pilot study. Ain Shams Maternity University Hospital. From fifteen December 2015 till fifteen December 2016. Fifteen (15) women with premenopausal bleeding were included in the current study and were subjected to surgery of total abdominal hysterectomy and bilateral salpingeoopherectomy.

Results: The biopsies taken for the study divided into 5 groups: Group 1: biopsies were taken at zero hour as a control to evaluate histological state by light microscope before vascular clamping, 4 Groups (warm ischemic groups): biopsies were taken every 1 hour with average 4 samples from vascular clamping for each case.

Conclusion: this study, the allowable warm ischemic time type 1 in human uterine myometrium under 3 hours and presence of positive correlation between increasing duration of warm ischemia over that time and significant ischemic injury by histopathological assessment.

Recommendation: Positive correlation between prolongation of warm ischemia and ischemic injury in human uterine myometrium and the allowable warm ischemic time type 1 in human uterine myometrium under 3 hours.

Keywords: Warm Ischemia Type 1, Human Uterine Myometrium, Basic Study Toward Uterine Transplantation

ACKNOWLEDGEMENT

First of all, thanks to Allah whose magnificent help was the main factor in completing this work.

No words can express my deep sincere feelings Towards Prof. Magdy Mohammed Mahmoud Abd El-Gawad, Professor of Obstetrics and Gynecology, Faculty of Medicine-Ain Shams University for his continuous encouragement, guidance and support he gave me throughout the whole work. It has been a great honor for me to work under his generous supervision.

I would like to express my deepest appreciation, respect and thanks to Prof. Magda Hassan Abd AL-Hamid, Professor of Pathology, Ain Shams University.

Finally I would present all my appreciations to Dr. Dina Yahia Mansour, Lecturer of Obstetrics and Gynecology, Faculty of Medicine, Ain Shams University, for her efforts, without she, this work could not have been completed.

Hany Salah Mohamed
2017

Contents

List of Abbreviations

ART : Assisted Reproductive Technology
ATP : Adenosine triphosphate
CTG : Cardiotocography
DD : Deceased Donors
DNA : Deoxyribonucleic acid
ECMO : Extracorporeal membrane oxygenation
EPH : Emergency peripartum hysterectomy
ET : Embryo transfer
FSH : Follicle stimulating hormone
GnRH : Gonadotrophin releasing hormone
GVHD : Growth versus-host disease
HBD : Heart beating donors
HLA : Human leukocyte antegin
HPV : Human papilloma virus
HSG : Hysterosalpingeography
I.V : Intravenous
I2/PGI2 : Prostacycline
ICG : Indocyanine green
ICU : Intensive care unit
IS : Immunosupression
IUAS : Intrauterne Adhesions
IUGR : Intrauterine growth restriction
IVF : In vitro fertilization
LD : Living Donors
LH : Luteinizing hormone
MMF : Myclophenolate mofetil
MRI : Magnetic Resonance Imaging
MRKH : Mayer-Rokitansky-Houser Syndrome
MURCS : Mullerian duct aplasia, Renal aplasis, Cervicothoracic Somite dysplasia
NGFβ : Nerve growth factor beta
NHBD : Non-heart beating donors
NTPR : National Transplantation pregnancy registry

PER : Perfadex solution
PGE2 : Prostaglandin E2
PGF2α : Prostaglandin f2 alpha
Qol : Quality of life
RIN : Ringer acetate

RNS : Reactive nitrogen species
ROS : Reactive Oxygen Species
TBARS : Thiobarbituric acid reactive species
UTX : Uterine transplantation
UW : University of Wisconsin

List of Figures

List of Tables

Warm ischemia type 1 in human uterine myometrium –Basic study toward uterine transplantation

Protocol of thesis

Submitted for Partial Fulfillment of the Master Degree in Obstetrics and Gynecology

BY

Hany Salah Mohamed Elnemr
M.B.,B.Ch.(2003)-Cairo University
Resident at El-Monira Hospital

Under the supervision of

Prof/ Magdy Mohammed Mahmoud Abd El-Gawad
Professor of Obstetrics and Gynecology
Faculty of Medicine-Ain Shams University
Prof/ Magda Hassan Abd AL-Hamid
Professor of Pathology-Faculty of Medicine
Ain Shams University
Dr/ Dina Yahia Mansour
Lecturer of Obstetrics and Gynecology
Faculty of Medicine-Ain Shams University

Faculty of Medicine
Ain Shams University
2015

Introduction

It is well known that infertility can interfere substantially with psychological health and that most subtypes of uterine infertility still remain untreatable **(Stanton et al., 2002**). Thus, patients lacking a uterus due to congenital uterine agenesis or previous hysterectomy (secondary to malignancy, large/inoperable myoma or life-threatening obstetric bleeding), or with untreatable uterine malformations or severe intrauterine adhesions still do not have a real possibility of achieving motherhood **(Goldfarb et al., 2000).**

Uterus transplantation (UTx) has been proposed as a possible temporary curative treatment for uterine factor infertility **(Sieunarine et al., 2005)**. The human UTx concept became a reality in the year 2000 in Saudi Arabia. Although a necrotic uterus was removed 99 days after surgery **(Fageeh et al., 2002),** this human patient motivated researchers to develop the UTx field towards establishing UTx as a feasible and safe procedure to be applied in human **(Branstromm et al., 2011)**. In Turkey 2011, the world's first uterus transplant from a deceased donor was conducted by a team of doctors at Akdeniz University Hospital **(Akar et al., 2013).**

In Turkey 2013, First clinical pregnancy after uterus transplantation was reported but this pregnancy unfortunately did not grow further and terminated at 8^{th} week **(Ozkan et al., 2013). I**n Sweden 2012, the first mother-to-daughter womb transplant was done by Swedish doctors at Sahlgrenska University Hospital at Gothenburg University led by Mats Brännström **(Brannstrom et al., 2012)**. In October 2014 for the first time, a healthy baby had been born to a uterine transplant recipient,

in an operation led by Dr. Brännström, Professor of Obstetrics and Gynecology at the University of Gothenbirg **(Brannstrom et al., 2015)**.

The success of an UTx procedure is dependent not only on immunological issues, but also on many other variables related to surgery and back-table preparation. Thus, data from studies of other types of solid organ transplantation suggest that ischemia–reperfusion injury is an important factor related to transplantation success. Long ischemic times are correlated with impaired post-transplantation perfusion, delayed graft function **(Quirog et al., 2006)** and increased frequency of acute and chronic rejection **(Diaz-Garcia et al., 2013).**

"Warm ischemia" is a term used to describe ischemia of cells and tissues under normothermic conditions. In the transplant setting, this term is used to describe two physiologically distinct periods of ischemia: (1) Ischemia during organ retrieval, from the time of cross clamping (or of asystole in non-heart-beating donors), until cold perfusion is commenced, and (2) Ischemia during implantation, from removal of the organ from ice until reperfusion **(Halazun et al., 2007).**

During the transplantation process, both cold ischemia and warm ischemia occur**.** The tolerance of a uterine graft to cold ischemia was previously evaluated in a syngeneic mouse model, and the findings were that cold ischemic preservation in University of Wisconsin preservation solution for 24 hour did not affect uterine functionality in terms of pregnancy potential but that cold ischemia for 48 hour resulted in graft necrosis upon transplantation **(El-Akouriet al., 2003).**

In vitro studies on cold ischemic tolerance of the human uterus demonstrated that small human myometrial tissue pieces that had been preserved in cold University of Wisconsin preservation solution or

Perfadex for 24 hour still showed spontaneous as well as prostaglandin F2α-induced contractility and were ultramorphologically normal **(Wranning et al., 2005)**. Similar results were also established in another study on possible uterine damage after retrieval of the uterus from brain-dead and heart-beating human multi-organ donors **(Sieunarine et al., 2008)**.

An experimental study analyzing the effect of warm ischemia in graft survival in UTx surgery was published in a rat model to mimic a time frame likely to occur in a human situation with results of evident signs of necrosis were seen in five of 10 animals in the warm ischemia group (in which UTx performed after exposure of the transplanted uterus for a four hours of extended period of warm ischemia) compared with only one of 10 in the control group (in which UTx performed immediately after uterus retrieval with all the ischemic time were minimized). Overall, uterine grafts from the warm ischemia group obtained poorer gross morphology scores, histological findings correlated with the surgical findings at inspections three and six days after surgery. Conclusion, an extended warm time has detrimental effects on the survival of the uterus after transplantation **(Diaz-Garcia et al., 2013).**

A human UTx attempt would involve time-consuming uterine retrieval surgery from donor with dissection of uterine vascular pedicle and uterine implantation with vascular anastomosis surgery in recipient, with at least four vascular anastomosis sites (two on each side) on the iliacs. Consequently, so it is of great interest to evaluate the tolerance of a uterine graft to warm ischemia **(Johannesson et al., 2012).**

Aim of the Work

The aim of this study is to evaluate the tolerance of human uterine myometrial tissue to warm ischemia type I.

Research hypothesis:

In women undergoing UTX, the success of the implantation may depend upon the duration of tissue or myometrial warm ischemia type I ?

Research question:

In women undergoing UTX, Does the success of the operation depend on the duration of myometrial warm ischemia type I ?

Patients and Methods

Type of the Study:

- Prospective Pilot study.

Place of the Study:

- Ain Shams Maternity University Hospital.

Sample size:

- Fifteen (15) women with premenopausal bleeding will undergo total abdominal hysterectomy and bilateral salpingeoopherectomy.

Inclusion criteria

- Healthy Women with premenopausal bleeding.

Exclusion criteria:

- No uterine pathology (fibroid or malignancy)
- Women with uterine hypoplasia.
- Vascular diseases.

Methodology

Each patient will be subjected to the following:

1-Informed consent after explanation will be obtained from every patient included.