A Study of the Effects of Clomiphene Citrate on the Histological Structure of the Fallopian Tube of Albino Rats: Possible Role of Isoflavone

Thesis

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Marwa Mohamed Safwat

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List of Abbreviations

Abb.	Full term
AMP	Ampulla
AR	Androgen receptors
CC	Clomiphene citrate
DES	Diethylstilbestrol
E	Estrus
E2	Estradiol
EE	Ethynylestradiol
ER	Estrogen receptor
ER α	Estrogen receptor alpha
ER β	Estrogen receptor beta
ESR	Estrogen receptor signaling
ESR2	Estrogen receptor-β
EV	Estradiol valerate
FSH	Follicular stimulating hormone
GnRH	Gonadotrophin releasing hormone
hCG	Human chorionic gonadotrophin
HDL	High density lipoprotein
ICLC	Interstitial cells Cajal-like cells
IL	Interleukin
INF	Infundibulum
IVF-ET	In vitro fertilization– embryo transfer cycles
LDL	Low density lipoprotein
LH	Luteinizing hormone

Abb.	Full term
LHRH	Luteinizing hormone releasing hormone
ND	Neonatal day
OCCs	Oocyte-cumulus complexes
OSG	Oviduct-specific glycoproteins
OVX	Ovariectomized
p	Protestrus
PCOS	Polycystic ovarian syndrome
PEs	Phytoestrogens
PR	Progesterone receptor
RER	Rough endoplasmic reticulum
SERM	Selective estrogen receptor modulator
TNF	Tumor necrotic factor
UTJ	Uterotubal junction

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Abstract

The mammalian oviduct or fallopian tube has fundamental roles in gamete transport, fertilization, and early embryo development that are facilitated by ciliated and secretory epithelial cells lining the lumen. Several experimental models had shown that oviductal development is regulated by the ovarian sex steroid hormones estrogen and progesterone. Sex steroids are involved in the control of gamete transport and ciliary activity in the fallopian tube. Estradiol (E2) treatment accelerates ovum transport from 1 h to 24 hours in rats, facilitates sperm migration and induces adhesion of spermatozoa to the oviductal epithelium. Aim: To study the effect of clomiphene citrate and isoflavone administration on the structure of the fallopian tube of prepubertal and adult albino rats to determine safe usage of clomiphene citrate in female infertility. Fallopian tube is a dynamic, steroid responsive tissue has fundamental roles in gamete transport, fertilization, and early embryo development. Clomiphene citrate (CC) is non steroidal synthetic hormone with estrogenic effect has been the first line therapy for induction of ovulation in women with anovulatory infertility. Phytoestrogens are a group of non- steroidal compound of plant origin that present structural and functional similarities with17 β estradiol. Isoflavones, are the most widely known category. There are different mechanisms of action of isoflavones accepted, although they may be considered as selective modulators of estrogen receptor.

Keywords: Oviduct, fallopian tube, clomiphene citrate, isoflavones.

Introduction

The mammalian oviduct or fallopian tube has fundamental roles in gamete transport, fertilization, and early embryo development that are facilitated by ciliated and secretory epithelial cells lining the lumen. Several experimental models had shown that oviductal development is regulated by the ovarian sex steroid hormones estrogen and progesterone **(Okada et al., 2001).**

Clomiphene citrate, a non steroidal synthetic hormone with estrogenic effect, has been the first line therapy for induction of ovulation in women with anovulatory infertility and in couples with unexplained infertility **(Bao et al., 2009; Fouda and Sayed, 2011).**

Its action seems to involve hypothalamic pituitary axis and ovaries **(Kilic et al., 2003; Hosie and Stewart, 2006)**. Although clomiphene citrate has limited dose-dependent side effects, prolonged treatment is associated with an increased frequency of tubal ectopic pregnancy in humans **(Costello, 2004; Pritts, 2010).** It causes apoptotic changes in the epithelium of the tube and block of oocyte **(Goldberg & Friedman, 1995).** It was reported that the damage was reversed with the administration of 17 beta-estradiol (E2) **(Shao et al., 2009).**

Clomiphene citrate is tissue-selective estrogen receptor modulator has antagonistic activity on estrogen receptor (ESR) signaling in female reproductive tissues such as rabbit fallopian tube **(Ali, 2013)** and cervical mucosa **(Sheehan, 2004).**

Sex steroids are involved in the control of gamete transport and ciliary activity in the fallopian tube. Estradiol (E2) treatment accelerates ovum transport from 1 h to 24 hours in rats, facilitates sperm migration and induces adhesion of spermatozoa to the oviductal epithelium **(Orihuela et al., 2001).**

Phytoestrogens are a class of plant estrogen that include isoflavones, flavones. Phytoestrogens are thought to have health benefits such as providing protection against breast cancer development **(Hilakivi-Clarke et al., 2001; Katadre et al., 2002)** and are potentially useful in the management of menopausal symptoms and also may be benefit to women with endometriosis **(Celec et al., 2004).**

Isoflavones, are found in many food stuffs especially soybean where they are found in high concentration**.** Its molecular structure has biochemical similarity with 17 β estradiol. These isoflavones may also bind to estrogen receptors **(Somjen et al., 2003).**

Previous experiments had demonstrated that the primary soy isoflavone as (genistein) induced proliferation of the chick oviduct. It was reported that genistein can function as a classical estrogen in the chick oviduct and that dietary exposures to genistein may alter oviduct development **(Stevenson et al., 2006).**

Aim of the Work

To study the effect of clomiphene citrate and isoflavone administration on the structure of the fallopian tube of prepubertal and adult albino rats to determine safe usage of clomiphene citrate in female infertility.

Review of Literature

Anatomy of the fallopian tube

The Fallopian tubes, also known as oviducts, uterine tubes, and salpinges (singular salpinx) which are formed of two very fine convoluted tubes lined with ciliated epithelium cells leading from the ovaries into the uterus, via the utero-tubal junction. The equivalent structures in non-mammalian vertebrates, called the oviducts **(Daftary and Chakravarti, 2011).**

Previously regarded as a simple transit zone, the oviduct is now regarded as a complex organ. As the fallopian tube is the intermediary between the ovary and the uterus, it is the seat of a variety of interactions.which play a major role in the quality of the conceptus. The oviducts support the transport and final maturation of gametes, and harbour fertilization and early embryo development. The tubal fluid is now better regarded to be the first interface between the mother and the embryo. The oviduct environment is regulated by ovarian steroids **(Koelle et al., 2010 & Halter et al., 2011).**

The oviduct of the adult rat, is compactly coiled tube. It is located close to the caudal pole of the ovary and the

cephalic extremity of the horn of the uterus. The length of the oviduct is approximately 2.5-3.0 cm **(Brenner, 1969).**

As regard the segmentaion of rat oviduct. It was decribed by **Lee et al. (1976).** He divided the rat oviduct into five segments. Segment I: which included the proximal portion of the preampulla and fimbria, Segment II: included the main portion of the ampulla, Segment III: included the distal portion of the ampulla and the proximal part of the isthmus Segment IV: this segment occupied the main portion of the isthmus and Segment V: which included the distal part of the isthmus and the intramural.

Arthur et al. (1996) also divided The oviduct of the human into four functional segments: the fringe-like fimbriae; which are funnel-shaped abdominal opening near the ovary, the infundibulum; the more distal dilated, major and the dilated portion of the uterine tube from the infundibulum to the isthmus is called the ampulla; and the narrow proximal portion of the oviduct connecting the oviduct with the uterine lumen, the isthmus, the interstitial (also known as intramural) part that transverses the uterine musculature.

The tubal ostium is the point where the tubal canal meets the peritoneal cavity, while the uterine opening of the

Fallopian tube is the entrance into the uterine cavity, called the utero-tubal junction **(Kodaman et al., 2004).**

Histology of the fallopian tube

The mammalian oviduct is a pair tubular organ lined with simple columnar epithelium formed by two types of cells, ciliated and secretory cells **(Kamaci et al., 1999; Steffl et al., 2004; Yániz et al., 2000, 2006).**

The ratio of these two cell types (ciliated and secretory) change during the reproductive cycle under the influence of sexual hormones. In the estrus period, the greatest density of fully developed ciliated cells is reached under-effect of estrogen hormone **(Reeder & Shirley, 1999)**.

The oviduct wall is composed of tunica mucosa, tunica muscularies, and tunica serosa. Tunica musclosa arranged lengthwise and circularly. While the mucosal layer is arranged into numerous folds **(Kenngott and Sinowatz, 2007)** made by ciliary and non ciliary epithelial cells and the layer of lamina propriae mucosae **(Uhrín, 1992).**

The oviductal mucosa is made of primary, secondary, and tertiary folds. The mucosa of the ampulla is

thrown into high، branched folds that decrease in height toward the isthmus and become low ridges in the uterotubal junction. The complex arrangement of these mucosal folds in the ampulla almost completely fills the lumen so that they act as a potential space **(Rajesh et al., 1997).**

The structure of the prepubertal oviduct:

The epithelium of the oviduct of the newborn to the old mouse was studied by **Komatsu (1979)**. He observed, the epithelial cells lining the ampulla of the mouse oviduct are simple columnar in shape and of one type in fine structure just after birth. Also, they contain numerous free ribosomes, an extremely poor rough endoplasmic reticulum, and a small Golgi complex. In the 3-day-old mouse, the epithelial cells are differentiated neither into ciliated or secretory cells. While, the ciliary cells differentiates 5 days after birth. Ciliogenesis is frequently observed at 5–10 days. The important role of the fibrous granules for ciliogenesis and that of the Golgi apparatus for membranogenesis of the cilia. He noticed that the secretory cells begin to differentiate at 23 days.

The microscopic examination of rat oviducts in the early postnatal period showed that the character of the epithelium remained relatively indifferent up to the 6th day.

which include an apical migration of the centrioles, with subsequent formation of solitary cilia; Ciliogenesis in the rat oviductal epithelium starts between the 8th and the 10th day. A continuous kinociliary apparatus is formed on the basis of centriole replication. The first secretory and peg cells appear at the end of the second week **(Jirsová and Vernerová, 1990).**

The oviducts of newborn mouse females were lined with a simple columnar epithelium which includes tall indifferent cells of uniform appearance in all tubal segments (the preampulla, the ampulla, and the isthmus). The transformation of indifferent cells into ciliated cells were observed in some of them and that's by ciliogenic activity. The presence of secretory cells or morphological marks of their differentiation were not obsereved during the first two weeks after birth **(Lauschová, 2003).**

The author also, added that the first secretory cells occurred in the epithelium of the ampulla and the isthmus of animals aged 14 days, the occurrence of mature secretory cells (both active and inactive) increased during sexual maturation so that they prevailed, while immature cells were only occasionally found in the oviductal epithelium of animals aged 35 days and more.