Relation of Inhibin to Male and Female Sex Hormones

Assay

Submitted for Partial Fulfillment of the Master Degree of

Clinical Pathology

By

Hanan Samwel El- Safy

M.B., B.Ch.

Supervised by

Prof. Dr. Hany sobhy Rufail

Prof. of Clinical Pathology

Ain Shams University

Dr. Ola Hamdy Demerdash

Ass. Prof. of Clinical Pathology

Ain Shams University

Dr. Mona Mohammed zaki

Lecturer of Clinical Pathology

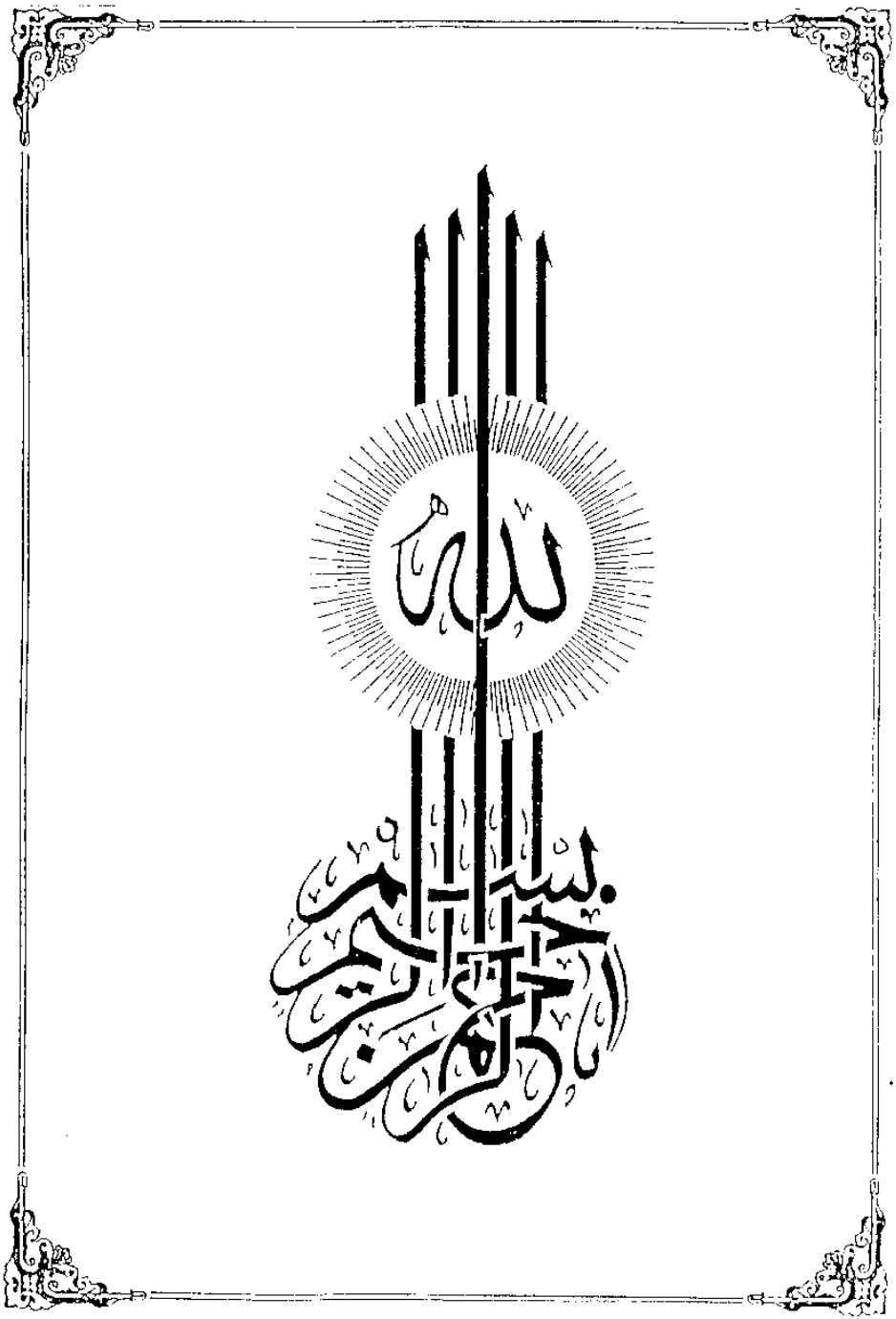
Ain Shams University

Faculty of Medicin

Ain Shams University

1995

CONTENTS:



Acknowledgement

I would like to express my unlimited gratitude and deep appreciation to prof. Dr. Hany Sobhy Rufail, Prof. of clinical Pathology, Ain Shams University for giving me the privelage of working under his supervision, and for his encourgement and eminent guidance and support.

My deepest thanks and sincere gratitude are due to Dr. Ola Demerdash, Ass. Prof. of Clinical Pathology, Ain shams University for her cooperation and assistance in performing this work.

I would also like to thank to Dr. Mona Zaki, lecturer of CLinicl Pathology, Ain Shams University, for her cooperation and continuous help in this work.

Lastly but not the least, I'm very thankful to my husband for his great help and continous encouragement. It is to him all this work is dedicated.

Above all, thankes to GOD for termination of this work.

Key Words :

- Inhibin.
- Sources and regulation.
- Methods of assay.
- Clinical applications.
- Theraputic applications.

List of Abbreviation

C.AMP	Cyclic adenosine monophosphate
EGF	Epidermal growth factor
ELISA	Enzyme linked immunosorbant assay
FSH	Follicle stimulating hormone
Gn RH	Gonadotropin releasting hormone
HCG	Human chorionic gonadotropin
IVF	In vitro fertilization
LH	luteinizing hormone
LHRD	Luteinizing hormore releasing hormone
PCC	Pituitary cell culture
PI	Prostatic inhibin
PIP	Prostatic inhibin Peptide
RIA	Radioimmunassay
RTF	Ram rete testis fluid
TGF	Transforming growth factor
TSH	Thyroid stimulating hormone.

INDRODUCTION

Over fifty years ago, a non steroidal factor of gonadal origin was postulated to inhibit pituitary follicular stimulating hormone(FSH) (Mc Cullagh, 1932). This substance was termed inhibin (Franchimont et al., 1979). Inhibinhas now been purified from bovine follicular fluid (Welschen et al., 1977) and procine follicular fluid (Ling et al., 1985). It is a glycoprotein consisting of two disulphide linked subunits. These peptides were subsequently cloned and sequenced (Mason et al., 1985), A recent surprising discovery was that dimers of smaller (B) subunit of inhibin isolated from procine follicular fluid stimulated FSH release from pituitary cells in vitro, in contrast to inhibin which inhibits FSH in vivo (Ling et al., 1986).

Recently inhibin has been demonstrated in the human placenta which suggests its production during pregnancy (Mc Lachlen et al., 1986). In addition its level was found to be elevated with complete hydatiform mole during early pregnancy (Yohkaichiya et al., 1989).

In male, it is secreted by sertoli cells under the influance of FSH (Bicsak et al., 1987). However, the serum concentration of inhibin in normal men does not differ significantly from those groups with

oligospermia, azoospermia or klinefelter's syndrome (Mc Lachlan, 1989).

Aim of Work :-

The aim of the present work is to give a detailed account on inhibin with respect to its origin, regulation, physico- chemical and immunological characheters, possible roles of inhibin and its assay methods. Furthermore, the possible future applications of inhibin will be discessed.

REVIEW OF LITERATURE

A. Introduction to Inhibin and Activin :-

Inhibin and activin are glycoprotein hormones which have been isolated and cloned in porcine, ovine, bovine, rat and human gonads. They were named inhibin and activin because they decrease and increase FSH release from cultured rat pituitary cells, respectively (Petraglia et al., 1991).

Inhibin is a gonadal hormone, which regulates the production of FSH in the anterior pituitary gland (Qe et al., 1991). Other names havbe been given to substances that have been isolated from a variety of biological fluids and possess similar properties to inhibin. For example Steinberger and Steinberger (1976) named the substance they isolated sertoli cell factor. In 1977 Schwartz and Channing named it folliculostatin.

Activin on the other hand is a dimer that could be found in either of two forms, activin A (B_A-B_A) and activin B (B_A - B_B). these were identified in follicular fluid, and in contrast to inhibin, stimulated FSH secration in vitro with minimal effects on LH (Val et al., 1986).

Moreover. Mclachlan et al. (1989) found that it stimulates FSH secretion in vivo.

B. Sources of Inhibin :

Inhibin is found in both sexes. In males, it has been detected and partially purified from human seminal fluid (Franchimont et al., 1975b), bovine seminal fluid (Chari et al., 1976), ram rete testis fluid (RTF) (Setchell and Sirinathsinghji, 1972), extracts of human spermatozoa (Lugaro et al., 1974) and human testicular extracts. On the other hand in females inhibin was found in ovarian extracts (Chappel et al., 1980), bovine follicular fluid (welschen et al., 1977), porcine follicular fluid (Lorenzen et al., 1978), and human follicular fluid (Chari et al., 1976). In vitro, inhibin could be produced and isolated from the culture medium of sertoli cells (Steinberger and Steinberger, 1976) as well as from that of granulos cells (Erickson and Hsuelr 1978).

1. Testicular Origin :

The testis is the source of inhibin, in males. The experiments of Steinberger and Steinberger (1976) have shown that sertoli cells are

directly involved in the synthesis and secretion of inhibin. In their study they called the hormone sertoli cell factor referring it to its site of production. Their proof for the source of inhibin was pituritary cell Co-cultured with isolated sertoli cells consistently released significantly less FSH than pituitary cells grown alone or cultured with spleen or kidney cells. Furthermore, the culture medium of viable sertoli cells alone inhibited the sponteneousand LH-RH induced FSH release in dispersed pituritary cell culture, thus inhibin appears to be synthesized by sertoli cells in vitro as well as in vivo.

Eddie et al. (1978) have also identified a substance produced by cultures of rat seminiferous tubules that suppressed the LH-RH induced secretion of FSH by pituitary cell cultures and inhibited the sectretion of LH to a lesser extent. This was also proved by Franchimont el al. (1979) who suggested that the site of production appears to be the seminiferous tubules because large quantities of the hormone are found in the rete testis, where the secretion of the seminiferous tubules accumulate.

Other evidence for the role of the sertoli cells in the secretion of inhibin has been provided by Demoulin et al. (1979a) In their study,

mice testes were maintained in organ culture and after 4 days the culture medium was removed and placed on dispersed rat pituitary cells. When the tests were cultered at 37°C, spermatogenesis was greatly altered by day 4, whereas the sertoli cells maintained their normal light microscopic apperance. This medium depressed the LH-RH induced FSH release without affecting LH release. After 8 days of culture, sertoli cells were also affected and the inhibitory effect on FSH secretion disappeared. Moreover, when testis was cultured at 31°C, spermatogenesis was altered histologically at day 8 where as sertoli cells remained in good condition for 20 days. The culture medium maintained its inhibin effect for 20 days of experiment. Thus, there was a relationship between the histological apperance of sertoli cells and inhibiting potency of the culture medium . In contrast alteration of gametogenesis did not have any effect on the inhibin activity of the culture medium.

Although there is no doubt that the sertoli cells are responsible for the secretion of inhibin, the possible role of spermatogensis in the induction of this secretion remains doubtful. Indeed, in some studies there was an inverse linear relationship between the level of FSH and the qualitative (Franchimont, 1972) and quantitative (De Kretser et al.,

1972) aspects of spermatogenesis observed in the testicular biopsies. In this aspect, inhibin activity was observed in extracts of human seminal plasma coming from oligospermic as well as normal subjects, but not from azoospermic individuals (Franchimont, 1972). On the other hand, in many other studies serum FSH levels, were exceptionally high when spermatid numbers were normal (Christiansen, 1975).

2. Follicular Origin :-

Inhibin found in the follicular fluid of different animals appears to be produced by granulosa cells. Erickson and Hsueh (1978) showed that the granulosa cells in culture secrete a substance that acts directly on pituitary cell cultures and preferentially suppressed FSH secretion. The inhibitory effect on FSH level by the culture medium was greater with more numerous granulose cells in the culture. Moreover granulosa cells acquire the ability to produce inhibin early in follicular development. A slight reduction of LH release is observed when the inhibitory effect of FSH reaches its maximum (Franchimont et al., 1979).