INTRODUCTION

The prediction of ovarian response before undertaking the expensive IVF treatment is quite important ***(Yingying et al., 2011)*** especially with assisted reproduction program in which the response of ovulating woman to exogenous gonadotropin therapy is often inconsistent ***(Fause et al., 1999)***. Patient characteristics, rather than the stimulation protocol seem to determine the individual response ***(Mayorga et al., 2000)***. In young ovulating women undergoing in vitro fertilization treatment, the standard stimulation protocol can result in either poor response or ovarian hyper stimulation syndrome ***(Elchala et al., 1997)***, the later, however, is one of the challenging complications ***(Abd-El-Maeboud, 2004).***

Poor ovarian response to gonadotropin stimulation results in small number of oocytes collected and thus a smaller number of embryos available for transfer, which therefore reduces the success rate of IVF ***(Bancsi et al., 2002).*** Advance identification of patient who will elicit a poor response or hyper response to standard treatment would be of great clinical advantage ***(Sheikhha et al., 2011)***.

Several parameters have been postulated as predictors of the ovarian response ***(Barnhart et al., 1998)*** all of which stripe to assist ovarian reserve ***(Taflan et al., 2011)***. Ideal ovarian reserve parameter should be easily measurable, minimally invasive, inexpensive, and should have a good predictive value ***(Sharara and Scott, 1997).***

The concentration of circulating FSH on the third day has been recognized as a better predictor of the ovarian response to hormonal stimulation and IVF outcome than age ***(Helsa, 1994).*** The number of primordial follicles available in the ovary influences the level of FSH, as the follicles are recruited; however they secrete estrogen, which in turn keeps the level of FSH low ***(Goher et al., 2004).*** Although response to stimulation reflects the quantity, pregnancy rate reflects both the quantity and quality of the oocytes ***(Scott et al., 1995).*** In one large study of IVF patients; it was evident that FSH>14.2 IU/L was associated with very low pregnancy rate (2.7%) which persisted even among women below age 35 ***(Levi et al., 2001).***

Ultrasonographic measurements of antral follicle count (AFC) and ovarian volume have also been explored as predictors of response to ovulation induction ***(Broekmans et al., 2006).*** The antral follicle count (AFC) is a minimally invasive, easily performed test provides a representation of remaining follicular pool levels to assess the probability of a positive response to controlled ovarian hyper stimulation (COH) and success of IVF ***(Maseelall et al., 2009).***

Antral Follicle Count is the sum of antral follicle in both ovaries, as observed with transvaginal ultrasonography during the early follicular phase ***(Mcllveen et al., 2007).*** Most studies have defined antral follicles as those measuring 2-10 mm in mean diameter in the greatest 2-dimensional (2-D) plane; some have defined antral follicles as those measuring 3-8 mm in

mean diameter ***(Bancsi et al., 2004).*** The AFC is a good predictor of the number of retrieved oocytes and rate of cancellation in IVF after COH ***(Kwee et al., 2007).*** It was also clear that women with fewer antral follicle needed longer duration and higher dosage of gonadotropin during the stimulation ***(Ernest et al., 2000)***.

In this study we will investigate if AFC is superior to basal Follicle stimulating hormone (bFSH) in predicting the ovarian response and thus predicting the outcome of oocytes and embryos obtained in IVF-ET cycles.

AIM OF THE WORK

To compare between bFSH and AFC in predicting the ovarian response in women undergoing super-ovulation with long protocol for assisted reproduction.

Chapter One

DEVELOPMENT AND PHYSIOLOGY OF HUMAN OVARIES

Introduction:

The development of a normal ovary during fetal life is essential for the production and ovulation of a high-quality oocyte in adult life ***(Aitken et al., 2011).*** The adult ovary is devoid of germline stem cells, thus the number of primordial follicles found in the ovary represents the entire ovarian reserve that a female will ever possess ***(Cohen and Holloway, 2010).*** The physiologic responsibilities of the ovary are the periodic release of gametes (oocytes) and the production of the steroid hormones, estradiol and progesterone ***(Speroff et al., 2011).*** Various genes and signals are implicated in germ and somatic cell development, leading to successful follicle formation and normal ovarian development ***(Kathryn and Richard, 2015).***

This chapter will briefly review the basic aspects of ovarian development, differentiation, anatomy, and physiological functions.

Ovarian Development and Differentiation

The Fetal Ovary:

Early in embryogenesis, the primordial germ cells (PGCs) migrate to and colonise the genital ridges ***(Mai and Ann, 2012).*** By the 6th gestational week, on completion of the

indifferent state, these primordial germ cells have multiplied by mitosis to a total of 10,000 ***(Speroff et al., 2011).*** Once the PGCs reach the bipotential gonad, the absence of the sex-determining region on the Y chromosome (SRY) gene and the presence of female-specific genes ensure that the indifferent gonad takes the female pathway and an ovary forms ***(Aitken et al., 2011).***

At 6–8 weeks, the first signs of ovarian differentiation are reflected in the rapid mitotic multiplication of germ cells starting from about 600,000 by the eighth week ***(Talwar, 2014),*** reaching a maximum number of about 6 million oogonia by 16–20 weeks ***(Selmo et al., 2012).*** From this point in time, germ cell content will irretrievably decrease until, about 50 years later; the store of oocytes will be finally exhausted ***(Speroff et al., 2011).***

At 11–12 weeks, the germ cells give rise to the oogonia by mitosis ***(Feng et al., 2014)***. The first meiotic division is initiated at about 15 week's gestation, signaling the transformation of oogonia to oocytes ***(Corrine et al., 2012).*** Follicle formation begins at around week 16–18 of fetal life ***(Marques et al., 2009).*** Oogonia are enveloped by somatic epithelial cells derived from genital ridge mesenchymal cells, forming primordial follicles ***(Falcone and Hurd, 2013).*** Then oogonia are transformed to primary oocytes as they enter the first meiotic division and arrest in prophase ***(Angela et al., 2012).***

By 4–5 months' gestation, the ovary has its maximum number of oocytes, between 5 and 8 million ***(Feng et al., 2014)***. This number decreases dramatically to 1–2 million at birth and less than 500,000 by puberty ***(Desai et al., 2013).*** At 18–20 weeks, the highly cellular cortex is gradually perforated by vascular channels originating in the deeper medullary areas, and this marks the beginning of follicle formation ***(Huber and Fieder, 2009)***.

Follicular growth is initiated in the fetus in a continuous pattern that is independent of gonadotropins and is apparently related to both the total mass of follicles and to factors released by atretic follicles ***(Peters et al., 2009).*** Follicles are capable of growth up to the pre-antral stage in the absence of gonadotropins, based upon studies of girls with congenital deficiency of GnRH (Kallmann syndrome) and anencephalic fetuses ***(Baker et al., 2009).***

Loss of germ cells takes place throughout all of these events: during mitosis of germ cells, during the various stages of meiosis, and finally, after follicle formation ***(Hansen et al., 2008).*** The massive loss of oocytes during the second half of pregnancy is due to regression of substantial numbers of oocytes during meiosis, and those oogonia that fail to be enveloped by granulosa cells undergo degeneration ***(Candace et al., 2009).*** Once all oocytes are encased in follicles (shortly after birth), the loss of oocytes will be only through the process of follicular growth and atresia ***(Speroff et al., 2011)***. The

atresia is a poorly understood process of follicular degradation occurring at any point during follicular growth and development ***(Morita et al., 2010).***

The Neonatal Ovary

The total cortical content of germ cells falls to 1–2 million by birth as a result of prenatal oocyte depletion ***(Fernanda et al., 2014)***. This huge depletion of germ cell mass (close to 4–5 million) has occurred over a short time as 20 weeks ***(The Mammalian Ovary from Genesis to Revelation)*** Because of the fixed initial endowment of germ cells, the newborn female enters life, still far from reproductive potential, having lost 80% of her oocytes ***(Speroff et al., 2011).***

Compartmentalization of the gonad into cortex and a small residual medulla has been accomplished by this time ***(Vegetti et al., 2006).*** In the cortex, almost all the oocytes are involved in primordial follicle units with varying degrees of maturation can be seen as in the fetal state ***(Telfer and Zelinski, 2013).*** There is a sex difference in fetal gonadotropin levels, where there are higher pituitary and circulating FSH and pituitary LH levels in female fetuses ***(Choi and Smitz, 2014)***. In infancy, the postnatal FSH rise is more marked and more sustained, whereas LH values are not as high as FSH ***(Speroff et al., 2011).*** After the postnatal rise, gonadotropin levels reach a nadir during early childhood at about 1–2 years in females and then rise slightly between 4 and 10 years ***(Main et al., 2002).***

The Ovary in Childhood

The childhood period is characterized by low levels of gonadotropins in the pituitary and in the blood, little response of the pituitary to GnRH, and maximal hypothalamic suppression ***(Hayes et al., 2013)***. The ovary is not quiescent during childhood, Follicles begin to grow at all times and frequently reach the antral stage ***(Anderson et al., 2014).*** The process of atresia with increase contribution of follicular remnants to the stroma causes progressive ovarian enlargement during childhood, about a ten-fold increase in weight ***(Thomas et al., 2013).*** Of course, the lack of gonadotropin support prevents full follicular development and function ***(Speroff et al., 2011).*** However, the oocytes during this time period are active, synthesizing mRNAs and protein ***(Semaan and Kauffman, 2015).***

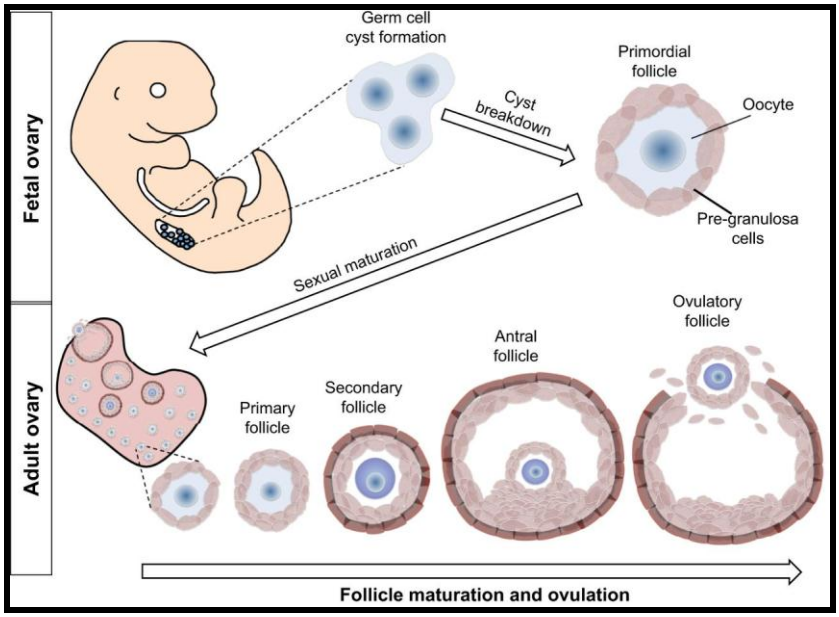

Fig. (1): Difference between fetal ovary and adult ovary ***(Grive and Freiman, 2015).***

The Adult Ovary:

At the onset of puberty, the germ cell mass has been reduced to about 500,000 units ***(Angela et al., 2012).*** During the next 35–40 years of reproductive life, 400 to 500 will be selected to ovulate ***(Nanette et al., 2011).*** In the last 10–15 years before menopause, there is an acceleration of follicular loss ***(Nikolaou and Templeton, 2003).*** This loss correlates with a subtle but real increase in FSH and decrease in inhibin B as well as insulin-like growth factor ***(Banerjee et al., 2010).***

The loss of oocytes (and follicles) through atresia is a response to changes in many factors such as gonadotropin stimulation and withdrawal, ovarian steroids, autocrine and paracrine factors ***(Carla et al., 2008).*** The consequence of these unfavorable changes is atresia which is a process called apoptosis, programmed cell death ***(Speroff et al., 2011).*** During the reproductive years, the typical cycle of follicle maturation, includes ovulation and corpus luteum formation that results from the complex sequence of hypothalamic-pituitary-gonadal interactions that are integrated to yield ovulation ***(Karl et al., 2011).*** For every follicle that ovulates about 1000 follicle will pursue abortive growth periods of variable length ***(Speroff et al., 2011).***

In the adult ovary, the follicular development stages observed in the prenatal period are repeated but to a more complete degree ***(Candace et al., 2009)***. Initially, the oocyte

enlarges and the granulosa cells proliferate markedly encasing the oocyte ***(Broekmans et al., 2009).*** It is now believed that the time elapses in progressing from a primary follicle to ovulation is approximately 85 days ***(Angela et al., 2012).*** The majority of this time passes in development that is independent of gonadotropins, achieving a state of readiness that will yield further growth in response to FSH stimulation ***(Speroff et al., 2011).***

Early in a menstrual cycle gonadotropin increments are available during which FSH-dependent stage of follicle maturation is seen, also the number of follicles that grow is dependent on the amount of FSH available to the gonad and the sensitivity of the follicles to the gonadotropins ***(Angela et al., 2009).*** FSH receptor expression is greatest in granulosa cells, but significant expression can be detected in ovarian surface epithelium and fallopian tube epithelium ***(Patel et al., 2013).***

A study evaluated the menstrual cycles, serum hormone concentrations, and oocyte viability in young women and older women who were still having regular menstrual cycles has revealed that oocyte viability declined in older women before they had any measurable decrease in serum or intrafollicular hormone concentrations ***(Klein et al., 2010).*** The isolated increases in serum (FSH) concentrations that are found in some normally cycling older women are probably caused by decreasing ovarian production of inhibin ***(Nanette et al., 2011).***

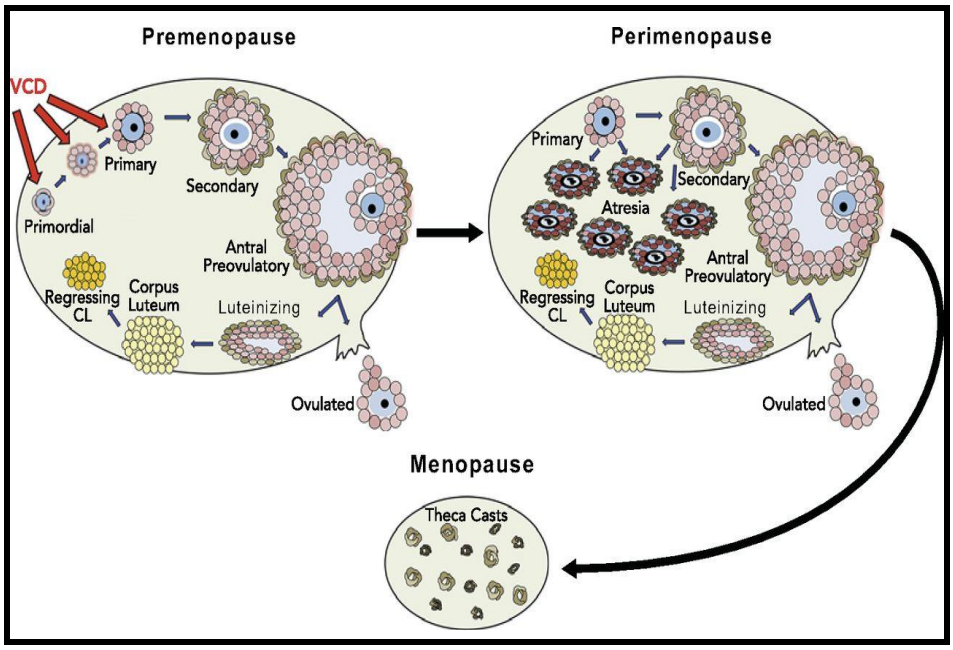

Fig. (2): Difference between premenopause (adult), perimoenopause and menopausal ovary ***(Brooks et al., 2016).***

The Menopausal Ovary:

Menopause occurs at a mean age of 51 years in normal women ***(Corrine et al., 2012).*** It is associated with a marked decline in oocyte number that is attributable to progressive atresia of the original complement of oocytes ***(Broekmans et al., 2009).*** Residual oocytes and differentiating follicles have been identified in the ovaries of some postmenopausal women, although the follicles are frequently atretic ***(Qian et al., 2012)***.

The decrease in developing follicles is reflected in a parallel decrease in the serum concentration of inhibin B, however, it is often viewed as a rather late marker of decreased follicle numbers ***(Burger et al., 2007).*** The rise in the serum

concentration of FSH in early menopause is also closely related to the fall in inhibin B, which plays an important role in the normal control of FSH secretion ***(Broekmans et al., 2009).***

Serum concentrations of Anti Mullerian Hormone (AMH]) may be a useful marker reflecting reproductive aging ***(van Disseldorp et al., 2008)***. In contrast to other markers, one of the main advantages of AMH is the stability of its serum level during the entire menstrual cycle ***(Qian et al., 2012)***. Nevertheless, serum AMH levels would invariably become undetectable near menopause and this low-value makes it difficult to predict menopause age accurately ***(Sowers et al., 2008).***

Some of the decline in ovarian function at the time of the menopause may be due to changes in the quantity or quality of the hormones secreted by the hypothalamus and pituitary or the pattern of their secretion, rather than to a primary loss of ovarian responsiveness ***(Wise et al., 2010).***

Chapter Two

PREDICTION OF OVARIAN RESPONSE FOR IVF CANDIDATES

Introduction:

In the field of assisted reproductive technologies one of the fundamental steps to reach the success is related to the number of eggs obtained after hormonal stimulation by gonadotropins in combination with GnRH analogues ***(Polyzos et al., 2014)***.

Despite the vast experience in that field, there are still women who respond poorly to gonadotropins resulting in few oocytes at retrieval ***(Maheshwari et al., 2007)*** reduced number of embryos for transfer and consequently unsatisfactory pregnancy rates ***(Oudendijk et al., 2012).***

A physiological decline of the "follicular heritage" can be observed in every woman over time with a marked increase in the rate of follicular disappearance from age 37 to 38 years onwards ***(Faddy et al., 2014).*** Different authors have suggested that poor responders "per se" do not represent a lower chance of success in IVF, with the age of the woman being the most important predictor of live birth rate ***(Ulug et al., 2014).***

However, very large studies have shown that this group of patients has reduced pregnancy rates compared with normal responders independently from the treatment protocol used

(Sunkara et al., 2014) and from the age of the patient ***(El-Toukhy et al., 2014).***

In this group of difficult patients, to adjust the clinical results in IVF it is needed to predict the ovarian reserve and to tailor the best stimulation protocol for optimizing the number of oocytes to be retrieved ***(Polyzos et al., 2014).*** Only with recent advent of new reliable biomarkers of ovarian reserve better strategies for the management of these patients have been suggested ***(Al-Azemi et al., 2011).***

Predictors of ovarian response:

While several groups have studied the use of various clinical, static and dynamic markers to assess ovarian reserve, no single parameter gives a satisfactory prediction ***(Bancsi et al., 2002).***

Clinical Marker:

Age is considered to be the single most important factor in determining quality and quantity of ovarian reserve ***(Bruno et al., 2012).***

Fecundability significantly declines since the early 30s ***(Nelson et al., 2013),*** and the prevalence of infertility increases significantly after the age of 35 years ***(Faddy et al., 2014)***. Fertility decline can be attributed to numerous events associated with advancing age, including changes in oocyte quality, frequency and efficiency of ovulation, sexual function,