INTRODUCTION

Some 30 years after the birth of the first 'test tube' baby, IVF has become a widely available treatment for most causes of subfertility. Despite ongoing advances in the associated assisted reproductive technologies (ART), pregnancy rates remain around 20–30% per started cycle. In order to compensate for inefficiencies in IVF procedures, high doses of exogenous gonadotrophins are administered to stimulate the development of multiple oocytes to mature in a single cycle. The use of such ovarian stimulation protocols enables the selection of one or more embryos for transfer, while supernumerary embryos can be cryopreserved for transfer in a later cycle ***(Macklon et al., 2006).***

The first in vitro fertilization (IVF) therapy was performed in a natural cycle. Gonadothropins are given to induce multiple follicular development and GnRH analogues are used for the prevention of premature LH surges in IVF. LH surges occur in about 20% of stimulated IVF patients. Preventing LH surges using GnRH analogues improves oocyte yielded with more embryos, allowing better selection and leading to an increase in pregnancy rate ***(Huirne et al., 2007).***

At the present time, a long gonadotrophin-releasing hormone (GnRH) agonist pituitary suppression regimen

combined with relatively high doses of exogenous follicle-stimulating hormone (FSH) remains the most frequently used stimulation protocol ***(Macklon et al., 2006).***

GnRH agonist administration causes gonadotrophin suppression via pituitary desensitization, after an initial short period of gonadotrophin hypersecretion. In contrast, GnRH antagonist accuses immediate and rapid gonadotrophin suppression by competitive occupancy of GnRH receptor and therefore is a choice to use in IVF for the prevention of premature LH surge ***(Tehraninejad et al., 2011).***

The GnRH antagonists were introduced in recent years as a new alternative for COH cycles in IVF-ET either in the form of multiple dose protocol or single dose protocol ***(Escudero et al., 2004)*** which have many advantages for patients and physicians with regard to convenience and flexibility of administration. The flexibility of administration led to interest in how best to use antagonists and compatible outcomes in pregnancy and implantation rates were achieved ***(Williams et al., 2002).***

Two GnRH antagonists, cetrorelix (Cetrotidew; Serono International S.A., Geneve, Switzerland) and ganirelix (Orgalutranw; Antagonw; Organon, Oss, The Netherlands), are currently commercially available for use in ovarian stimulation ***(Griesinger et al., 2005).***

Several potential advantages of antagonists are suggested over GnRH agonists. Among these advantages are shorter duration of injectable drug treatment, decreased gonadotropin requirement percycle, and lower overall treatment cost ***(Shapiro et al., 2003).***

Although GnRH antagonists have been used successfully in normal responders in clinics worldwide, most clinics currently use them mainly in patients with unfavourable prognoses (e.g. older patients) or those in whom previous cycles have been unsuccessful ***(Griesinger et al., 2005).*** A wide variety of GnRH antagonist protocols have been proposed, reflecting the fact that the protocol is still undergoing refinement. The 'ideal' protocol is yet to be determined ***(Macklon et al., 2006; Huirne et al., 2007).***

AIM OF THE WORK

To compare the efficacy of GnRH antagonist with standard GnRH agonist long protocol as regard the total number of oocytes retrieved as a primary outcome and the pregnancy rate as a secondary outcome in controlled ovarian hyperstimulation (COH) in patients undergoing ICSI.

Chapter 1
PHYSIOLOGY OF OVARIAN FUNCTION RELEVANT TO OVARIAN STIMULATION

Endocrine control of follicular development

The three major organs that regulate human reproduction are the hypothalamus, the pituitary and the ovary. The central nervous system- pituitary complex determines and direct the chronology of developmental events within a reproductive ovary. However the menstrual cycle is controlled by the sex-steroid and peptides produced within the follicle destined to ovulate ***(Dixson, 2001)***.

A- Hypothalamus

The hypothalamus is the part of diencephalons at the base of the brain just above the junction of the optic nerves which forms the floor of the third ventricle and part of its lateral walls ***(Sherwood et al., 2005).***

GnRH secretion and its regulation:

GnRH is a decapeptide derived from a large precursor molecule (12 amino acids) the biological activity of GnRH is very short with a half life of 4-6 minutes and secreted in a pulsatile pattern at 60 to 90 minutes interval during the follicular phase and 4 hours interval during the mid luteal

phase. Its secreting cells are neurons of the arcuate nucleus whose axons traverse the median eminence of the mediobasal hypothalamus where it is synthesized, stored and transported to the portal capillaries via the neural axons ***(Adam Osterzensky, 2002)***.

The molecule of GnRH binds specifically to a receptor on membrane of gonadotropic cells of the pituitary gland leading to synthesis and regulation of follicle stimulating hormone (FSH) and lutenizing hormone (LH) ***(Roberts et al., 2005)***.

Besides its well-known endocrine function, GnRH may directly regulate some extrapituitary reproductive tissues such as endometrium, ovary and placenta ***(Grundker et al. 2004, Choi et al. 2006).***

Since its discovery, about 1000 GnRH analogues have been identified and widely studied ***(Cheung et al., 2006).***

B- Pituitary

The pituitary gland is located in the sella tursica in relation to optic chiasma, and formed of anterior, intermediate and posterior lobes. The gonadotropic cells which present in the anterior lobe are responsible for synthesis of gonadotropins FSH and LH ***(Melis et al., 1998).***

In the menstrual cycle, the prime role of adenohypophysis is to allocate gonadotropins FSH and LH. GnRH controls and regulates the synthesis, storage and secretion of these hormones. Because GnRH is secreted in a pulsatile manner, FSH and LH respond accordingly with a pulsatile mechanism. But pituitary suppression occurs with giving GnRH agonist in a non-pulsatile manner which is commonly used during IVF downregulation ***(Jarvela et al., 2003)***.

Control of the reproductive cycle depends on the constant release of GnRH. The function depends on the complex and coordinated relationship among these releasing hormones, other neurohormones, the pituitary gonadotropins and the HMG steroids. The interplay among this substance is governed by feedback effects both stimulatory and inhibitory. The long feedback loop refers to the feedback effects of circulating levels of target gland hormones, and this occurs both in the hypothalamus and the pituitary, positive feedback is illustrated by the midcycle effect of estradiol (E2) and progesterone on the LH, and negative feedback refers to the effect of inhibin on FSH. The short feedback loop indicates a negative feedback of pituitary hormones on their own secretion, presumably via inhibitory effects on releasing hormones in the hypothalamus. Ultra short feedback refers to inhibition by releasing hormone on its own synthesis ***(Ben- Jonathan et al., 2001)***.

C-Ovaries

Adult ovaries are ovoid structure lies in the ovarian fossa. A mesovarium attaches the ovary to the posterior wall of the broad ligament, while the posterior margin is free. The peritoneum does not cover the ovary proper, which is covered by germinal epithelium. The hilus is the base of the ovary; at this point the ovarian blood vessels enter. The ovarian arteries arise from the abdominal aorta just below the renal arteries. They pass downward across the pelvic brim, cross the external iliac artery, and traverse the infundibulopelvic fold of peritoneum. Branches go to the ureter, round ligament, and tube and anastomose with the uterine artery. At the hilus venous drainage forms a pampiniform plexus, which consolidates to form the ovarian vein. On the right side the ovarian vein drains into the inferior vena cava, while the left ovarian vein drains into the left renal vein. The ovarian as well as the uterine blood supply frequently is anomalous ***(Sokol, 2011)***.

The nerve supply derives from a sympathetic plexus accompanying the vessels of the infundibulopelvic ligaments. The plexus arises at the level of the tenth thoracic segment, but fibers from renal and aortic plexuses as well as from the mesenteric and celiac ganglia are present ***(Sokol, 2011)***.

The earliest recognizable stage of an ovum is primordial germ cell at about 24 days of embryonic life and the primordial germ cells are situated in the wall of the yolk sac outside the actual embryo. During the fourth or the fifth

week of gestation these cells migrate to the HMG region where the gonads will develop, there are about 1000 to 2000 germ cells at that time ***(Gregory, 2003)***.

During development into an ovary the number of germ cells increases greatly by division. The germ cells are changed during this process via oogonia into primary oocytes. At about the seventh month of gestation the number of germ cells reach its maximum, about seven million. All the germ cells are then transformed into oocytes. The number of oocytes then drops sharply again to about 2 million at birth and to 300,000 at the age of seven years ***(Gougeon, 2004)***. At the beginning of puberty there are only about 40,000 remaining and not more than 400 to 500 of these will be released by means of ovulation ***(Erickson, 2000)***.

A. Morphology and Physiology of folliculogenesis

Folliculogenesis begins with the recruitment of a primordial follicle into the pool of growing follicles and ends with either ovulation or death by atresia. Follicles are present in the ovary at different stages of development, and large numbers of follicles of different sizes can be observed at any given point of the menstrual cycle ***(Gougeon, 2004)***.

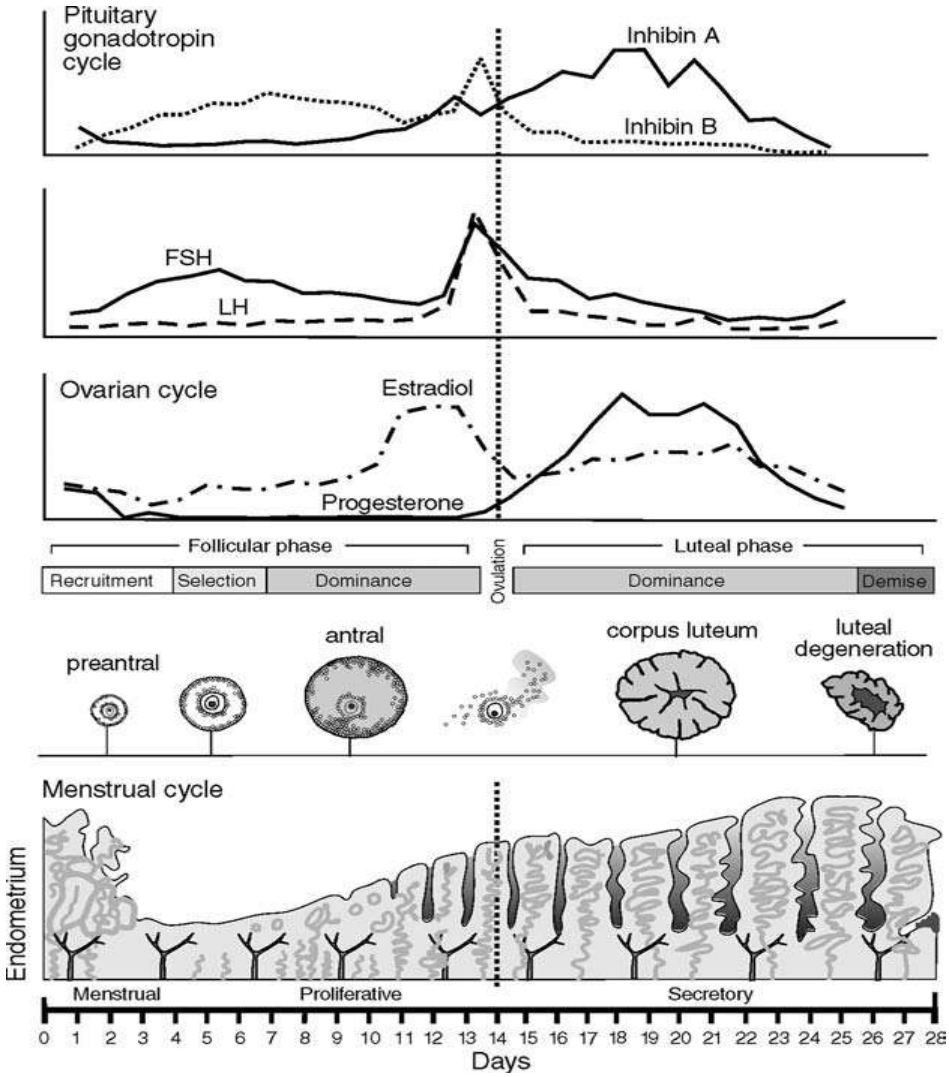

Figure (1): Schematic representation of the ovarian and menstrual cycles ***(David, 2007)*.**

Human follicular development consists of several stages, which include the gonadotropin-independent recruitment of primordial follicles from the resting pool and growth of these follicles into the antral stage. This process appear to be under the control of locally produced growth factor ***(Aaltonen et al., 1999: Hreinson et al., 2002)***.

These factors are produced by the oocytes, suggesting that the early steps in follicular development are in part of oocyte controlled. As the antral follicles develop, the surrounding stromal cells are recruited in a yet-to-be defined mechanism to become thecal cells. Although not required for early stages of follicular development, follicle stimulating hormone FSH is required for further development of large antral follicles ***(Hillier, 2001)***.

During each ovarian cycle, a group of antral follicles, known as a cohort, begin a phase of semisynchronous growth as a result of their state of maturation at the time of the FSH rise during the late luteal phase of previous cycle. This FSH rise leading to the development of follicles is called the selection window of the ovarian cycle. Only the follicles progressing to this stage develop the capacity to produce estrogen. During the follicular phase, estrogen levels rise in parallel to the growth of the dominant follicle and the increase in its number of granulosa cells ***(Macklon and Fauser, 2001)***.

The granulosa cells are the exclusive site of FSH receptor expression. The increase in circulating FSH during the late luteal phase of the previous cycle stimulates an increase in FSH receptors and subsequently the ability to aromatize thecal cells that respond to lutenizing hormone (LH) and granulose cells that respond to FSH represents the two-gonadotropin ***(Cunninghem et al., 2005)***.

After the appearance of LH receptors, the preovulatory granulose cells begin to secrete small quantities of progesterone. The preovulatory secretion of progesterone, although somewhat limited, is believed to exert positive feedback on the estrogen-primed pituitary to either cause help or augment release of LH. In addition, during the late follicular phase, LH stimulates thecal cells production of androgens, particularly androstenedione, which are then transferred to the adjacent follicles where they are metabolized to estradiol. During the early follicular phase, the granulose cells also produce inhibin B, which can feedback on the pituitary to inhibit FSH release ***(Groome et al., 1996)***.

As the dominant follicle begins to grow, the production of estradiol and the inhibin increases, resulting in a decline of follicular phase FSH. This drop in FSH is responsible for the failure of other follicles to reach preovulatory status – the Graffian follicle stage- during any one cycle. Thus 95 percent of plasma estradiol produced at this time is secreted by the dominant follicle, which is destined to ovulate. The contra lateral ovary is relatively inactive ***(Cunninghem et al., 2005)***.

B. Ovulation:

The onset of the gonadotropin surge resulting from increasing secretion of estrogen by preovulatory follicles is a relatively precise predictor of the time of ovulation, occurring some 35 to 36 hours before the release of the

ovum from the follicle. The peak of LH secretions occurs 10 to 12 hours before ovulation and stimulate the resumption of the meiosis process in the ovum with the release of the first polar body. At that time a protrusion of the follicular wall (the stigma) develops. Following a hormonal stimulus from the pituitary gland the follicle rupture to release the oocyte together with its surrounding cumulus cells and this process is known as ovulation ***(Godwin, 2001)***.

Current studies suggest that in response to LH, increased production of progesterone and prostaglandins activates members of both plasminogen activator and matrix metalloproteinases. Activation of these proteases is likely to play a pivotal role in the weakening of the follicular basement membrane and ovulation ***(Ny et al., 2002)***.

C. Luteal phase of the ovarian cycle

Following ovulation, under the influence of luteogenic hormones, the corpus luteum (CL) develops from the remnants of the ovulated ovarian follicle. The basement membrane separating the granulosa-lutein and theca-lutein cells breaks down, and by day 2 postovulation, blood vessels and capillaries invade the granulosa cell layer. The rapid neovascularization of the once a vascular granulosa may be due to a variety of angiogenic factors. These include vascular endothelial growth factor and others

produced in response to LH by the theca-lutein cells and granulosa-lutein cells. During lutenization, these cells undergo hypertrophy and increase their capacity to synthesize hormones ***(Albrecht and Pepe, 2003)***.

This process called luteinization and the stimulus for its initiation, the preovulatory LH surge, are common among species. The morphological events underlying this process involve intense reorganization of constituent cells, particularly granulosa cells, phenomena that includes varying cell-matrix interactions. These events, however, are poorly characterized. After expulsion of the oocyte, the blood capillaries of the theca rapidly invade the granulosa, thereby provoking the transformation of these cells (luteinization) and the formation of the CL. The blood vessels completely traverse the granulosa and open up in the follicular cavity. The granulosa cells are transformed into large luteal cells whose ultrastructure is the same as that of steroidogenic cells. The main hormone product of the CL is progesterone, which induces the necessary endometrial modifications required for the acquisition of a receptive state, an anticipation of embryo implantation. The life span of the CL is limited. In a nonfertile cycle, corpora lutea regress at the end of the menstrual cycle and are eliminated by a process called luteolysis ***(Mohri, 1996).***

Within the corpus luteum, luteolysis is characterized by a loss of luteal cells due to an increase in apoptotic cell death ***(Vaskivuo et al., 2002)***.

If pregnancy does occur, regression must be inhibited since the CL is the main source of steroidogenesis, supporting the establishment and maintenance of a successful pregnancy ***(Mohri, 1996).***