Welcome to chapter 1.

The following chapter is called "Ovarian Stimulation Protocols: General Overview".

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ART or IVF is a 4-stage procedure, consisting of:
- Controlled ovarian stimulation
- Oocyte retrieval
- Laboratory procedures, including insemination, fertilization of the gametes, and embryo culture
- Embryo transfer

The objective of this presentation is to review the first stage in this process, controlled ovarian stimulation also known as controlled ovarian hyperstimulation.
The process of follicular stimulation for patients undergoing assisted reproductive technologies (ART) is referred to as controlled ovarian hyperstimulation (COH). The goal of treatment is the development of multiple follicles and hence the production of a sufficient number of mature oocytes to be used for in vitro fertilization (IVF).

To accomplish this it is necessary to use specific medications to stimulate follicular development. These medications are called gonadotropins.
This slide summarizes the various stages of follicular growth. The follicles become responsive to gonadotropins in the early antral stages. It takes about 3 months for a primordial follicle to reach the antral stage.
In this drawing are illustrated the various stages of the follicular reproductive units scattered throughout the ovarian cortex. In the cortex it is possible to identify the primordial follicles, the primary and secondary follicles and the antral follicle. Once the antral follicle reaches a diameter of about 16-20mm, the process of ovulation takes place and the oocyte is ovulated surrounded by the cumulus cells (corona radiata). The now empty follicular unit develops into a corpus luteum. Once the corpus luteum ceases its hormonal function (mainly production of progesterone) it is called corpus albicans.
In order to achieve controlled ovarian hyperstimulation we use gonadotropins that can be extracted from biological fluids or produced through recombinant technology.

The specific gonadotropins are follicle stimulating hormone (FSH), luteinizing hormone (LH) and human chorionic gonadotropin (hCG), which are all glycoprotein hormones. This figure provides the tridimensional protein structure of the 3 glycoproteins (FSH, LH and hCG).

In the female, FSH induces the recruitment and growth of ovarian follicles, while LH is responsible for final follicular maturation and resumption of meiosis in the oocytes. hCG is generally secreted by the trophoblastic tissue, hence it indicates pregnancy.

During COH, hCG is used to mimic the natural LH surge which insures final follicular maturation and detachment of the oocyte from the follicular wall.

Originally the gonadotropins FSH and LH were extracted exclusively from the urine of post-menopausal women while hCG was derived from the urine of pregnant women. However, in recent years gonadotropins are produced with recombinant technology (rFSH; rLH and rhCG).
The first type of gonadotropin medications introduced for COH were purified from the urine of post-menopausal women.

Still available today, these products are referred to as human menopausal gonadotropins. Some contain both FSH and LH activity, while others contain primarily FSH with very low levels of LH activity.

These products are available as freeze-dried powder which has to be solubilized in diluent.
In the mid-1990s, the first recombinant gonadotropin became available (recombinant human FSH).

Thereafter, both recombinant human LH and recombinant human chorionic gonadotropin were introduced.

These products are available as multidose vials, prefilled syringes or pen devices.

The benefits of recombinant medications include the elimination of contaminating urinary proteins, greater batch-to-batch consistency and higher bioactivity versus the urine-derived products.
When comparing urine-derived and recombinant gonadotropins, the risk of Ovarian Hyperstimulation Syndrome and multiple births are similar.

Pregnancy outcomes with the different gonadotropin medications are also similar.
The foundational tenets of COH are derived from our knowledge of normal female reproduction. The human ovulation cycle is of high complexity, so that small deviations can disrupt it and prevent ovulation.

This slide summarizes the normal cycle which consists of an ovarian component, comprised of the follicular and luteal phases, and an endometrial component, comprised of the proliferative and secretory phases.

The normal reproductive cycle is approximately 28 days.

The regulation of the cycle is controlled by the anterior pituitary gonadotropins, FSH and LH. The secretion of these hormones is influenced by gonadotropin releasing hormone (GnRH), a product of the hypothalamus and by hormonal levels of estrogen produced from the ovary.

Ovulatory disorders are caused by: a) deficiency in one or more controlling hormones (most common cause); b) ovarian resistance to normal levels of these hormones; c) ovarian damage or disease (for example, chemotherapy or radiotherapy).
FSH and LH are glycoprotein hormones comprised of two subunits, alpha and beta.

The alpha subunit is common to both FSH and LH while the beta subunit is unique to each gonadotropin and is responsible for the gonadotropin's specific biological functions.

The release of FSH is associated with follicular growth and the production of the ovarian hormones estrogen and progesterone.

The illustration shows that in the first half of the follicular phase the release of LH is minimal. It then increases until it peaks at mid-cycle (also called the LH-surge) which corresponds with ovulation on or around day 14 of a normal 28 day cycle.
The synthesis and release of LH and FSH from the anterior pituitary are induced by the physiologic actions of GnRH.

The number of GnRH receptors (GnRHr) on the pituitary gonadotrope cells can be modified by both pulsatility and the concentration of GnRH.

This provides an additional mechanism through which GnRH may regulate LH and FSH secretion.

In addition, GnRH has been detected in the placenta, ovary, testes, breast, lymphocytes and possibly in the pituitary itself. The physiologic role of these extra hypothalamic sources remains unclear.
The structure of GnRH shown here reveals the decapeptide structure and the areas responsible for the physiological actions: a) activation of the GnRH receptor on the pituitary cells (amino acids [aa] 1-3); b) regulation of GnRH receptor affinity (aa 5-6); and c) regulation of biologic activity (aa 9-10).

These three areas of the molecule can be modified to change the properties of synthetic produced GnRH analogs.
As mentioned before, GnRH is the native decapeptide which initiates the reproductive cascade.

GnRH analogs are synthetic versions of GnRH with various amino acid substitutions. These substitutions serve to increase their half-life (time in circulation before they are broken down by enzymes) and to increase their affinity for the GnRH receptor.

There are two types of analogs: GnRH agonists and GnRH antagonists.

GnRH agonists act like GnRH and therefore stimulatory to the LH/FSH production.

GnRH antagonists block the effects of GnRH immediately and are inhibitory.

The most common GnRH agonists and antagonists are listed on this slide.
The amino acid sequences of native GnRH, a synthetic GnRH agonist (leuprolide acetate) and a synthetic GnRH antagonist (ganirelix acetate) are illustrated on this slide.

For leuprolide acetate, the glycine in position 6 has been replaced by D-leucine and the glycine in position 10 has been removed.

The substitution at position 6 helps to protect against enzymatic degradation.

For ganirelix acetate there are many more substitutions with unnatural amino acids at position 1, 2, 3, 6, 8 and 10.

Substitutions at position 1, 2 and 3 are important for the antagonistic function.

The substitution at position 6 helps to protect against enzymatic degradation and enhances aqueous solubility.

In concert with substitutions at positions 8 and 10 it also helps to reduce the histamine release effects that were caused by earlier generations of GnRH antagonists.
The actions of GnRH analogs are illustrated on this slide.

The agonist, on the left, stimulates FSH and LH release initially and then becomes inhibitory.

On the right, the antagonist causes immediate blockade of FSH and LH action resulting a decrease of estradiol and progesterone production by the ovary.
Due to the binding of GnRH agonists to the receptors located on the surface of the pituitary gland agonists have a stimulating effect, resulting in an increase of endogenous LH and FSH and in turn a rise of E2. This initial stimulatory phase is called “flare up effect”.
However, after about 2 weeks of continuous GnRH agonist action, the number of receptors on the surface of the pituitary gonadotrope decreases, while the remaining receptors will become less sensitive (phenomenon known as desensitization or down regulation). Both effects cause a decline in FSH, LH and E2.

Before a COH treatment is initiated, laboratory testing has to reveal that the hormone levels (FSH and LH) are below the normal values (down regulation).
This slide shows the initial response of the reproductive axis caused by daily administration of GnRH agonists - the so-called “flare-up effect” which retains about 14 days.

The stimulatory effects of the GnRH agonist regimen are more pronounced in the follicular phase than in the luteal phase.
Antagonists are administered after a few days of stimulation with Gonadotropins alone.

The GnRH antagonist is structurally very similar to the GnRH receptor. The competitive binding with the native receptor immediately causes a decline of FSH and LH levels, without the flare-up effect of the GnRH agonist.
In contrast to the initial stimulatory effects of GnRH agonists, the initial response of the reproductive axis to the daily administration of GnRH antagonists is suppression. This suppression occurs within hours and is sustained as long as levels of GnRH antagonist are sufficient to occupy the GnRH receptors, i.e. preventing native GnRH from exerting its biological actions.
Prior to the wide-spread use of GnRH agonists, up to 35% of ART cycles were cancelled due to premature ovulation resulting from a premature LH surge.

Use of GnRH analogs in ART prevent a premature LH surge and avoid cycle cancellation.

If a cycle is not cancelled despite a premature LH surge, the number of oocytes retrieved would be lower and result in a poor prognosis for pregnancy and cause financial loss.

According to the World Collaborative Report on IVF, in 1995, 85% of ART cycles worldwide were treated with GnRH agonists and gonadotropins.
Factors that influence the choice of COH protocol are largely driven by the patient’s prognosis and factors concerning the ART centers themselves.

Patient factors include:
- The age of the woman in addition to her baseline levels of FSH and E2
- Her antral follicle count and body weight
- The etiology of infertility
- The patient’s history of prior stimulation outcome (normal response versus poor or hyper-response).
- Recently, in addition to FSH and E2, Anti-Müllerian hormone (AMH) has been added to the baseline work up to assess ovarian reserve.

ART centers may also select specific protocols due to the flexibility of their staff and facilities, the experience of the physicians and whether or not they offer embryo or oocyte cryopreservation.

Different COH protocols are used to accommodate all these factors.
When initiating gonadotropin therapy, the dose of the gonadotropins is usually kept constant for the first 4-5 days of stimulation.

The patient’s initial response to this dose is then assessed by transvaginal ultrasound (to visualize the number and size of developing follicles) and serum estradiol.

If the follicular response is adequate, the dose will not be changed.

If not, the dose will be increased or decreased respectively.

When an appropriate number of mature follicles have developed and criteria for hCG administration have been met, hCG is administered.
LH is not commercially available in adequate strength to serve as the “final LH surge”; hence hCG is used as a substitute.

If a urine-derived product is used, a dosage of 5,000 to 10,000 IU has to be applied.

If recombinant hCG is used, the dosage is set to 250 micrograms (equivalent to approximately 6,500 IU of hCG).

Within 35 to 36 hours of hCG administration, the oocytes are retrieved from the follicles using transvaginal-guided ultrasound aspiration.
This slide shows two examples of various combinations of complex COH protocols (not meant to be a comprehensive overview):

1) The first example is pre-treatment with GnRH agonist ± oral contraceptive pill (OC) for patients with polycystic ovaries. Since patients with PCOS are more prone to develop OHSS, the use of OC in the month prior to the ovarian stimulation may decrease the risk for OHSS. As an example, the OC is started on menstrual cycle day 2 of the month prior to stimulation and then during the last 7 days of OC the GnRH agonist (leuprolide acetate) is added (indicated in the slide as 7 day overlap). Patients will usually menstruate 3-5 days after stopping OC and will then be instructed in the use of their particular dose of gonadotropins.

2) The second COH example is the use of antagonist (cetrorelix in the example) and gonadotropins for patients who are poor responders. After an initial stimulation with high doses (450IU/day) of gonadotropin alone (for about 6 days), patients are instructed to add cetrorelix to the protocol, either at daily doses of 0.25mg or with a single dose of 3mg (which lasts approximately 7 days) when the leading follicle has reached a mean diameter of about 13 mm.

For both protocols the doses of gonadotropins are adjusted, if necessary, after the initial 5-6 days based on the follicular response. The administration of hCG (rhCG in the diagram) is ordered when at least two follicles have reached a mean diameter of 18-20 mm.
One of the most popular protocols utilizes a GnRH agonist initiated in the luteal phase prior to the onset of gonadotropin stimulation.

This procedure is based on evidence that the developing follicular cohort is more synchronized.

Known as the long or luteal phase GnRH agonist protocol, treatment is initiated on day 21 (the luteal phase) of the prior cycle.

The goal of pre-treatment is suppression (also known as down-regulation) of FSH, LH, and estradiol by the time of menses onset.

Once suppression is confirmed (on day 2 or 3 of menses, the estradiol level should be <50 pg/ml) gonadotropin treatment is initiated.

The GnRH agonist treatment is continued until the day of hCG administration to prevent a premature surge of endogenous LH.

The protocol at the bottom of the previous slide is an example of the luteal GnRH agonist protocol.
Another protocol utilizing GnRH agonists is called the short or follicular phase protocol. In this protocol the GnRH agonist is initiated on the first day of the menstrual cycle.

On day 2 of the cycle gonadotropin treatment is commenced and GnRH agonist treatment is continued until the day of hCG administration to prevent a premature LH surge.

This treatment is used in patients with a known or expected poor response to COH.
Another protocol used in patients with known or expected poor response is known as the "mini-dose GnRH agonist protocol".

The goals of this protocol are to increase the stimulatory response as well as prevent an LH surge.

As a result, cycle cancellations are decreased.

The GnRH agonist is started on day 2 of the cycle along with gonadotropins if the patient’s endogenous FSH level is <15 IU/L and the estradiol level is <50 pg/ml.

A much lower dose of GnRH agonist is used (50 micrograms of leuprolide acetate) and the drug is administered twice a day.
An alternative to the GnRH agonist protocol is utilizing a GnRH antagonist.

Another method to prevent a premature LH surge in COH protocols is using a GnRH antagonist.

When using a GnRH antagonist, administration of gonadotropins is started on day 2 or 3 of the cycle and on day 5 or 6 of simulation or when the leading follicle is at least 14 mm, the GnRH antagonist treatment is initiated.

For the treatment of GnRH antagonists two dosing options are available:
- A daily 0.25 mg dose which is administered until the day of the hCG injection or
- a single 3 mg dose which is equivalent to 4 days of LH suppression. If additional days of LH suppression are required, administration of the daily 0.25 mg dose is recommended.
GnRH antagonists and agonists are equally effective at preventing spontaneous LH surge.

GnRH antagonists are associated with a lower risk of ovarian hyperstimulation syndrome (OHSS).

Additionally, lower amounts of gonadotropins are needed for follicular stimulation which may result in lower costs for patients.

However, there is an ongoing debate about the slightly lower pregnancy and implantation rates with GnRH antagonists versus GnRH agonists.
There are some differences when comparing the GnRH antagonist and agonist cycles:
There is no cyst formation when using the GnRH antagonist protocol (since there is no flare up effect) and no hormonal withdrawal symptoms (since pituitary suppression is achieved after ovarian stimulation has been started and not before).

The concentration of gonadotropins necessary is lower and the treatment duration is shorter. Furthermore, the treatment is more physiologic, since integration in the natural menstrual cycle is possible.