IVF in PCOS patients

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- Polycystic ovary syndrome (PCOS) is a common endocrine disorder of women, which occurs in 5% to 13% in women of reproductive age.
- PCOD characterized by **a heterogeneous** presentation of **hyperandrogenism** (increased body hair or hirsutism, acne), ovulatory dysfunction (irregular or absent periods, abnormal or absent ovulation and infertility) and
- **increased body weight/body mass index** (BMI) at the reproductive age is often associated with infertility and clinical and metabolic disorders.

- Hirsutism is defined as a score of 8 or more on the modified Ferriman-Gallway index (Ferriman and Gallwey, 1961).
- Oligomenorrhea is also one of the clinical manifestations of PCOS. Oligo/amenorrhea cycles are defined as 8 or less cycles per year and biochemical androgen measurements should be fulfilled in follicular phase in patients with preserved menstrual cycles
- heterogeneity seems to be adjusted by several factors, such as **genetic factors, nutritional condition in the uterus, prenatal androgen exposure, insulin resistance, exaggerated adrenarche, and body weight changes**

Polycystic ovary syndrome (PCOS) is a common polygenic multifactorial condition affecting a wide population who recognized that **enlarged ovaries, amenorrhea, infertility, and hirsutism could be collated together**.

This collection of symptoms has been widely researched and many conclusions made, yet it continues to prove to be a difficult condition to treat.

PCOS is now recognized as a spectrum disorder ranging from ultrasound features of polycystic ovarian morphology to anovulatory infertility.

Obesity, hyperandrogenemia, and insulin resistance are all key factors that influence the expression and symptoms of the condition

- Environmental status and factors, such as obesity, appear to exacerbate the underlying genetic predisposition. PCOS is characterized by *increased levels of circulating androgen, polycystic ovarian morphology (PCOM), arrested follicle development, and anovulatory infertility.*

- PCOS is commonly associated with **insulin resistance, hyperinsulinemia, components of the Metabolic Syndrome, and oligo anovulatory cycles**
- Although some of the clinical symptoms and presentations of PCOS is dependent on age, ovarian failure and hyper androgenism (HA) are common characteristics at any age

- While during childhood first signs of the syndrome can be perceptible, the unique features of PCOS in puberty are not yet clear.
- Despite all of these difficulties, PCOS early diagnosis has great and undeniable importance, because its presence is related to a **greater risk of future infertility, disease which is related to cardiovascular system, diabetes mellitus (type II),** MetS (metabolic syndrome).
- The PCOS diagnosis in puberty can be **difficult,** because anovulation is common in young girls (in the first two years of menarche half of menstural cycles are anovulatory), and ***multiple follicles display on ultrasound is also a fairly common finding during puberty***
- Thus, the main findings at present which indicate diagnosis of the syndrome at this age are biochemical hyperandrogenism or clinical hyperandrogenism with hair excess.

- PCOS is a disease that often presents during adolescent, but there is **an overlap between** features of PCO syndrome and physiological findings observed during the normal progression of puberty, and this matter makes the diagnosis more complicated in this age group
- Prevalence of the syndrome varies according to diagnostic consensus used, with estimates ranging from 9% according to National Institutes of Health consensus, up to 18% with the Rotterdam consensus

- Studies on PCOS reported multiple relatives and siblings in families with **autosomal dominant inheritance**.
- The prevalence of PCOS in the first-degree relative of the proband that was found in nearly **55–60% in several small** families supported the hypothesis of autosomal dominant inheritance of PCOS. Later on, **monogenic causes of hirsutism and oligomenorrhea in PCOS** women and male-pattern baldness were identified.
- **Twin studies** in small cohorts of mono- and dizygotic twin pairs suggested that PCOS is neither an autosomal dominant nor a monogenic disease; rather, it is an **X-linked polygenic disorder**.
- Moreover, twin studies estimaited 72% variance in risk of PCOS to be genetic in basis, highlighting the genetic involvement.

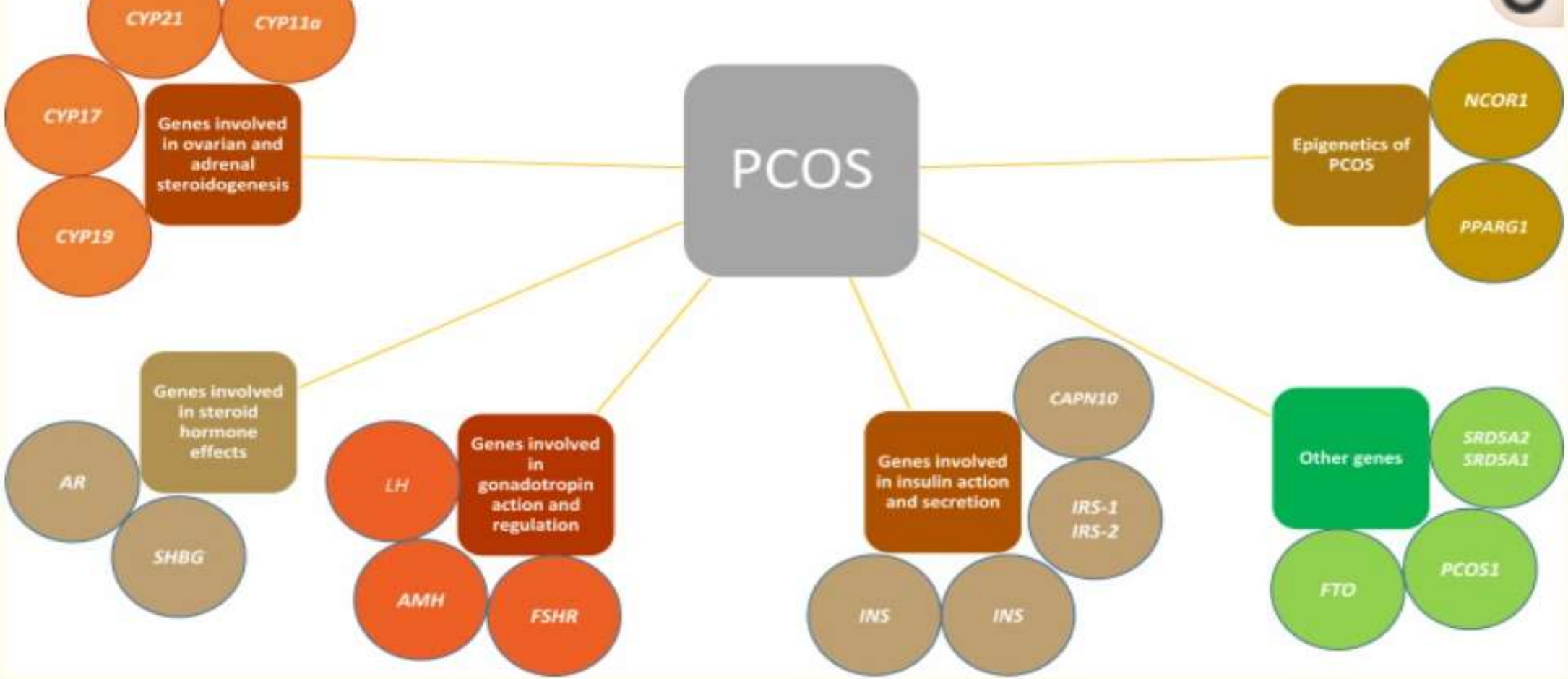


Figure 1

Summary of the genes involved in PCOS highlights the complexity of the disease.

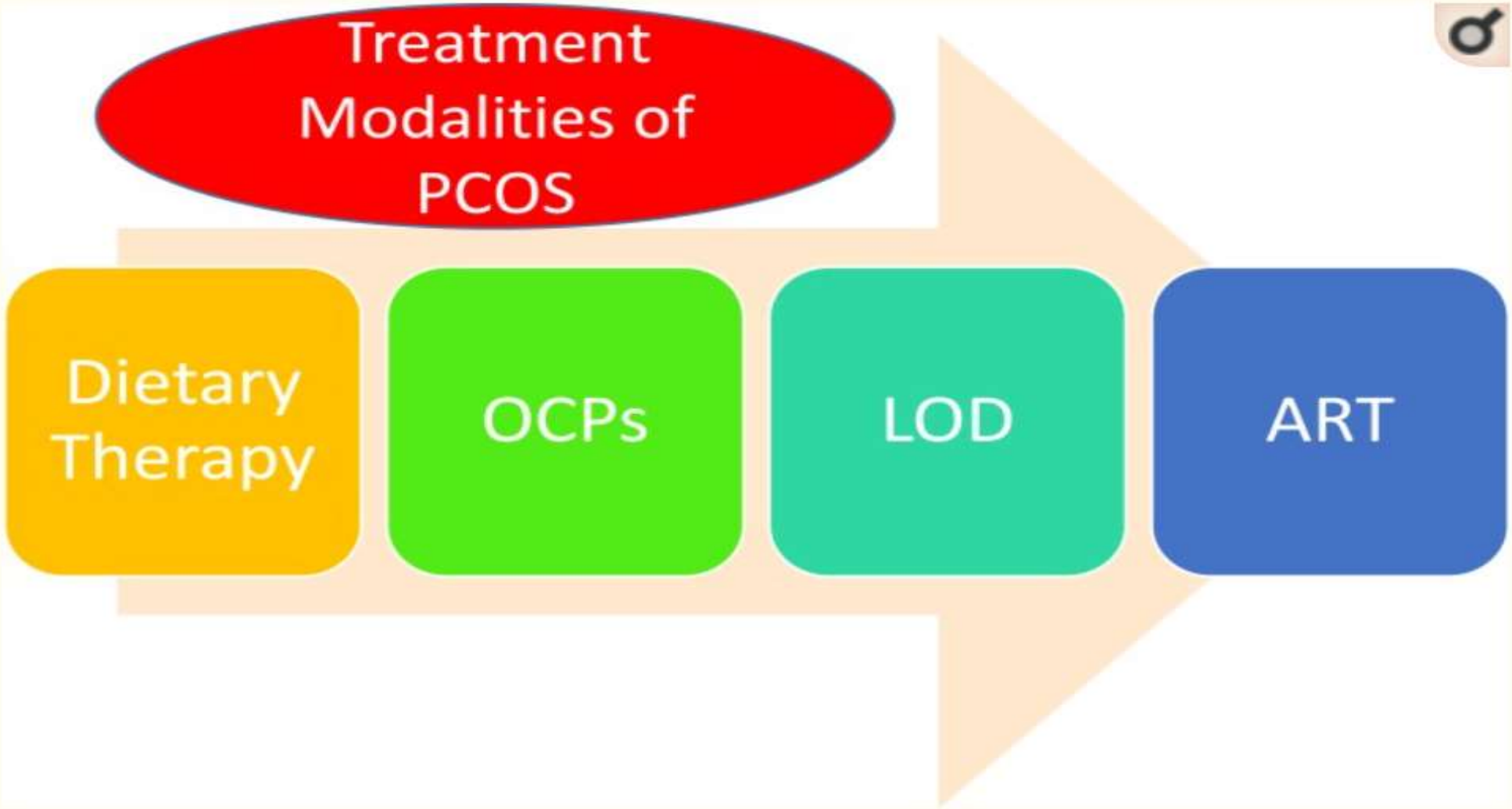
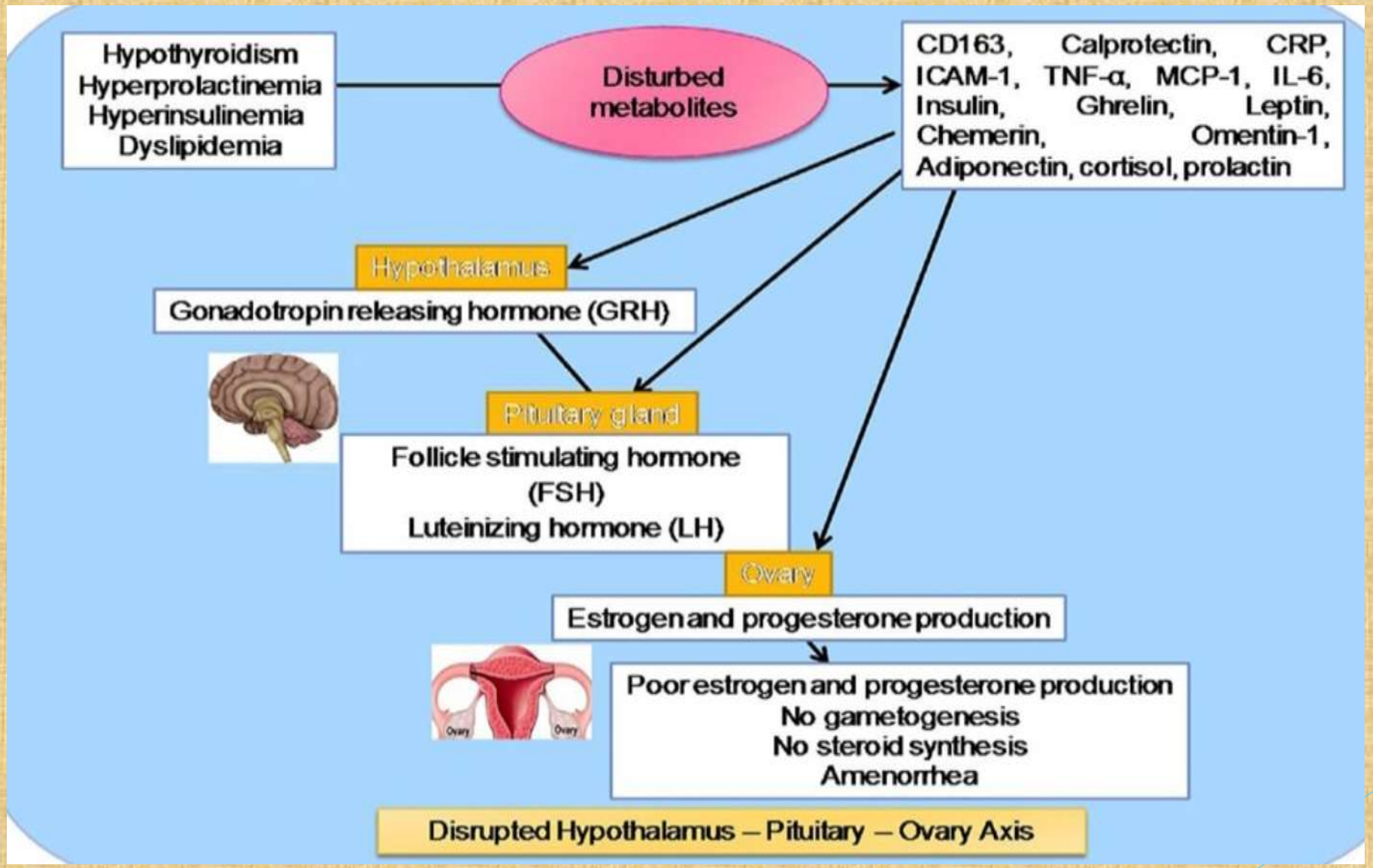


Figure 2

Summary of treatment modalities of PCOS.


Hypothyroidism
Hyperprolactinemia
Hyperinsulinemia
Dyslipidemia
Disturbed metabolites
CD163, Calprotectin, CRP, ICAM-1, TNF-α, MCP-1, IL-6, Insulin, Ghrelin, Leptin, Chemerin, Omentin-1, Adiponectin, cortisol, prolactin
Hypothalamus
Gonadotropin releasing hormone (GRH)
Pituitary gland
Follicle stimulating hormone (FSH)
Luteinizing hormone (LH)
Ovary
Estrogen and progesterone production
Poor estrogen and progesterone production
No gametogenesis
No steroid synthesis
Amenorrhea
Disrupted Hypothalamus – Pituitary – Ovary Axis

- The ovaries of women with PCOS contains *multiple micro-cysts often arranged like a "string of pearls" immediately below the ovarian surface (capsule*), interspersed by an overgrowth of ovarian connective tissue (stroma). PCOS has its unique properties such as **increased antral follicle count, serum AMH (Anti-Mullerian hormone) and LH/FSH ratio.** The low FSH concentration combined with high LH probably shows a well-preserved ovarian reserve and is associated with high pregnancy rates in IVF/ICSI cycles.
- Antral follicle count evaluates by ultrasonography and the AMH level considered a diagnostic or even prognostic marker of PCOS.

- AMH is *a dimeric glycoprotein of the transforming growth factor-β (TGF-β)* family produced in the ovary by granulosa cells of pre-antral and small antral follicles of less than **4 mm** diameter.
- AMH is considered a useful marker of ovarian reserve, and even clinical outcome of IVF.
- PCOS Patients greater oocyte production and are more likely to experience ovarian hyperstimulation syndrome (OHSS) and miscarriages.
- However, the rates of pregnancy are equivalent to those in patients with other causes of
- Many other studies have demonstrated that oocyte quality and embryo development may be affected by PCOS. However, it is still obscure whether these deleterious effects can induce developmental arrest of early embryos during IVF.

- Many studies have been conducted to evaluate the IVF/ ICSI outcome in PCOD and non-PCOD patient
- Swanton et **Al** found that significantly **increased number of retrieved oocyte and decreased rate of fertilization rate in the PCOD** group as compare to non-PCOD group in first IVF or ICSI cycle however, they did not found any significance differences in clinical pregnancy rates.

The Place of In Vitro Maturation in PCO/PCOS

- Various treatment modalities are used for treatment of PCOS-related infertility, including **lifestyle modification as a first-line treatment** for obese and overweight women with anovulation, ovulation induction with either oral *agents or gonadotrophins and laparoscopic ovarian drilling as second-line therapy*
- However, a subset of these patients will either be resistant to treatment or will fail to conceive despite ovulation induction treatment and will eventually need controlled ovarian stimulation (COS) and in vitro fertilization (IVF) [3].

- Additionally, they may have compromised fallopian tube function or male factor infertility and require IVF from the start. However, when undergoing IVF treatment, women with PCOS are predisposed to developing ovarian hyperstimulation syndrome (OHSS) due to their high antral follicle count; this facet also make them ideal for in vitro maturation (IVM) treatment
- OHSS is a significant cause of discomfort, distress, hospitalisation, and even mortality for women undergoing IVF treatment, due to the extravasation of fluid out of the vascular system leading to the development of ascites and potentially pleural effusion and thromboembolic phenomena

- In vitro maturation of oocytes has been suggested as an alternative approach to conventional IVF as it completely avoids the risk of OHSS .
- IVM treatment typically involves a relatively short duration of gonadotrophin stimulation and the retrieval of oocytes from follicles at a much smaller diameter than with conventional IVF treatment, often without the use of a trigger injection and oocyte maturation occurs in vitro .
- The process of IVM involves the collection of immature oocytes at the germinal vesicle (GV) or metaphase I (MI) stages of meiosis, retrieved from small ovarian follicles, by transvaginal oocyte retrieval.
- Subsequently, these oocytes undergo resumption of meiosis and maturation to metaphase II (MII) oocytes in the laboratory.

- The *in vivo* preparation for IVM treatment is a source of contention, and it has been suggested that cycles involving both gonadotrophin and an ovulation trigger should instead be referred to as “truncated” or “minimal stimulation” IVF
- By the administration of a human chorionic gonadotrophin (hCG) trigger prior to oocyte collection, “hCG priming,” the resumption of meiosis begins and subsequently oocytes are collected that may be at varying stages of the maturation process; GV, MI, or MII oocytes. In turn, this makes *in vitro* culture, fertilization, embryo culture timing, and embryo transfer logistically difficult, as the oocytes need to be treated individually according to their stage of development.
- In agreement with De Vos, it is our view that the true classification of **IVM should be restricted to cycles without the use of a hCG trigger**, with the process of germinal vesicle breakdown and resumption of meiosis completed “*in vitro*.” Hence, true IVM involves the culture of germinal vesicle (GV) oocyte in vitro culture.

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- Anovulation is common among women with PCOS and accounts for 80%–90% of World Health Organization (WHO) group II anovulatory subfertility.
- Treatment has centered **on weight management and lifestyle modification** followed by ovulation induction with clomiphene citrate
- The cumulative pregnancy rate with *clomiphene after six months of treatment is between 40% and 50%*
- Women who remain anovulatory can be stimulated with low-dose gonadotropins.
- Ovarian diathermy has been suggested as an effective alternative in ovulation induction
- For those who *remain refractory to these treatments* or with coexisting pathologies, assisted reproductive techniques can be employed within aclosely supervised setting to produce the desired outcome of pregnancy with the challengingly sensitive polycystic ovary (PCO)

DIAGNOSIS AND PREVALENCE

- Two of the following are required:
  1. Oligo and/or anovulation
  2. Clinical and/or biochemical signs of hyperandrogenism
  3. PCO morphology on ultrasound

  Exclusion of other etiologies (such as congenital adrenal hyperplasia, androgen-secreting tumor, or Cushing's syndrome) must be elucidated by appropriate investigation as indicated.

  Twelve or more follicles measuring 2–9 mm or increased ovarian volume over 10 cm3 offers the best specificity (99%) and sensitivity (75%) for the diagnosis of PCOS

- The distribution of follicles and description of the stroma are not required.

- This prompted a hypothesis that the hyperandrogenic microenvironment resulted from an increased recruitment of growing follicles followed by their arrest at 6–9 mm

- Since the Rotterdam consensus, there has been further debate about the definitions of both the syndrome and the morphology of the PCO. More recently, it has been suggested that the definition of PCO should be

  increased to 25 follicles per ovary

Further population studies have confirmed a common finding of PCO in 21%–23%, although a significant proportion (25%) were without any clinical features of PCOS

The highest reported prevalence of PCO is 52% in South Asians living within the U.K.

REPRODUCTIVE HEALTH IN PCOS

- In addition to anovulation, there may be other factors that *contribute to subfertility in women with PCOS, including the effects of obesity and metabolic, inflammatory, and endocrine abnormalities on oocyte quality and fetal development.*
- Oocytes from PCOs may exhibit reduced developmental competence, with a reduced ability to complete meiosis, achieve fertilization, and develop into a normal embryo.
- **Ovarian hyperandrogenism and hyperinsulinemia may promote premature granulosa cell luteinization**;
- furthermore, **paracrine dysregulation of growth factors** may disrupt the intramolecular environment, alter granulosa cell-oocyte interactions, and impair cytoplasmic and/or nuclear maturation of oocytes

- PCOS is associated with metabolic disturbances that include impaired insulin signaling and glucose metabolism in ovarian follicles
- It is likely that the metabolic lesion in the follicle precipitates an altered metabolic milieu throughout oogenesis, which may have *downstream consequences for oocyte energy generation*.
- Altered expression of key genes associated with chromosome alignment and segregation has also been attributed to hyperandrogenemia
- Indeed, it has been shown that differences in metabolism exist in oocytes derived from women with PCOS, and this is associated with chromosomal pre-division; that is, premature separation of sister chromatids
- During early pregnancy, the embryo may be exposed to androgen excess *in utero*, which may have long-term effects, particularly on female offspring. Fetal hyperandrogenism may disturb epigenetic programming, particularly those genes regulating reproduction and metabolism
- the potential **influences of hyperinsulinemia and its effect on the intrauterine environment.**

- significantly higher risk of developing gestational **diabetes mellitus (GDM**; **pregnancy-induced hypertension** pre-eclampsia and preterm birth
- Their babies had a significantly higher risk of admission ***to a neonatal intensive care unit*** and a higher perinatal mortality unrelated to multiple births (25).
- In addition, GDM may also result in fetal macrosomia. Obesity in its own right is associated with several adverse pregnancy outcomes, including spontaneous miscarriage, pre-eclampsia, GDM, congenital anomalies (e.g., cardiac and spina bifida), and fetal mac

HYPERANDROGENEMIA

- Hyperandrogenism, in conjunction with **hyperinsulinemia,** is a cardinal feature of PCOS.
- It is plausible that the ~~follicular microenvironment is related to oocyte quality.~~ Teissier et al. showed that **follicular testosterone levels** were significantly elevated in PCOS, especially in meiotically incompetent oocytes .
- High androgen levels may therefore contribute to the **lower fertilization** rate among the oocytes retrieved from a PCO.
- Conversely, a positivecorrelation exists **between testosterone concentration and the number of antral follicles (2–5 mm in** diameter).
- Pretreatment with an ***aromatase inhibitor increases the ovarian androgen level, improving ovarian response in the low responder within an in vitro fertilization (IVF) treatment*** **an increased recruitment of primordial follicles form the resting pool.**

- On the day of human chorionic gonadotropin (hCG) administration, the PCOS subject will have a **higher estradiol concentration**. **increase in androgen substrates and aromatase activity**
  Under *in vitro* conditions, granulosa cells from a PCO exhibit an increased response to follicle-stimulating hormone (FSH) stimulation compared to size-matched controls
- potentially due to the higher number of FSHreceptors attributed to the stimulatory effects of androgen on FSH receptor synthesis
- Androgen enhances the production of ovarian steroids in response to gonadotropin stimulation whilst promoting the ***expression of IGF-1 and IGF-1 receptor genes*** in the growing follicles up to the small antral stage

- **Serum androgen** levels rise during ovarian stimulation and are higher in PCOS patients. The higher levels are suggested to **negatively impact** on pregnancy outcome
- negative correlation exists between androgen concentration and uterine placental **protein (PP14,** also known as glycodelin) in women with PCOS and recurrent miscarriage**.** **Glycodelin** is an important secretory protein from the endometrium and is a marker of endometrial receptivity
- It is increased in successful conception IVF cycles**.** *Androstenedione causes a dose-dependent reduction* in glycodelin and endometrial cell proliferation which are inhibited by administration of an anti-androgen, **cyproterone.**
- A reduction in sex hormone-binding globulin in the endometrial stroma in PCOS increases the bioavailability of androgens
- With an overall increase in expression of **endometrial androgen receptors**, high levels of estrogen and androgen up-regulate this expression, whilst progesterone has the opposite effect
- **αVβ3 integrin**, a cell adhesion molecule, is suppressed at the time of implantation by high androgen levels, further reflecting endometrial reduced receptivity

OBESITY

- **Central obesity** is a major factor influencing outcomes of both treatment of symptoms and infertility in women with PCOS.
- Obesity is seen in 38%–66% of those with PCOS, with body mass index (BMI) correlating with severity of the phenotypical features.
- Clinical pregnancy rates are significantly **lower in the obese** in both natural and assisted conception cycles
- This also translated to a **reduced live birth rate**, although this failed to reach significance
- A reduced fertilization rate, fewer oocytes, and reduced peak estradiol level highlight the impaired follicular and oocyte response in the morbidly obese. Other problems related to obesity include miscarriage and cancellation of assisted reproductive cycles
- Fedorcsak et al. concluded that obesity, independent of insulin resistance, is associated with an increase in **miscarriage and gonadotropin resistance and a reduction in oocyte**

- Increased FSH is needed in the obese to stimulate the ovary, but once the threshold has been reached, the subsequent response can be dramatic, leading to an uncontrolled response and a risk of ovarian hyperstimulation syndrome (OHSS)
- This may be related in part to insulin resistance. We should not purely focus on conception as the main problem of the obese.
- the importance of maternal health should remain paramount.
- The triennial confidential enquiry into maternal death—"Saving lives, improving mothers' care"—is sobering reading, with obesity implicated in 27.1% of maternal deaths in the U.K.
- Both obesity and PCOS increase the risk of developing GDM, pre-eclampsia, and preterm birth. Obesity also increases the need for operative delivery, with the ensuing problems of wound infection and venous thromboembolism. Targeted preconceptional counseling is paramount to reducing the spiraling problems related to obesity, fertility, and childbirth.
- The national **guidance of needing to obtain a BMI of less than 30 kg/m**2 in order to be eligible for National Health Service (NHS) fertility treatment within the U.K. goes a small way to promoting these salient facts.

OVARIAN STIMULATION RESPONSE IN PCOS

- Ovulation induction for women with PCOs requires a different approach to that for women with normal ovaries. The response is often initially slow, but then may spiral rapidly to a picture of over-response, with a significant risk of OHSS and cyst formation
- Dor et al.
  showed a significant **increase in oocyte number recovered** per cycle when compared with tubal factor infertility but a lower fertilization rate (40.4% vs.67.6%, $p < 0.001$)
- significantly less human menopausal gonadotropin (hMG), but still reached a significantly higher
  estradiol level on the ovulation trigger day with hCG
- **a higher incidence of embryo transfer cancellation due** to failed fertilization
  and OHSS .

- Contrary to earlier theories, these follicles are not atretic, but rather there is an **increased cohort of selectable antral follicles sensitive to exogenous gonadotropins. Anti-Mullerian hormone** (AMH), a dimeric glycoprotein produced from the granules cells of the pre-antral and antral follicles, is elevated in PCOS
- AMH has been implicated in two stages of the follicle dysfunction that leads to the development of PCOS
- Excessive follicle recruitment from the primordial pool is followed by defective selection of the lead follicle, culminating in anovulation.
- The usual inhibition of follicle recruitment is lost, perhaps due to limited paracrine control from reduced expression of AMH in the primordial follicle pool
- There is now significant evidence supporting the correlation between **AMH, oligo-anovulation, and hyperandrogenemia**
- Circulating insulin, insulin-like growth factor, and androgen concentrations are all implicated in the higher rate of recruitment
- **Elevated AMH can predict the occurrence of OHSS and** can help to tailor treatment protocols for those at high risk of over-response

OVARIAN HYPERSTIMULATION SYNDROME

- OHSS is the most serious iatrogenic complication of IVF treatment
- In the most severe cases, hypovolemia, thromboembolism, hemoconcentration, ascites, hydrothorax, pericardial effusion, or adult respiratory distress syndrome can occur.
- incidence is hard to quantify, ranging from 10% to 18%
- Higher estradiol concentrations and oocyte numbers occur in those who develop OHSS
- The cardinal feature of the pathogenesis of OHSS is an increased capillary permeability
- Vascular endothelial growth factor (VEGF) is a potent angiogenic endothelial cell mitogen and a key mediator of OHSS
- Serum and follicular levels are higher in those women with PCOS and those who develop OHSS on the day of egg collection (75). Estradiol and VEGF levels positively correlate on the day of hCG administration, with VEGF being the best predictor of OHSS
- Miele et al. demonstrated that **insulin and IGF-1 increase VEGF mRNA expression (80). The synergistic effect of insulin with gonadotropin and hCG was**

SUPEROVULATION STRATEGIES

- Pituitary desensitization with a gonadotropin-releasing hormone (**GnRH)** agonist has become a universal concept within assisted conception regimens.
- Reversible hypogonadotropic hypogonadism allows enhanced control of **follicular development and improved pregnancy rates** in IVF cycles
- Suppression of endogenous luteinizing hormone (LH) by GnRH agonists may be **advantageous for** the sensitive PCO, allowing follicular development to occur without the adverse effects of high LH concentrations
- These oocytes appear to **fertilize better than** those obtained in cycles without pituitary desensitization.
- A **prolonged pituitary desensitization (30 days rather than 15**) avoids the initial surge of gonadotropins and the resultant ovarian steroid release seen with shorter treatments.
- Androgen levels may be reduced and an increase in exogenous gonadotropin dosage is not required. Although pregnancy rates are not improved, ovarian hyperstimulation is reduced

- The pros and cons of different gonadotropin preparations have been debated for years.
- After the initial use of hMG and then highly purified urinary FSH came the recombinant preparations of FSH (rFSH), LH, and hCG. Overall, there appears to be **little difference in outcomes** when all studies are combined.
- Teissier et al. demonstrated that women with PCOS undergoing IVF using hMG compared with rFSH have a **higher testosterone and estradiol level due to higher LH**
- A meta-analysis has shown no difference in outcome between hMG or rFSH when used in conjunction with a long GnRH agonist protocol
- There was *no significant difference in number of oocytes retrieved, live birth rates, miscarriage, multiple pregnancy, or OHSS.*

- GnRH antagonists do not activate the GnRH receptors and produce a rapid suppression of gonadotropin secretion within hours
- This offers the potential for shorter treatments compared with the long protocol using a GnRH agonist.
- Previously, it was suggested that the antagonist protocol led to a reduction in clinical and live birth rates
- In contrast, a Cochrane review showed no evidence of a statistically significant difference in live birth rate (nine randomized controlled trials [RCTs]; OR 0.86, 95% CI: 0.69–1.08).
- There was a **statistically significant lower incidence of OHSS in the GnRH antagonist group** (29 RCTs; OR 0.43, 95% CI: 0.33–0.57)
- Consideration has recently been given to use of a **GnRH antagonist within an ovulation induction protocol to reduce the risk of premature luteinization**.

- In another study, patients with PCOS undergoing IVF in a GnRH antagonist protocol were found to have earlier follicular growth and higher estradiol concentration during rFSH stimulation compared with those on a long GnRH agonist regimen
- Despite a shorter stimulation phase, the number of oocytes and the fertilization and clinical pregnancy rates were not different, ***but the risk of OHSS was significantly lower.***
- Despite the reduction in those experiencing OHSS, overall there remains a significant majority with OHSS who have PCOS, even when using an antagonist protocol (65.2% vs.8.1%; $p < 0.05$) (98).

- Native GnRH or a GnRH agonist can **displace the antagonist from the pituitary GnRH receptors**.
- This realizes the potential to use a ***GnRH agonist as the final trigger for the LH surge and subsequent oocyte maturation***
- Cochrane review by Al-Inany et al. (92), who demonstrated an inferior live birth rate (OR 0.47, 95% CI: 0.31–0.70; five RCTs, 532 women), but **the reduction in OHSS (OR 0.15, 95% CI: 0.05–0.47; eight RCTs, 989 women) was certainly an advantage** .
- The compromised live birth rate is believed to be due to **deficient luteal-phase support.**
- **LH's shorter half-life is less able to support the corpus luteum in a developing pregnancy**.
- Optimized luteal-phase support with a combination of **low-dose hCG, estradiol, and progesterone has been shown to improve the clinical outcome and equal the rates achieved with hCG**
- authors achieved a comparable **pregnancy rate per embryo transfer (GnRH agonist 40.7% vs. hCG 35.0%),** with reductions in freeze-all cycles and incidence of mild to moderate OHSS (GnRH agonist 16.2% vs. hCG 31.0%) in favor of the GnRH agonist trigger (106)

INSULIN RESISTANCE AND METFORMIN

- Insulin resistance is a key factor coupled with hyperandrogenemia in the pathophysiology of PCOS.
- Insulin resistance is thought to arise **from aberrant phosphorylation of tyrosine and serine residues on the insulin receptor,** resulting in increasing insulin resistance and compensatory hyperinsulinemia.
- As insulin ***binds IGF-1 receptors, it augments the response of theca cells to LH, resulting in disordered steroidogenesis and excess androgen production.***
- Hyperinsulinemia results in reduced hepatic synthesis of sex hormone binding globulin and insulin-like growth factor binding protein-1.
- In turn, this increases the bioavailability of both androgens and IGF-1 and -2, which are important regulators of ovarian follicular maturation and steroidogenesis
- Insulin resistance has been stipulated as a risk factor for cardiovascular disease in high-risk populations such as those with PCOS
- **Measurement of insulin resistance in this population is best screened for using a traditional oral glucose tolerance test or hemoglobin A1c**

- With hyperinsulinemia being well recognized in women with PCOS, it is reasonable to assume that the use of insulin-sensitizing drugs should improve many aspects of the syndrome, with respect to both *metabolic and reproductive function*.
- *Metformin, an oral biguanide*, is the most widely researched agent in this category. Metformin **reduces hepatic gluconeogenesis, increasing peripheral glucose utilization and mediating receptor kinase activity within numerous cells, including the theca and granules cells.**
- A recent systematic review of insulin-sensitizing agents concluded that there was no evidence to suggest
  metformin used alone improved live birth rates
- Furthermore, there was no improvement in live birth rate when used in combination with clomiphene versus clomiphene only (seven RCTs, 907 participants; OR 1.16, 95% CI: 0.85–1.56).

- The total dose and duration of metformin use is not standardized, ranging **from 500 mg twice a day to 850 mg three times a day taken for up to 16 weeks**,
- usually up to hCG trigger. Fleming et al. demonstrated that a protracted **treatment of metformin over four monthsmay decrease the antral follicle count and AMH levels;** however, this was not **shown to improve the number of oocytes retrieved or fertilization** rates.
- Tang et al. reported a *significant improvement in live birth rates for those taking metformin* over a much shorter period of time (from the commencement of GnRH agonist to the day of hCG in a long protocol), with rates of 32.7% versus 12.2% in the placebo arm
- The lower-than-expected birth rate in the placebo group is difficult to explain, and may be secondary to **subtle effects on oocyte/embryo quality or endometrial development**.
- Kjotrod et al. corroborated the findings of Tang et al. by suggesting that the live birth rate may be improved in lean women with PCOS (118,119). Furthermore, a study of 112 women with a BMI <28 kg/ m2 showed that the live birth rate was also higher when metformin was given over 12 weeks (48.6% vs. 32.0%; 95% CI: 1.1–32.2, p = 0.0383)

- The consistent advantage of using **metformin appears to be a reduction in OHSS** (OR 0.29, 95% CI: 0.18–0.49; 798 women),
- as shown in a recent Cochrane review The use of GnRH antagonists in IVF protocols also reduces the risk of OHSS. Doldi et al. presented their findings of a reduction in OHSS from 15% with placebo to 5% with metformin
- Metformin has been observed to ***reduce serum testosterone concentration (1.96 vs. 2.52 nmol/L, p = 0.269) and free androgen index (FAI)*** (2.43 vs. 3.34) on the day of hCG administration
- A negative correlation exists between day-12 post-embryo transfer hCG levels and FAI. Through speculation, alleviation of **hyperandrogenism and insulin resistance at the ovarian level may improve folliculogenesis and therefore the developmental potential of the embryo.**
- **Serum VEGF and estradiol concentrations on the day of hCG administration are also greatly reduced in those on metformin**.
- By ameliorating the expression of VEGF, the risk of OHSS can be reduced.
- Thus, whilst there is variable data on whether metformin improves the “take home baby rate” after IVF,

IN VITRO MATURATION

- **Immature oocytes are** retrieved transvaginally from antral follicles from either unstimulated or minimally stimulated ovaries
- The oocyte matures *in vitro* in a ***specially formulated medium for 24-48 hours.***
- The oocyte is then fertilized, usually with intracytoplasmic sperm injection (ICSI), and the selected embryo(s) are transferred two to three days later. Although more labor intensive, the potential clinical advantage is that patients generally require less monitoring and, most importantly, **avoid the risk of OHSS.**
- For those with PCOS, IVM offers a promising alternative to conventional IVF
- The maturation rate of oocytes retrieved from patients with PCOS has been lower than those with normal ovaries
- However, priming with hCG or gonadotropin before the retrieval has been shown to improve maturation
rates from unstimulated PCOs

- IVM compared with conventional IVF yields ***significantly fewer mature oocytes*** (7.8 vs. 12.0, $p < 0.01$), with significantly lower implantation rates
- The lower implantation rates may be due to a **reduced oocytepotential, a higher frequency of abnormal meiotic spindles and chromosomal alignment, or reduced endometrial receptivity**
- It is important to ensure that infants born through such treatment remain healthy in the long term.
- A prospective observational study on 41 pregnancies showed no increase in preterm birth, birthweight, or major structural malformation as compared with pregnancies achieved through conventional IVF (129). However, much larger studies are required to provide robust safety data on this new technology.
  In a Cochrane systematic review, Siristatidis et al. concluded that no RCTs exist upon which to base practice recommendations regarding IVM before IVF or ICSI inwomen with PCOS (130). Whilst continued improvement in culture medium and synchrony between endometrial and embryonic development may result in improved success rates, IVM as a treatment has yet to be adopted as a routine clinical entity, and may remain purely a research interest.