Predictors of ovarian response: progress towards individualized treatment in ovulation induction and ovarian stimulation

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ABESTRACT

Background: Polycystic ovary syndrome is often associated with overweight/ obesity.Obesity may result in insulin resistance and a compensatory hyperinsulinemia, which will exacerbate hyperandrogenism by stimulation of theca cell androgen synthesis, in this review, the methodology behind predictive factors and prediction models and their potential clinical applicability across ovulation induction and ovarian stimulation are explored.

Summary: Predicting and managing the variability between patients is a significant clinical challenge in mono- or multifollicular ovarian stimulation protocols. Research into predictive factors and the construction of multivariate models are the first steps towards evidence-based individualized treatment. As yet, however, predictive models have a limited use in clinical practice because of their limited power and the need for validation.

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INTRODUCTION

Polycystic ovary syndrome is often associated with overweight/ obesity. Obesity may result in insulin resistance and a compensatory hyperinsulinemia, which will exacerbate hyperandrogenism by stimulation of theca cell androgen synthesis **(1).**

Theoretically, PCOS is a result of abnormal interaction between different behavioral, environmental, and genetic factors. PCOS is associated with long -term metabolic abnormalities and cardiovascular risks like insulin resistance, dyslipidemia and endothelial dysfunction **(2,3).**

Usually, in healthy women, the ratio between LH and FSH usually lies between 1 and 2. In polycystic ovary disease women, this ratio becomes reversed, and it might reach as high as 2 or 3 **(4).**

Anti-estrogen therapy with clomiphene citrate (CC) has for many years been used as first-line therapy for anovulatory PCOS. CC is thought to bind and block the estrogen receptors in the hypothalamus for prolonged periods, thereby decreasing the normal ovarian-hypothalamic feedback. Letrozole is now recommended as a first-line pharmacological treatment for ovulation induction and may soon replace CC. Recent

ovaries. From this initial description, the term Stein-Leventhal syndromewas originally used to identify other similarly affected women **(6).**

PCOS is the most common endocrine disorder of reproductive- aged women and affects approximately 4 to 12 % in general population**.** Moreover, it is present with ovarian dysfunction and endocrine problems and is also associated with hyperinsulinemiaand metabolic disease. The incidence of infertility in women with PCOS was noted to be about 8%. The etiology of PCOS is unknown but it is a heterogeneous disorder which is mostly genetic **(7).**

studies indicate an improvement in pregnancy and live birth rates without affecting miscarriage or multiple pregnancy rates compared to CC (**5).**

In this review, the methodology behind predictive factors and prediction models and their potential clinical applicability across ovulation induction and ovarian stimulation are explored.

Definitions of PCOS: Polycystic ovarian syndrome (PCOS) was originally described in 1935 by Stein and Leventhalas a syndrome manifested by menorrhea, hirsutism and obesity associated with enlarged polycystic

Table (1): Definition of Polycystic Ovarian Syndrome**(8)**

Two of the three: Clinical and / or biochemical hyperandrogenism Oligo-/ anovulation Polycystic ovaries
To include both: Clinical and / or biochemical hyperandrogenism Oligo-/ anovulation
To include both: Clinical and / or biochemical hyperandrogenism Oligo-/ anovulation and/or polycystic ovaries

community-based studies suggesting that between 6% and 10% of women in developed countries are PCOS. An estimated prevalence of 20-30% in developing countries,Including up to 52% of South Asian immigrant women in Britain**.** Approximately 20 % of women of reproductive age demonstrate ultrasound picture of polycystic ovaries with half that number having clinical or biochemical signs of anovulation or androgen excess **(11)**

II. PATHOGENESIS AND ETIOLOGY OF PCOS:

At the time the diagnosis is made, PCOS presents as a phenotype that reflects a self-perpetuating vicious cycle involving neuroendocrine, metabolic, and ovarian dysfunction. Over the years, several hypotheses regarding the proximal physiological origins of PCOS have been proposed **(12)**

Normally only a few follicles develop at the one time and they are scattered throughout your ovary. PCO is diagnosed if the follicles behave abnormally. This can be characterised by a greater number of follicles developing at the same time and their positioning. Polycystic ovaries are also larger and have a slightly different appearance.PCO does not cause pain in the pelvic area. If you are experiencing pain, this is more likely due to a cyst, which usually disappears without treatment. Surgical treatment is only required if cysts persist. PCO does not need to be treated surgically (**9).**

I. CLASSIFICATION:

At least there are 2 subtypes of PCOD; high-LH low-insulin (Non –insulin resistant) and low-LH high-insulin (Insulin resistant) **(10).**

Several attempts have been made to quantify the prevalence of polycystic ovaries in

blood sugar levels are variably reported and depend on type of COCP used, duration of use,.COCPs come in a variety of combinations, as well as oestrogen and progestin formulations with different pharmacological and clinical effects **(19)**

2. **Metformin:**

Metformin is a low-cost, generally available medicine that has been widely utilised as an insulin sensitizer in DM2 and PCOS for over seven decades. Insulin resistance has been shown in 75% of lean women and 95% of overweight women in clamp studies, and addressing this has been the driving force behind the use of metformin in PCOS **(19)**

3. **Anti-androgen pharmacological agents:**

Hirsutism, acne, and androgen-related alopecia are the most prevalent androgen-related symptoms of PCOS. Cosmetic and COCP therapy are the first-line therapies for female hirsutism, including PCOS. There are few studies on the use of anti-androgen pharmacological drugs in the treatment of PCOS (**19)**

4. Inositol:

Inositol (myo-inositol and di-chiro inositol) is a nutritional supplement that acts as a second messenger and has been shown to play a role in insulin signaling transduction. Previous studies have focused on insulin resistance and hormonal profiles and gestational diabetes in women with PCOS. Currently, given the lack of evidence, international guidelines recommend that inositol should be considered an experimental therapy in PCOS **(19), (20).**

III. Assessment and treatment of infertility

1. Drug Therapy for anovulation:

❖ **Letrozole**: An alternative first line therapy to stimulate ovulation could be letrozole**(16)**

Aromatase inhibitors (AI) are effective as ovulation-inducing agents, including letrozole and anastrozole, with letrozole being the most widely used **(19).**

It is still unclear if these four phenotypes represent a broad spectrum of the same condition, that is, PCOS. Not enough work has been done to study the different PCOS phenotypes **(13).**

Clinical picture and diagnostic work up of PCOS: Diagnostic criteria for PCOS according to the 1990 NIH conference, the revised criteriafrom the ESHRE/ASRM sponsored consensus meeting (2013) and the criteria of the Androgen Excess Society (2016)**(14)**

MANAGEMENT OF POLYCYSTIC OVARY SYNDROME

I. **Lifestyle changes:**

Promoting weight loss, are the first-line treatment recommended for women with PCOS. A good diet and regular physical activity are known to aid in the reduction of IR and hyperandrogenism, as well as the optimization of hormonal imbalance, lipid profile, and cardiovascular health. According to one study, losing just 5% of one's baseline body weight can restore normal menses and increase sensitivity to ovulation-inducing and reproductive medicines (**15).** The reduced glycemic load by reducing sugar content and more complex carbohydrates, avoid fatty meals plus thirty minutes per day of brisk exercise is advised to maintain health **(16).** The British Fertility Society guidance suggests that treatment should be deferred until BMI less than 35Kg/m2 **(17).**

II. Pharmacological treatment for non-fertility indications:

1. Combined contraceptives:

First-line medical therapy usually consists of an oral contraceptive to induce regular menses. The COC not only inhibits ovarian androgen production but also increases sex hormone-binding globulin, decreasing androgen bioavailability, COC reduce terminal hair growth, although many patients still require concomitant mechanical hair removal **(18).**

The effects of COCPs on menstrual cycle, hirsutism, weight loss, waist/hip ratio, testosterone concentrations, lipid profile and

Table (2): Recommendations for inducing ovulation with letrozole or clomiphene **(21)**

	Letrozole	Clomiphene
Initialregimen	2.5mgdailyoncycleday3−7(5days)	50mgdailyoncycleday3−7(5days)
Indication forincrease	Absence ofovulation	Absence ofovulation
How much toincrease	2.5 mg dailyincrement	50 mg dailyincrement
Maximum dailydose	7.5 mgdaily	150 mgdaily
Treatmentduration	6 ovulatorycycles	6 ovulatorycycles
Confirmationofovulation	Serumprogesterone>10nmol/Lat cycle day21−23	Serumprogesterone>10nmol/Lat cycle day21−23

utilized therapeutic procedure for management of infertility **(22)**.

Indications of ovulation induction: Ovarian stimulation with fertility drugs is used for treatment of:

1- Ovulatory disorders:

Approximately 40% of all female infertility problems are results of ovulatory disorders. According to the World Health Organization (WHO), ovulatory dysfunctions are classified into, three groups: group I; hypothalamic-pituitary failure, group II; hypothalamic-pituitary dysfunction, group III; ovarian failure and group IV; hyperprolactinemic anovulation **(23)**.

2- To improve ovulation in subfertile women:

Women with apparently normal cycles have subtle cycle abnormalities such as luteal phase abnormalities, hyper-prolactinaemia and abnormal FSH and LH patterns and luteinized unruptured follicle syndrome. So induction of ovulation can improve such abnormalities **(24)**.

3- Empirical treatment to maximize chances of conception:

With or without IUI in male infertility, endometriosis and unexplained infertility **(25)**.

4- As a fundamental adjunct to increase the success of treatment with the Assisted Reproductive Technology (ART) (26).

Monitoring of ovulation induction

Values of monitoring: Evaluate the ovarian response during the stimulation period so adjustment can take place if the response is insufficient or too strong.The monitoring will identify those who have not responded adequately or poor responders.Detect women at risk of OHSS.Evaluate follicular and endometrial maturation, aiming to find the

❖ **Gonadotrophins**

Gonadotrophin therapy is suitable for improving infertility in women with PCOS in specialist care. Gonadotropin therapy provides better per cycle and cumulative pregnancy and live birth rates compared with the use of oral anti-oestrogens and or no therapy in anovulatory women with PCOS; and there is no evidence of teratogenicity.

2. **Laparoscopic ovarian drilling:**

In last years, the rapidly expanding field of operative laparoscopy has led to a renewed interest for surgical treatment for PCOS. Several methods of laparoscopic treatment have been studied, including biopsy, electro-cautery and laser treatment.

3. **Vitamin D:**

The prevalence of vitamin D insufficiency or deficiency in reproductive age women is 45% to 90%. PCOS and vitamin D deficiency are both associated with IR. A study found that vitamin D deficiency in women with PCOS who underwent ovarian stimulation for the treatment of infertility was associated with significantly diminished rates of ovulation, of pregnancy, and ultimately a reduced chance of live birth**.**

4. **Assisted reproductive technologies (ART):**

ART, mainly in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI) and in vitro maturation (IVM), have a role in PCOS after failure to respond to pharmacological ovulation induction or if there are other indications such as tubal damage or male factor infertility **(19)**

Induction of ovulation

Ovulation induction is a process of promotion of follicular growth and development culminating in ovulation and frequently

ultrasound tests including antral follicle count, ovarian volume, ovarian stromal peak systolic velocity including waveform and pulsatitlity index and ovarian follicular vascularity, also histological tests like ovarian biopsy **(29)**.

Female age:

Increasing female age is associated with decline in natural fecundity and pregnancy rates, fertility is known to decline in women after the age of 30 years and striking decreases are observed after 35 years of age**(30)**.

Anti-Mullerian Hormone (AMH):

Serum AMH levels are lower in female compared with males, and it declines with advancing female age, after puberty and onset of menstrual cycle, serum AMH decreases progressively until it becomes undetectable around menopause **(31).**

Several studies have shown that AMH is an excellent marker to determine ovarian responsiveness. Hormone measurements in the early follicular phase (day 3 of spontaneous cycle), revealed that AMH are lower in patient with poor ovarian response than in women with normal response **(32)**.

Total Antral Follicle Count (AFC): Antral follicles are defined as early graafian follicles that are 2-10 mm in size, round to oval, echlucent fluid filled structures, easily imaged by ultrasonography in early follicular phase. It represents a cohort of follicles awaiting further recruitment and stimulation by gonadotropin. There is clear association between the number of growing follicles and number of resting follicles in the follicle pool **(33)**.

Predictors of CC ovulation induction: They have worked extensively on assessing possible predictors of treatment outcomes based on initial screening characteristics. Using multi-variate prediction models they could show that factors such as body mass index, high androgen levels, female age, and insulin resistance are fairly good predictors of both ovulation and pregnancy chances. Such approaches may help to render ovulation induction more patient tailored, helping to optimal time for triggering ovulation with HCG **(27)**.

Methods of monitoring:

1. Serum E_2 level:

Ovulation induction was first monitored by serum E_2 level (mature follicle gives 150-200pg/ml E_2 serum level). However, it was not possible to draw conclusion from such measurements on how many mature follicles would ovulate.

2. Transvaginal Ultrasonography (TVS):

The first ultrasound measurement should be performed in a stimulated cycle between days 5-7, but this may vary depending on the protocol used and if the patient is at risk of OHSS the additional number of measurements is also dependent on the stimulation protocol and the reason for ovulation induction.

Also three dimensional ultrasound and color Doppler identify and quantify blood flow in small vessels of the follicular wall to study ovulation as well as the uterine artery for prediction of endometrial receptivity **(28)**.

3. Endometrial development:

It is also monitored during stimulation by measuring the endometrial thickness. Although multiple studies have examined the prognostic value to endometrial thickness and echo texture in ART cycles, the issue remains controversial. Many have suggested that results are best when endometrial thickness measures 8-9 mm or greater or appears "trilaminar", and poor when the endometrium is less than 6-7 mm in thickness or appears homogeneous on the day of hCG administration.

Prediction and detection of ovarian response:

Various tests have been developed to assess ovarian reserve and to predict the response to ovarian stimulation, they include clinical variables as age and history of cancelled cycles, basal blood tests as Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), FSH:LH ratios, Estradiol (E_2), inhibiin B, Antimullerian Hormone (AMH). Dynamic tests are as clomiphene citrate challenge test, gonadotropin agonist stimulating test, exogenous FSH ovarian reserve tests,

conventional hMG protocol. They stated thatthere was no significant difference in pregnancy rates between the two groups. However, in the metformin group, significant improvements in menstrual function and ovulation rate of 46.7% with a significant decrease in fasting insulin levels were reported.

4. Weight loss and lifestyle modifications

Obese women with PCOS are more likely than thin women with PCOS to suffer from anovulation. Lifestyle modification is the first line treatment in an evidence based approach for the management of the majority of PCOS women who are overweight **(38)**.

Alvarez-Blasco et al. (39)reported that a 6-week intervention of structured exercise training and a hypocaloric diet was effective in restoring menstrual cycles, increasing the probability of ovulation and pregnancy under CC treatment in overweight and obese CCR-PCOS patients.

5. Third-generation aromatase inhibitors

Letrozole has better ovulation and pregnancy rates in comparison to CC and placebo in patients with CC- resistant PCO., exemestane) are approved adjuvants for treatment of estrogen-receptor–positive breast cancer that were first used in ovulation induction in anovulatory women in 2001 **(40)**.

6. Oral contraceptives

Combined oral contraceptives (COC) (0.03 mg of ethinyl estradiol and 0.15 mg of desogestrel) used for 2 months followed by CC, at dosage of 100 mg/day on days $5^{th}$to $9^{th}$of the cycle, improved ovulation and pregnancy rates in CC resistant women in comparison with repeated cycles of CC alone **(41)**.

7. N-acetyl-cysteine

N-acetyl-cysteine (NAC) is a mucolytic drug. Long term NAC treatment (1.8 g/d for 5–6 weeks) was associated with significant increase in insulin sensitivity and reduction in insulin levels, testosterone and free androgen index in hyperinsulinemic PCOS

8. Dexamethasone therapy

choose the best treatment option and individualize dosing**(34).**

Clomiphene CitrateResistance (CCR):CCR defined as failure to ovulate after receiving 150 mg of CC daily for 5 days per cycle, for at least three cycles, is common and occurs in approximately 15 to 40% in women with PCOS**.**Insulin resistance, hyperandrogenemia, and obesity represent the major factors involved in CC resistance; avert the ovaries from responding to raised endogenous FSH levels following CC therapy.

Management of CCR (Second-line infertility treatments):

1. Gonadotropins:

Exogenous gonadotrophins with conventional high-dose protocols applied in the 1960s and 1970s, ovulation rates were excellent but at the price of unacceptably high rates of ovarian hyperstimulation syndrome (OHSS) and multiple pregnancy **(34)**.

Cumulative ovulation rates are as high as 90%, with pregnancy rates of 50–70%, multiple pregnancy rates up to 15% and OHSS 2%. Cumulative singleton live birth rates as high as 71% after 24 months of ovulation-induction treatment with CC as first line, followed by gonadotrophins as second-line treatment, have been described **(35)**

2. Laparoscopic Ovarian Surgery (LOS):

LOSrepresents a single surgical procedure (under general anaesthesia) which may replace months of daily hormone injections and frequent hospital visits. Multiple ovarian punctures, either by diathermy or laser, are performed at laparoscopy **(36)**.

3. Insulin-sensitizing drugs

Approximately 50%-70% of all women with PCOS have some degree of insulin resistance. Metforminis now the most widely insulin sensitizer used for ovulation induction in women with CCR- PCOS. In these women, it appears to affect ovarian function in a dual mode, through the alleviation of insulin excess acting upon the ovary and through direct ovarian effects**(36)**.

Penzias et al. (37) compared sequential treatment of metformin and CC with

incorporate into daily work and to include only variables that are routinely measured.

Despite problems in using the current predictive tests in clinical practice, the wide variation in patients' characteristics mean that individualized, patient-tailored approaches remain mandatory for safe and effective ovarian stimulation. The current practice of individualized treatment is based only on clinical experience and has poor reproducibility. The challenge is to design studies to identify better response prediction and further test the added value of individualized approaches.

References

1. Calcaterra, V., Verduci, E., Cena, H., et al. (2021). Polycystic Ovary Syndrome in Insulin-Resistant Adolescents with Obesity: The Role of Nutrition Therapy and Food Supplements as a Strategy to Protect Fertility. Nutrients, 13(6), 1848.
2. Conway, G., Dewailly, D., Diamanti-Kandarakis, E., et al. (2014). The polycystic ovary syndrome: a position statement from the European Society of Endocrinology. Eur J Endocrinol. Oct; 171(4): P1-29.
3. Saadia, Z. (2020). Follicle stimulating hormone (LH: FSH) ratio in polycystic ovary syndrome (PCOS)-obese vs. Non-obese women. Medical Archives, 74(4), 289.
4. Mitrašinović-Brulić, M., Buljan, M., &Suljević, D. (2021). Association of LH/FSH ratio with menstrual cycle regularity and clinical features of patients with polycystic ovary syndrome. Middle East Fertility Society Journal, 26(1), 1-9.
5. Kriedt, K. J., Alchami, A., & Davies, M. C. (2019). PCOS: diagnosis and management of related infertility. Obstetrics, Gynaecology& Reproductive Medicine, 29(1), 1-5.
6. Jin, P., & Xie, Y. (2018). Treatment strategies for women with polycystic ovary syndrome. *Gynecological Endocrinology*, *34*(4), 272-277.

A high-dose short course of dexamethasone for inducing ovulation inCCR-PCOSpatients even they have normal DHEAS levelsshowed significantly higher ovulation and pregnancy rates in those who received 200mg of CC (days 5–9) and 2mg of dexamethasone (days 5–14) compared with CC alone **(17)**.

Bromocriptine

PCOS and hyperprolactinaemia are two distinct entities without a patho-physiological link. Bromocriptine administration provided no benefit in CC-resistant PCOS patients with normal prolactin levels**(42)**.

9. Sequential use of induction agents without a progesterone withdrawal bleeding

One such protocol involves starting CC at the typical 50 mg dose. If inadequate follicular growth is noted, the dose can be increased by 50 mg for another round of treatment, without an intervening menses, until ovulation is achieved or the maximal dose of150 mg daily is used**(43).**

Conclusions

Predicting and managing the variability between patients is a significant clinical challenge in mono- or multifollicular ovarian stimulation protocols. Research into predictive factors and the construction of multivariate models are the first steps towards evidence-based individualized treatment. As yet, however, predictive models have a limited use in clinical practice because of their limited power and the need for validation.

Predictive power will improve when more factors are identified, particularly genetic factors. Validation will improve with further studies that apply the prediction model prospectively in a different patient population but with similar characteristics to that in which the model was developed. Only when these criteria have been met can the validation be trusted. So far, the results from validation studies that have met these criteria have been encouraging. Practical considerations also need attention: it is important for a prediction model to be simple enough for physicians to remember and

7. Escobar-Morreale, H. F. (2018). Polycystic ovary syndrome: definition, aetiology, diagnosis and treatment. *Nature Reviews Endocrinology*, *14*(5), 270-284.
8. Pasquali, R., & Gambineri, A. (2018). New perspectives on the definition and management of polycystic ovary syndrome. *Journal of Endocrinological Investigation*, *41*, 1123-1135.
9. Morgante, G., Troìa, L., & De Leo, V. (2021). Coronavirus Disease 2019 (SARS-CoV-2) and polycystic ovarian disease: Is there a higher risk for these women?. *The Journal of Steroid Biochemistry and Molecular Biology*, *205*, 105770.
10. Dapas, M., Lin, F. T., Nadkarni, G. N., Sisk, R., Legro, R. S., Urbanek, M., ...& Dunaif, A. (2020). Distinct subtypes of polycystic ovary syndrome with novel genetic associations: An unsupervised, phenotypic clustering analysis. *PLoS medicine*, *17*(6), e1003132.
11. Skiba, M. A., Bell, R. J., Herbert, D., Garcia, A. M., Islam, R. M., & Davis, S. R. (2021). Use of community-based reference ranges to estimate the prevalence of polycystic ovary syndrome by the recognised diagnostic criteria, a cross-sectional study. *Human Reproduction*, *36*(6), 1611-1620.
12. Rosenfield, R. L. (2020). Current concepts of polycystic ovary syndrome pathogenesis. *Current opinion in pediatrics*, *32*(5), 698.
13. **Sachdeva, G., Gainder, S., Suri, V., Sachdeva, N., & Chopra, S. (2019).** Prediction of responsiveness to clomiphene citrate in infertile women with PCOS. Journal of Reproduction & Infertility, 20(3), 143.
14. Williams, T., Mortada, R., & Porter, S. (2016). Diagnosis and treatment of polycystic ovary syndrome. *American family physician*, *94*(2), 106-113.
15. RCOG. (2016): Green top guideline no.33: long term consequence of polycystic ovary syndrome.London, RCOG; 5-8
16. Carson, S. A., & Kallen, A. N. (2021). Diagnosis and management of infertility: a review. *Jama*, *326*(1), 65-76.
17. **Balen, A. H., Morley, L. C., Misso, M., Franks, S., Legro, R. S., Wijeyaratne, C. N., ...& Teede, H. (2016).** The management of anovulatory infertility in women with polycystic ovary syndrome: an analysis of the evidence to support the development of global WHO guidance. Human reproduction update, 22(6), 687-708.
18. Goodman, N. F., Cobin, R. H., Futterweit, W., Glueck, J. S., Legro, R. S., & Carmina, E. (2015). American Association of Clinical Endocrinologists, American College of Endocrinology, and androgen excess and PCOS society disease state clinical review: guide to the best practices in the evaluation and treatment of polycystic ovary syndrome-part 1. *Endocrine Practice*, *21*(11), 1291-1300.
19. Teede, H. J., Misso, M. L., Costello, M. F., Dokras, A., Laven, J., Moran, L., ...& Norman, R. J. (2018). Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. *Human reproduction*, *33*(9), 1602-1618.
20. Showell, M. G., Mackenzie-Proctor, R., Jordan, V., & Hart, R. J. (2020). Antioxidants for female subfertility. *Cochrane Database of Systematic Reviews*, (8).
21. Mejia, R. B., Summers, K. M., Kresowik, J. D., & Van Voorhis, B. J. (2019). A randomized controlled trial of combination letrozole and clomiphene citrate or letrozole alone for ovulation induction in women with polycystic ovary syndrome. *Fertility and sterility*, *111*(3), 571-578.
22. McKnight K and McKenzie L J (2016): Evaluation of infertility, Ovulation Induction and Assisted reproduction. Clinical Policy: Infertility and Fertility Preservation; 28(2):218-27.

Ovarian Response in Controlled Ovarian Stimulation for IVF Patients. A first pilot study in the Indian population. *J .Obstet .Gynecol India* ;63(4):268–72.

33. Owj M, Tehrani Nejad ES, Amirchaghmaghi E et al.,(2020): The effect of coasting period on the outcome of IVF, Eur J Obstet Gynecol reprod Biol;15: 8.
34. Fauser, B. C. (2019). Medical approaches to ovarian stimulation for infertility. In *Yen and Jaffe's reproductive endocrinology* (pp. 743-778). Elsevier.
35. Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Consensus on infertility treatment related to polycystic ovary syndrome. Hum Reprod. 2018;23(3):462–477.
36. Hanem, L. G. E., Stridsklev, S., Júlíusson, P. B., Salvesen, Ø., Roelants, M., Carlsen, S. M., ... & Vanky, E. (2018). Metformin use in PCOS pregnancies increases the risk of offspring overweight at 4 years of age: follow-up of two RCTs. *The Journal of Clinical Endocrinology & Metabolism*, *103*(4), 1612-1621.
37. Penzias, A., Bendikson, K., Butts, S., Coutifaris, C., Falcone, T., Fossum, G., ...& Vernon, M. (2017). Role of metformin for ovulation induction in infertile patients with polycystic ovary syndrome (PCOS): a guideline. *Fertility and sterility*, *108*(3), 426-441.
38. Blackmore, R., Boyle, J. A., Fazel, M., Ranasinha, S., Gray, K. M., Fitzgerald, G., ...& Gibson-Helm, M. (2020). The prevalence of mental illness in refugees and asylum seekers: A systematic review and meta-analysis. *PLoS medicine*, *17*(9), e1003337.
39. Alvarez-Blasco, F., Botella-Carretero, J. I., San Millán, J. L., & Escobar-Morreale, H. F. (2006). Prevalence and characteristics of the polycystic ovary syndrome in overweight and obese women. *Archives of internal medicine*, *166*(19), 2081-2086.
40. Nagini, S. (2017). Breast cancer: current molecular therapeutic targets and new players. *Anti-Cancer Agents in Medicinal*

23. Rebar, R. (2018): Evaluation of amenorrhea, anovulation, and abnormal bleeding. *International journal of reproductive medicine*;90(1):219-23
24. Guzick DS and Hoeger K (2020): Clinical Updates in Women's Health Care: Polycystic Ovary Syndrome;8(1).
25. Takeuchi S, Minoura H, Shibagara T et al., (2020): In vitro fertilization and intracytoplasmic sperm injection for couples with unexplained infertility after failed direct intraperitoneal insemination. J Assist Reprod Genet; 17: 515.
26. Ben-Ami M, Perlitz Y and Haddas (2017): A color Doppler sign for ovulation induction outcome, Ultrasound Obestet Gynecol; 20: 96-7.
27. Engel JB, Ludwig M, Fleberaum R et al., (2016): Use of cetrolix in combination with clomiphene citrate in ovulation induction suitable approach to friendly IVF, Hum Reprod; 17: 2022-26.
28. El-Garhy EA, and El-Sebaey A (2018): Anti-müllerian hormone: Correlation of early follicular, ovultory and midluteal levels with ovarian response and cycle outcome in intrayctoplasmic sperm injection patients. Fertil Steril; 89: 1670.
29. Balasch J, Wang PT, Lee RK, et al., (2016) : Cessation of low dose gonadotropin releasing hormone agonist therapy followed by high- dose gonadotropin stimulation yields a favorable ovarian response in poor responders. J Assist Reprod Genet;19:1.
30. Baird DT (2012): Ovulation induction: current status and future prospects of gonadotropins therapy. In: Adashi EY, Laung PCK, Eds. The ovary; New York: Raven: 529-54.
31. La Marca A, Broekmans FJ, Volpe A et al., (2019): ESHRE Special Interest Group for Reproductive Endocrinology–AMH Round TableAnti-Mullerian hormone (AMH): what do we still need to know? , *Hum Reprod* ;24 :2264-75.
32. Singh N, Malik E, Banerjee A et al., (2013): Anti-Mullerian Hormone: Marker for

42. Su, H.W.; Chen, C.M.; Chou, S.Y.; Liang, S.J.; Hsu, C.S. & Hsu, M.I. Polycystic ovary syndrome or hyperprolactinaemia: a study of mild hyperprolactinaemia. Gynecological Endocrinology 2011, Vol. 27, No.1, pp.55-62.
43. Fedotcheva, T. A. (2021). Clinical Use of Progestins and Their Mechanisms of Action: Present and Future. *Современные технологии в медицине*, *13*(1 (eng)), 93-106.

Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents), *17*(2), 152-163.
41. Al Khalifah, R. A., Florez, I. D., Dennis, B., Thabane, L., & Bassilious, E. (2016). Metformin or oral contraceptives for adolescents with polycystic ovarian syndrome: a meta-analysis. *Pediatrics*, *137*(5).