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REVIEW ARTICLE

New Insights into Infertility Associated with Polycystic Ovarian Syndrome

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ABSTRACT

Polycystic ovarian syndrome (PCOS) is a common endocrine disorder which was thought to be reasonably well understood. However, new diagnostic criteria have evolved over the years just as has the management of associated infertility. Our review looks at the various diagnostic criteria for PCOS and what has prompted the need for a constant change in these. We also analyze the need for various investigations and what new evidence is out there for optimal treatment for infertility associated with this condition.

Keywords: Diagnosis, Hyperandrogenism, Infertility, Polycystic ovarian morphology, Polycystic ovarian syndrome.

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A Medline search was done using the above subject headings. Searches were limited to studies in humans and articles in English between the years 1995 and 2014. If referenced articles had relevant information, earlier articles were also included.

THE DIAGNOSTIC JOURNEY

When Stein and Leventhal in 1935 described 'a small group of women complaining of long periods of amenorrhea, sterility and hirsutism' little did they realize that the heated discussions that arose in its wake, would not cease even in the next century.[1] Polycystic ovarian syndrome (PCOS) as it is popularly known was first defined by National Institute of Child Health and Human Development (NICHD) in 1990, as the combination of androgen excess and oligo-anovulation in the absence of all other causes for anovulatory infertility.[2] The primary etiology was believed to be androgen excess leading to menstrual derangements. Polycystic appearance of the ovaries was not considered a prerequisite for diagnosis. This was an important first step but the criteria were based on opinions rather than evidence.[3] In 2003, European Society of Human Reproduction and Embryology (ESHRE) and American Society for Reproductive Medicine (ASRM) met in Rotterdam and introduced polycystic ovaries as a third diagnostic criterion, allowing a diagnosis of polycystic ovarian syndrome if two of the three criteria were met (Table 1). The syndrome was, thus, deemed to be more a problem of ovarian dysfunction (reflected as menstrual irregularities or polycystic ovarian morphology).[4]

The freedom to choose two out of the three criteria meant that this syndrome could be diagnosed in the absence of hyperandrogenism or menstrual dysfunction—both of which had been the etiopathological basis for the previous classifications![5] The idea of dropping androgen excess as a prerequisite was difficult to digest for many. In 2006, the Androgen excess Society Taskforce came up with their new diagnostic criteria which acknowledged androgen excess as key and coupled it with menstrual irregularity/polycystic morphology to diagnose this condition (Table 1). This message was more or less reiterated in their task force report in 2009. Having gone around in full circle, the National Institute of Health (NIH) Evidence-based Methodology Workshop on PCOS in December 2012 recommended that Rotterdam classification was the most inclusive in the global context but suggested that a less 'ovary focused' name should be sought.[6] This workshop proposed classifying PCOS patients into four distinct phenotypes in any future research so as to get more meaningful information for comparison between various ethnicities. The phenotypes proposed were:

a. Androgen excess + ovulatory dysfunction
b. Androgen excess + polycystic ovarian morphology
c. Ovulatory dysfunction + polycystic ovarian morphology
d. Androgen excess + ovulatory dysfunction + polycystic ovarian morphology

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Table 1: Comparison of diagnostic criteria for PCOS

1990 National Institute of Child Health and Human Development (NICHD) Guidelines
Patient demonstrates both:
• Clinical and/or biochemical signs of hyperandrogenism
• Oligo- or chronic anovulation
Exclusion of other etiologies of androgen excess and anovulatory infertility is necessary
2003 European Society for Human Reproduction and Embryology and American Society for Reproductive Medicine (ESHRE/ASRM or Rotterdam) Guidelines
Patient demonstrates two of three criteria:
• Oligo- or chronic anovulation
• Clinical and/or biochemical signs of hyperandrogenism
• Polycystic ovaries
Exclusion of other etiologies of androgen excess and anovulatory infertility is necessary
2006 Androgen Excess Society (AES) Guidelines
Patient demonstrates both:
• Hirsutism and/or hyperandrogenemia
• Oligo-anovulation and/or polycystic ovaries
Exclusion of other etiologies of androgen excess and anovulatory infertility is necessary

With the use of the above phenotypically descriptive categories one feels that a near perfect system has been devised. But is it really so?

On closer look, one finds that the evaluation of each of these diagnostic criteria poses its own challenges.

EVALUATION OF PCOS

Hyperandrogenemia: Hyperandrogenemia may be present in 60 to 80% women with PCOS.[5] As mentioned by all major bodies above, other causes of androgen excess need to be ruled out before attributing it to PCOS. However, there is lack of agreement on what androgen to measure, when to measure, the normal range and the assay technique to be used.[7,8] It is usually the free testosterone that is increased in PCOS. However, in the absence of robust methods to measure this, total testosterone is recommended as the first simple test.[9] Very high levels with history of rapid virilization can help identify a neoplastic source of testosterone secretion. Though liquid or gas chromatography with mass spectrometry is considered as gold standard, most laboratories use a direct method for measuring total testosterone before extraction.[9] This can then be used to calculate free androgen index (FAI) which is nothing but the ratio between total testosterone and sex hormone binding globulin (SHBG) multiplied by 100. Though currently it is the recommended method, it is not completely foolproof as the SHBG levels are reduced in obese women.

Testosterone level in PCOS is usually <150 ng/dl (5.2 nmol/l). A value of >200 ng/dl (6.9 nmol/l) raises suspicion of ovarian or adrenal tumor.[10] Dehydroepiandrosterone-sulphate (DHEA-S) should then be requested. A value >800 mcg/dl (21.7 mcmol/l) is highly suggestive of adrenal tumor. If the DHEAS is normal then androgen secreting tumor of ovarian origin or ovarian hyperthecosis may be responsible. Imaging of the ovaries is essential. Late onset 21-hydroxylase deficiency must be ruled out. A morning fasting 17-hydroxyprogesterone of <200 ng/ml (6 nmol/l) reliably rules out this condition. If Cushing's syndrome is suggested by clinical examination a dexamethasone suppression test is advised. All these values can be affected by oral contraceptive pills which should ideally be stopped at least 3 months prior to evaluation.

Total testosterone may also increase due to increase in SHBG levels either due to estrogenic effects of drugs (e.g. tamoxifen) or due to liver dysfunction (portal hypertension with biliary cirrhosis) or hyperthyroidism.[11]

Role for Assessing other Hormones

a. *Thyroid function tests:* Menstrual irregularities were seen in 35% women with severe hypothyroidism and in 10% with mild-moderate disease in a study by Kakuno et al.[12] Hyperthyroidism is more likely to present as hypomenorrhea and secondary amenorrhea. Moreover, women presenting with infertility as their primary symptom are more like to have hyperprolactinemia and/or thyroid disorders.[13] Hence, these hormones remain crucial initial investigations.
b. *Prolactin:* Hyperprolactinemia (defined as serum prolactin >25 ng/ml) can occur in 3 to 10% women with PCOS and in up to 40% patients with primary hypothyroidism.[14] Levels can be marginally raised (to <50 ng/ml) due to conditions, like non-fasting sample, exercise and medications. It may present with or without galactorrhea. If high levels are documented in two fasting early morning samples, then treatment may be necessary. If associated with hypothyroidism, thyroid replacement itself may be sufficient. If not, bromocriptine or cabergoline should be given. Prolactin levels normalize in a week and

ovulation/menstrual dysfunction reverts in 4 to 8 weeks.

c. *LH and FSH:* Is there any relevance in measuring LH or determining the LH/FSH ratio? Elevated LH with a normal or low FSH, in the early follicular phase, is a common finding in women with PCOS. Hendriks et al[15] compared oligo/amenorrhoeic women with and without PCOS. They found that using a receiver operating curve, an LH ≥ 6.5 IU/l was 84% sensitive and 78% specific in diagnosing PCOS accurately in 93% cases.[15] The LH pulse frequency in women with PCOS is higher compared with normal women regardless of their BMI. LH pulse amplitude on the other hand is negatively affected by obesity which means that lean PCOS tend to have a higher LH.[16] Arroyo et al,[16] therefore, suggested that assessment of LH and LH/FSH becomes meaningful only if the BMI is taken into consideration. Moreover, LH and FSH were once measured by radioimmunoassay (RIA) method using polyclonal antibodies and a ratio of ≥ 3 was considered diagnostic for PCOS. In early 1990, the RIA method was replaced by monoclonal antibodies with a ratio of > 1 as most reliable to differentiate the PCOS from the non-PCOS.[17] However, this change was not adopted by all. Due to the confusion ESHRE/ASRM group in the Rotterdam conference did not recommend measurement of LH or the ratio for diagnostic purpose. However, LH, FSH measurements do help in ruling out premature ovarian failure as a cause for oligo/amenorrhea, and hence should be advised from that point of view.

   There is a correlation between high LH levels in the follicular phase and pregnancy outcomes. High tonic rise in the LH concentration in the late follicular phase is proposed to induce premature maturation of oocyte resulting in faulty fertilization and miscarriages. When Homburg et al[18] tried to stimulate women with PCOS they found that the late follicular LH levels were significantly lower in women who ovulated and conceived than who did not. In another study, a high basal follicular LH (> 10 IU/ml) in regularly menstruating women was also associated with higher rates of failure to conceive and miscarriages.[19] Reducing the high LH levels with oral contraceptive pills or gonadotropin releasing hormone analogues (GnRHa) before starting IVF is shown to improve IVF outcomes, another reason why some clinicians may want to assess LH levels.[20]

d. *AMH:* This is a peptide produced by granulosa cells of follicles, and hence correlates with the number of ovarian follicles. With the raging debate about the follicle number per ovary (FNPO) for diagnosis of PCOM, researchers have turned their attention towards AMH as diagnostic tool for PCOS.[21,22] Dewailly et al[22] suggested that an AMH > 35 pmol/l (> 5 ng/ml) was highly sensitive and specific in diagnosing PCOS. When this threshold value was tested against Rotterdam criteria to find out the prevalence of PCOS in a cohort of Danish women, Lauritsen et al[23] found that AMH performed well as a diagnostic marker but they stressed the need to validate AMH threshold levels. Moreover, AMH can be affected by obesity and long-term use of oral contraceptive pills. As AMH assays continue to evolve an international cut-off value is yet to be agreed upon.[24] From the present evidence, AMH cannot be recommended as a diagnostic test but developments in this field need to be watched closely.

Ovulatory dysfunction: Menses occurring at intervals of greater than 35 days or less than eight menstrual bleeds a year is defined as oligomenorrhea.[25] Menstrual irregularity in PCOS women is usually due to anovulation but can occur even in the presence of regular ovulation. Hence, efforts must be made to establish or refute ovulation. A mid-luteal progesterone of > 30 nmol/l is suggested as a fairly standard method to predict ovulation[26] as is the demonstration of ovulation on ultrasound. Urinary LH is also understood to correlate with ultrasound diagnosis of ovulation.[27] Application of these tests in women with irregular menstruation is, however, not easy.

Polycystic ovarian morphology: This aspect in diagnosis of PCOS has turned out to be the most contentious. The Rotterdam workshop introduced polycystic ovarian morphology as the presence of ≥ 12 follicles between 2 to 9 mm in the entire ovary and/or an ovarian volume of > 10 cm^3. A single ovary with these characteristics was deemed sufficient for diagnosis. Studies subsequently questioned the application of this criterion which did not take into the account the effect of age[28] or ethnicity[29,30] on the follicle count. Lately with huge improvements in the ultrasound technology it is not uncommon to detect higher number of follicles in absence of any other features suggesting PCOS. Consequently, it is being debated whether we should increase the number of follicles required per ovary (FNPO) to fulfill the criteria of being 'polycystic'. Based on receiver operating curve analysis, two recent studies have suggested an FNPO of ≥ 19 or ≥ 26.[22,31] Lujan et al[31] in their elegant study have also compared three parameters namely FNPO, follicles number per section (FNPS) and the ovarian volume (OV) for diagnosing PCOS. They found that FNPO of ≥ 26 had a better diagnostic potential than an FNPS of ≥ 9 or an

OV of >10 ml[31] though the interobserver variability in all three methods was more or less similar. It is recommended that situations where imaging facilities do not allow FNPO estimation, ovarian volume must be used.[9]

TREATMENT OF PCOS ASSOCIATED INFERTILITY

The ESHRE/ASRM-Sponsored PCOS Consensus Workshop in Thessaloniki, Greece in 2007 tried to clarify the treatment of infertility in women with PCOS.[32] However, this has not proven to be straightforward.

Lifestyle modifications: The androgen excess in PCOS causes obesity especially abdominal obesity, which in turn worsens insulin resistance and leads to compensatory hyperinsulinemia.[9] Weight loss and dietary modification, therefore, remain the crucial first steps in management. Lifestyle changes (exercise, diet and behavioral modifications) have shown to reduce androgens, improve menstruation as well as insulin sensitivity.[33,34] Reducing the energy intake by as little as 500 Kcal/day can help the weight drop by 10% in a year. The composition of dietary intake doesn't appear to have any significant influence as long as it is associated with weight loss.[35] Robust evidence regarding the effect of lifestyle interventions on reproductive outcomes is, however, lacking.[34] Again there is not a specific type of exercise that can be recommended for weight loss in PCOS women. A systematic review of eight studies evaluated moderate intensity exercise (aerobics and/or resistance training) over a period of 12 to 24 months and found improvement in ovulation and insulin resistance but emphasized the need for further well designed studies.[36]

Some evidence suggests that weight loss is better maintained with the addition of weight loss medications than with exercise and diet alone.[37] There are currently three drugs (Orlistat, Lorcaserin, phentermine/topiramate combination) approved by FDA in the US for weight loss. All three are contraindicated in pregnancy and till recently had not been studied specifically in infertile obese women with PCOS.[38] Orlistat is comparable with metformin in causing weight loss and ovulation induction[39] and more recently also shown to yield better conception rates with lesser side effects when compared with metformin.[40]

In obese women (BMI >30) where simple measures fail, bariatric surgery has shown to improve menstrual function and ovulation status as well as fertility outcomes.[41,42] Though often used as a last resort due to the risks of surgery, there is a rising demand that these surgeries should be offered earlier, particularly in young PCOS women with metabolic syndrome.

OVULATION INDUCTION

Clomiphene citrate remains the first drug of choice for ovulation induction due to its low cost, oral route of administration, few side effects and good safety record. This selective estrogen receptor modulator binds to estrogen receptors on the hypothalamus and releases it from the negative feedback inhibition which results in increase in pituitary gonadotrophin release and increased ovarian follicular activity. An ovulation rate of 75 to 80% and a conception rate of 22% are expected per cycle with clomiphene.[43,44] This discrepancy between the ovulation and conception rates may be explained by the antiestrogenic effects seen on the cervical mucus, endometrium, ovum and embryo. However, there is no clear evidence that any of the above effects present any real hindrance in most women.[45] Enclomiphene preparations were introduced into the markets as it was believed that zuclomiphene, due to its androgenic property, can thin the endometrium and thicken cervical mucus. A small study comparing the traditional clomiphene citrate with enclomiphene could not show any improvement in endometrium or pregnancy rates with the latter.[46] If the endometrium responds poorly, tamoxifen could be used as it stimulates the endometrial proliferation but is not licensed for this use.[47]

Aromatase inhibitors work by inhibiting the action of enzyme aromatase which converts androgens into estrogen and by doing so decrease the estrogen levels, thus reducing the negative feedback inhibition on hypothalamus and consequently increasing the gonadotrophin release. The common drugs from this group, letrozole and anastrozole are again not licensed for this purpose. Anastrozole has proven to be less effective than clomiphene so the focus has shifted to letrozole.[48] Letrozole, however, attracted adverse publicity due an association with congenital defects prompting many bodies (including Drugs Controller General of India) to ban its use in infertility. The Pregnancy in Polycystic Ovary Syndrome II (PPCOSII) study comparing Letrozole with clomiphene in PCOS women found a significantly higher ovulation and live birth rate with letrozole.[49] The birth defects in the two groups were similar. Whether these findings give a new leash of life to Letrozole in infertility, remains to be seen.

Metformin, a biguanide (FDA category B drug) is known to decrease hyperinsulinemia and androgen excess. According to the Thessaloniki consensus, metformin should be used in PCOS women with glucose intolerance only.[32]

Clomiphene was found superior to metformin for ovulation, conception, pregnancy and live birth rates.[50]

Though the combination of clomiphene and metformin had superior ovulation rates compared to either of them alone, live birth rates did not improve. Moreover, the miscarriage rates did not decrease with the addition of metformin to clomiphene. What was interesting to note was that women with BMI <30 had much higher live births whether on clomiphene or metformin. The same point was reiterated by Johnson et al[51,52] who concluded that in women with BMI <30–32 kg/m^2, metformin and clomiphene had comparable clinical pregnancy rates. They proposed that in this group of women metformin maybe a suitable alternative to clomiphene specially due to its numerous advantages—no effect on the endometrial lining, no increase in multiple pregnancies, no risk of OHSS and no long-term risk of ovarian cancer.

A Cochrane review assessing the effect of metformin on pregnancy outcomes in PCOS women undergoing IVF has also concluded that in comparison with placebo/no treatment, metformin increased the clinical pregnancy rates and decreased the OHSS rates though there was no significant effect on live birth rate.[53]

Other insulin sensitizers (pioglitazone and rosiglitazone) are class C drugs and have not been extensively studied.

Role of Antioxidants: Myoinositol, melatonin, n-acetylcystiene and vitamin D are new kids on the block in PCOS management. Though being marketed enthusiastically a recent Cochrane review reported no significant improvement in clinical pregnancy or live birth rates with any of these antioxidants.[54] It is worth noting that the quality of studies in this review was reported as poor.

Myoinositol (MI) an insulin sensitizer exerts its effect by acting as a precursor for the synthesis of phosphatidylinositol polyphosphates, which in turn are involved in the regulation of several cellular functions.

Chiu et al have demonstrated that high levels of MI in follicular fluid are important for follicular maturation and embryonic development.[55] Impaired availability of MI is proposed to be one of the causes for insulin resistance in PCOS.[56] Hence, it was not surprising that treatment with MI improved erratic menstruation and ovulation in women with PCOS.[57] In a systematic review on the effect of MI on women with PCOS, the authors have provided level 1 evidence about its effectiveness in improving various hormonal disturbances in PCOS.[58] The main mechanism of action is proposed to be improvement of insulin sensitivity of tissues resulting in restoration of ovulation, production of competent oocytes and reduction in hyperandrogenism and dyslipidemia. Patients undergoing controlled ovarian hyperstimulation with MI and gonadotropins showed significantly lower estradiol levels, decreasing the risk of ovarian hyperstimulation and cycle cancellations. Moreover, no side effects were seen in any of the studies in the systematic analysis and a dose of 4 gm/day has been suggested to ensure complete benefits.

Melatonin a lipophilic indoleamine is synthesized by pineal gland. It is a free radical scavenger mopping up the reactive oxygen and nitrogen species generated during ovulation. Melatonin is shown to improve the yield of high quality oocytes and high quality embryos and may prove promising particularly in cases of poor responders.[59] The studies so far have been small and unable to recommend a suitable dose. Consequently, a placebo-controlled dose-response trial is currently underway to study the effect of melatonin on IVF outcomes.[60]

N-acetylcysteine (NAC) had shown promising results in clomiphene resistant PCOS in terms of ovulation and pregnancy rates.[61] However, more recent studies have been unable to show the same significant effect.[62]

Vitamin D deficiency is common in PCOS women particularly the obese type and has been correlated with insulin resistance, menstrual dysfunction and poor fertility outcomes. Though some small studies have shown improvement in menstrual dysfunction with vitamin D supplementation, there is a lack of robust evidence for the effect of vitamin D supplements on fertility outcomes in women with PCOS.[63]

Role of gonadotropins: Gonadotrophins have been used in clomiphene resistant PCOS. There is a higher risk of multifollicular stimulation, and hence a low starting dose of 37.5 to 50 IU/day is recommended.[32] Step-up regimen have been found easier to monitor and safer in terms of avoiding OHSS as compared to the step-down regimen. Though a few sporadic studies have talked about combining clomiphene with gonadotrophins to improve conception rates, adequate evidence is not yet available.[64]

Laparoscopic ovarian drilling has been recommended to clomiphene resistant PCOS women for ovulation induction and has the advantage of avoiding the risk of ovarian hyperstimulation and multiple pregnancies. It offers an alternative to gonadotrophin stimulation but is not recommended outside fertility setting.[32]

IVF Stimulation: According to ESHRE (2007), the ideal stimulation protocol in PCOS is not clear. The cycle cancellations are higher in these women either due to poor response or hyper-response and risk of OHSS.

A recent meta-analysis compared agonist with antagonist stimulation in PCOS women and concluded that the pregnancy rates in the two groups were comparable although the OHSS rates were significantly lower with the latter.[65] It is also well established that the gonadotropin

usage and number of days of stimulation is reduced with the antagonist protocol making it easier to use. Unless there is a persistent high LH not responding to suppression with OCPs, the authors prefer antagonist stimulation in women with PCOS.

CONCLUSION

Polycystic ovarian syndrome associated infertility remains a fascinating subject for reproductive endocrinologists and infertility experts. Rotterdam criteria are currently the most accepted method for establishing a diagnosis but the number of follicles required per ovary to fulfill the 'polycystic morphology' criteria are under review. Similarly, AMH has been explored as a diagnostic tool but awaits universal consensus. Researchers are encouraged to classify the PCOS women into the four phenotypic groups discussed earlier. Weight loss and ovulation induction with clomiphene remain the cornerstone of management. Though off-license, there is some compelling data supporting letrozole use for ovulation induction. The numerous antioxidants offer promise in improving fertility results but robust evidence is awaited.

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