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Case Study

# Series of 18 Cases of Clomiphene Resistant Anovulatory Women with Polycystic Ovary Syndrome and Altered Response to FSH Stimulation

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**Abstract**

The outcome of ovulation induction in anovulatory polycystic ovarian syndrome (PCOS) may depend, in part, on the pharmacologic compounds used, but also on individual patient characteristics, such as age, body mass index (BMI), hyper- androgenism, luteinizing hormone (LH), hyper-secretion, anti mullerian hormone (AMH) levels and possibly antral follicle count (AFC) with ovarian volume of these women. There exists a subset of clomiphene citrate (CC) resistant PCOS women who require stimulation of ovulation with high doses of human menopausal gonadotropin (hMG), after not having responded to chronic low dose step up regimes of recombinant follicle stimulating hormone (r-FSH).

**Keywords:** Polycystic ovarian syndrome; Hyperstimulation

## Case Represewntation

We report a case series of 18 such PCOS women selected from 100 CC resistant women undergoing r-FSH stimulation with the purpose of ovulation induction.

All patients presented to the outpatient department where their clinical examination was done by a clinician which included measurement of body weight, height, waist and hip circumferences and blood pressure. Height and weight were measured with subjects in light clothes and without shoes, using a standard apparatus. Weight was measured on a calibrated beam scale. The height and WC were measured to the nearest 0.5 cm with a measuring tape. Waist was measured midway between the lower rib margin and the iliac crest at the end of a gentle expiration. BMI was calculated as the weight in kg divided by the height in meters squared (kg/m$^2$).

For biochemical and hormonal measurements, overnight fasting blood samples were taken from each subject. Oral glucose tolerance test was done by drawing blood in EDTA-treated test tubes in fasting status and then after 2 h of ingesting 75 g of glucose, by an enzymatic colorimetric method with hexokinase. Lipid measurements including total cholesterol (TC), triglycerides (TGs) and HDL-C were obtained using commercial assay kits. TGs were assayed using enzymatic colorimetric tests using triglycerides GPO blank. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH), and serum estradiol were measured by chemiluminescence method by Diasorin, LIAISON, Italy. AMH was measured by chemiluminscene method using Beckman-coulter kit.

All study subject's follicular growth were monitored by trans-vaginal ultrasound scans LOGIQ P5GE Healthcare with 6.5 MHz trans-vaginal transducer and all scans were performed and assessed by a single sonographer. Ultrasound was performed till adequate follicular growth was obtained.

In all these CC resistant cases, ovulation induction was started with injection of r-FSH 50/75 IU which was increased by 25 units every 5 to 7 days according to low dose step up protocol. Follicular response was monitored by serum estradiol (E2) levels and ultrasound (USG) follicle monitoring (FM). 18 PCOS were selected from this cohort of women as they showed no ovulatory response in terms of rise in serum E2 levels (at least rise of 30 pg/ml from baseline serum estradiol levels (20-80 pg/ml) or presence of a follicle of 13 mm or larger, even after 18-25 days of stimulation with incremental doses of r-FSH of up to 150 IU.

These women were labeled as 'FSH resistant PCOS' and were further stimulated with high doses of injection hMG (Humog Bharat Serum, India). The starting dose of hMG was 150 units which were subsequently increased to 225 units if there was no response after 6 days in terms of rise in S. E2 and USG evidence for dominant follicle. Surprisingly all patient responded or hyper responded within 10 days of stimulation.

We looked for characteristics common to this subset of PCOS women so that we could possibly identify these FSH resistant PCOS' in advance and treat them differently from the beginning. Phenotypic, laboratory and ultrasound features were noted. These included age, BMI, waist circumference (WC), oral glucose tolerance test (OGTT), triglyceride (TG) levels, AMH, basal FSH, basal LH, ovarian volume and AFC. All these women has clinical features of hyper-androgenism hence testosterone levels were not tested. Observations were made for this subset of women and all of them had almost equal characteristics in terms of high BMI and WC, very high AMH and high ovarian volume with AFC. Interestingly, no patient was a LH hypersecretor.

Therefore, it appears there exists a definite group of anovulatory PCOS, who appear to be resistant to ovulation induction when treated with small doses of FSH alone for the purpose of ovulation induction. There are 2 possible explanations to this resistance to injection rFSH when given in low doses for the purpose of ovulation induction. Firstly, very high levels of AMH have an inhibitory effect on follicular recruitment under the influence of exogenous FSH [1]. Addition of LH possibly increases follicular sensitivity of granulosa cells by increasing FSH receptors on them [2] so that they could respond to lower doses of FSH which are used for ovulation induction in non IVF cycles. Therefore, it appears addition of LH may help to overcome resistance

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| S. No. | Characterstics | Min | Max | Mean | SD | Normal physiological index |
|---|---|---|---|---|---|---|
| 1 | BMI (kg/m$^2$) | 29 | 36 | 33 | 3.32 | 18-24.9 |
| 2 | WC (cm) | 98 | 124 | 108 | 5.0 | <80 |
| 3 | AMH (pmol/L) | 60 | 145 | 106 | 37.7 | 7.7-22.5 |
| 4 | FSH (mIU/ml) | 4 | 8 | 5.6 | 1.09 | 3-10 |
| 5 | LH (mIU/L) | 3.3 | 12 | 6.4 | 6.12 | 3-10 |
| 6 | Oral GTT (2 h value) | 151 | 198 | 178 | 13.47 | <140 |
| 7 | Lipid profile (TG in mg/dl) | 170 | 220 | 193 | 14.27 | <150 |
| 8 | Ovarian volume (cc) | 12 | 21 | 18 | 3.7 | 3-10 |
| 9 | Antral Follicle Count | 30 | 56 | 42 | 10.17 | 5-10 (in each ovary) |

**Table 1:** Characteristics evaluated to identify features common to this group of FSH resistant PCOS.

of follicular recruitment by FSH alone, which is a known possibility in PCOS women with concomitant high levels of AMH. The second explanation for FSH resistance observed could be presence of FSH receptor polymorphism which makes the follicles resistant to lower doses of FSH. To overcome this receptor polymorphism one may require higher doses of FSH to be able to go above the serum threshold levels of FSH required to select dominant follicles. It is well researched that frequency of FSH-R Ser680 variant is high in hypo-responders and consumption of FSH is higher in carriers of this polymorphism [3]. It is also interesting to note that ovarian stimulation for IVF where higher doses of FSH are used at the initiation for controlled ovarian stimulation, may overcome both of these inhibitory effects, whether it is the inhibitory effect of high AMH on follicular growth or the FSH receptor polymorphism.

By using monkey as an experimental model, it has been found that alternation of DNA methylation patterns might lead non-human primates predispose to polycystic ovary syndrome (PCOS) [4]. In addition, in granulosa cells that derived from patients, 12245 differential methylated CpG sites were detected in the PCOS groups [5], which suggested that loci specific and/or global DNA methylation alteration may play a direct role in the initiation and development process of PCOS. Since DNA methyl-transferase (DNMTs) are the major enzymes for the depositing and protection of DNA methylation [6] and some other histone modifiers also play a role for the maintenance of DNA methylation at specific loci [7,8] aberrant express of these enzymes may also trigger the occurrence of PCOS in humans. Therefore, study the expression levels and catalytic activity of these enzymes may create a new direction for the investigation of PCOS and shed light on the further treatment of this disorder.

## Conclusion

A subset of obese PCOS with very high AMH levels who otherwise appear to be severe hyper-responders may require the addition of LH to higher doses of FSH for inducing ovulation, possibly because of presence of FSH receptor resistance or polymorphism (Table 1).

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