The effect of Laparoscopic ovarian drilling on the serum levels of AMH, FSH, LH and Testosterone hormones, in patients with PCOS

Ali F. Al-Assadi¹, Dhamia S. Al-Haroon², Alaa Hefdhi Al-Rubaye³ & Reem Ghaleb Doshan⁴

ABSTRACT

Objective: This study was performed to evaluate the effect of laparoscopic ovarian drilling on the serum levels of anti-Müllerian hormone (AMH), Follicle Stimulating hormone (FSH), Lutenizing hormone (LH) and testosterone hormones, in patients with PCOS.

Methods: A prospective observational study carried out on 50 patients with PCOS who underwent LOD, as a treatment for anovulatory infertility between June 2015 and June 2016. This study was conducted in AL Basrah Hospital for maternity and children. We assessed the serum levels of anti-Müllerian hormone (AMH), Follicle Stimulating hormone (FSH), Lutenizing hormone (LH) and testosterone hormone (test.) one month before laparoscopic surgery, one day after and 3 months after the surgery.

Results: Among 50 patients (2 patients were lost from follow up and they were excluded from the study), 31 (64.6%) started to menstruate regularly and 26 (54.2%) ovulated spontaneously and 9 (18.8%) of them conceive spontaneously after LOD within 3 months of doing the operation. The serum levels of hormones, before, one day after and 3 months after surgery, for AMH were 8.9 ± 3.5 ng/ml, 7.3 ± 2.9 ng/ml and 7.4 ± 2.1 ng/ml, (P value = 0.000), respectively. For the FSH were 5.2 ± 1.8 IU/L, 6.5 ± 2.0 IU/L and 7.1 ± 1.9 IU/L, (p value = 0.000), respectively. For the LH were 10.2 ± 21 IU/L, 10.7 ± 4.5 IU/L and 7.2 ± 2.2 IU/L, (p value = 0.000), respectively. For the testosterone levels were 1.16 ± 0.7 ng/ml, 0.44 ± 0.2 ng/ml and 0.34 ± 0.1ng/ml, (P value= 0.000), respectively.

Conclusion: LOD operation is an effective treatment for infertile PCOS patient who are not responding to medical treatment (clomiphene citrate and gonadotrophine resistant cases or cases who can’t offer the gonadotrophines). If it is done properly it will not affect the ovarian reserve. The resultant hormonal changes after the operation (decrease LH and testosterone) infavoure the continuation of pregnancy and lower the miscarriage rate. In our locality it represents a cost-effective and offered able alternative to medical treatment of PCOS.

Key words: PCOS and laparoscopic ovarian drilling.

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INTRODUCTION

The first recognition of the polycystic ovarian disease goes back to 1935 by American gynecologists (Stein and Leventhal) from whom its original name of Stein-Leventhal Syndrome is taken.\[^1\] The polycystic ovary syndrome (PCOS) is a heterogeneous collection of signs and symptoms that aggregated together consequent to ovarian, endocrine and metabolic dysfunction affecting (5-10%) of women of the reproductive age which can be mild in some but sever in others.\[^2,3\]\[^4\] It is the major cause of ovulation related infertilities, accounting for about 80% of cases with anovulatory infertility.\[^4\] The European Society for Human Reproduction and Embryology (ESHRE) and American Society for Reproductive Medicine (ASRM) defined PCOS as the need of presence of two out of the following three criteria:\[^5\]:

1. **Oligo - and/or amenorrhea.**
2. **Hyperandrogenism (clinical and / or biochemical).**
3. **Polycystic ovaries assessed by ultrasound.**

The morphology of PCOS has been subjected to redefinition as an ovary with 12 or more follicles measuring 2-9 mm in diameter and / or increased volume more than 10 cm:\[^6\] even after these criteria have been met, other potential causes of hyperandrogenism must be excluded like congenital adrenal hyperplasia, Cushing syndrome and presence of any androgen secreting tumors.\[^7\] Polycystic ovary syndrome (PCOS), is due to combination of enviromental and genetic factors.\[^8\] There are considerable heterogeneity of sign and symptoms among women with polycystic ovarian syndrome and these could change with time. PCOS may be inherited in various aspect and can exist without clinical signs and can be expressed in certain cases.\[^9\] Menstrual disturbance, obesity, infertility, acne, hirsutism, androgenic alopecia and acanthosis nigricans are among the commonest features. The biochemical features include high fasting insulin level. (50 patients) total and free testosterone levels may be elevated,\[^10\] increased Dehydroepiandrosterone sulfate. increase LH, decrease FSH and
Elevated LH / FSH ratio ≥ 2, Decreased SHBG, increase free androgen index (FAI), serum prolactin increased in 30-40% of PCOS women. Antimullerian hormone (AMH). AMH is increased in women with PCOS, and may become part of its diagnostic criteria. Management of unovulation in PCO patient:

Management of unovulation in PCO patient:

Perales-Puchalt & Legro. Steroids 2013; 78:767-772

Laparoscopic ovarian drilling (LOD): The pregnancy rate following LOD is 66% in clomiphene citrate-resistant PCOS related infertility. LOD could be predicted to result in poor reproductive outcome in clomiphene citrate resistant PCOS women when: obese (BMI > 25 kg/m2), long duration of infertility > 3 years, marked biochemical hyperandrogenism (testosterone levels ≥ 4.5 nmol/L, free androgen index > 15) and high basal AMH ≥ 7.7 ng/mL.

Complications:
One of the main short coming of LOD is iatrogenic adhesion due to bleeding from the ovarian surface of premature contact between the ovary and bowel after cauterization, Adhesion rates range from (0-100%). Another potential risk is premature ovarian failure, especially if the ovarian blood supply is damaged inadvertently or if large number of punctures are made, leading to excessive destruction of ovarian follicular pool or production of anti-ovarian antibodies. The risk of premature ovarian failure can be reduced by minimizing the thermal damage to the ovary, this achieved by reducing the number of punctures, using only very short bursts of cutting current and lavaging of the ovary to cool it down.
PATIENTS, METHODS AND MATERIAL
This study is a prospective observational study, fifty patients aged from 20-35 with an ovulatory infertility problem. Patients who were diagnosed to have PCOS by using Rotterdam criteria and resistant to medical treatment for ovulation induction (like clomiphene citrate, gonado tropin or letrozole) for varying periods. We advise them to stop metformin and myoinositol if they were using them at least 3 months before the first evaluation. This study was performed in AL Basrah Hospital for maternity and children for the period from June 2015 to June 2016. Body mass index was calculated as weight in Kg/height in m² and the women were categorized as lean (BMI ≤ 19.9), normal weight (BMI 20.0-25.0), over weight (BMI 25.1-29.9), or obese (BMI ≥ 30.0) LOD was performed using monopolar diathermy at 40 watts making 4-6 punctures according to the size of the ovary for 4 seconds for both ovaries. The ovary was cooled after drilling by Ringer lactate solution and at the end of the procedure, repeated suction irrigation of the pelvis with 500cc of Ringer lactate solution was done. We assessed the serum level of AMH, FSH, LH and testosterone before, 1 day after and 3 months after laparoscopic surgery in all patients, hormonal assay were measured on day 2 of the menstrual cycle. Before surgery, about 5cc blood sample was taken from each patient and maintained in tubes containing clot activator material (serum separation). The samples were centrifuged with 3000 rpm and the serum was collected at 2ml microtubes and stored at-20° C freezer until subsequent analysis. AMH was measured using a commercially available enzyme-linked immunosorbent assay kit (Immuno tech, Beckman Coulter USA Ltd). FSH, LH and testosterone levels were measured using an Enzyme Immunoassay method by using TOSOH kit.

Statistical analysis: Analysis was performed using SPSS version 17. Data were presented as number and percent for non-parametric variables and as mean ± SD (standard deviation) for parametric variables. For comparison between more than two study groups, we use ANOVA (POST HOC) test. We use ANOVA (Friedman) test to assess the difference between means of the study groups. In all cases, P value ≤ 0.05 was considered as significant.

RESULTS
A total of 50 infertile patients with PCOS were studied (who were within the inclusion criteria), 2 patients were lost from follow up and they were excluded from the study.

### Table 1. Patient’s characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NO.</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>48</td>
<td>24.25</td>
<td>3.34</td>
<td>20-33</td>
</tr>
<tr>
<td>Infertility (number)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infertility Duration (yr)</td>
<td>6.18</td>
<td>2.7</td>
<td>1.5-11</td>
<td></td>
</tr>
</tbody>
</table>

BMI: normal 10, overweight 25, obese 13 (total = 48) The mean age was 24.25 ± 3.34.

(46) of them were between 20-30 years and (2) of them were more than 30 years old. 43 patients were complaining of primary infertility and only 5 of them had secondary infertility. The mean duration of infertility was 6.18 ± 2.7 years. Regarding the BMI, no patient was in the lean group, 20.8% of them were having normal weight, 52.1% were overweight and 27.1% were obese.
Table 2. The effect of body weight on the hormonal levels

<table>
<thead>
<tr>
<th>Hormones</th>
<th>Lean Bmi ≤ 19.9</th>
<th>Normal Bmi 20.0 -25.0</th>
<th>Overweight Bmi 25.1-29.9</th>
<th>Obese Bmi ≥ 30.0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>P-value</td>
<td>Mean</td>
<td>P-value</td>
</tr>
<tr>
<td>AMH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMHa</td>
<td>0</td>
<td>0</td>
<td>10.704</td>
<td>0.044</td>
</tr>
<tr>
<td>AMHb</td>
<td>0</td>
<td>0</td>
<td>8.8140</td>
<td>0.068</td>
</tr>
<tr>
<td>AMHc</td>
<td>0</td>
<td>0</td>
<td>8.8100</td>
<td>0.26</td>
</tr>
<tr>
<td>FSH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSH a</td>
<td>0</td>
<td>0</td>
<td>4.9100</td>
<td>0.424</td>
</tr>
<tr>
<td>FSH b</td>
<td>0</td>
<td>0</td>
<td>6.0300</td>
<td>0.447</td>
</tr>
<tr>
<td>FSH c</td>
<td>0</td>
<td>0</td>
<td>6.4900</td>
<td>0.479</td>
</tr>
<tr>
<td>LH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH a</td>
<td>0</td>
<td>0</td>
<td>9.7630</td>
<td>0.620</td>
</tr>
<tr>
<td>LH b</td>
<td>0</td>
<td>0</td>
<td>10.207</td>
<td>0.543</td>
</tr>
<tr>
<td>LH c</td>
<td>0</td>
<td>0</td>
<td>7.1800</td>
<td>0.689</td>
</tr>
<tr>
<td>Test.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TEST. a</td>
<td>0</td>
<td>0</td>
<td>1.5860</td>
<td>0.035</td>
</tr>
<tr>
<td>TEST. b</td>
<td>0</td>
<td>0</td>
<td>0.4090</td>
<td>0.855</td>
</tr>
<tr>
<td>TEST. c</td>
<td>0</td>
<td>0</td>
<td>0.3470</td>
<td>0.936</td>
</tr>
</tbody>
</table>

(a)= before laparoscopic ovarian drilling; (b)= day 1 after laparoscopic ovarian drilling; (c)= day 90 after laparoscopic ovarian drilling.

(Table-2), shows the effect of the patients' body mass index on the level of serum hormones under study (AMH, FSH, LH and Testosterone). From the above data and after application of (POST HOC) test, there was no fixed statistically significant effect of the BMI on the serum levels of the hormones with only statistically significant effect at 4 occasions and even at these occasions there was no specific pattern of association. 9 patients in our sample get pregnant after doing the LOD operation within the 1st 3 months and they were excluded from hormonal analysis at day 90 after operation because of the effect of pregnancy on the level of these hormones.

Table 3. The effect of LOD on the levels of AMH, FSH, LH and Testosterone Hormones.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Before (mean) ± SD</th>
<th>Day 1 (mean) ± SD</th>
<th>90 day (mean) ± SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMH(ng/ml)</td>
<td>8.9000 ± 3.5792</td>
<td>7.3713 ± 2.9111</td>
<td>7.4362 ± 2.1511</td>
<td>0.0007</td>
</tr>
<tr>
<td>FSH(IU/L)</td>
<td>5.2564 ± 1.8966</td>
<td>6.5231 ± 2.0712</td>
<td>7.1164 ± 1.9348</td>
<td>0.0007</td>
</tr>
<tr>
<td>LH(IU/L)</td>
<td>10.2177 ± 4.2120</td>
<td>10.7000 ± 4.548</td>
<td>7.2077 ± 2.2676</td>
<td>0.0007</td>
</tr>
<tr>
<td>Test.(ng/ml)</td>
<td>1.1638 ± 0.79484</td>
<td>0.4459 ± 0.2067</td>
<td>0.3403 ± 0.1739</td>
<td>0.0007</td>
</tr>
</tbody>
</table>

*P-value was significant.

This table shows what happen to the level of the 4 hormones under study after doing the LOD operation within 90 days. Regarding the AMH, there was a significant reduction in its level with the lowest value at day 1 post operation (7.37). The FSH show a significant progressive increase with the time and being highest after 90 days, whereas the LH level was increased at day 1 compare to the base line value and then was significantly decreased at day 90. Testosterone shows a significant progressive reduction with time and it was lowest at day 90 after surgery.
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this study was reported and this difference in

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PCOS is considered to be a significant cause of anovulatory infertility; it is responsible for 80% of cases of infertility due to anovulation." Lod is offered as second - line treatment in PCOS patients, especially those who failed to respond to medical methods of ovulation induction. Lod in this study 96% of patients were at age group 20-30 years which is the typical age of presentation, and 79.2% of them were overweight (BMI 25.1 – 29.9) kg/m² and obese (BMI ≥ 30) kg/m² and this is similar to the findings of Misbah Sultana in a study of patients with PCOS, 77% of them were presented with high BMI with mean of 27.8 kg/m² while Lei etal, a BMI of 25.5 kg/m² in his study was reported and this difference in

(Table-4) depicts the outcome of Lod in patients with PCOS in term of cycle regularity, ovulation and pregnancy. Thirty one (64.6%) patients resumed regular menstruation after Lod whereas 17 (35.4%) patients failed to do so. After Lod spontaneous ovulation occurred in 26 (54.2%); whereas 22 (45.8%) did so after induction of ovulation by clomiphene citrate or by gonadotrophin use. Pregnancies achieved after Lod were 9 (18.8%) patients within 1st 3 months of doing the operation. The patients were followed up for 1 year only, there were 4 additional pregnancies between 6 and 12 months but they were not included in the result.

DISCUSSION

PCOS is considered to be a significant cause of anovulatory infertility; it is responsible for 80% of cases of infertility due to anovulation. Lod is offered as second - line treatment in PCOS patients, especially those who failed to respond to medical methods of ovulation induction. Lod in this study 96% of patients were at age group 20-30 years which is the typical age of presentation, and 79.2% of them were overweight (BMI 25.1 – 29.9) kg/m² and obese (BMI ≥ 30) kg/m² and this is similar to the findings of Misbah Sultana in a study of patients with PCOS, 77% of them were presented with high BMI with mean of 27.8 kg/m² while Lei etal, a BMI of 25.5 kg/m² in his study was reported and this difference in

selected samples may be attributed to the difference in societies. In the current study, the mean of preoperative AMH level was (8.9 ng/ml) which is high when compared with normal value of women in reproductive age with normal menstruation, this agree with Broer et al[25] who found that the serum level of AMH for PCOS patients is more than (4.0 ng/ml) while the normal level of serum AMH is (1.5 - 4.0 ng/ml). Other previous studies as Weerakiet et al,[26] Maheshwari et al,[27] Haadsma, et al[28] and Butt F.[29] showed similar finding; that women with PCOS have 2-3 times increased level of their serum AMH concentration which was related to increment in the number of small follicles. In this study, we reported a significant reduction in the AMH level after doing the LOD operation and this is due to reduction in the number of AFC caused by thermal destruction to the ovarian tissue, this is similar to that reported by Amer, et al[30] in patients with PCOS after laparoscopic ovarian diathermy and showed that serum level of AMH was 6.1 ng/ml which was reduced to 4.7 ng/ml after one week and remain at the same level, reduction in the AMH levels may be due to decrease in the number of AFC as a result of using bilateral diathermy technique. Also Kandi and Salim[31] examined AMH level after treatment with clomiphene, unilateral and bilateral LOD and showed that only after bilateral LOD ovarian reserve reduced significantly which support our study while Api, et al[32] showed that ovarian reserve of patients with PCOS does not change significantly after LOD. In our study 90 days after LOD, serum level of AMH was found to be lower than its level before LOD, and this level stayed higher than normal when compared with normal women without PCOS, is in agreement with Chang, et al[33] and Farzadi, et al[34] who conclude that, LOD had normalized ovarian function which is a significant factor in the follicular recruitment and their maturation. Therefore, LOD has no negative effect on ovarian supply. This mean that if the operation was done properly, it will not result in reduction

<table>
<thead>
<tr>
<th>Outcome measure</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Menstrual cycle</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regular</td>
<td>31</td>
<td>64.6</td>
</tr>
<tr>
<td>Irregular</td>
<td>17</td>
<td>35.4</td>
</tr>
<tr>
<td><strong>Ovulation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spontaneous</td>
<td>26</td>
<td>54.2</td>
</tr>
<tr>
<td>By Induction</td>
<td>22</td>
<td>45.8</td>
</tr>
<tr>
<td><strong>Pregnancy</strong></td>
<td>9</td>
<td>18.8</td>
</tr>
</tbody>
</table>

Table 4. Outcome of LOD for PCOS.
of ovarian reserve which is in agreement with Amer, et al\textsuperscript{[35]} who conclude that laparoscopic ovarian puncture does not change durably AMH serum level in women with PCOS, but differ from that reported by Elmashad’s, et al\textsuperscript{[36]} in which a significant decrease in serum levels of AMH, and AFC were detected at 1\textsuperscript{st}, 3\textsuperscript{rd} and 6\textsuperscript{th} cycle after laparoscopy. This could be explained by possible damage to the ovarian parenchyma after bipolar electrocaogulation during laparoscopy. The reduction in AMH level was a little bit lower at day 1 compare to day 90, which is similar to that reported by Farzadi, et al\textsuperscript{[34]} in which serum AMH level was reduced in the 1\textsuperscript{st} week after surgery and then recovered 1 and 3 months after surgery to approximately 65\% of the preoperative level which conclude that LOD had no adverse effect on ovarian reserve in patient with PCOS. Regarding FSH and LH level, before surgery LH level was double that of FSH (typical for PCOS LH : FSH =2) after LOD, there is progressive increase in FSH level and progressive decrease in LH level and after 3 months both FSH and LH levels are almost the same (LH:FSH ratio approximately equal to 1) and these findings are similar to that reported by Zahir, et al\textsuperscript{[37]} Buttram\textsuperscript{[38]} and Greenblatt E.\textsuperscript{[39]}

In this study, D1 after surgery showed an increase in serum level of LH which is a little bit higher than preoperative value, these results are in agreement with M. Suni, et al\textsuperscript{[40]} in which serum level of LH found to be higher in responders group to bilateral LOD in the 1\textsuperscript{st} month of followup in a comparative study between dose-adjusted unilateral diathermy versus fixed dose bilateral LOD in patients with PCOS. In the present study, serum testosterone level was progressively decrease with time after surgery and it was significantly lowest after 90 days and this is similar to that reported by Zahiri et al\textsuperscript{[37]} and E.M. Seyma et al,\textsuperscript{[41]} the mechanism by which LOD results in decrease androgen level is similar to that of ovarian wedge resection surgery which produced by destruction of androgen producing tissues and this lead to decrease in peripheral conversion of androgen to estrogen (112). A decrease in serum level of androgen and LH and increase FSH level have been demonstrated after LOD similar to that reported by Api M, \textsuperscript{[32]} Elmashad A,\textsuperscript{[36]} Zahiri, et al\textsuperscript{[37]} and Mitra S. et al\textsuperscript{[42]} A change in endocrine function converts the androgen dominant intrafollicular enviroment to estrogenic one which was in agreement with Issam L. et al\textsuperscript{[22]}, Mitra S.et al\textsuperscript{[42]} and Aakvaag A. et al.\textsuperscript{[43]} It affects ovarian-pituitary feedback mechanism, \textsuperscript{[37,44]} so both intraovarian and systemic endocrinial effects may induce ovulation and improved reproductive outcome in these patients after LOD.\textsuperscript{[42, 45-47]} LOD may also increase ovarian blood flow, allowing high delivery of Gonadotropins and post-surgical local growth factors. An improvement in insulin sensitivity after LOD has also been suggested (32, 48, 49). In our study, we found there is no significant effect of BMI on the hormonal levels, these finding were supported by Fernandez et al,\textsuperscript{[50]} Niraji et al \textsuperscript{[51]} and Van wely et al.\textsuperscript{[52]} Pignyand et al \textsuperscript{[53]} found that AMH levels were lower in obese than non-obese women, but the difference was not statistically significant. Other studies that suggested no correlation between BMI and AMH levels in women with PCOS and control subjects done by E.M. Seyam\textsuperscript{[41]} and Piltonen et al.\textsuperscript{[54]} Regarding the impact of LOD on cycle regularity, ovulation and pregnancy rate. We saw that among 48 PCOS women, 31 (64.6\%) resumed regular menstruation and 26 (54.2\%) started to ovulate spontaneously, this is differ from that reported by Farquhar et al \textsuperscript{[55]} and Ibrahim Abd El gafor\textsuperscript{[56]} which have higher ovulation rate about 80\%. Difference in response may be attributed to the serum level of AMH, women who ovulated subsequent to LOD had significantly low pretreatment AMH levels than those who did not ovulate.\textsuperscript{[35]} Successful outcome, as pregnancy occuring within 3 months after LOD was 9 (18.8\%) which is low in comparison to other study like Misbah Sultana et al\textsuperscript{[23]} who reported 21(42.8\%)
pregnancy rate in his study which may be due to more than one reason. First shorter period of the follow up (3 months), in this study there were 4 additional pregnancies between (6 - 12 months), but they were not included in the results. Another cause may be due to several factors which influence the efficacy of ovarian drilling such as pretreatment elevated LH concentration > 10 IU/L, < 3 years of infertility and younger age less than 25 years.[23,52] In our country (Iraq), the single gonadotrophin (recombinant FSH) cost the patient 25 US Dollars while the LOD operation can cost the patient 400 US Dollars at the general hospital so it is probably more offer able to the patients than to continue on ovulation induction with gonadotrophine, specially to those no feasible for follow up of intensive monitoring and for those who showed exaggerated response to gonadotropins. Other advantage of this technique is provide an opportunity to assess the pelvis for other potential causes of subfertility which could be treated at the same time.

CONCLUSION

LOD operation is an effective treatment for infertile PCOS patient who are not responding to medical treatment (clomiphene citrate and gonadotrophine resistant cases). If it is done properly it will not affect the ovarian reserve. The resultant hormonal changes after the operation (decrease LH and testosterone) infavoure the continuation of pregnancy and lower the miscarriage rate. In our locality it represents a cost effective and offered able alternative to medical treatment of PCOS especially for those who can't offer the gonadotrophins.

RECOMMENDATION

1. It is the policy of the unit to encourage obese women to lose weight by life style modification before undergoing LOD surgery. Loss of weight helps in a change in hormonal milieu as well as making operation free of anesthetic and surgical risks.
2. A detailed knowledge of the clinical and hormonal profile of the patients may be useful in a careful selection of cases likely to respond to LOD.
3. If 12 ovulatory cycles after LOD there is no conception one should proceed for assisted reproductive techniques rather than waiting.
4. Since LOD improves ovarian responsiveness to CC and gonadotropins like recombinant FSH (r FSH), these may be considered after 3 months and 6 months, respectively after LOD failure, especially in those who are less hyperandrogenic and less insulin-resistant.

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