Short-term changes in hormonal profiles after laparoscopic ovarian laser evaporation compared with diagnostic laparoscopy for PCOS

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STUDY QUESTION: Which reproductive endocrine changes are attributed exclusively to laparoscopic ovarian drilling in polycystic ovarian syndrome (PCOS)?

SUMMARY ANSWER: Laser evaporation-specific endocrine effects were the prevention of an immediate increase in inhibin B and a sustained decrease in testosterone, androstenedione and anti-Müllian hormone (AMH).

WHAT IS KNOWN ALREADY: All ovarian drilling procedures result in reproductive endocrine changes. It is not known which of these changes are the result of ovarian drilling and which are related to the surgery per se.

STUDY DESIGN, SIZE, DURATION: This prospective controlled study was performed at an outpatient academic fertility clinic. Between 2007 and 2010, a total of 21 oligo- or amenorrheic PCOS patients were included.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Included were oligo- or amenorrheic PCOS patients with all three of the Rotterdam criteria and luteinizing hormone (LH) >6.5 U/l. All PCOS patients had an indication for diagnostic surgery due to subfertility. There were 12 PCOS patients who chose to undergo ovarian laser evaporation (CO₂ laser, 25 W, 20 times/ovary) and 9 PCOS who chose a diagnostic laparoscopy only (controls). Reproductive endocrinology was measured before, and until 5 days after, surgery, and four gonadotrophin-releasing hormone (GnRH) ‘double pulse’ tests were included. The main outcome measures were changes in reproductive endocrinology and pituitary sensitivity/priming to GnRH after laser evaporation compared with diagnostic laparoscopy only.

MAIN RESULTS AND THE ROLE OF CHANCE: In the first hours after surgery, both groups showed an increase in LH, follicle stimulating hormone, estrogen and a decrease in testosterone, androstenedione, AMH and insulin growth factor-1 (P<0.05). Inhibin B increased in the laparoscopy only group (P<0.05). In the first days after surgery, testosterone, androstenedione and AMH remained at lower than baseline levels exclusively in the laser group (P<0.05). Pituitary sensitivity/priming to GnRH was not altered after either laser evaporation or laparoscopy only.

LIMITATIONS, REASONS FOR CAUTION: The limitations of this study are the short follow-up period and the relatively small groups.

WIDER IMPLICATIONS OF THE FINDINGS: The strength of this study is the integrally measured endocrine profiles in combination with an optimal control group of PCOS patients undergoing diagnostic laparoscopy only. Interestingly, most of the immediate endocrine changes after laser evaporation could be related to the surgical context and not to the ovarian drilling procedure itself.

STUDY FUNDING/COMPETING INTEREST(S): The study was funded by the Foundation of Scientific Research in Obstetrics and Gynaecology and the study medication, Lutrelef, was donated by Ferring, The Netherlands, Hoofddorp. There were no conflicts of interest mentioned by the authors.

Key words: ovarian drilling / PCOS / reproductive endocrinology / GnRH tests / laser evaporation
Introduction

Ovarian surgery by wedge resection or ovarian electrocaugulation has been used for years now as a treatment option for anovulatory women with polycystic ovarian syndrome (PCOS) (Stein and Leventhal, 1935; Gjonnaess, 1984). In spite of its use for over seven decades, the exact working mechanism of ovarian surgery/drilling is still not fully understood. All types of ovarian drilling applied in PCOS share a common assumed effect of creating ovarian damage and, from an endocrine point of view, can be seen as equivalent procedures (Cohen, 1996). During the last 30 years, many papers have been published about the endocrine consequences of these procedures, resulting in many theories for the cause of the re-establishment of menstrual cycles after ovarian drilling. Originally the removal of a mechanical barrier was postulated as the cause of the re-establishment of menstrual cycles after ovarian drilling. This altered endocrine situation seems to concord with increased ovarian sensitivity to follicle stimulating hormone (FSH) (Hendriks et al., 2001). This was done (i) by measuring all the currently known potentially attenuating factor (GnSIF/AF) activity (Danforth et al., 2001). All PCOS patients underwent GnRH ‘double pulse’ tests on four different occasions, namely cycle Days 5–7, the day of surgery (before the laparoscopy), the first day afterwards and 5–7 days after the procedure. The regularly ovulating controls underwent one GnRH ‘double pulse’ test on cycle Days 5–7. On all occasions, 25 μGnRH (Lutrelef, Ferring B.V., The Netherlands) was administered twice through an IV canula with a 120 min interval. Blood was drawn for LH analysis before and at 30 and 60 min after both GnRH doses (Rommler et al., 1993). The LH increment 30 min after the first GnRH dose is an indication of pituitary sensitivity.

Materials and Methods

Patients

Patients of the outpatient fertility clinic of the VU University Medical Center and patients referred to us from the Onze Lieve Vrouwe Gasthuis in Amsterdam, the Netherlands, were screened for eligibility and asked to participate from January 2007 until December 2010. Approval for the study was obtained from the Medical Ethics Board of the VU University Medical Center and patients gave written informed consent.

For this study, women were diagnosed as PCOS when all of the Rotterdam criteria were present: (i) oligo- or amenorrhea (cycle >35 days), (ii) biochemical/clinical hyperandrogenism (defined as a Ferriman–Gallwey score of ≥8, testosterone >2.5 nmol/l and/or androstenedione >9.0 nmol/l) and (iii) polycystic ovarian morphology (>12 follicles (2–9 mm) in at least one ovary) (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). Exclusion criteria were previous ovarian surgery, oral contraceptive use within the last 3 months, mechanical or male subfertility and co-existing endocrine diseases (diabetes mellitus, estrogen-dependent tumors, thyroid disease, Cushing’s syndrome or congenital adrenal hyperplasia). All PCOS patients had an indication for surgery (clopophene resistance up to 150 mg/day and/or after six cycles of ovulation induction with FSH). Patients were fully informed by the first author and were allowed to choose freely between a diagnostic laparoscopy only (Laparoscopy Only Group) or laparoscopy in combination with ovarian laser evaporation (Laser Group). This trial was deliberately not set up as a randomized controlled trial, as we found it not ethical to randomize between such different treatment modalities and its consequences for future treatment.

Furthermore, an extra control group was used for the GnRH tests (see below). These were women between 18 and 45 years with a regular menstrual cycle (25–35 days). Exclusion criteria were cycle Day 3 FSH levels >10 IU/l, or use of an oral anticonceptive or levonorgestrel-releasing intrauterine system.

GnRH tests

GnRH ‘double pulse’ tests were performed to measure pituitary sensitivity and as an indirect measurement for gonadotrophin surge inhibiting/attenuating factor (GnSIF/AF) activity (Danforth et al., 1987; den koping et al., 2001). All PCOS patients underwent GnRH ‘double pulse’ tests on four different occasions, namely cycle Days 5–7, the day of surgery (before the laparoscopy), the first day afterwards and 5–7 days after the procedure. The regularly ovulating controls underwent one GnRH ‘double pulse’ test on cycle Days 5–7. On all occasions, 25 μGnRH (Lutrelef, Ferring B.V., The Netherlands) was administered twice through an IV canula with a 120 min interval. Blood was drawn for LH analysis before and at 30 and 60 min after both GnRH doses (Rommler et al., 1973; Gjonnaess and Norman, 1987; Campo et al., 1993). The LH increment 30 min after the first GnRH dose is an indication of pituitary sensitivity. The ratio between the first and second LH response on GnRH is seen as an indirect indication of GnSIF/AF activity. The larger the difference between the first and the second LH response on GnRH, the more GnSIF/AF activity is present (den koping et al., 2001). For analysis, the ratio of the absolute LH increment 30 min after the second compared with the first GnRH dose was used.

Endocrine measurements

To evaluate the endocrine changes after both procedures, blood was drawn from all PCOS patient on multiple occasions. Blood samples were taken on the day of surgery before the laparoscopy, and then after surgery for 5 h, followed by daily samples for 5 days. Ovulatory status was evaluated in the Laser Group by measuring progesterone levels 28 days after surgery, and a value

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above 3.6 nmol/l indicated ovulation. All blood samples were centrifuged at 3000 Hz for 10 min. The obtained serum was stored at −20°C until the assays.

The serum was analyzed by the endocrine laboratory of the VU University Medical Center. Plasma LH and FSH levels were determined by immunometric assay (Delfia, Wallac oy, Turku, Finland), with a lower detection limit of 0.5 U/l for both hormones. For LH and FSH, the intra-assay and inter-assay coefficients of variance (CV) were 3 and 6–7%, respectively. Estradiol and progesterone were determined by competitive immunassay (Luminescence Architect, Abbott Laboratories, Illinois, USA), with a lower detection limit of 150 pmol/l for estradiol and 2 nmol/l for progesterone. Intra-assay and inter-assay CVs were 2 and 5%, respectively, for progesterone, and 3–9 and 10%, respectively, for estradiol. Sex hormone binding globuline (SHBG) was determined by immunometric assay (Luminescence Immulite 2500, Siemens Medical Solutions Diagnostics, USA), with a lower limit of 2 nmol/l and intra-assay and inter-assay CVs of 2–3 and 4%, respectively. Testosterone was determined by radioimmunoassay (Coat-A-Count, Siemens Medical Solutions Diagnostics, USA), with a lower detection limit of 1 nmol/l and intra-assay and inter-assay CVs of 6–8 and 10%, respectively. Androstenedione was determined by radioimmunoassay (coated tubes DSL, Webster, TX, USA), with a lower detection limit of 0.5 nmol/l and intra-assay and inter-assay CVs of 6–8 and 10%, respectively. AMH was determined by immunoassay (Luminex Immulite 2500, Siemens Medical Solutions Diagnostics, USA), with a lower detection limit of 3.2 nmol/l of intra-assay and inter-assay of CVs 5 of 8%, respectively. AMH was determined by immunoassay (Luminex Immulite 2500, Siemens Medical Solutions Diagnostics, USA), with a lower detection limit of 3.2 nmol/l of intra-assay and inter-assay of CVs 5 of 8%, respectively. AMH was determined by immunoassay (Luminex Immulite 2500, Siemens Medical Solutions Diagnostics, USA), with a lower detection limit of 3.2 nmol/l of intra-assay and inter-assay of CVs 5 of 8%, respectively. AMH was determined by immunoassay (Luminex Immulite 2500, Siemens Medical Solutions Diagnostics, USA), with a lower detection limit of 3.2 nmol/l of intra-assay and inter-assay of CVs 5 of 8%, respectively. AMH was determined by immunoassay (Luminex Immulite 2500, Siemens Medical Solutions Diagnostics, USA), with a lower detection limit of 3.2 nmol/l of intra-assay and inter-assay of CVs 5 of 8%, respectively. AMH was determined by immunoassay (Luminex Immulite 2500, Siemens Medical Solutions Diagnostics, USA), with a lower detection limit of 3.2 nmol/l of intra-assay and inter-assay of CVs 5 of 8%, respectively. AMH was determined by immunoassay (Luminex Immulite 2500, Siemens Medical Solutions Diagnostics, USA), with a lower detection limit of 3.2 nmol/l of intra-assay and inter-assay of CVs 5 of 8%, respectively. AMH was determined by immunoassay (Luminex Immulite 2500, Siemens Medical Solutions Diagnostics, USA), with a lower detection limit of 3.2 nmol/l of intra-assay and inter-assay of CVs 5 of 8%, respectively. AMH was determined by immunoassay (Luminex Immulite 2500, Siemens Medical Solutions Diagnostics, USA), with a lower detection limit of 3.2 nmol/l of intra-assay and inter-assay of CVs 5 of 8%, respectively.

Statistical methods

The power of this study was based on a previous publication using ‘double pulse’ GnRH tests, showing a 50% decrease of pituitary sensitivity after laparoscopic laser surgery in women with PCOS (Rossmanith et al., 1991). Calculating with a decrease in pituitary sensitivity of 25 and 90% power, 12 patients per group would be needed.

The independent samples T-test was used to compare the baseline values. Generalized estimating equations (GEEs) analyses were used to assess the longitudinal relationship between the hormones within and between both groups. GEE is a type of regression analysis which takes into account the correlation of the repeated measures within a person; it includes subjects regardless of missing values and it has the advantage of handling longitudinal data on subjects with unequally spaced observations (Liang and Zeger, 1993; Twisk, 1997). In case of a skewed distribution a natural logarithm (ln) transformation was performed.

The GnRH tests were analyzed with the independent samples T-test in case of normal distribution and in the other cases an independent samples Mann–Whitney U-test was performed to examine the differences between the groups. To analyze the results within the groups, paired samples t-tests (in case of normal distribution) or related samples Wilcoxon Signed Rank Test was used.

GEE-analyses were performed with STATA 10.0. All other analysis were performed with SPSS (version 20.0, Inc., Chicago, IL, USA). A P-value of <0.05 was considered significant.

Results

In total, 21 PCOS patients were included of which 12 patients underwent laparoscopic laser evaporation and 9 underwent a diagnostic laparoscopy. Inherent to the non-randomization nature of this study, there was a risk of disproportionate distribution between the two groups. After the completion of the 12 patients in the Laser group, the study was stopped as new patients were not able to choose anymore between the two treatment modalities.

The median (± interquartile range) length of the surgery was 45 min (± 18) in the Laser group and 36 min (± 12) in the Laparoscopy Only group (P = 0.2). Potentially fertility obstructing factors were found in two patients (16.7%) of the Laser group (uterus septum and mild unilateral adhesions, both of which were treated) and in two patients (22.2%) in the Laparoscopy Only group (unilateral tubal factor in both patients).

Endocrinology

Baseline levels

Baseline endocrine levels were comparable between both the PCOS groups (Table I). The median (± interquartile ranges) values in the regular cycle controls were 4.2 U/l (± 2.5) for LH, 5.8 U/l (± 1.6) for FSH, and they had an average cycle length of 28 days (± 1) and BMI of 24.5 (± 7.4).

After surgery, 1–5 h

In the first 5 h after surgery, significant increases in LH, FSH and estradiol and a significant decreases in testosterone, androstenedione, AMH and IGF-1 compared with baseline values were seen in both groups (Fig. 1). There were no significant differences between the two groups. Progesterone remained stable and was comparable between both groups. Inhibit B increased in the Laparoscopic controls only, and there was a significant difference between the Laser and the Laparoscopic Only group in the first hours after surgery.

| Table I Patient characteristics and median (±interquartile ranges) of the hormonal levels at baseline (day of surgery). |
|-----------------|-----------------|-----------------|-----------------|
| Laser (n=12)    | DLS (n=9)       | P-value         |
| Average cycle length (days) | 180 (105) | 55 (141) | 0.16 |
| BMI (kg/m²)     | 31.0 (8.6)  | 26.0 (9.4) | 0.08 |
| FSH (U/l)       | 5.9 (3.0)   | 5.9 (2.2)  | 0.95 |
| LH (U/l)        | 8.0 (7.1)   | 7.4 (3.4)  | 0.42 |
| Estradiol (pmol/l) | 165 (74) | 175 (230) | 0.35 |
| Progesterone (nmol/l) | 0.65 (0.5) | 0.8 (0.2) | 0.74 |
| Testosterone (nmol/l) | 2.1 (0.9) | 2.1 (1.0) | 0.32 |
| FAI (%)         | 7.3 (11.0)  | 3.7 (4.4)  | 0.11 |
| Androstenedione (nmol/l) | 11.8 (4.0) | 9.5 (4.8) | 0.38 |
| AMH (µg/l)      | 7.8 (3.0)   | 5.6 (11.8) | 0.76 |
| IGF-1 (nmol/l)  | 22.4 (7.4)  | 22.5 (12.2) | 0.29 |
| Inhibin B (ng/l) | 86 (58)    | 105 (27)  | 0.68 |

Baseline levels were compared with an independent samples t-test. Laser, Laser group; DLS, Diagnostic Laparoscopy Only group.
Figure 1 Measured hormones before and after surgery, in relation to baseline levels before surgery (time = 0). GEE analysis was performed. Laser, Laser group; DLS, Diagnostic Laparoscopy Only group. §Significant difference (P < 0.05) between the Laser and Laparoscopy Only group. *Significant change (P < 0.05) of hormone compared with before surgery (time = 0) in Laser group. #Significant change (P < 0.05) of hormone compared with before surgery (time = 0) in Laparoscopy Only group.
After surgery, 1–5 days
From Days 1 to 5 after surgery LH, FSH and IGF-1 returned to baseline levels in both groups and no significant differences between the two groups was observed. The Laser group showed significantly lower levels of estradiol, testosterone, androstenedione, inhibin B and AMH levels compared with baseline (on ≥1 days), whereas the levels of these hormones returned to baseline in the Laparoscopy Only group. Testosterone, androstenedione and AMH remained significantly lower in the Laser group compared with the Laparoscopy Only group at ≥2 days. Estradiol and inhibin B did not show significant differences between the two groups (Fig. 1).

GnRH tests
Compared the regularly cycling controls (2.15, ± 0.53), PCOS patients from the Laser (ratio 1.35, ± 0.70) as well as the Diagnostic Laparoscopy Only groups (1.43, ± 0.65) showed a significantly lower ratio of absolute LH change after the second compared with the first GnRH injection (P = 0.04 and 0.02, respectively) (Fig. 2a). The ratio did not differ between the two PCOS groups on any of the four test days. Within the Laser and Laparoscopy Only groups, no significant differences in the ratio were seen after surgery compared with before surgery (Fig. 2b and Table II).

The LH response 30 min after the first GnRH dose can be considered as the measure of pituitary sensitivity. On cycle Days 5–7, the absolute LH response was significantly higher in the Laser group (16.9 U/l, ± 13.1) compared with the regularly ovulating controls (6.9 U/l, ± 2.5) (P < 0.01) (Fig. 2a). Within the two PCOS groups, no significant difference in the LH response was seen after surgery compared with before surgery. No significant differences were found between the two PCOS groups, except on the first day after surgery when a higher LH response was seen in the Laparoscopy Only group compared with that in the Laser group (Fig. 2b and Table II).

Follow-up
Five patients (41.7%) became ovulatory within 28 days after ovarian laser evaporation. In the Laparoscopy Only group, spontaneous ovulation

![Figure 2](https://academic.oup.com/humrep/article-abstract/29/11/2544/2428369/2548)
was not awaited as these women started or continued ovulation induction treatment with gonadotrophins some weeks after the surgery.

**Discussion**

The endocrine changes seen after the ovarian drilling procedures in PCOS patients are usually attributed to morphological changes of the ovaries caused by the ovarian drilling (Hendriks et al., 2010). The possible endocrine influence of anesthesia (on central endocrine regulation, (para)sympathetic nervous system and circulation) and the manipulation of the intra-abdominal organs, including the ovaries, is largely unknown.

This study evaluated, in an integral way, the reproductive endocrine profile before and after laser evaporation in PCOS women and for the first time compared this to an optimal control group of PCOS women undergoing diagnostic laparoscopy only, providing the possibility to isolate changes specifically caused by the laser evaporation. Remarkably most immediate endocrine changes after laser evaporation can be regarded as related to the surgical context and not to the ovarian drilling procedure. Laser specific changes appeared to be (i) the prevention of an immediate increase in inhibin B and (ii) the sustained decrease in testosterone, androstenedione and AMH. Pituitary priming/sensitivity did not change after surgery.

**The response of pituitary hormones**

In the present study, LH and FSH increased in the first hours after surgery in both groups, followed by a return to baseline levels already after 1 day. Our data confirm some but not all literature (Hendriks et al., 2007). Since gonadotrophin secretion was increased in both the ovarian drilling and control group, it is likely that these changes result from a combination of anesthesia and the surgical procedure and not from the ovarian drilling per se. There are no data on the effect of general anesthesia on LH and FSH secretion in PCOS patients, but in regularly cycling women the gonadotrophins remain stable or show a temporary increase (Adashi et al., 1980; Hagen et al., 1980; Messinis et al., 1999). This would to some extent support the role of general anesthesia in the gonadotrophin increase, most likely mediated through neuroendocrine mechanisms possibly via activation of the sympathetic-adrenomedullary system (Hagen et al., 1980; Adams and Hempelmann, 1991). This is supported by the finding that blocking the sympathetic signals with spinal anesthesia results in a decrease in gonadotrophin secretion (Hagen et al., 1980; Adams and Hempelmann, 1991). Alternatively, but less likely, the laparoscopic procedure including the non-destructive manipulation of the internal organs may have been of influence. We recently observed an immediate drop in gonadotrophin secretion after mechanical ovarian manipulation in PCOS patients undergoing ovulation induction with FSH (Hendriks et al., 2013). Although this change in gonadotrophins is contrary to the observed increase in the current study, it nevertheless indicates the possibility that mechanical manipulation of the internal organs/ovaries can influence the gonadotrophin secretion. It should furthermore be noted that in the current study no hormonal treatment was given whereas in this other study ovarian manipulation took place while patients underwent ovulation induction with r-FSH. Furthermore, the observed endocrine changes may simply be a recovery to a normal levels, as a pre-operative increases or decreases in endocrine levels may have occurred due to stress, as has been recently suggested for androgens (Ott et al., 2014).

**The response of ovarian hormones**

There was a sharp decrease in testosterone, androstenedione and AMH seen directly after surgery in both groups. These hormones remained lower in the first days after surgery in the Laser group, contrary to the return to baseline in the Laparoscopy Only group. Since both groups showed similar changes, the decrease of the androgens in the first hours after surgery cannot be explained by the cellular destruction after laser evaporation. In support of this, a recent study which showed that the intra-operative decrease in androstenedione after ovarian drilling was not a predictor for spontaneous ovulation, contrary to the pre-operative androstenedione level (Ott et al., 2014). The longer term production of testosterone and androstenedione was significantly reduced in only the Laser group and therefore likely to have been
caused by destruction of ovarian tissue (Aakvaag and Gjonnaess, 1985; Hendriks et al., 2007). In support of this is the sustained reduction of the ovarian reserve marker AMH reflecting irreversible ovarian tissue damage (Amer et al., 2009; Elmashad, 2011). In this study, we applied 20 punctures per ovary as commonly recommended (Daniell and Miller, 1989; Keckstein et al., 1990; Verhelst et al., 1993). This number of punctures is likely to cause the required ovarian ‘damage’ in order to obtain a substantial ovulation rate and indeed all patients showed a sustained decline in AMH. On theoretical grounds, it is possible that less punctures would have inflicted less ovarian damage and consequently less of an AMH suppressing effect.

Estradiol showed an increase in both groups immediately after surgery. Although not significant, it seemed less marked in the Laser group. This was followed by a small decrease in estradiol in the first days after surgery in only the Laser group, which probably reflects granulosa cell destruction (Hendriks et al., 2007).

Inhibin B increased in the first hours after surgery in the Laparoscopy Only group. Apparently the Laser evaporation prevented the rise of inhibin B and during the days after surgery this was even followed by a small decrease in the Laser group, conforming with literature observations (Kovacs et al., 1991; Lockwood et al., 1998).

Progesterone seemed to remain (globally) stable after surgery. Obviously, progesterone levels were low prior to surgery, because according to the protocol all women were measured in the follicular phase. These results conform to the literature showing stable progesterone levels after ovarian drilling (Hendriks et al., 2007).

The combined observations of an immediate decline in testosterone, androstenedione and AMH in both groups after surgery and at the same time an increase in estradiol and inhibin B is puzzling. An all-embracing explanation is difficult. Possibly, a strong increase in aromatase activity mediated through the higher FSH levels directly after surgery, resulting in a decrease in the androgens and a concomitant increase in estradiol, may be responsible. The higher FSH levels could have stimulated the inhibin B production. Inhibin B and estradiol tended to be lower in the Laser group, thus laser evaporation/ovarian destruction may have prevented some of the increase. Furthermore, we cannot exclude an effect of the anesthesia and the manipulation of the internal organs on the autonomous nerve system with so far unknown effects on (PCOS) ovarian steroid production. Since the immediate endocrine shifts were seen in both groups, destruction of ovarian tissue is not likely involved in these short-term changes.

The laser evaporation-specific ovarian hormone changes emerging in the Laser group, as the target of 12 patients was not reached. The complexity and costliness of the study furthermore limited the number of patients and duration of follow-up.

Other hormones

IGF-I modulates ovarian function through control of ovarian androgen production together with LH (Fowler et al., 2000). In the present study, both groups showed a similar decrease of IGF-I shortly after surgery, followed by a recovery to baseline. Thus, the laser evaporation per se did not seem to influence the IGF-I levels, which conforms to the available literature (Tiihinen et al., 1993; Amin et al., 2003; Wu et al., 2004). This makes it unlikely that IGF-I has a role in the restoration of ovulatory cycles after ovarian drilling.

Pituitary sensitivity and GnRH priming

Patients with PCOS have a higher LH response to the GnRH ‘double pulse’ test at the first and second GnRH dose, compared with regularly cycling women, which we confirmed in the present study (Rossmanith et al., 1991). It has been argued that relatively low GnSIF/AF concentrations cause this primed pituitary and the higher LH levels in PCOS (Balen and Jacobs, 1991; de koning et al., 2001). The difference in LH response on the GnRH ‘double pulse’ tests in the present study supports the role of GnSIF/AF in PCOS. GnSIF/AF is an ovarian hormone which suppresses LH secretion by reducing pituitary sensitivity and antagonizing GnRH (Messinis et al., 1991; de koning et al., 2001; Fowler et al., 2003). In our study, the LH response on the GnRH ‘double pulse’ test did not change over time in the Laser group, suggesting no change in GnSIF/AF production directly after the laser evaporation. Theoretically, with GnSIF/AF being an ovarian product, a decrease could be expected in the days after surgery, as we have demonstrated in this study with most other ovarian hormones. On the other hand, the GnSIF/AF concentration might have been already very low before surgery, allowing no further measurably decrease. The stable LH and FSH levels from Day 1 after laser evaporation are also in line with the unaltered GnRH test.

The LH response on the first GnRH dose as a measure of pituitary sensitivity is influenced by multiple factors, such as estradiol, progesterone, inhibin and GnSIF/AF (Lasley et al., 1975; Wang and Yen, 1975). Both groups showed no significant difference in the LH response over the study period indicating an absence of change in pituitary sensitivity in relation to surgery.

Thus, a laparoscopy with or without ovarian laser evaporation does not influence pituitary priming and sensitivity in the short term. Information about pituitary priming and sensitivity in the long term is very limited. One other publication exists on the pituitary response on a GnRH ‘double pulse’ test after laser evaporation, showing an attenuated LH response on the first and second GnRH dose in the early follicular phase of a resumed second menstrual cycle (Rossmanith et al., 1991). Nowadays, this change in the pituitary response on the GnRH ‘double pulse’ test can be attributed to increased GnSIF/AF production secondary to becoming ovulatory. Combining the results of this and the present study, it can be argued that GnSIF/AF has no or a limited role in inducing ovulatory cycles after ovarian drilling, but increases in response to the initiation of an ovulatory cycle.

Strength, weaknesses and future perspectives

The strength of this study is the integrally measured endocrine profiles in combination with an optimal control group of PCOS patients undergoing a diagnostic laparoscopy only.

Limitations are the short follow-up period and the relatively small groups. The limited number of patients means that only overt endocrine changes would have been significant and smaller changes may not have been noticed. This is particularly the case in the Laparoscopy Only group, as the target of 12 patients was not reached. The complexity and costliness of the study furthermore limited the number of patients and duration of follow-up.

Furthermore, only 42% of the PCOS patients became ovulatory after the laser vaporization. This may be somewhat lower than expected.
Possibly, even stronger endocrine alternations could have been registered if the laser treatments had led to more ovulation. Unfortunately, follow-up of natural ovulation in the Laparoscopy Only group was not planned, since patients normally continue immediately with the routine ovulation induction. Therefore, a possible effect of this procedure on the ovulation rate could not be studied, which would have been interesting in retrospect after seeing the major short-term effects on the reproductive endocrine status.

Future studies will have to show for how long and which of the observed endocrine changes will hold. Furthermore, future studies analyzing markers of sympathetic nervous activity after surgery, such as adrenaline, noradrenaline, anti-diuretic hormone, adrenocorticotropic hormone and cortisol may potentially shine light on the role of this system herein (Adams and Hempelmann, 1991).

**Conclusion**

This study evaluated for the first time, in an integral way, the reproductive endocrine profile before and after laser evaporation in PCOS compared with an optimal control group of PCOS undergoing diagnostic laparoscopy only. Remarkably, most immediate endocrine changes after laser evaporation can be regarded as being related to the surgical context and not to the ovarian drilling procedure itself. All observed short-term endocrine changes after diagnostic laparoscopy only were transient, as all hormones returned to pre-surgical levels after 1 day. A comprehensive conclusion on the specific cause of these endocrine shifts cannot be made. The anesthesia could have an effect through changes in the central endocrine regulation, (para)sympathetic nervous system and circulation. Intra-abdominal manipulation of the organs could have endocrine effects directly or indirectly through the (autonomous) nerve system and FSH induced aromatase activity may be boosted. Furthermore, the observed changes may reflect a recovery to a normal levels, as a pre-operative changes in endocrine levels may have occurred due to stress. Laser evaporation-specific endocrine effects were the prevention of an immediate increase of inhibin B and the sustained decrease in testosterone, androstenedione and AMH. These changes are likely attributed to the destruction of ovarian tissue and the morphological changes caused by the ovarian drilling. At this time, it cannot be established which of these pathways are instrumental in causing ovulation in patients undergoing surgical treatment.

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**Authors’ roles**

M.L.H. was the investigator and writer of the article; T.K. was also an investigator; T.K. coordinated the blood samples; I.M., J.D., V.M. and P.G.A.H. were the surgeons; R.S. and E.M.K. participated in patient inclusion; R.H. co-designed the study; J.W.R.T. performed the statistics and C.B.L. was the senior investigator and co-designer of the study.

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**Conflict of interest**

There were no conflicts of interests mentioned by the authors.

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