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Hendriks, M.L.

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Chapter 8

Short-term changes in hormonal profiles after laparoscopic ovarian laser evaporation compared to diagnostic laparoscopy in PCOS: A prospective controlled study

Marja-Liisa Hendriks
Tamar König
Ted Korsen
Ingeborg Melgers
Judith Dekker
Velja Mijatovic
Roel Schats
Peter G.A. Hompes
Roy Homburg
Eugenie M. Kaaijk
Jos W.R. Twisk
Cornelis B Lambalk

ABSTRACT

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 of Inhibin B and the sustained decrease of testosterone, androstenedione and anti-Müllerian 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 surgical context.

Study design, size, duration: This prospective trial was performed at an outpatient academic fertility clinic. Between 2007-2010 a total of 21 oligo- or amenorrhic PCOS patients were included.

Participants/materials, setting, methods: Included were oligo- or amenorrhic PCOS patients with *all* 3 of the Rotterdam criteria and luteinising hormone (LH)>6,5U/L. All PCOS patients had an indication for diagnostic surgery due to subfertility. Twelve PCOS chose to undergo ovarian laser evaporation (CO₂ laser, 25W, 20 times/ovary) and 9 PCOS controls a diagnostic laparoscopy only. Reproductive endocrinology was measured before until 5 days after surgery, including 4 gonadotropin-releasing hormone (GnRH) 'double pulse' tests. Main outcome measures were changes in reproductive endocrinology and pituitary sensitivity/priming to GnRH after laser evaporation compared to diagnostic laparoscopy only.

Main results and the role of chance: The first hours after surgery both groups showed an increase of LH, follicle stimulating hormone (FSH), estrogen and a decrease of testosterone, androstenedione, AMH and insulin growth factor (IGF)-1 ($P<0.05$). Inhibin B increased in the Laparoscopy Only Group ($P<0.05$). The first days after surgery testosterone, androstenedione and AMH remained lower than baseline exclusively in the Laser Group ($P<0.05$). Pituitary sensitivity/priming to GnRH was not altered after laser evaporation.

Limitations, reasons for caution: Limitations 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 a diagnostic laparoscopy only. Interestingly, most immediate endocrine changes after laser evaporation can be related to the surgical context and not to the ovarian drilling procedure itself.

INTRODUCTION

Ovarian surgery by wedge resection or ovarian electrocoagulation has been and is used for years now as a treatment option for anovulatory patients with polycystic ovarian syndrome (PCOS) (Gjonnaess, 1984; Stein and Leventhal, 1935). 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, namely 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 has been postulated and reducing the size of the ovary was thought to allow gonadotropins to act more effectively (Ben Shlomo et al., 1998; Katz et al., 1978). Others have suggested that surgery may cause increased blood flow to the ovaries, resulting in increased delivery of gonadotropins (Cohen, 1996; Takeuchi et al., 2002). Studies showed that ovarian drilling results in less (free) androgens, lower luteinizing hormone (LH) levels and lower anti-Müllerian hormone (AMH) production (Elmashad, 2011; Hendriks et al., 2007). This altered endocrine situation seems to concord with increased ovarian sensitivity to FSH (Hendriks et al., 2007). Furthermore, changes in pituitary sensitivity and state of priming have been suggested to play a role (Rossmannith et al., 1991).

Interestingly, so far it was not known if the mentioned endocrine changes are a direct result of the actual intervention (ovarian drilling) or might be (partly) caused by the surgical procedure including mechanical manipulation/movement of the ovaries. Recently we have demonstrated that mechanical movement of the ovaries resulted in significantly altered LH levels in PCOS patients only (Hendriks et al., 2013). Multiple studies used regularly ovulating women undergoing (various types of) surgery as controls, which is not ideal, as it only shows the endocrine effects of the narcotics used and only in a non-PCOS group.

This study aimed to isolate endocrine effects of ovarian drilling per se. This was done by 1) measuring all the currently known potentially relevant hormones for the first time integrally in one study, including gonadotropin-releasing hormone (GnRH) tests, and 2) through the use of an optimal control group of PCOS patients undergoing diagnostic laparoscopy without ovarian drilling. This control group offers the unique possibility to differentiate between mechanical/ surgical and ovarian drilling influences.

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 (OLVG) 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.

Women were diagnosed as PCOS when *all* of the Rotterdam criteria were present: 1) Oligo- or amenorrhea (cycle >35 days), 2) biochemical/clinical hyperandrogenism (defined as a Ferriman-Gallwey score of ≥ 8 , testosterone >2.5 nmol/L and/or androstenedione >9.0 nmol/L) and 3) polycystic ovarian morphology (≥ 12 follicles (2-9mm) in at least one ovary) (, 2004). Furthermore, LH level was >6,5 U/l on cycle day 3-21 (with the exception of the LH surge) (Hendriks et al., 2008). 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 (clomifene resistance up to 150mg/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 in combination with ovarian laser evaporation (Laser Group). This trial was deliberately not set up as a randomised controlled trial, as we found it not ethical to randomise between such different treatment modalities and its consequences for future treatment.

Furthermore, an extra control group was used for the GnRH tests (see further). Included 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, oral contraceptive or levonorgestrel-releasing intrauterine system use.

Surgery

Around cycle day 14 (after a progesterone induced withdrawal bleeding) laparoscopy was performed in all PCOS patients. All surgeries were performed in the VU University medical center by experienced surgeons (I.M., J.D. S.T. and V.M.). Tubal patency was assessed with Methylene Blue and the macroscopic aspect of the uterus, tubes and ovaries were examined and possible fertility obstructing abnormalities were removed in both groups. In the Laser Group a carbon dioxide (CO₂) laser (Lumenis) was used on continuous firing mode on 25W, applied 20 times on both ovaries for 6 seconds at a time (Daniell and Miller, 1989; Keckstein et al., 1990; Verhelst et

al., 1993). A CO₂ laser was used as it was the preference of the surgeons, due to the superficial and the limited amount of inflicted ovarian damage (Hendriks et al., 2010).

GnRH tests

GnRH 'double pulse' tests were performed to measure pituitary sensitivity and as an indirect measurement for gonadotropin surge inhibiting/attenuating factor (GnSIF/AF) activity (Danforth et al., 1987; de koning et al., 2001). All PCOS patients underwent GnRH 'double pulse' tests on 4 different occasions, namely cycle day 5-7, the day of surgery (before the laparoscopy), the first day and 5-7 days after the procedure. The regularly ovulating controls underwent one GnRH 'double pulse' test on cycle day 5-7. On all occasions 25 µg GnRH (Lutrelaf, Ferring B.V., The Netherlands) was administered twice through an IV canula with a 120 minutes interval. Blood was drawn for LH analysis before and after 30 and 60 minutes after both GnRH doses (Campo et al., 1993; Gjonnaess and Norman, 1987; Rommler et al., 1973). The LH increment 30 minutes 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 (de koning et al., 2001). For analysis the ratio of the absolute LH increment 30 minutes after the second compared to the first GnRH dose was used.

Endocrine measurements

To evaluate the endocrine changes after both procedures blood was drawn in all PCOS patient on multiple occasions. Blood samples were taken on the day of surgery before the laparoscopy, followed by hourly blood samples after surgery for 5 hours and daily samples for 5 days. Ovulatory status was evaluated in the Laser Group by measuring progesterone levels 28 days after surgery, a value above 3,6 nmol/l indicated ovulation. All blood samples were centrifuged at 3000 Hz for 10 minutes. The obtained serum was stored at -20 degrees Celsius until assay.

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), lower detection limit of 0.5 U/L for both hormones. For LH and FSH the intra-assay coefficient of variance (CV) was 3% and inter-assay 6-7%. Estradiol and progesterone: competitive immunoassay (Luminescence Architect, Abbott Laboratories, Illinois, USA), lower detection limit of 150 pmol/L for estradiol and 2 nmol/L for progesterone. Intra-assay CV for progesterone was 2% and inter-assay 5%, for estradiol 3-9% and 10% respectively. SHBG: immunometric assay (Luminescence Immulite 2500, Siemens Medical Solutions Diagnostics, USA), lower limit 2 nmol/L. Intra-assay CV was 2-3% and inter-assay 4%. Testosterone: radioimmunoassay (Coat-A-Count, Siemens Medical Solutions Diagnostics, USA), lower detection limit 1 nmol/L.

Intra-assay CV 6-8% and inter-assay of 10%. Androstenedione: radio-immunoassay (coated tubes DSL, Webster Texas USA), lower limit of detection was 0.5 nmol/L. Intra-assay CV 6-8%, inter-assay 9-12%. Insulin growth factor (IGF)-1: immunometric assay (Luminescence Immulite 2500, Siemens Medical Solutions Diagnostics, USA), lower limit 3.2 nmol/L. Intra-assay CV 5%, inter-assay 8%. AMH: Elisa (GEN II AMH elisa, Beckman Coulter), lower limit of 0.2 µg/L. Intra-assay CV 10%, inter-assay 8%. Inhibin B: non-competitive assay (Beckman Coulter, Woerden, The Netherlands) , lower detection limit 18 ng/L. Intra-assay CV 9% , inter-assay 8%. FAI (free androgen index) was calculated using the formula: $FAI(\%) = (\text{testosterone}/SHBG) \times 100$.

Power calculation

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 (Rossmann et al., 1991). Calculating with a decrease of pituitary sensitivity of 25% and 90% power, twelve patients per group would be needed.

Statistical methods

The independent samples t-test was used to compare the baseline values. Generalized estimating equations (GEE) 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 independent samples t-Tests 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 analyse 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 a diagnostic laparoscopy. Inherent to the non-randomisation nature of this study there was a risk of disproportionately 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 minutes (+/- 18) in the Laser Group and 36 minutes (+/- 12) in the Laparoscopy Only group ($p=0.2$). Potentially fertility obstructing factors were found in 2 patients (16,7%) of the Laser Group (uterus septum and mild unilateral adhesions, both were treated) and 2 (22.2%) in the Laparoscopy Only Group (unilateral tube factor in both patients).

Endocrinology

Baseline levels

Baseline endocrine levels were comparable between both PCOS groups (Table 1). The median of LH (+/-interquartile ranges) in the regularly controls was 4.2 U/L (+/-2.5), FSH 5.8 U/L (+/-1.6), average cycle length 28 days (+/-1) and BMI 24.5 (+/-7.4).

Table 1. 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/m ²)	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

Laser: Laser Group

DLS: Diagnostic Laparoscopy Only Group

Baseline levels were compared with an independent samples t-test.

1 to 5 hours after surgery

The first five hours after surgery a significant increase of LH, FSH and estradiol and a significant decrease of testosterone, androstenedione, AMH and IGF-1 compared to baseline values was seen in both groups (Figure 1). There were no significant differences between the two groups. Progesterone remained stable and comparable between both groups. Inhibin B increased in the

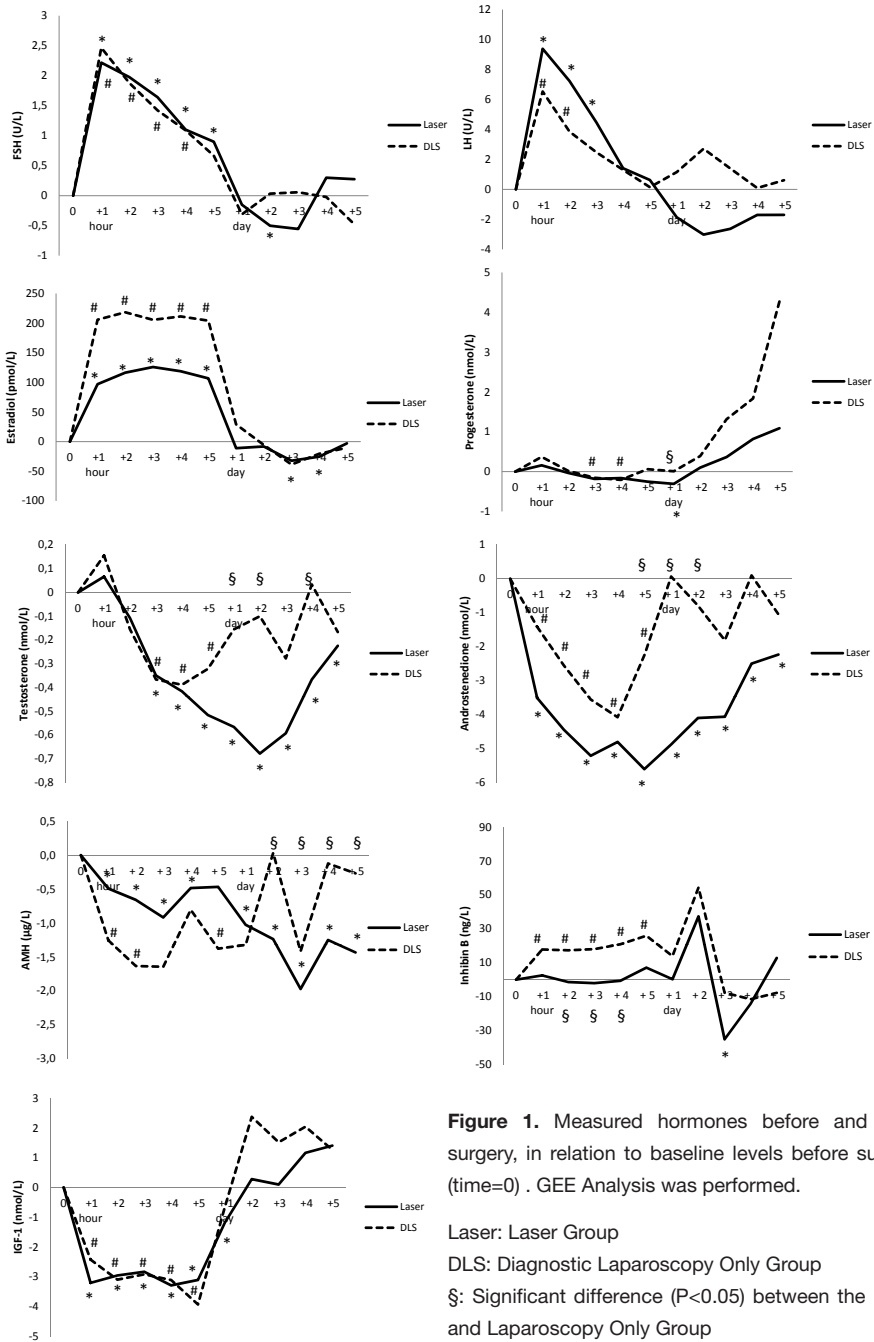


Figure 1. Measured hormones before and after surgery, in relation to baseline levels before surgery (time=0) . GEE Analysis was performed.

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 to before surgery (time=0) in Laser Group
 #: Significant change (P<0.05) of hormone compared to before surgery (time=0) in Laparoscopy Only Group

Laparoscopic controls only, with a significant difference between the Laser and the Laparoscopy Only Group in the first hours after surgery.

Day 1 to 5 after surgery

From day one to five 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 significant lower estradiol, testosterone, androstenedione, Inhibin B and AMH levels compared to baseline (at one or more 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 two or more days. Estradiol and Inhibin B did not show significant differences between the two groups (Figure 1).

GnRH tests

PCOS patients from the Laser (ratio 1.35, +/-0.70) as well as the Diagnostic Laparoscopy Only Group (1.43, +/-0.65) showed a significantly lower ratio than the regularly cycling controls (2.15, +/-0.53) ($P=0.04$ and 0.02 respectively) (Figure 2a). The ratio did not differ between the two PCOS groups at any of the four tests days. Within the Laser and Laparoscopy Only Group no significant differences were seen after surgery compared to before surgery. (Figure 2b and Table 2).

The LH response 30 minutes after the first GnRH dose can be considered as the measure of pituitary sensitivity. At cycle day 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) ($P<0.01$) (Figure 2a). Within the two PCOS groups no significant change was seen after surgery compared to before surgery. No significant differences were found between the two PCOS groups, except the first day after surgery showing a higher LH response in the Laparoscopy Only Group compared to the Laser Group. (Figure 2b and Table 2)

Follow-up

Five patients (41.7 %) became ovulatory within 28 days after ovarian laser evaporation. In the Laparoscopy Only Group spontaneous ovulation was not awaited as these women started or continued ovulation induction treatment with gonadotropins some weeks after the surgery.

DISCUSSION

The endocrine changes seen after the ovarian drilling procedures in PCOS patients are usually attributed to the morphological changes of the ovaries caused by the ovarian drilling. (Hendriks et al., 2010) The possible endocrine influence of anaesthesia (on central endocrine regulation,

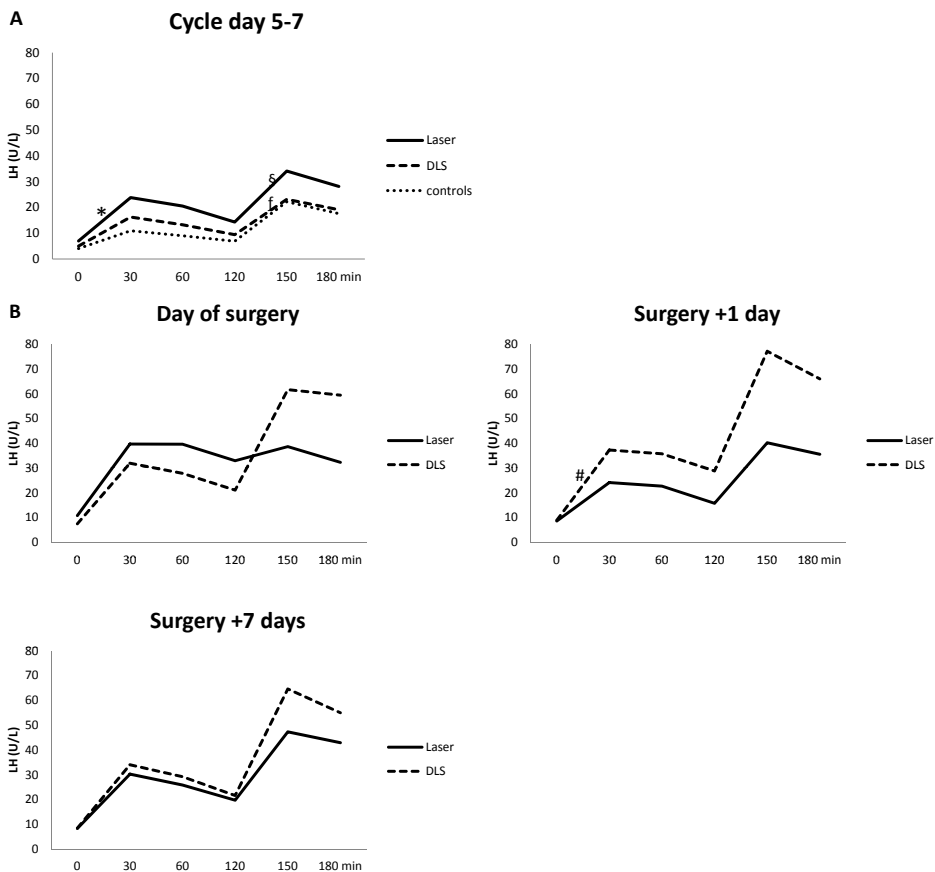


Figure 2a and b. GnRH ‘double pulse’ tests. 25µg GnRH was given at start (0 minutes) and 120 minutes on cycle day 5-7 (figure 2a) and before surgery, one and seven days after surgery (figure 2B)

Laser: Laser Group

DLS: Diagnostic Laparoscopy only Group

Controls: regularly cycling controls

*: $P < 0.05$, LH increment (0 until 30 minutes) after GnRH injection in Laser Group compared to regularly ovulating controls (Mann-Whitney U test).

§ and F: $P < 0.05$, Mean ratio of absolute LH change after the second compared to the first GnRH gift in Laser and Laparoscopy Only Group compared to regularly ovulating controls (Mann-Whitney U test).

#: $P < 0.05$, LH increment (0 until 30 minutes) after GnRH injection in Laser Group compared to Laparoscopy Only Group (independent samples t-Test).

Table 2. Mean ratio of absolute LH response after the second compared to the first GnRH injection (+/- SD) (top) and mean LH response 30 minutes after the first GnRH dose (+/- SD) (bottom).

	Laser (n=12)	DLS (n=9)	Laser After surgery versus before P-value*	DLS After surgery versus before P-value*	Laser versus DLS P-value **
Mean ratio					
Day of surgery	1.53 (0.65)	1.35 (0.61)	n.a.	n.a.	0.60
Surgery + 1 day	1.61 (0.56)	1.56 (0.72)	0.26	0.09	0.87
Surgery + 7 days	1.40 (0.53)	1.82 (1.03)	0.77	0.28	0.24
LH response to 1st GnRH (U/L)					
Day of surgery	28.9 (38.1)	24.5 (11.4)	n.a.	n.a.	0.74
Surgery + 1 day	15.6 (6.5)	28.3 (12.6)	0.26	0.18	0.02
Surgery + 7 days	21.9 (9.5)	25.6 (11.5)	0.60	0.68	0.43

*Paired samples t-Test

**Independent samples t-Test

Laser: Laser Group

DLS: Diagnostic Laparoscopy Only Group

n.a.: not applicable

(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 and for the first time compared this to an optimal control group of PCOS 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 1) the prevention of an immediate increase of Inhibin B and 2) the sustained decrease of 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 the first hours after surgery in both groups, followed by a return to baseline levels already after one day. Our data confirm some but not all literature (Hendriks et al., 2007). Since gonadotropin secretion is increased in both the ovarian drilling

and control group it is likely that these changes result from a combination of anaesthesia and surgical procedure and not from the ovarian drilling per se. There are no data on the effect of general anaesthesia on LH and FSH secretion in PCOS patients, but in regularly cycling women the gonadotropins 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 anaesthesia in the gonadotropin increase, most likely mediated through neuroendocrine mechanisms possibly via activation of the sympathetic-adrenomedullary system (Adams and Hempelmann, 1991; Hagen et al., 1980). This is supported by the finding that blocking the sympathetic signals with spinal anaesthesia results in a decrease of gonadotropin secretion (Adams and Hempelmann, 1991; Hagen et al., 1980). 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 of gonadotropin secretion after mechanical ovarian manipulation in PCOS patients undergoing ovulation induction with FSH (Hendriks et al., 2013). Although this change in gonadotropins 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 gonadotropin 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.

The response of ovarian hormones

There was a sharp decrease of testosterone, androstenedione and AMH seen directly after surgery in both groups. These hormones remained lower 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 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 intraoperative decrease of 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 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 wanted ovarian “damage” in order to obtain a substantial ovulation rate and indeed all patients showed sustained decline of AMH. On theoretical grounds it is possible that less punctures would have inflicted less ovarian damage and consequently less AMH suppressing effects.

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 of estradiol in the first days after surgery in only the Laser Group and is probably reflecting 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 seems to remain (globally) stable after surgery. Obviously, progesterone levels were low prior to surgery, because according to protocol all women were measured in the follicular phase. Results conform to the literature showing stable progesterone levels after ovarian drilling (Hendriks et al., 2007).

The combined observations of an immediate decline of testosterone, androstenedione and AMH in both groups after surgery and at the same time increasing estradiol and Inhibin B is puzzling. An all-embracing explanation is difficult. Possibly, a strong increase of aromatase activity mediated through the higher FSH levels directly after surgery, resulting in a decrease of the androgens and a concomitant increase of estradiol may be responsible. The higher FSH levels could have stimulated the Inhibin B production. Inhibin B and estradiol tend 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 anaesthesia and the manipulation of the internal organs on the autonomous nerve system with so far unknown effects on (PCOS) ovarian steroid production. Since all these 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 particular from day one to five after surgery such as the decreasing testosterone, androstenedione, AMH, estradiol and Inhibin B, seem to have resulted from the inflicted theca and granulosa cells destruction with lower production of their endocrine products as a consequence. In addition, through a paracrine route the lower Inhibin B levels could cause a further decrease in theca cell androgen production (Hillier et al., 1991).

Other hormones

IGF-1 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-1 shortly after surgery, followed by a recovery to baseline. Thus the laser evaporation per se did

not seem to influence the IGF-1 levels, conforming to the available literature (Amin et al., 2003; Tiitinen et al., 1993; Wu et al., 2004). This makes it unlikely that IGF-1 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 to regularly cycling women, which we confirmed in the present study (Rossmannith 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 antagonising GnRH (de koning et al., 2001; Fowler et al., 2003; Messinis et al., 1991). 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, GnSIF/AF being an ovarian product, a decrease could be expected 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 one 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, like estradiol, progesterone, Inhibin and GnSIF/AF (Lasley et al., 1975; Wang and Yen, 1975). Both groups showed no significant difference between the LH response over the study period indicating absence of change of 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 (Rossmannith 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 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 amount of patients means that only overt endocrine changes will be significant and smaller changes may not be noticed. This is particularly the case in the Laparoscopy Only Group, as the target of twelve patients was not reached. Only 42% of the PCOS patients became ovulatory after the laser evaporation. 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 routinely continue with ovulation induction immediately. Therefore, a possible effect of this procedure on ovulation rate could not be studied, which would have been interesting in retrospect seeing the major short term effects on the reproductive endocrine status. The complexity and costliness of the study furthermore limited the number of patients and duration of follow-up.

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, ADH, ACTH 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 to an optimal control group of PCOS undergoing diagnostic laparoscopy only. Remarkably, most immediate endocrine changes after laser evaporation can be regarded as 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 anaesthesia 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 change might be a recovery to a normal level, as a pre-operative increase/decrease might have occurred due to stress, as was recently suggested for androgens (Ott et al., 2014).

Laser evaporation specific endocrine effects were the prevention of an immediate increase of Inhibin B and the sustained decrease of 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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