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Hendriks, M.L.

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Chapter 7

Influence of ovarian manipulation on reproductive endocrinology in polycystic ovarian syndrome and regularly cycling women

Marja-Liisa Hendriks
Tamar König
Remi S. Soleman
Ted Korsen
Roel Schats
Peter G.A. Hompes
Roy Homburg
Cornelis B. Lambalk

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ABSTRACT

Objective: Little is known about the function of the ovarian neuronal network in humans. In many species, copulation influences endocrinology through this network. As a first step, the possible influence of ovarian mechanical manipulation on pituitary and ovarian hormones was evaluated in polycystic ovarian syndrome (PCOS) and regularly cycling women.

Design: Prospective case–control study (2008–2010).

Methods: Ten PCOS women (Rotterdam criteria) undergoing ovulation induction with recombinant-FSH and ten normal ovulatory controls were included in an academic fertility clinic. In the late follicular phase blood was drawn every 10 min for 6 h. After 3 h the ovaries were mechanically manipulated by moving a transvaginal ultrasound probe firmly over each ovary ten times. Main outcome measures were LH and FSH pulsatility and ovarian hormones before and after ovarian manipulation.

Results: All PCOS patients showed an LH decline after the ovarian manipulation (before 13.0 U/l and after 10.4 U/l, $P < 0.01$), probably based on a combination of a longer LH pulse interval and smaller amplitude ($P = 0.07$). The controls showed no LH change (before 9.6 U/l and after 9.3 U/l, $P = 0.67$). None of the ovarian hormones (estradiol, progesterone, anti-Müllerian hormone, inhibin B, androstenedione and testosterone) changed in either group.

Conclusions: Ovarian mechanical manipulation lowers LH secretion immediately and typically only in preovulatory PCOS patients. The immediate LH change after the ovarian manipulation without any accompanying ovarian hormonal changes point to nonhormonal communication from the ovaries to the pituitary. A neuronal pathway from the ovaries communicating to the hypothalamic–pituitary system is the most reasonable explanation.

INTRODUCTION

The most frequent cause of anovulation in women is polycystic ovarian syndrome (PCOS), characterized further by polycystic ovaries and hyperandrogenism (1). Multiple treatments are available for infertility associated with oligo- or anovulation. Commonly used are clomifene citrate and (recombinant)-follicle-stimulating hormone (r-FSH), but a surgical procedure is also an alternative. Wedge resection or, more recently, 'ovarian drilling' by electrocoagulation or laser-evaporization leads to (temporary) restoration of ovulatory cycles in most patients. These ovarian drilling procedures result in multiple endocrine alterations (2) and it is predominantly assumed that this is the result of the induced ovarian damage. However, it cannot be ruled out that the manipulation of the ovaries by itself during the surgery plays a role.

In many species, functional neurological pathways exist from the ovary to the CNS. These neurological pathways are stimulated during mating, sending signals to the gonadotropin-releasing hormone (GnRH) center in the hypothalamus. Subsequently, a pituitary luteinizing hormone (LH) surge is induced leading to ovulation. This is called 'reflex ovulation' and is the most efficient form of reproduction because of the optimal synchronization. Some species, for example rabbits, only ovulate after mating (reflex ovulators). Others, for example rats, are spontaneous ovulators, but can also ovulate after copulation (facultative reflex ovulators) (3). In humans, it is presumed that intercourse has no influence on the endocrine environment, follicular development or ovulation (4, 5, 6). The cycle is coordinated through interaction between the hypothalamic GnRH center, pituitary and the ovaries. Little is known about the nerve innervations of the ovary in humans and their possible influence on ovarian activity, even though the presence of intraovarian nerves was reported more than a century ago (7, 8).

It is remarkable that PCOS as a frequently occurring anovulatory disorder has a very high heritability rate (9). In evolutionary terms one would expect rapid extinction of such disorders, unless, at certain times in these women, anovulation could be overcome under natural conditions. We now know that one of these natural conditions may be aging, as many if not all anovulatory patients with PCOS become regular ovulators around the age of 40 (10). Another explanation could be that natural ovarian manipulation as a result of intercourse may introduce endocrine conditions that contribute to recurrence of ovulation. This led to the hypothesis that rudimentary neuronal interaction between ovary and brain exists in humans. This could play little or no role in the reproductive physiology of normal women, but such activated pathways could be employed as a salvage mechanism for restoration of ovulation in chronic anovulatory PCOS patients. In this study, as a first step, we evaluate the possible existence of such neuronal pathways in patients with PCOS and normal controls by measuring pituitary LH and FSH secretions and various ovarian hormones before and after mechanically moving the ovaries (mechanical ovarian manipulation).

MATERIALS AND METHODS

Patients

Patients attending the outpatient fertility clinic of the VU University Medical Centre (Amsterdam, The Netherlands), were asked to participate in the study (March 2008 until May 2010). Approval for the study was obtained from the Local Ethical Committee and patients gave written informed consent.

Ten PCOS patients and ten controls between 18 and 45 years were included. Patients were diagnosed as PCOS when besides oligo- or amenorrhea (cycle >35 days), biochemical/clinical hyperandrogenism (defined as a Ferriman–Gallwey score of ≥ 8 , testosterone >2.5 nmol/l and/or androstenedione >9.0 nmol/l) and/or polycystic ovarian morphology (≥ 12 follicles (2–9 mm) in at least one ovary and/or ovarian volume >10 ml) were present (1). The PCOS patients underwent ovulation induction with r-FSH (Puregon, Schering-Plough, Oss, The Netherlands), which was administered in the evening.

The controls had a regular cycle between 25 and 35 days and the tests were performed in a natural cycle of intrauterine insemination. Excluded were women with a history of ovarian surgery or pathology, WHO III imminent or premature ovarian failure (FSH cycle day 3 ≥ 10 U/l) or endocrine diseases.

Tests

In both groups the mechanical ovarian manipulation and endocrine tests were performed in the late follicular phase, the day the largest follicle was 17–20 mm in diameter. Blood was drawn every 10 min for 6 h (starting in the morning) through an i.v. canula. After 3 h of blood sampling a transvaginal pelvic ultrasound was performed. The size of the largest follicle(s) and endometrial thickness were measured. Furthermore, the ovaries were mechanically manipulated by moving the tip of the ultrasound probe firmly over both ovaries (ten times per ovary, from left to right and back, frequency ~15 times/min). The blood sampling and ultrasound were performed by two experienced doctors (ML Hendriks and T König). After the mechanical ovarian manipulation, blood sampling was continued for three more hours.

Endocrine measurements

The serum samples were 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 0.3 U/l for LH and 0.5 U/l for FSH. LH had an intra-assay coefficient of variability (CV) of 3–4% and interassay of 6–7%, and for FSH the intra-assay CV was 3–5% and interassay 6–7%. Estradiol (E2) and progesterone: competitive immunoassay

(Luminescence Architect, Abbott Laboratories), lower detection limit of 150 pmol/l for E2 and 2 nmol/l for progesterone. Intra-assay CV of E2 was 3–9% and interassay 10%, CV of progesterone was 2 and 5% respectively. Anti-Müllerian hormone (AMH): immunometric assay (DSL, Webster, TX, USA), lower detection limit of 0.1 mg/l, intra-assay CV of 5% and interassay of 8%. Inhibin B: immunometric assay (colorimetric, Serotec Limited, Oxford, UK), lower detection limit 15 ng/l, intra-assay CV of 5% and interassay of 9%. Prolactin: immunometric assay (Luminescence Advia Centaur, Siemens Medical Solutions Diagnostics, Malvern, PA, USA), lower limit of quantitation 0.05 U/l, intra-assay CV of 3–4% and interassay of 6%. Testosterone: RIA (Coat-A-Count, Siemens Medical Solutions Diagnostics), lower detection limit 1 nmol/l, intra-assay CV of 6–8% and interassay of 7–10%. SHBG: immunometric assay (Luminescence Immulite 2500, Siemens Medical Solutions Diagnostics), lower limit 2 nmol/l, CV intra-assay 2–3% and interassay 4%. Cortisol: competitive assay (Luminescence, Advia Centaur, Siemens Medical Solutions Diagnostics), lower limit of quantitation 30 nmol/l, intra-assay CV of 3% and interassay of 6–8%. Androstenedione: RIA (coated tubes DSL), lower limit of detection was 0.5 nmol/l, intra-assay CV of 6–8% and interassay of 9–12%.

LH and FSH were determined in duplicate in all samples. E2, progesterone, AMH, inhibin B, testosterone, androstenedione, SHBG, prolactin and cortisol were determined twice before the ovarian manipulation (at start and after 3 h) and twice after (directly after the ultrasound and after 3 h at the end of the test). Free androgen index (FAI) was measured using the following formula: $FAI (\%) = (\text{testosterone} / \text{SHBG}) \times 100$.

Statistical analysis

Statistical analysis was performed using SPSS Software (version 15.0, SPSS, Inc.). Baseline characteristics were analyzed with the independent T-test. The nonparametric Wilcoxon's signed-rank test was performed comparing the data before and after the ovarian manipulation. A P value <0.05 was considered to be statistically significant.

Pulse analysis

LH results were individually plotted and used for LH pulse detection. Pulse analysis was performed with the validated pulse detection method from Lambalk et al. (11). The algorithm used in this pulse analysis is valid for replicate repeated measurements of LH with a chance of <5% to indicate nonexistent pulses. Nadirs preceding the pulses are indicated as marker points, rather than the pulses themselves. The LH pulse interval was calculated by measuring the average time between the pulses. The average LH amplitude was assessed by calculating the difference between the nadir and peak value of each individual pulse.

RESULTS

Ten patients with PCOS and eight control patients were included in the final analysis. In retrospect we excluded two of the regularly ovulating controls because they showed postovulatory progesterone values during the test.

The baseline characteristics are shown in Tables 1 and 2 (mean levels before ovarian manipulation). All characteristics in Table 1 were comparable except for the length of the menstrual cycle and the cycle day on the day of the tests. Both were significantly longer in the PCOS group, as could be expected. Endocrinologically the PCOS group and the controls showed comparable baseline LH, FSH, E2, progesterone, inhibin B, cortisol and prolactin levels on the day of ovarian mechanical manipulation ($P=0.06$, 0.38 , 0.67 , 0.20 , 0.16 , 0.36 and 0.26 respectively). Baseline AMH, testosterone, androstenedione and FAI were significantly higher in the PCOS group ($P=0.006$, 0.04 , 0.02 and 0.001 respectively).

The mean hormone levels before compared with after the ovarian manipulation are shown in Table 2. All ten PCOS patients showed a decline in LH and FSH levels after the mechanical ovarian manipulation (Figure 1). The mean LH decreased from 13.0 U/l before the mechanical ovarian manipulation to 10.4 U/l ($P=0.005$) after the manipulation. The average FSH levels decreased from 5.6 to 5.1 U/l ($P=0.005$) in the PCOS. In contrast, the controls did not show any significant changes in LH and FSH levels (mean LH before: 9.6 U/l and after 9.3 U/l, $P=0.67$ and mean FSH levels before 4.8 U/l and after 4.6 U/l, $P=0.3$; Table 2 and Figure 2). The PCOS patients injected their r-FSH for ovulation induction in the evening, thus potentially the FSH decrease seen in the PCOS group reflects the decline expected during the day by clearance.

LH pulse analysis showed nonsignificant increases in both groups of the mean LH pulse interval after the ovarian manipulation (PCOS, $P=0.31$; controls, $P=0.18$). The average LH pulse amplitude was 4.1 U before the ovarian manipulation and 3.8 U ($P=0.61$) after the procedure in the PCOS, and 1.6 and 2.4 U respectively in the controls ($P=0.12$). The LH pulse frequency times amplitude tended to be significant ($P=0.07$) in the PCOS group whereas it did not show any change in the controls ($P=0.4$).

All measured ovarian hormones did not change significantly in either group before compared with after the mechanical ovarian manipulation (Table 2). Cortisol decreased in both groups significantly after the ovarian manipulation compared with before ($P=0.009$ in the PCOS group and 0.012 in the controls).

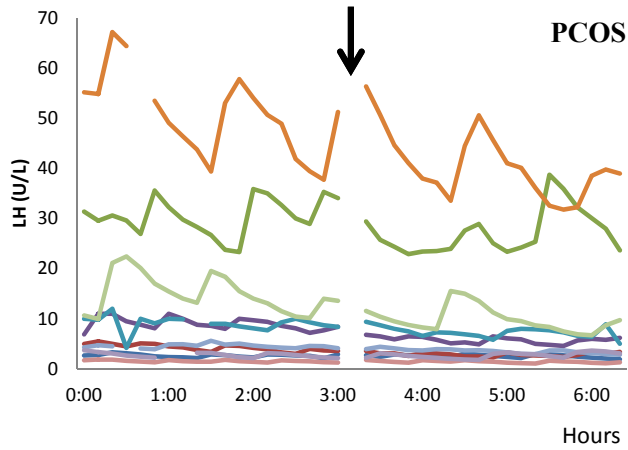


Figure 1. LH levels before and after the ovarian manipulation of all PCOS patients. The arrow indicates the time of mechanical ovarian manipulation.

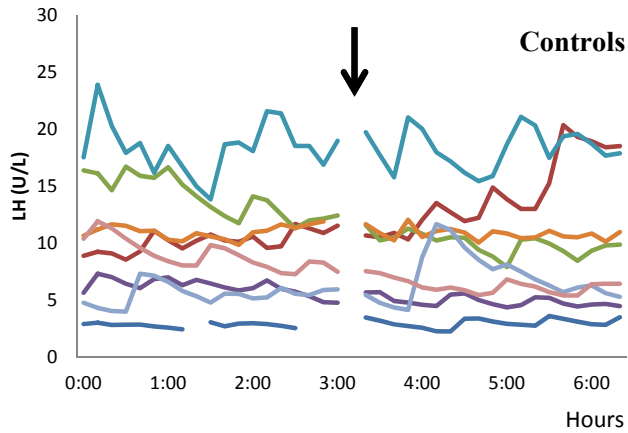


Figure 2. LH levels before and after the ovarian manipulation of all regularly ovulating controls. The arrow indicates the time of mechanical ovarian manipulation.

Table 1. Baseline characteristics of the PCOS patients and controls.

	PCOS (+/- SD) (n=10)	Controls (+/- SD) (n=8)	p-value
Age (years)	30.2 (4.2)	33.6 (5.0)	0.15
BMI (kg/m ²)	29.2 (5.5)	23.2 (3.1)	0.06
Average cycle length (days)	70 (44)	27 (1)	0.01
Size of largest follicle (mm)	17.4 (1.8)	17.4 (1.4)	0.96
Endometrial thickness (mm)	8.8 (1.6)	8.4 (2.3)	0.66
Cycle day (test day)	19.0 (4.0)	11.1 (1.4)	<0.01
Mean coital frequency/week	1.8 (1.1)	1.5 (0.6)	0.44

Values are mean (+/- 1 S.D.). Size of the largest follicle and endometrial thickness were measured on the test day in the late follicular phase.

DISCUSSION

Our study shows that mechanical manipulation of the ovaries, in patients with PCOS in the late follicular phase undergoing ovulation induction with r-FSH, results in an immediate decline of LH secretion. This decrease is likely the result of a combined decline in LH pulse frequency and LH pulse amplitude. At the same time none of the ovarian hormones that could be of any influence changed. In regularly ovulating women, around the same time of follicle development in a natural cycle no changes in LH secretion were observed.

Our findings support the possibility that procedures like laparoscopic ovarian electrocoagulation in PCOS may not only affect as is commonly assumed, via the feedback circuitry via destruction of hormone producing ovarian tissue (2), but also via ultrarapid (neuronal) pathways that are of influence on the hypothalamic–pituitary system. The immediate change in the LH secretion as we have demonstrated here is suggestive for the existence of such pathways.

For several reasons this first experiment was carried out in the late follicular phase and not under follicle recruitment. In the first place, we were forced to choose a clinical condition that would allow us to perform the invasive mechanical manipulation without major ethical objections. This brought us to ovulation induction in PCOS patients and intrauterine insemination treatments in regularly ovulating patients. In both clinical settings, vaginal ultrasound evaluation is the standard procedure. The second and more important reason was to measure PCOS patients and controls under similar conditions of follicle development with as much as possible similar reproductive (endocrine) conditions. Finally, it was expected that the most likely moment to observe any sign of a rudimentary reflex ovulation mechanism would be around the time of ovulation.

Table 2. Mean hormonal values before compared with after mechanical ovarian manipulation (\pm 1 S.D.) in PCOS patients and controls.

	PCOS		Controls		p-value
	Mean level before ovarian manipulation (n=10) (\pm -SD)	Mean level after ovarian manipulation (n=10) (\pm -SD)	Mean level before ovarian manipulation (n=8) (\pm -SD)	Mean level after ovarian manipulation (n=8) (\pm -SD)	
LH (U/L)	13.0 (15.7)	10.4(13.1)	9.6 (5.0)	9.3 (5.1)	0.67
LH pulse interval (min)	58 (13)	68 (26)	53 (25)	65 (21)	0.18
LH pulse amplitude (U)	4.1 (5.0)	3.8 (4.6)	1.6 (1.0)	2.4 (1.6)	0.12
Mean number of LH pulses / 3 hours	3.1 (0.7)	2.7 (0.8)	3.3 (1.3)	2.6 (0.5)	0.24
Number of LH pulses times LH amplitude	12.8 (15.0)	8.2 (8.9)	5.5 (4.5)	6.4 (4.6)	0.40
FSH (U/L)	5.6 (2.6)	5.1 (1.9)	4.8 (1.6)	4.6 (1.8)	0.33
Estradiol (pmol/L)	865 (805)	842 (713)	736 (289)	758 (267)	0.48
Progesterone (nmol/L)	2.1 (0.1)	2.1 (0.1)	2.0 (0.0)	2.0 (0.0)	1.0
AMH (μ g/L)	11.0 (6.4)*	10.8 (6.2)	3.6 (2.0)*	3.5 (1.9)	0.52
Inhibin B (ng/L)	275.1 (373.3)	287.8 (384.4)	77.1 (13.1)	66.1 (14.8)	0.09
Androstenedione (nmol/L)	12.3 (6.8)*	12.8 (5.4)	6.0 (1.7)*	6.5 (1.7)	0.14
Testosterone (nmol/L)	1.59 (0.88)*	1.57 (0.69)	0.86 (0.21)*	0.69 (0.34)	0.62
FAI (%)	5.12 (2.46)*	5.09 (2.34)	1.50 (1.06)*	1.51 (1.23)	0.67
Cortisol (nmol/L)	233.2 (87.7)	185.2 (81.8)	269.6 (75.5)	188.2 (75.1)	0.012
Prolactin (U/L)	0.20 (0.04)	0.20 (0.04)	0.18 (0.06)	0.17 (0.06)	0.34

*P<0.05, comparing baseline levels (before ovarian manipulation) of PCOS to the baseline levels of controls.

The key questions to be answered are via which mechanisms ovarian manipulation alters episodic pituitary LH secretion and why only in preovulatory PCOS patients. The neuronal pathway is the most likely route in accordance with the immediateness of the induced change and since no change of any relevant feedback hormones could be observed.

Neuronal pathways

What do we know from existing ovarian neuronal pathways? In reflex ovulating species (like rabbits, camels and cats) genital-somatosensory signals from copulation result in activation of midbrain and brainstem noradrenergic neurons (3, 12). Consequently, the hypothalamus is activated to release GnRH and this leads to a pituitary LH surge that results in ovulation (12). The rise in LH can already be seen 3 min after ejaculation (13).

Spontaneous ovulatory animals with also the potential to induce a reflex ovulation (facultative reflex ovulators), like rats, have autonomic nerves (parasympathetic and sympathetic) and visceral sensory fibers innervating the ovary (14, 15). Copulation in the spontaneous ovulating rats can trigger or hasten ovulation and cervicovaginal stimulation increases the output of eggs (12, 16). Furthermore, it seems that the ovarian nerves can influence the hormonal activity and interfere with cyclicity (14). Transsection of the efferent parasympathetic fibers in rats (vagotomy) altered the length of the estrous cycle (17) and caused a delay of the onset of puberty (18). It seems that both efferent and afferent ovarian nerves in rats can influence ovarian function and that ovarian function in normally spontaneous ovulating rats is not only controlled by the hypothalamic–pituitary axis but also by neural influence.

In the human little is known about the ovarian nerve innervations and their possible influence on ovarian activity. What we do know is that the extrinsic innervation of the human ovary is composed of (at least) both sympathetic and sensory fibers. Furthermore, nerve fibers are distributed through the different ovarian compartments, such as blood vessels, ovarian stroma and follicle wall (7, 19). PCOS ovaries have a higher density of nerve fibers than ovaries from cyclic women (20). The presence of extrinsic innervations and intrinsic neurons in the human ovary raises the possibility that they may be engaged in regulating ovarian function (19).

But how can the difference between the regularly ovulating controls and the PCOS patients be explained? We know that the PCOS ovaries have a higher nerve density (20), so potentially there might also be a difference in the presence or the amount of afferent nerve fibers. These could both result in altered afferent feedback in the PCOS after ovarian mechanical manipulation. Another explanation could be a higher ovarian sympathetic tone. In rats an increased sympathetic tone seems essential for the existence and maintenance of PCOS, as restoration of cyclicity can be

achieved by transection of the sympathetic innervations (21, 22, 23, 24). In humans there is also evidence that PCOS is associated with an increased sympathetic nerve activity compared with controls (25). It is well known that PCOS patients have more central obesity, an increased chance of developing hypertension and other cardiovascular diseases and diabetes. All these aspects of PCOS are also features of increased sympathetic tone (26, 27, 28). The data of the animal and human studies both support the involvement of sympathetic neuronal influence in the existence and maintenance of PCOS. Combining the results from our study with this information, one could speculate that ovarian manipulation may temporarily inhibit/normalize the higher ovarian sympathetic nerve activity. Subsequently, this information is sent through afferent nerve fibers directly to the hypothalamus or indirectly via other locations of the CNS, resulting in a decline in pituitary LH secretion.

The same could be true for ovarian drilling and ovarian wedge resection. These surgical procedures result in changes of ovarian and pituitary hormones (2). The primary reason for the hormone changes is believed to be destruction of ovarian tissue. But disruption of nerve connections within the ovary or disruption of the afferent/efferent (sympathetic) nerve fibers could also play a part (24, 29). Furthermore, the effect of ovarian drilling is usually temporary. Transition after months to years from ovulatory cycles to anovulation is hard to explain when the basis of the primary recovery of ovulatory cycles is (permanent) ovarian damage. It has been suggested that re-innervation of the ovary could be the cause for the progress to anovulation after months to years after the ovarian drilling procedures (24). Ovarian sympathetic innervations seem to recover quickly after transplantation of the ovaries to an ectopic site (30).

Interestingly, the LH decreased after the ovarian manipulation in PCOS, contrary to the LH increase seen after copulation in reflex ovulators. The reason for this difference is unclear. In patients with PCOS the LH pulse frequency and amplitude are relatively high (31, 32, 33, 34). Potentially, the LH frequency and amplitude are 'normalized' after ovarian manipulation, thus restoring it to the normal (non-PCOS) situation. Could it be a salvage mechanism for the otherwise anovulatory PCOS patients? Or is the (temporary) lowered/normalized LH a trigger for the induction of follicle growth? Future studies can hopefully answer these important questions.

Ovarian hormones

In this study all measured ovarian hormones remained stable after manipulation in both groups. Thus the observed decrease of LH in the PCOS group is not likely to be due to a different ovarian hormonal feedback. But, other less or yet unknown ovarian hormones could play a role in the LH change seen after ovarian manipulation, for example gonadotropin surge inhibiting/attenuating factor (GnSIF/AF). GnSIF/AFs cannot be measured directly, because its molecular

structure has not been fully identified yet. GnSIF/AF normally influences LH through inhibition of the GnRH-induced LH secretion and antagonizing the self-priming effect of GnRH (35, 36, 37). All measurable ovarian hormones in this study did not change, thus it is not likely that GnSIF/AF would be the only ovarian hormone that would change after the manipulation. Furthermore, GnSIF/AF protein synthesis takes time and LH levels show an immediate decline.

Pituitary sensitivity

No direct information about pituitary priming before or after ovarian manipulation is available from this study, because the nature of the current experiment did not allow it to combine with GnRH tests. Nevertheless, analyzing the LH pulse data shows that the LH decrease in PCOS after ovarian mechanical manipulation seems to be based on a combination of a lower LH pulse frequency and a lower amplitude. The combination of the quick LH change and the decreasing LH pulse frequency suggests a centrally induced LH decline, making a change in the pituitary priming less likely.

Other possible ways to influence LH secretion

Besides the above-mentioned possible pathways to influence LH secretion after ovarian manipulation, one could expect pain stimuli or stress stimuli to be of influence on the LH secretion (38). Both groups in our study received the same interventions and therefore it can be assumed that neither stress nor pain is the reason for the LH change seen. This is supported by the observed cortisol levels, which decreased after the ovarian manipulation in both groups. The decrease of the stress hormone cortisol is very likely the reflection of the cortisol night–day pattern, which shows a peak in the morning (39). Furthermore, the start of the test day with an i.v. canula and an intravaginal ultrasound could have induced stress with a subsequent decrease over time, reflecting in lower cortisol levels. The fact that cortisol shows a comparable decrease in both groups suggests that it is not the explanation for the observed difference in pituitary LH output in the PCOS group.

No circadian rhythm is present for LH and FSH in regularly ovulating women (40). PCOS women on the other hand do show a diurnal pattern with the highest LH levels in the late afternoon (41). The data from our study show an LH decrease during the day in PCOS, which does not resemble the known day pattern of LH. Thus the circadian pattern of LH is not the explanation for the lower LH seen after the ovarian manipulation.

Strengths, weaknesses and future perspectives

This is the first study to show direct changes in the pituitary secretion after manipulation of the ovaries in PCOS and this raises more questions than answers. Although the group sizes in this study are small, the fact that all ten PCOS patients individually show an LH decline after the ovarian manipulation is suggestive of a real phenomenon.

Obviously, the results of this study have to be confirmed by larger and more extended studies. Multiple issues should be addressed in these future experiments, for example, a longer period of endocrine measurements to evaluate how long the effect of ovarian manipulation holds and testing in different phases of the cycle. Furthermore, GnRH tests should be performed to determine if pituitary sensitivity alters after ovarian manipulation. Unfortunately, within this study the FSH analysis and conclusions were limited, due to the fact that PCOS patients received r-FSH to induce their menstrual cycle and the regularly ovulating women did not. Thus important information can be gathered from a PCOS group without ovulation induction and a PCOS group undergoing ovulation induction without ovarian mechanical manipulation. Future experiments have to indicate whether the mechanical manipulation of the ovaries in PCOS patients can contribute to initiation of their arrested follicle growth. So far we have only seen that there might be pathways that exist that may be employed as such. Hopefully future studies will answer the questions raised and provide information about the biological background of these pituitary changes.

Conclusion

Ovarian mechanical manipulation influences pituitary LH secretion immediately and typically only in preovulatory PCOS patients. The LH decrease might result from a combination of a lower LH pulse frequency and a lower amplitude. The immediate LH change after the ovarian manipulation without any accompanying ovarian hormonal changes points to nonhormonal communication from the ovaries to the pituitary. A neuronal pathway from the ovaries communicating to the hypothalamic-pituitary system is the most reasonable explanation. These neuronal pathways are present in many species. This is the first indication that neuronal interaction between ovary and brain may also exist in the human and that these pathways could be activated as a salvage mechanism for restoration of ovulation in the otherwise anovulatory PCOS. The mode of action could be via (temporary) normalization of the high sympathetic tone innervating the PCOS ovaries.

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