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Hendriks, M.L.

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Chapter 9

General discussion and summary

GENERAL DISCUSSION AND SUMMARY

The aim of this thesis was to acquire a further understanding of the neuroendocrine control of PCOS. Today, some years and studies later after we started the project various new and innovating aspects have come up, which shed some new light on the reproductive endocrinology in PCOS. In the coming paragraphs the results of the performed studies will be discussed.

Fertility treatment in PCOS

PCOS is common and has a prevalence of 5-16% depending on the population and criteria used (1;2). Many PCOS patients with a child wish do not visit a fertility specialist, as they have sufficient ovulations to conceive naturally within a few years. Besides, by losing around five percent of their bodyweight many overweight PCOS patients resume ovulatory cycles. So there must be many lean PCOS patients without cycle disturbances who will not need fertility assistance unless they increase in weight (3). Thus PCOS patients who need ovulation induction in the fertility departments are a selection of the existing PCOS patients. If ovulation induction is necessary clomifene citrate is accepted as first choice method, as it is cheap, easy to use with a low check-up frequency, offers good ovulation (75-80%) and acceptable cumulative conception rates after six months (38-66%) (4;5). The preference for clomifene citrate comes from an era when ovulation induction with gonadotropins was a challenge. Nowadays, the precisely dosed FSH injections and the chronic low dose step-up strategy offers an improved (in terms of less multiple pregnancies) and safer (in terms of less ovarian hyperstimulation syndrome) treatment (6). In **chapter 6** the hypothesis that pregnancy and live birth rates are higher after ovulation induction with low-dose FSH than with clomifene citrate as first-line treatment was tested. This study showed indeed that reproductive outcome (cumulative pregnancy and live birth rate) was superior after three cycles of ovulation induction with FSH compared to clomifene citrate (52 versus 41% and 47 versus 37 %, respectively). Furthermore, this was accompanied by an acceptable twinning rate of 3.4% with FSH and an extremely low rate (0%) with clomifene. These results are in line with another publication, showing a cumulative clinical pregnancy rate after three treatment cycles of 43% with FSH and 24% with clomifene (7). This does not immediately imply FSH as the first line treatment, since costs, risks and convenience should also be taken in to account. Nevertheless, FSH could be considered as first line treatment in PCOS woman seeking fertility assistance, when sufficient monitoring experience and financial potency is available as pregnancy is achieved faster and more often.

In case of clomifene resistance or after various ovulation induction cycles with FSH ovarian drilling is nowadays a treatment option for oligo- or amenorrheic PCOS patients (8;9). However, remarkably the various ovarian drilling techniques were used without sufficient knowledge about the amount of possible permanent ovarian damage. In **chapter 5** the grade of ovarian damage

caused by the most frequently used ovarian drilling procedures (CO₂ laser, monopolar and bipolar electrocoagulation) was evaluated by testing these techniques on bovine ovaries. The bovine ovary resembles the human ovary, as size, cellular morphology and physiology approximately match (10). Due to the high resemblance it gives a good indication of the amount of expected ovarian damage by the various ovarian drilling procedures in human. Especially the commonly advised bipolar electrocoagulation resulted in substantial ovarian tissue loss. Extrapolating it to the human PCOS ovary approximately one fourth of the ovary would be destroyed, when using the usually applied number of punctures. The other two evaluated techniques, CO₂ laser and monopolar electroagulation, would damage approximately 5% of an average PCOS ovary. Damaging up to a quarter of both ovaries will invariably reduce the ovarian reserve. This was confirmed by multiple studies which showed lower antral follicle count, inhibin B, AMH and higher FSH levels after ovarian drilling (11-15). These results point to ovarian damage in, at least, the short term. The long term effects of ovarian drilling are largely unknown now. It is important to know if the ovarian drilling causes reduction of the onset age of menopause or even premature ovarian failure. The interval between the procedure and the normal onset of menopause is 20-30 years, which makes long term follow-up a challenge. So far data on the follow up of patients up to 12 years after ovarian drilling are reassuring and showed no premature ovarian failure (8;16). The coming decade will probably bring more long term follow-up data of ovarian drilling including endocrinology, so 'time (and money) will tell'.

Neuro-endocrinology in PCOS

PCOS is a complex disorder with heterogeneity of clinical and endocrine features. Elevated LH is common in PCOS, but formally no part of the diagnostic criteria (17). In many, if not all, reports LH concentrations are assessed in the early follicular phase (around cycle day 3) when LH is still suppressed by the previous luteal phase or progesterone induced withdrawal bleeding (18;19). The reported prevalence of elevated LH (35-77%) in literature is therefore presumably an underestimation (20-23). In **chapter 3** LH was measured during the so called 'specific oligomenorrhoeic phase' (23). This phase is between at least 2 weeks from the start of the menstruation and at least 3 weeks prior to a next menstruation and only exists in cycles longer than 35 days. Under these conditions LH is least under influence of a previous luteal phase or progesterone treatment and an upcoming hectic midcycle hormonal event. In this study elevated LH levels were found in 84% of the PCOS women (defined according to the Rotterdam criteria) (17), when using the optimal cut-off point of ≥ 6.5 IU/l (sensitivity 84%, specificity 78%). For a subgroup of patients with oligo/amenorrhoea and hyperandrogenism (1990 NIH criteria), a sensitivity of 85% and specificity of 78% was found. The high prevalence of elevated LH as measured under these conditions makes it an interesting feature that should be used as an additional diagnostic test. Recent studies have shown that elevated AMH could also function

as a tool for the diagnosis of PCOS, with a sensitivity of 92-95% (NIH criteria) (24;25). Elevated levels of these hormones will be especially helpful in cases when differentiation with other causes of oligo- or anovulation is difficult to make and/or when transvaginal ultrasound is not possible or desirable.

It seems that not only the prevalence, but also the pathophysiological importance of LH in PCOS is underestimated. LH seems an essential hormone in understanding PCOS. It is known that LH levels reduce with age in PCOS, simultaneously with decreasing ovarian reserve and the resumption of regular menstrual cycles (26;27). Regaining regular menstrual cycles through ovarian drilling is also accompanied by decreasing LH levels (28). Furthermore, follicle number, ovarian volume, cycle disturbances and infertility rate are positively correlated with LH (22;29;30). All the above suggests that the level of LH correlates with the severity of PCOS. The question remains if the higher LH levels are part of the cause of anovulation or an expression of inadequate folliculogenesis. The higher LH levels found in PCOS are a consequence of increased amplitude and frequency of pituitary LH pulses (31). It is postulated that the higher pituitary response on GnRH is caused by insufficient ovarian gonadotropin surge inhibiting/attenuating factor (GnSIF/AF) production due to the lack of normal follicle growth in PCOS (32-34). Thus, from this point of view resumption of regular menstrual cycles should lead to lower LH through normalization of ovarian GnSIF/AF production. This was seen in a study which showed altered pituitary priming (lower response to GnRH suggesting increased GnSIF/AF levels) in combination with lower LH levels in a second ovulatory cycle after ovarian drilling in PCOS (35). Unfortunately, further investigation is hampered by the difficulty in measuring GnSIF/AF. In **chapter 4**, purification of GnSIF/AF was described and two new putative GnSIF/AF bioactive molecular weight forms were isolated. In total seven potential GnSIF sequences have been published, but so far the 'true' GnSIF remains unknown (36-40). The found putative GnSIF/AF forms in our study were the first that showed influence on intracellular processes involved in GnRH self-priming and the biological action of GnSIF/AF was preserved in the produced synthetic peptide. These results provide strong evidence that the identified candidate proteins are true GnSIF/AF. Future research will hopefully identify the full molecular structure of GnSIF/AF and create the possibility to produce an assay for direct measurement of GnSIF/AF levels. This would boost the knowledge on GnSIF/AF and hopefully answer the remaining questions.

Endocrine implications of ovarian drilling

Ovarian drilling has been used in PCOS since decades for the induction of regular ovulatory cycles. It is invariably reported that it alters the endocrine profile, but the mechanisms behind the reversal of the endocrine dysfunction in PCOS remained incompletely understood. In **chapter 2** the literature was reviewed and all available papers were systematically combined to identify

the endocrine changes after ovarian drilling, in order to glean more knowledge of the possible mechanisms involved. During the first days after ovarian drilling, a rapid reduction of all ovarian hormones was seen, in combination with increased secretion of pituitary LH and FSH. With initiation of folliculogenesis an increase in estrogen was seen, simultaneously with a reduction of pituitary LH and FSH. Subsequent ovulatory cycles after ovarian drilling occurred in an environment with less androgens, lower LH and FSH levels compared to pretreatment levels. After publication of this review information was published on AMH and Inhibin B levels after ovarian drilling including our own study (**chapter 8**), showing reduction of both ovarian reserve markers (34;41-44).

The endocrine changes seen after ovarian drilling surgery are usually attributed to the implied ovarian damage, but could (also) be the result of various other processes, namely the manipulation of the internal (including genital) organs during surgery and the anesthesia with all its potential effects. To distil the endocrine changes caused by the ovarian drilling procedure itself a prospective trial was performed. In **chapter 8** the endocrine changes in PCOS patients undergoing an ovarian drilling procedure and PCOS controls who underwent a diagnostic laparoscopy only were compared. The only difference between both groups was the ovarian drilling procedure (by CO₂ laser evaporation), which made it possible to extract the endocrine changes that were attributed exclusively to ovarian drilling. Laser evaporation in PCOS resulted in a sustained decrease of testosterone, androstenedione and AMH and prevented an increase of inhibin B in the first hours after surgery. All other endocrine shifts after surgery, namely an increase of LH, FSH, estrogen and a decrease of testosterone, androstenedione, AMH and IGF-1 during the first hours after surgery occurred in both groups and could be regarded as surgery related (Figure 1).

The laser specific endocrine effects could be part of the essential endocrine environment necessary for the induction of ovulatory cycles. Possibly, the combination of permanently lower androgens and inhibin B restores feedback to the hypothalamus and pituitary, resulting in appropriate gonadotropin secretion. Furthermore, the lower AMH levels could cause the FSH to act more effectively. There are animal studies that indicate that AMH reduces the sensitivity of the ovary to FSH (47). The effects of GnSIF/AF seemed limited directly after ovarian surgery, as pituitary sensitivity and priming state were not altered. High levels of androgens, AMH and inhibin B are all known and cardinal features of PCOS. Lower levels of these hormones through other routes than ovarian drilling, for example in association with aging, are also accompanied with the induction of regular ovulatory cycles (48-50). This supports the general idea that such changes are essential for induction of folliculogenesis.

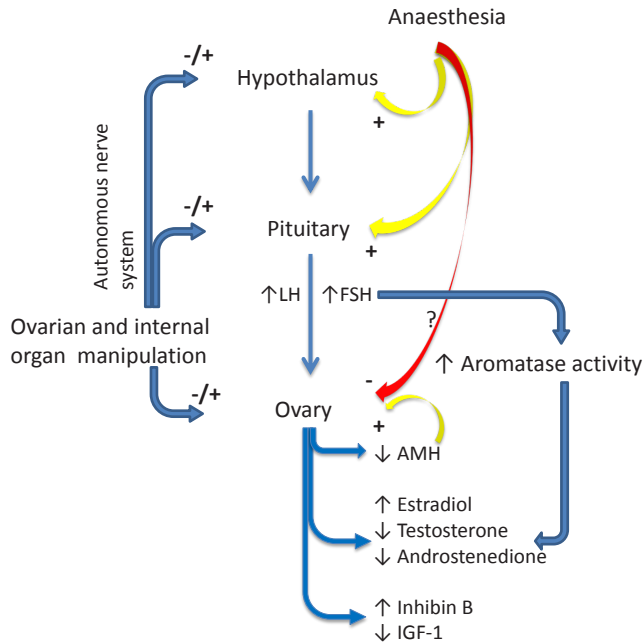


Figure 1. Potential mechanism behind the endocrine effects of diagnostic laparoscopy only.

The temporary endocrine shifts seen the first hours after diagnostic laparoscopy are a combination of the effects of anaesthesia and the surgical procedure itself. Hypothetically, anaesthesia could have a direct or indirect effect on the pituitary and hypothalamus, mediated through neuroendocrine mechanisms possibly via activation of the sympathetic-adrenomedullary system (45;46) Furthermore, manipulation of the internal organs could affect the autonomous nerve system with so far unknown consequences on hypothalamus, pituitary and ovarian function. Overall, LH and FSH increased after diagnostic laparoscopy. The higher FSH levels could stimulate Inhibin B production and aromatase activity, the latter resulting in a decrease of the androgens and an increase of estradiol.

Furthermore, ovarian drilling may disrupt/alter the function of the ovarian nerve fibers. Afferent ovarian neurological pathways are present in many animals including primates (51). In humans we know that the ovary is innervated by both sympathetic and sensory fibres, distributed throughout the ovary (52). Ovaries from PCOS women have a higher density of nerve fibers than regularly cyclic women (53). Animal studies show that the ovarian nerves can influence hormonal activity and interfere with cyclicity, for example transection of the superior ovarian nerve in PCOS rats (unilateral or bilateral) restored follicle growth and ovulation from both ovaries (54-56). Ovarian drilling damages the ovarian tissue and consequently likely disrupts the ovarian nervous fibers. Consequences of such disruption on menstrual cycle and reproductive endocrine regulation are unclear. Possibly, the chronic general sympathetic overactivity in PCOS is locally decreased after ovarian drilling (57).

Figure 2 and 3 show a compilation of the potential mechanisms involved after ovarian drilling in short and long term.

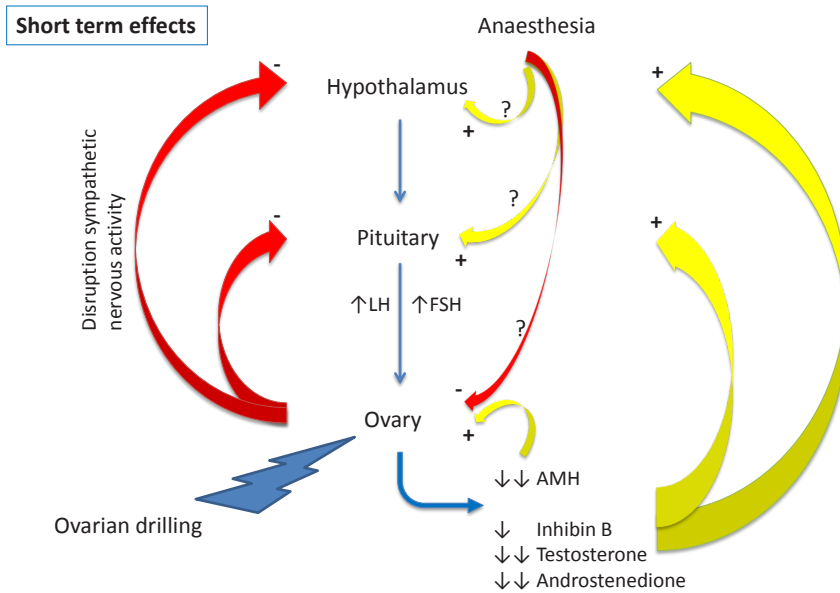


Figure 2. Potential mechanism of ovarian drilling in short term.

Ovarian drilling causes ovarian damage and result an instant disruption of the ovarian endocrine output, resulting in lower levels of AMH, Inhibin B, testosterone and androstenedione. This acute drop in the ovarian hormones results in a decrease of the tonic inhibition at the hypothalamus and pituitary level. The lower AMH causes the ovary to act more sensitive to FSH. Simultaneously the ovarian sympathetic nervous activity is disrupted, leading to hypothalamus and pituitary inhibition. Anaesthesia might influence hypothalamus and pituitary through activation of the sympathetic-adrenomedullary system and could have unknown effects on the ovarian endocrine production. The net effect on the hypothalamus-pituitary axis will be a higher LH and FSH level.

Given the net endocrine effects of ovarian drilling now known, the possible effect of mechanical manipulation of the ovaries is the following matter of concern. In **chapter 7** the effects of mechanical ovarian manipulation in pre-ovulatory PCOS patients (during ovulation induction cycle with FSH) and regularly cycling women (spontaneous cycle) were evaluated. Ovarian mechanical manipulation lowered LH secretion immediately and typically only in pre-ovulatory PCOS patients. The lower LH was probably based on a combination of a longer LH pulse interval and smaller amplitude, indicating influence of ovarian manipulation on hypothalamus as well as pituitary level. The immediate LH change after the ovarian manipulation was not accompanied by changes of any of the ovarian hormones (see Figure 4).

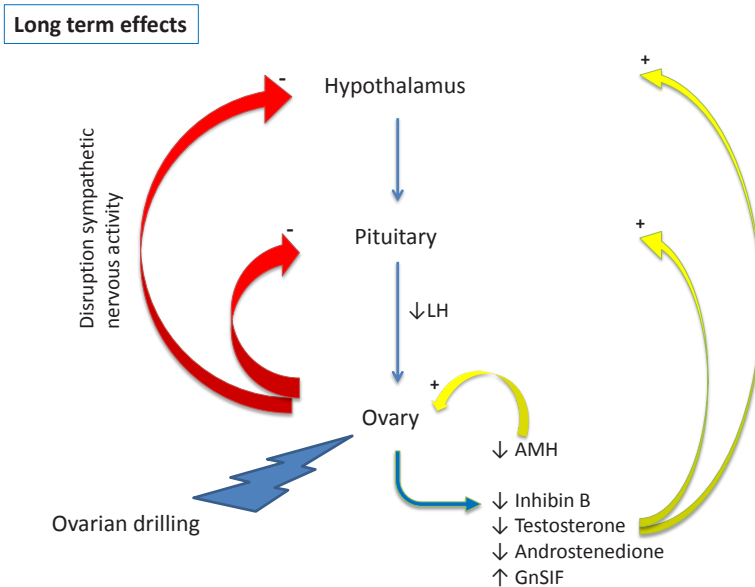


Figure 3. Potential mechanism of ovarian drilling in long term.

The inflicted ovarian damage will continue to lead to lower levels of AMH, Inhibin B, testosterone and androstenedione. GnSIF/AF will increase in response to normal follicle growth. Overall, some decrease of the inhibition at the hypothalamus and pituitary level is present in the long term in combination with a increased ovarian sensitivity to FSH. The disruption of the sympathetic nervous activity will continue to inhibit the hypothalamus and pituitary. The net effect in the long term will be lower LH levels. FSH will stay comparable to presurgical levels, but because of the higher ovarian sensitivity to FSH it will be sufficient for the induction of folliculogenesis.

A very interesting finding from this study is that mechanical manipulation of the ovaries in PCOS results in an immediate response of the pituitary apparently independent from ovarian hormonal feedback, as all the measured ovarian hormones remained stable. How can the ovaries communicate to the hypothalamic/pituitary region when the ovarian hormones do not change? Is neuronal ovarian-hypothalamic/pituitary feedback possible? It is known that in many species ovarian nerves have an important role in the regulation of ovulation and timing of the LH surge. Based on the results from this study we speculate about (rudimentary) afferent nerves / nerve fibers in human, which could be activated when the ovaries are manipulated for example during sexual intercourse where the ovaries communicate with other regions of the body through non-hormonal pathways. This could offer new potential possibilities in the treatment of PCOS. Interestingly the LH decreased in the PCOS, whereas in reflex ovulatory species LH normally increases in reaction to coitus, resulting in the LH peak (58). Ovarian manipulation may temporarily inhibit/normalize the higher ovarian sympathetic nerve activity. Possibly, the lower sympathetic

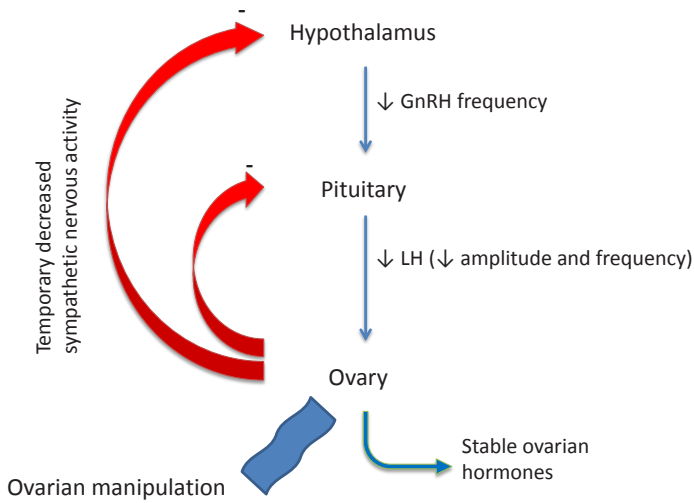


Figure 4. Potential mechanism of LH decrease directly after ovarian manipulation.

Ovarian manipulation could temporarily decrease the sympathetic nervous activity, leading to inhibition at the hypothalamus and pituitary level. This results in an lower GnRH frequency and lower pituitary sensitivity to GnRH and as a net effect transient lower LH levels. The ovarian hormones are not influenced directly after the ovarian manipulation.

activity results in a ‘normalized’ LH frequency and amplitude after ovarian manipulation, which could potentially lead to a reset. This might be essential as a trigger for the induction of follicle growth. As discussed previously, high LH levels seem to be correlated with the severity of PCOS and regaining regular menstrual cycles is accompanied by decreasing LH levels (28).

Future perspectives

After answering one question, ten new ones come up. Having finished this thesis, many questions remain unanswered, old and new. The most pressing issue is the possible existence of non-hormonal communication between the ovaries and the hypothalamus-pituitary and the role of the sympathetic nervous system. Future experiments should indicate through which ways mechanical manipulation of the ovaries in PCOS patients works and if it can contribute to initiation of the arrested follicle growth. So far we have only indications for the presence of nervous pathways that exist that may be employed as such. New experiments should include ovarian manipulation in PCOS patients without FSH supplementation to rule out the unlikely possibility that the observed LH drop was influenced by this and also to see if FSH levels are influenced by ovarian manipulation. Furthermore, ovarian manipulation should be performed in different phases of the cycle and in combination with GnRH tests to determine if pituitary sensitivity alters. Hopefully future studies will answer the questions raised and provide information about the biological background of the observed pituitary changes.

Another interesting development is the discovery of kisspeptin. The hypothalamic produced kisspeptin has a crucial role in initiating GnRH secretion at puberty. Kisspeptin administration results in increase of the pituitary LH pulse frequency and it has a role in the onset of the LH surge (59;60). Kisspeptin receptors are found in many different tissues/organs, for example the ovaries, fat tissue and pituitary (61). Due to the role in the gonadotropin regulation and the high LH levels found in many PCOS it can be speculated that aberrant kisspeptin release/secretion could play a part in PCOS (60-63). Limited data are available yet.

Last, but not least is the discovery of nerve growth factor in semen (64). This has been found in spontaneous and induced ovulatory species, including humans. It seems a highly conserved trait, which implies an important role in reproduction (64). Interestingly, the nerve growth factor found in semen plasma is essential for ovulation in lama's (an induction ovulating species). It furthermore seems to directly influence the female hypothalamus-pituitary-ovarian axis in cows (causes higher FSH levels, shortens the ovulation cycle and stimulates the development of the corpus luteum) (65;66). The fact that a seminal substance has a direct effect on the female brain and reproductive cycle is intriguing. The importance of nerve growth factor in human still needs to be established, but it could play a role in (in)fertility. The direct effect of nerve growth factor on the hypothalamus-pituitary-ovarian axis makes it an interesting potential hormone for future treatment in anovulatory PCOS.

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