Chapter 1

General introduction, aim and outline of the thesis

GENERAL INTRODUCTION

Diagnosis

Polycystic ovarian syndrome (PCOS) was first described in 1935 by two American gynaecologist Stein and Leventhal (1). They reported a cluster of symptoms; amenorrhea, signs of hyperandrogenism and found enlarged polycystic ovaries. Since then PCOS became a recognisable entity in gynaecology. Almost 80 years after the first publication it is clear that they described the cardinal features of PCOS, as we still use these symptoms (oligo- or amenorrhea, hyperandrogenism and/or polycystic ovaries) for the diagnosis (2). Diagnosing patients with PCOS has proven to be difficult, as it has a notorious variable clinical presentation and biochemical features (3). Furthermore, PCOS is a syndrome and as such no single diagnostic criterion is sufficient for the diagnosis (2). There has been an ongoing debate about the diagnostic criteria of PCOS and it remains controversial (4-7). Currently the 2003 Rottterdam criteria are used, defining PCOS by two out of the three following features: a) oligo- and/or anovulation, b) clinical and/or biochemical signs of hyperandrogenism and c) polycystic ovaries on ultrasound.

Neuro-endocrine aspects

PCOS is associated with endocrine abnormalities, including high testosterone, androstenedione, luteinising hormone (LH) and hyperinsulinemia (8-10). The current diagnostic criteria only include hyperandrogenism and do not incorporate the other endocrine features. Elevated LH levels are common in PCOS and a good predictor for PCOS, as no other condition shows high LH levels in combination with normal FSH levels (4;9;11). In spite of this, LH has been left out of the diagnostic criteria. This is probably due to the large variety in reported prevalence (from 35 till 77%) and the intercycle variability (3;12;13). The timing of LH sampling is essential. LH is usually measured in the early follicular phase, in which it is still suppressed by the progesterone from the previous luteal phase (14;15). This timing in the beginning of the menstrual cycle results in an underestimation of the real prevalence of elevated LH levels in PCOS patients (16). Measuring LH in a non-suppressed period, namely between the early follicular and peri-ovulatory phase, is expected to result in a higher frequency of elevated LH levels.

The cause of higher LH level in PCOS is not fully understood. A disturbance in the hypothalamic-pituitary-ovarian axis regulation is seen, with a higher amplitude and frequency of LH pulses and an increased pituitary response to GnRH (17;18). Insufficient gondadotropin surge inhibiting / attenuating factor (GnSIF/AF) secretion in PCOS may play a role (19). GnSIF/AF is an ovarian hormone, made by granulosa cells in response to FSH (20;21). An intercycle variation is observed, showing detectable GnSIF/AF levels during the early and mid-follicular phase and a decrease in the late follicular phase (22;23). GnSIF/AF reduces the pituitary responsiveness to GnRH by antagonising the GnRH self-priming, inhibits GnRH stimulated LH secretion and is of

influence on the timing of the LH surge (24-26). Adequate ovarian GnSIF/AF production seems of major importance for a regular ovulatory cycle. In PCOS the distorted follicle growth could lead to inadequate GnSIF/AF production and consequently higher pituitary sensitivity. This is supported by the fact that an induced ovulatory cycle in PCOS women leads to a lower pituitary response to GnRH (27;28), suggesting higher GnSIF/AF activity.

It is still unclear to which extent aberrant GnSIF/AF levels play a role in PCOS (19;29). A major barrier for GnSIF/AF research is that the molecular structure is still not fully elucidated. Only indirect measurements of its activity in vitro by bioassay or in vivo by the so called GnRH 'double pulse' method are available.

Therapeutic options

Therapeutic options for oligo- or amennorheic PCOS exist since the first report on PCOS in 1935, as it was discovered by coincidence that partial removal of the ovaries resulted in resumption of ovulatory cycles (1). Until 1960 the so called 'wedge resection' was the only treatment option. It fell into disuse when two other therapies became available, clomifene citrate and gonadotropins. Both medication work by increasing the level of follicle stimulation hormone (FSH), either indirectly (clomifene) or directly (gonadotropins). This higher FSH level will lead in the majority of patients to induction of follicle growth and subsequently ovulation. With the introduction of a laparoscopic version of the ovarian wedge resection in the 1980's, the popularity of the surgery started to increase again. Various types of ovarian surgery (for example, electrocoagulation, laser evaporation and multiple ovarian biopsies) have been performed to induce ovulation, for which the term ovarian drilling is used. Nowadays ovarian drilling is usually performed in clomifene resistant patients, but is also used in PCOS who hyper-respond to gonadotropin treatment (30).

The therapeutic armamentarium in PCOS has recently been expanded by the discovery that insulin-sensitizing drugs like metformin and aromatase inhibitors which block estrogen production from all sources have ovulation induction capacities (31-34).

Nowadays, the usual treatment strategy to induce ovulation in PCOS patients is clomifene citrate, followed by ovarian drilling in clomifene resistant patients or gonadotropins in a low-dose step-up scheme. The order of the different therapeutic options is mostly determined by financial and practical reasons, as clomifene is cheap and easy to use, and less by therapeutic effectiveness.

Mode of action of ovarian drilling

Controversy about the mode of action of ovarian drilling in PCOS continues to this day, but the high efficacy of the surgery has led to its indiscriminate use. In 1976 the first reports about

changes in the endocrine environment after ovarian wedge resection were published (35;36). Since then many publications followed, but the cause for restoration of ovulation after ovarian drilling is not fully elucidated. Ovarian drilling is the only therapy in PCOS which gives immediate endocrine alterations and therefore an ideal way to analyse which of the endocrine factors is essential to induce ovulation.

Effect of ovarian drilling on ovarian reserve

In spite of many decades experience with ovarian drilling procedures, the extent of ovarian damage inflicted by most of these techniques is not quantified and the long-term effects are not fully known. There are signs of a significant reduction of the ovarian reserve after ovarian drilling, as lower ovarian volume, antral follicle count, inhibin B, anti-Müllarian hormone levels and higher FSH concentrations are seen after surgery (37-41). Consequently, there are concerns for the long term fertility prognosis, although the first results are reassuring (42). Given the same clinical effectiveness of the various ovarian drilling procedures it is essential to use the technique with the lowest (but still effective) ovarian damage. To make a considered decision the amount of ovarian damage caused by the various techniques must be quantified. The minimally needed ovarian damage to induce ovulation after ovarian drilling is unknown. There have been a few studies which show a dose response relationship, but no optimal dose/damage is assessed (43-45).

Besides the ovarian damage, the ovarian manipulation that occurs during surgery might theoretically also cause endocrine changes. Ovarian afferent and efferent nerves are known in many animals and have an important function in the control of the ovulatory cycle and timing of the LH peak (46;47). Little is known about the nerve innervations of the ovary in human and their possible influence on ovarian activity, even though the presence of intra-ovarian nerves was reported more than a century ago (48;49).

AIM OF THE THESIS

To acquire a more profound understanding about the neuroendocrine control of PCOS, in particular which endocrine changes are essential for the induction of an ovulatory cycle. Furthermore, obtain more knowledge about the possible existence of a non-hormonal feedback mechanism in PCOS.

This was assessed by

a) reviewing literature on the reported reproductive endocrine changes after ovarian drilling in PCOS

b) studying the endocrine effects of ovarian drilling and comparing it to the effects of diagnostic laparoscopic surgery without ovarian drilling in PCOS

c) assessing the role of aberrant GnSIF/AF secretion in PCOS by analysing the influence of ovarian drilling on GnSIF/AF levels

d) analysing the prevalence of elevated LH levels in PCOS and evaluate its importance as a diagnostic tool

e) identifying if mechanical movement/manipulation of the ovaries has influence on the endocrine environment and hypothalamic-pituitary-gonadal axis regulation

OUTLINE OF THE THESIS

Chapter 2 Provides an overview of literature on the endocrine changes after various ovarian drilling procedures in PCOS. Furthermore, it addresses the most likely explanations for the resumption of the spontaneous ovulatory cycle after the procedure.

Chapter 3 Reports the prevalence of elevated LH levels in PCOS patients in adequately timed samples and evaluates the importance of LH as a diagnostic tool.

Chapter 4 Presents a purification and molecular identification attempt of GnSIF/AF, showing two new putative GnSIF/AF proteins and its influence on an intracellular process involved in GnRH self-priming.

Chapter 5 Shows the extent of ovarian damage caused by the most frequently used ovarian drilling techniques.

Chapter 6 Reports the results of a randomized controlled multinational trial comparing Clomifene citrate or low-dose FSH for the first-line treatment of infertile oligo-amenorroeic women with PCOS.

Chapter 7 Presents the results of a prospective study on the endocrine effects of mechanical ovarian manipulation in PCOS compared to regularly ovulating controls.

Chapter 8 Presents integrally measured reproductive hormone profiles and GnRH tests before and after laparoscopic ovarian laser evaporation in PCOS in a prospective controlled study.

Chapter 9 Provides an overview and general discussion of the findings in this thesis.

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