Pituitary down-regulation in IVF/ICSI: consequences for treatment regimens
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The ‘Brave New World’ of Aldous Huxley may be nearer realization. Pincus and Enzmann have started one step earlier with the rabbit, isolating an ovum, fertilizing it in a watch glass and reimplanting it in a doe other than the one which furnished the oocyte and have thus successfully inaugurated pregnancy in the unmated animal. If such an accomplishment with rabbits were to be duplicated in the human being, we should in the words of ‘flaming youth’ be ‘going places.’

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Introduction

The flaming youth, who were they?

The first successful in vitro fertilization of a human oocyte was achieved in the USA, at The Free Hospital for Women in Boston by the gynaecologist John Rock and lab-technician Miriam Menkin in 1944. For six years, they had carried out numerous attempts on more than 800 in vivo matured human oocytes. They had obtained these mature oocytes from patients who had to undergo a gynaecological operation and were pre-ovulatory at the same time. Rock and Menkin experimented with several external factors, like culture conditions, concentration of sperm suspensions, and duration of sperm incubation.

In 1944 they finally succeeded, when they observed -by chance- after an unusually long sperm incubation time, two human eggs reaching a two-cell stage in a Petri dish.

Rock and Menkin could not elaborate in the success of these preliminary IVF attempts, since Menkin, due to personal circumstances, had to quit her job and move to North Carolina. Rock, unable to proceed in this field of research without Menkin, gave up the in vitro fertilization experiments, and went on working on the birth control pill and male infertility.

In 1953 Landrum Shettles, also an American gynaecologist, succeeded in replicating the Rock-Menkin experiment and in 1955, working at the Colombian University, New York, he managed a human embryo to reach the morula-stage in culture. His professional aim was “to try and find out everything I could” about human fertilization. In the 1960s, a decade before anyone else, he transferred one of those fertilized eggs into the uterus of a woman who was scheduled to have a hysterectomy. When the uterus was removed two days later, Shettles claimed the embryo had implanted, thus demonstrating that human IVF was possible.

Meanwhile, at the other site of the ocean, Robert Edwards, geneticist, Edinburgh, UK, was experimenting with artificial insemination of mice. From 1955 onward, he enlarged his knowledge on the reproductive cycle of mice and the effect of various fertility drugs on ovarian stimulation. In 1964 he started preliminary work on fertilizing rabbit eggs in vitro. Having children of his own, he empathized with a befriended childless couple. Motivated by their silent
yearning, he began devoting his time fertilizing and culturing human eggs\textsuperscript{13}. In 1965 he described some unsuccessful attempts on maturing in vitro of human oocytes, receiving only little ovarian tissue from his wife's gynaecologist, who he knew from the delivery of his own daughters\textsuperscript{3,13}. In need of much more ovarian tissue, he went to the Johns Hopkins Hospital in Baltimore, summer 1965, to expand his work on in vitro maturation human oocytes. There he collaborated with the gynaecologists Howard and Georgeanna Jones from whom he obtained small strips of ovarian tissue after bilateral ovarian wedge resection in women with the Stein-Leventhal-syndrome. At the end of 1965 he was optimistic enough to launch the idea to utilize this in vitro maturation technique as future treatment of couples with tubal infertility in a publication\textsuperscript{14}. He went on to fertilize the in vitro matured human eggs with in vitro capacitated sperm, however with little success. Finally, in 1969 he was able to report on seven in vitro matured eggs that showed signs of pronuclei, sperm tails and polar bodies as evidence for in vitro fertilization.\textsuperscript{15}

After this key publication, Edwards met Patrick Steptoe, gynaecologist in Oldham, Lancashire in 1968. Steptoe had developed an aspiration device to retrieve human oocytes by laparoscopy, thus creating for the first time the opportunity to obtain human oocytes via a minimally invasive procedure\textsuperscript{16}. As Edwards knew from his previous animal experience, rabbit blastocysts obtained after fertilization of in vitro matured oocytes all fail to grow to term, while blastocysts obtained after fertilization of in vivo matured oocytes, lead to full term (rabbit) fetuses. Edwards combined this knowledge on the viability of vivo matured oocyte with the new minimal invasive technique and finally was able to report on 8 cell-stage embryos, however still without proceeding to a genuine embryo re-implantation as it was then called\textsuperscript{17,18}.

In 1971 a milestone in the history of in vitro fertilization was reached, rather remarkably at exactly the same time, by both Edwards and Shettles. Both reported on, in the January's edition of Nature, the first human blastocysts grown in culture\textsuperscript{19,20}.

It was Shettles who performed the first real IVF attempt as we now know it, i.e. oocyte retrieval, in vitro fertilization and embryo “re-implantation”, on a patient with blocked fallopian tubes\textsuperscript{3}. However, just hours before the actual re-implantation, he was halted by Raymond van de Wiele, chief of the department of Obstetrics and Gynaecology Columbia-Presbyterian Hospital in New York. Van de Wiele considered the IVF procedure unsafe and unethical. He ordered
the test tube to be removed from the incubator and send to him, exposing its contents to room temperature. Shettles had to resign and his days of pioneering work on IVF were over. Van de Wiele was later sued by the patient who had volunteered for this first test tube embryo attempt ever for “intentional infliction of emotional distress”.

Edwards was now the only IVF pioneer left. To obtain some control over the menstrual cycle and oocyte maturation, he made use of ovarian hyperstimulation combined with ovulation triggering by human chorionic gonadotrophin (hCG) and reported on 14 successful cervical embryo transfers, although none had implanted. Edwards thought that the ovarian stimulation itself was the cause of this failure. The abnormal steroid production would derange the luteal phase, interfering with implantation. Ovarian stimulation was therefore abandoned and Edwards and Steptoe returned to the natural cycle. Finally, in 1978, after nearly 20 years of experimenting, Edwards and Steptoe could report on the first live birth after re-implantation of a human embryo retrieved in a natural cycle IVF.

Towards a mainstream procedure

This success triggered other groups to start with IVF as well. At first they all followed ‘the Oldham procedure’, i.e. IVF in the natural cycle, which meant obtaining one mature oocyte, mid cycle, by laparoscopy and fertilizing the oocyte in vitro. In those days, the natural cycle was monitored by measuring estrogen and luteinizing hormone in 3-hourly urine samples. Women were admitted to hospital, their fluid intake was strictly controlled and 24 to 26 hours after a significant rise in urinary LH values, a laparoscopic egg collection was carried out. The risk of failure, ie no oocyte or non fertilization of the one oocyte, was high, and the subsequent live birth rate of a mere 8% was disappointing.

During the 1980’s several changes were made to the Oldham procedure. Ultrasound techniques were introduced for follicle imaging. This made a re-introduction of ovarian hyperstimulation possible, because follicle growth could be monitored and controlled.

To achieve ovarian stimulation, human menopausal gonadotropin (hMG) was used. The first hMG preparation, containing a mixture of FSH and LH, was registered for clinical use in 1950. This hMG was derived from urine of postmenopausal women. During the sixties, 120,000 liters per year were collected to meet worldwide demand. This entailed 600-1000 urine donors per year. In the
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eyearly nineties, this demand for hMG increased 100-fold\textsuperscript{31}. Research on how to acquire more pure preparations, had started in the early 1970’s. The World Health Organization had announced the desirability of an international standard for quality control, and, as a consequence, an International Unit for consistency was defined\textsuperscript{32}. The first highly purified FSH preparations (hpFSH, FSH-HP) entered the market. These new products were virtually devoid of contaminating urinary proteins and LH, which led to a consistent batch-to-batch quality. Still, several other shortcomings of urine extractions continued to be of great concern to the pharmaceutical industry. Appropriate quality control was impossible, since the vast amount of urine donors were not traceable. The time had come to start to invest in recombinant FSH\textsuperscript{33}.

Ovarian stimulation protocols typically recovered two, three, maximally four oocytes per cycle\textsuperscript{34}. Soon it was recognized that pregnancy rates depended upon the total number of retrieved oocytes and subsequently the total number of embryos transferred. The pregnancy rate increased from 7% to 27% with the transfer of three embryos\textsuperscript{34,35,36}. In order to increase the yield of oocytes, the ovarian stimulation protocols had to be improved.

In 1984 a publication appeared on eleven low responder women who were selected to receive a pretreatment with a GnRH analogue during at least 15 days followed by ovarian stimulation with HMG or hpFSH. A significantly higher number of oocytes could be retrieved, when using this ovarian stimulation protocol\textsuperscript{37}. This was the starting point of the extensive world-wide use of GnRH analogues in IVF treatment cycles.

At first, mainly GnRH agonists were used. Studies showed indeed a significant increase in the total number of retrieved oocytes after downregulation with GnRH agonists\textsuperscript{38,39}. In addition, the inconvenient occurrences of LH surges during hyperstimulation were prevented, by which a decrease in cancellation rates was accomplished\textsuperscript{39}. It became possible to delay oocyte retrieval for at least 24 hours, thus avoiding oocyte collecting during the weekends\textsuperscript{40}. The use of GnRH agonists became the gold standard in an IVF treatment cycle, provided that some form of exogenous luteal phase support was added to overcome the derangement of the corpus luteum as a consequence of the use of an GnRH agonists\textsuperscript{41}.

In 1999 the first GnRH antagonist attained market approval. Due to its limited water solubility, localized and systemic histamine release, difficulty in formulation for long term administration, and the requirement for larger doses of antagonist to suppress the LHRH receptor, it had taken almost 20 years to
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develop a GnRH antagonist suitable for clinical use\textsuperscript{42,43,44}. It was expected that GnRH antagonists would rapidly replace GnRH agonists in clinical practice. The immediate and direct suppression working mechanism would decrease treatment length and costs, and increase patient’s convenience\textsuperscript{45}. However, when all available data from randomised trials was pooled in a Cochrane meta-analysis, a significant lower ongoing pregnancy rate and live birth rate was found in women treated with GnRH antagonists compared to women treated with GnRH agonists (0.87 95% CI 0.68-0.97) \textsuperscript{45}. Nowadays, IVF is considered a mainstream procedure. The International Committee for Monitoring Assisted Reproductive Technologies (ICMART) reported on 460,000 IVF/ICSI performed cycles world wide in the year 2000 leading to an estimated births of 197,000 to 220,000 babies \textsuperscript{46}. In 2005, one out of 43 Dutch newborns was an IVF/ICSI child\textsuperscript{47}

Background of the research of this thesis

Pituitary down regulation has played an important role in the improvement of IVF-ICSI success. In this thesis, we focus on adjustments of pituitary down regulation itself but also on the clinical consequences of any form of pituitary down regulation in IVF-ICSI. Albeit unintentionally, the pituitary suppression does extend to the follicular phase, resulting in aberrant controlled hyperstimulation, it does extend to the pre-ovulatory phase affecting the decision making of planning an oocyte collection, and it does extend to the luteal phase, resulting in a luteal phase deficiency.

After more than 60 years of experience in \textit{in vitro} fertilization new stimulation protocols are still being developed, for instance mild stimulation protocols or mini IVF or modified natural IVF protocols, mostly in combination with single embryo transfer (SET)\textsuperscript{48;49;50}. These forms of mild stimulation are preferably carry out with GnRH antagonists, since GnRH antagonists intrinsically yield significant lower number of oocytes compared to GnRH agonists, as shown in a Cochrane meta-analysis\textsuperscript{45}. However, this meta-analysis also shows a decrease in treatment effect of almost 5% in ongoing pregnancy rates. This difference in treatment effect needed further assessment. In an attempt to optimize the use of GnRH antagonists, we compared two strategies in using a GnRH antagonist. GnRH antagonists as a rule are starting after 5 or 6 days of ovarian stimulation with recombinant FSH, without taken the individual follicle growth into account. Therefore an alternative strategie was investigated in a randomized
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clinical trial, in which the GnRH antagonist was started when the dominant follicle reaches ≥15 mm, entailing variable starting moments during the stimulation phase. Next, we assessed patients’ preference for GnRH agonist or antagonist in a discrete choice experiment (DCE) (Chapter 3). The goal was to gain more insight on the relative weight patients place on various aspects of the down-regulation treatments and the treat-offs they make between the specific characteristics of the two different medications.

Various preparations containing FSH can be used for controlled ovarian hyperstimulation (COH). The two commonly used forms are urinary hMG (which contains FSH and LH activity) and recombinant FSH (which contains just FSH)\(^51\). When a gonadotrophin-releasing hormone (GnRH) agonist is used in combination with rFSH, the growing follicles are completely deprived of LH, since the GnRH analogue blocks the output of LH for at least 10 days after cessation\(^52\). The question has arisen whether the lack of LH on growing follicles would have a negative impact, since a meta-analysis comparing urinary hMG versus rFSH for COH following an agonist long down-regulation protocol in IVF or ICSI treatment showed a significant increase in live birth rate (relative risk, RR = 1.18, 95% CI: 1.02–1.38, P = 0.03). If the clinical superiority of hMG is due to the LH it contains, then it may be possible to add recombinant LH to rFSH to achieve better results. Several studies have therefore assessed the effect on pregnancy rate of adding rLH to rFSH for COH. To establish the effectiveness of this co-administrating of rLH all studies identified and pooled in systematic meta-analysis with ongoing pregnancy as outcome measure (Chapter 4).

We subsequently assessed the timing of oocyte collection according to follicle maturation. This subject had not received much attention in previous studies on stimulation protocols in IVF. It is a widely agreed that follicles need to reach at least 17 mm in diameter before administrating hCG for follicular maturation for oocyte collection\(^53\). This view, however dates from a period in which the GnRH agonists were not yet introduced. Before that time, premature LH surges were very common and it was noticed that these premature LH surges had a deleterious effect on pregnancy rates. Cycles with premature LH surges were therefore cancelled. To lower this cancellation rate, hCG was administered at follicle diameters of 17 mm or less. Since this was no longer necessary when using a GnRH analogues we aimed to seek more evidence on the impact of delaying oocyte collection on pregnancy rates. We therefore compared the effect of planning oocyte collection when the leading follicle had a diameter of 18 mm versus planning oocyte collection when the leading follicle had a
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diameter of 22 mm on ongoing pregnancy rates (Chapter 5). To compensate for the failing of the corpus luteum, it is necessary to give luteal support. Two forms of luteal support are available, i.e. HCG, which mimics LH activity, and progesterone, the end-product of the corpus luteum itself. We evaluated therefore firstly, whether the addition of HCG to progesterone would be beneficial to progesterone alone (chapter 6). Progesterone transforms the proliferative endometrium into secretory-phase and creates an optimal surface for the embryo to implant. In the natural cycle, the embryo usually arrives at the uterine cavity 72–96 h after ovulation. The in vitro fertilized embryos arrive earlier in the uterine cavity since most clinicians transfer the embryos 72 hour after oocyte retrieval. It may be possible that the transformation of the endometrium lags behind the optimal implantation window, which may have a deleterious effect on implantation. Therefore, we also assessed the effect of bringing forward the starting point of progesterone on ongoing pregnancy rate (Chapter 7).

Outline of this thesis

Chapter 2 reports on the results of a comparison of two different GnRH antagonists protocols in a multi-centre randomised clinical trial. Patients were treated with rFSH, starting on cycle day 2 or 3, followed by a GnRH antagonists, starting either on cycle day 6 or on the day when the dominant follicle reaches 15 mm. The primary outcome was number of retrieved oocytes.

Chapter 3 is a discrete choice experiment, in determining patients’ preferences for GnRH antagonist or GnRH agonist in couples who were scheduled to undergo IVF/ICSI. The goal was to gain more insight on the relative weight patients place on various aspects of the down-regulation treatments and the treat-offs they make between the attributes.

Chapter 4 provides a systematic review of published literature in which the addition of rLH to rFSH is compared to rFSH alone, for ovarian hyperstimulation for IVF or ICSI, in GnRH agonist and antagonists down-regulated normogonadotrophic women. The primary outcome was live birth rate.
Chapter 5 reports on the results of a multi-centre randomised clinical trial, in which the timing of oocyte collection is planned, according to fixed criteria of the size of diameter of the dominant follicle assessed by ultrasound. Patients were treated with a long, luteal started, GnRH agonist protocol. The primary outcome was ongoing pregnancy rate.

Chapter 6 reports on the results of a single center randomised clinical trial, that compared two different forms of luteal support, i.e progesterone combined with HCG and progesterone alone. Patients were treated with a short flare-up protocol; i.e. starting a GnRH agonist on the first day of the cycle, followed by HMG on the fifth day of the cycle. The primary outcome was clinical pregnancy rate.

Chapter 7 reports on the results of a single center randomised clinical trial, that compared three different starting points of luteal support with a fixed embryo transfer day i.e: three days after the oocyte retrieval. Patients were treated with a long GnRH agonist protocol, followed by HMG or hpFSH or rFSH. The primary outcome was ongoing pregnancy rate.

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