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Artificial Reproductive Technologies (ART) and Controlled Ovarian Hyperstimulation

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1. BACKGROUND HISTORICAL DEVELOPMENT IN THE UNDERSTANDING OF OVULATION

If a couple cannot conceive and carry out a baby we speak of "infertility Infertility, as an unwanted condition, that has been well known since ancient times. However, the first known gynecologic medical text, the Kahoun papyrus (2200-1950 B.C), describes infertility as a disruption in the continuity between the reproductive organs and the digestive tract.

Infertility throughout the millennia started to be better understood when Hippocrates (1) described several causes of infertility and potential therapies. He prescribed "when, a cervix is closed too tightly the inner orifice must be opened using a special mixture of red nitre, cumin, resin and honey…or it could be dilated by inserting a hollow leaden probe into the uterus enabling emollient substances to be poured in". Vesale in the time of Renaissance, much later, published an anatomic atlas, **Humani Corporis Fabrica** in 1543, in which he included anatomic cross sections of female genital organs. Early developments of understanding ovulation and follicular function were slow and went over centuries. Table 1 specifies some early developments in the meaning of ovulation and follicular function as well its use to avoid fertilization.

Since the early successful days of Artifical Reproductive Technologies (ART) after the birth of Luis Brown in 1978 (1) still in a spontaneous cycle at that time, controlled ovarian hyperstimulation became the advised concept for a successful outcome of any ART treatment. This concept my change, once optimal ways of single embryo development and transfer in consecutive cycles to the ovum pick up cycle, will prove the same save outcome with healthy children and mothers in spontaneous ovulatory cycles. It is the aim of the present paper to specify the understanding of ovulation and controlled ovarian hyperstimulation, also referred to as controlled ovarian superovulation.

De Graaf (2) described in De Mullerium Organis * already in the year 1672 the ovarian and follicular function, although he mistakenly interpreted the follicle to be the egg. Martin Naboth, in 1707, published De Sterilitate, in which he claimed that ovarian sclerosis and tubal blockage caused infertility. In 1769, Morgani added follicular absence or agenesis as a cause for infertility. While a greater understanding of the etiology of infertility was gained during this time, the Renaissance physician continued to struggle to find an effective treatment to offer their patients.

The 19th and 20th century were marked with numerous advances which now define what is known as modern medicine today. Reproductive endocrinology was born with the discovery of gonadotropins and their regulation of the menstrual cycle. In 1927, Ascheim and Zondek (8) isolated human chorionic gonadotropin (hCG) from the urine of pregnant women. Soon thereafter in 1930, Cole and Hart discovered equine chorionic gonadotropin, which became known as pregnant mare serum gonadotropin (PMSG)(9). From these discoveries, the technique of superovulation was born. Methods were developed to superovulate immature mice using PMSG followed by hCG to induce estrus and ovulation. Foreseeing the ethical and medical complications of ovarian stimulation, Edwards and Fowler described an increase in liter size and fetal mortality during superovulation of mice (10). In spite of already discovered in the late 1920ies to early 1930ies, it took over 40 years for the technique to be adviced as a treatment of anovulation in infertility.

Table 1: Chronology in understanding ovulation, fertilization and avoiding pregnancies

- 1672: De Graaf. *De Mullerium Organis* (follicular functions in the female ovary -thinking that the follicle was the egg-) (2)
- 1707: Martin Naboth *De Sterilitate*, (ovarian sclerosis and tubal blockage provoked infertility) (3)
- 1796: Morgani (the reason of infertilityis follicular absence or agenesis) In 1905 Theodoor Hendrik van de Velde, (women only ovulate once per menstrual cycle) (4)
- 1920s: Kyusaku Ogino and Hermann Knaus, (ovulation occurs about fourteen days before the next

menstruation). Ogino used his discovery to develop a formula aiding infertile women to time intercourse to achieve pregnancy (5)

- 1930: John Smulders used Herman Knaus discovery to create a method for *avoiding* pregnancy. His work called the rhythm method was published with the Dutch Roman Catholic medical association, being the first published system for periodic abstinence. Scientist (6)
- 1950: Gregory Pincus developed the first oral contraceptives in 1950. (7)

2. CONTROLLED OVARIAN HYPERSTIMULATION

Only in the 20th century medical research brought up an understanding of the pituitary gonadal axis and the role of gonadotropins in the control of ovulation and reproduction. After testing these hormones in different animal species the use of these hormones for ovulation induction in humans was tested. A real achievement in the history of ovulation induction was the isolation and purification of human gonadotropins.

At the beginning the amount of urine required and the extraction procedure for human gonadotropins was too complex to serve as a stimulation procedure. It was thought to better derive gonadotropins from the pituitary gland. Table 2 specifies the consecutive chronology in understanding details in ovulation induction. In spite of intense work in the first half of the 20th century ovulation induction started only with GEMZELL in 1958 to be effective in the human (11).

Table 2: Chronology in understanding ovulation induction

- 1927, Ascheim and Zondek isolated human chorionic gonadotropin (hCG) from the urine of pregnant women.(8)
- 1930, Cole and Hart discovered equine chorionic gonadotropin, which became known as pregnant mare serum gonadotropin (PMSG). From these discoveries, the technique of superovulation was born.
- 1953 Watson and Crick description of the double double helical structure of DNA
- 1959 Edwards and Fowler: Superovulation, was first described by an increase in liter size and fetal mortality during superovulation of mice. It took 40 years from 1920ties till 1959 for the technique to be described as a treatment of anovulation in humans
- 1958 Gemzell was the first to report induction of ovulation with pituitary derived gonadotropins (11).
- 1960 Buxton and his colleagues at Yale University confirmed Gemzell's work in the United States in 1960.
- 1964 Donini extraction of human gonadotropins (HMG) from human menopausal urine in sufficient quantities; he stated to use of HMG for ovarian stimulation as a treatment for amenorrhea (12).
- 1972 Walter Fiers: first complete gene sequence of a nucleotide was obtained and published by Walter Fiers.
- 1974 Shome and Parlow proposed the amino acid sequence of FSH. This was soon followed by the cloning and DNA sequencing of the common alpha subunit then the hormone specific B subunit. These early studies led the way to the expression of human FSH by Chinese hamster ovary cells, providing a source of bioactive FSH not only for experimental use but also for clinical application.
- 1971 GnRH was discovered by Shally, Arimura and Kastin (13)
- In the 1980ties followed the introduction of GnRH analogues (agonists and antagonists) for medical use in various fields. In Human Reproduction they are till today a basic entity in every ovulation induction program. Their names usually end in -relin. The most well-known and widely used GnRH analogues are as GnRh agonist leuprorelin (brand name Lupron) and as GnRh antagonist triptorelin (brand name Decapeptyl). The application is performed intra muscular, sub-cutaneously daily or as depot preparations once a month or every 3 months, daily or as a nasal spray and recently also orally.

Gemzell's theory was confirmed by Buxton and his colleagues in the USA. This initiated the United States Public Health Service and the Veterans Administration to recruit a large number of hospitals for the extraction and purification of the pituitary tissue. In different centers, induction of ovulation was performed using pituitary extracts, which had primarily FSH activity, and hCG as a substitute for LH. Although successful in stimulating follicular development, the technique was very expensive and had problems. Pituitary glands from 10 individuals were needed to yield sufficient quantities of gonadotopin to stimulate one patient for one cycle.

In 1969 as a young gynecologist, interested in endocrinology and infertility, I wrote to Andrew Shally, who was at that time in Houston, Texas that I want to buy a few grams of Human pituitary extracts with FSH activity for ovulation stimulation for my patients in Kiel, Germany. The answer was: "dear young doctor, fine, but how many million dollars you have available for this purchase?" Well, the concept of using GnRH, after its synthesis as decapeptide, and after understanding its rhythmical release from the pituitary let to the treatment of hypothalamic amenorrhea with the GnRh pump as normally GnRH is released in pulses at intervals of about 90 to 120 minutes from the hypothalamus. The releasing hormone must be administered in pulses by a pump to increase serum gonadotropin concentrations in patients with GnRH deficiency.

The "extraction of human gonadotropins "(HMG) from human menopausal urine in sufficient quantities was described by Donini in 1964; and later introduced as HMG for ovarian stimulation as a treatment for amenorrhea. In these studies, he identified forms of hyperstimulation and described the untoward side effect of multiple pregnancies as a result of the use of HMG and hCG for ovulation induction. The development of the technique to extract urinary derived gonadotropins provided a safer and more easily available

source of FSH and LH, compared to the pituitary extracts but the purity of the product was still low.

The high levels of co-purified contaminant proteins resulted in hypersensitivity and injection site reactions. While comparable to purified pituitary many problems appeared with batch-to-batch variations and standardization of the dose from one batch to the next

The desire to develop a monotherapy with FSH alone was driven by the hopes to provide a more consistent and better-defined dose. This led to the development of urinary FSH with minimal LH activity by Serono. They marketed the product under the name Metrodin. This formulation was used successfully in patients with PCOS and later for superovulation in IVF. Reproductive endocrinologists continued to isolate, purify, and study the regulation and function of hormones and the field of molecular and genetic science exploded. In 1953 Watson and Crick published their classic paper that first described the double helical structure of DNA (14). The central dogma of molecular biology, a gene is transcribed to mRNA and mRNA is translated to protein, was proposed by Crick in 1958. These two monumental theories ushered in the molecular genetic age. In 1972, the first complete gene sequence was obtained and published by Walter Fiers. Shome and Parlow suggested the amino acid sequence of FSH in 1974. The cloning and DNA sequencing of the common alpha subunit was possible before the hormone specific B subunit. The expression of human FSH by Chinese hamster ovary cells, providing a source of bioactive FSH not only for experimental use but also for clinical followed these studies. I very well remember our trials in the late 1980ties. Using recombinant human FSH for ovarian stimulation in in vitro fertilization was only possible after 1992 (15,16).

After immune affinity purification and high-performance liquid chromatography urinary derived gonadotropins could be obtained with low levels (<1%) of co-purified non-gonadotropin proteins. A repeatable standard dose of combined human gonadotropins is today giving us multiple gonadotropin formulations for ovulation induction with recombinant FSH and LH in addition to combined gonadotropins purified from urine.

As similar results are today achieved with all these stimulation patterns, the choice is not easy, depending on financial issues and knowledge of the physician. Although downregulation with and without flare up protocols for GnRh agonists and antagonists are available a stimulation with purified FSH appears to be the leading strategy. Let us not forget oral agents to introduce ovulation. In 1967 already clomiphen was approved by the FDA for ovulation induction. Today also Tamoxifen and Letrozol are used as effective medications and show some benefits.

3. OVULATION INDUCTION (STIMULATION) IN ARTIFICIAL REPRODUCTIVE TECHNOLOGIES

This brings us to ovulation induction or stimulation, to ovarian hyperstimulation or superovulation within our field of Artificial Reproductive Technologies (table 3). With all the progress in genetics it is still not possible to decide upon an ovarian hyper stimulation formula according to the human genome of the individual “to be mother”. In September 1981 at our first Bourn Hall meeting: Patrick STEPTOE, Robert EDWARDS and Jean Purdy invited their friends, clinicians and researchers from around the world to Bourne HALL/CAMBRIDGE and we established 5 contents that are still valuable today. These consents are still guiding all IVF protocols till today:

1. Stimulated cycles are better than natural cycle for better number of oocytes and better prediction of ovulation and pregnancies
2. US to monitor growth of follicles
3. Focus on Embryo Transfer (ET) / Progesterone for luteal phase support- start on the day of follicular puncture
4. Control of culture media and laboratory procedures is essential
5. Effect of CO-2 on oocyte quality with laparoscopy? The first oocyte retrievals were done for years by laparoscopy.

Only between 1982 and 1988 transvaginal ultrasound guided follicular puncture was developed. Many publications followed this meeting (17).

Since diminished ovarian reserve is diagnosed by blood tests measuring hormone levels, and not by counting eggs, it’s hard to determine the average number of oocytes of someone who has this condition. Stimulation protocols have to be adjusted to poor, normal, moderate, low and high ovarian responders. Nowadays, the use of IVF has improved the prospects of infertility treatment. The expected outcome of IVF depends greatly on the effectiveness of controlled ovarian hyperstimulation (COH), where exogenous gonadotrophins are used to induce folliculogenesis.

The response to stimulation varies substantially among women and is difficult to predict. Several predictive markers of COH outcome are available (e.g. maternal age and ovarian reserve) but no genetic markers have as yet been well defined for routine use.

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The response to stimulation varies substantially among women and is difficult to predict. Several polymorphisms in genes involved in FSH signaling, estrogen biosynthesis, folliculogenesis, folate metabolism and other aspects influence the response to exogenous gonadotrophin administration, resulting in differences in COH and IVF outcomes.

Nevertheless, the most studied

polymorphism FSHRAsn680Ser is practically the only genetic marker, together with ESR1 PvuII T/C, that could be applied in clinical tests. Although data are accumulating with evidence suggesting that the ovarian response to COH is mediated by various polymorphisms, the optimal biomarkers and the efficacy of the tests still remain to be evaluated. Even as to date, some results obtained by different research groups are promising, research involving useful predictive panels of genetic markers in COH is still in its infancy.

In addition to the large sample size, these genetic association studies should focus on optimal selection of study samples, which are controlled for ethnicity and geographic origin in order to minimize phenotype heterogeneity. A well-designed study requires also power calculations for the sample size optimization and optimal marker selection The relatively slow uptake of pharmacogenetics in clinical practice is a result of several factors, including the additional cost of genetic tests, the need for specific laboratory equipment, the lack of proof of the utility of pharmacogenetic tests, difficulty in interpretation of test results and a lack of knowledge amongst health professionals and also patients regarding pharmacogenetics. However, here is the easy way:

Basic parameters for monitoring a controlled ovarian hyperstimulation in 2024 still are:

1. Follicular size, vascularization and numbers in vag. US,

2. Serum oestradiol measurement (LH, FSH and progesterne occasionally)

3. Endometrial thickness.

Table 3: Chronology of IVF and ICSI development

- 1890: Heape isolated and briefly cultured embryos from an Angora female rabbit, transferred the embryos into the reproductive tract of a Belgian hare recipient, and obtained offspring from the transfer.
- 1944: John Rock published the first report claiming successful in vitro fertilization and cleavage of a human oocyte (18).
- 1955: Shettles repeated Rocks work and reported successful culture to the morula stage in vitro of a human embryo (19).
- In 1976: Steptoe and Edwards achieved their first pregnancy with IVF and ET, however it was an ectopic pregnancy. By this time they had performed well over 100 stimulated cycles, and were able to transfer embryos into approximately 70 women without success (20).
- 1978: Steptoe and Edwards were concerned that their failure in achieving pregnancies was related to the hormonal stimulation and the resulting luteal phase defect. This made them to return to use the natural cycle and then they published the delivery of the first IVF baby, Louise Brown (21).
- 1981: Bourne Hall meeting: In September 1981 Patrique Steptoe, Robert Edwards and Jean Purdy invited some 25 clinicians and scientists from around the world to build up basic guidelines for IVF and ET
- 1992: The first pregnancy using recombinant human FSH during ovarian stimulation for in vitro fertilization (22)
- 2023: ESHRE - Copenhagen, Denmark - At least 12 million babies since the first IVF birth in 1978

4. POSSIBLE INFLUENCE OF IVF ON THE HEALTH OF DESIGNATED MOTHERS AND BORN CHILDREN

Influence of superovulation on the health of designated mothers

Here I summarize only that we have to think about questions like, if we decrease the ovarian reserve by a super ovulation, if we may induce pregnancy pathologies, increased risk of intrauterine fetal death, increased risk of malformations, multiple pregnancies. Of course, disease conditions of the born children, cardio vascular diseases and the oncologic risk of the born children may also influence the mothers. The risks are minimal but should be mentioned although the benefits fully verify the treatment (22 23).

Concerning the influence of superovulation on the health of born children assisted reproductive techniques, e.g. in-vitro-fertilization, intracytoplasmic sperm injection and cryoconservation of zygo- tes and embryos have become routinary clinical procedures yielding high pregnancy and birth rates. ART-children represent in between 2,3 % and 4 % of all children born in European countries. However, the question if ART-children may perform different regarding their health issues is still to be clarified in depth. Within the frame of hyperstimulation and other steps within in vitro fertilization, all our efforts to imitate natural culture conditions, must be summarized as being suboptimal: These are Controlled ovarian super ovulation or hyperstimulation, mechanical opening of the follicles, aspiration of the cumulus oocyte complexes, in vitro fertilization as ISCI or natural, embryo or blastocyst culture, and the transcervical embryo transfer. All these are procedures that do not occur in physiological conception and therefore could principally influence the life of the later born individuals.

Preimplantation conditions of the embryos in vitro may influence child health according to the Barker-hypothesis. All data available so far indicate higher incidences of pregnancy associated pathology as well as incidence of malformations in born children, but in very small absolute numbers. No higher susceptibility for infectious diseases could be observed. The discussion regarding cardiovascular diseases and a possible oncological impact in human beings born after ART is still ongoing. In summary ART-children perform very similar to spontaneously conceived children. However, continuous follow-up is mandatory (24,25,26).

5. CONCLUSIONS

Together with advances in other sections of our medical field in the last century till today we reached wonderful improvements and a good understanding of infertility treatment. The development of ART went hand in hand with Infection prophylaxis and treatment as well as medicine production. Concerning Covid? Well, we are getting closer with the vaccines from Pfizer/Biontech, Moderna, Astra Zeneca, Oxford vaccine-Covishield, Covaccine (Bharat) etc. Imaging techniques have made a great advance. Today we have Robot sounds to test for gastric ulcer and cancer, intraoperative MRI, intraoperative navigation, MIS-Micro invasive Surgery, robot assisted surgeries and much to come. In prenatal medicine we have huge progress and finally we are also getting ahead in cancer diagnosis and therapy in many fields.

Ovulation induction together with spermiogenesis and sperm genetics still form the basis of every infertility treatment Controlled ovarian hyperstimulation remains to be important, as already stated in the 1981 Bourne Hall meeting, for ovulation induction and in vitro fertilization. Luckily technologies were developed to monitor and decrease ovarian hyperstimulation side effects or pathologies and optimizations for one singleton life birth are on the way. New ethical and medical challenges that have come take well care of all these factors. With increasing numbers of embryo transfers in only slightly modified natural cycles and application of Artificial Intelligence (AI) selected systems for embryo selection better pregnancy rates are already there to be used more widely in the field of ART.

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