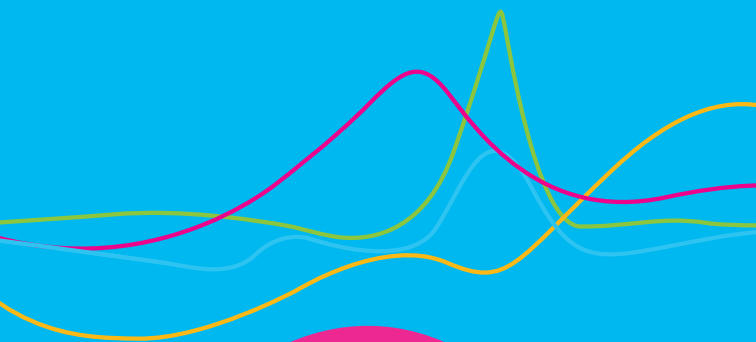




BIOMÉRIEUX

HORMONE ASSAYS

Making a key contribution to Assisted
Reproductive Technologies (ART)



PIONEERING DIAGNOSTICS

PREFACE



Professor Johan Smitz, MD, PhD.

Over the past 60 years, the therapeutic arsenal to treat infertility has substantially increased. The development of medication to regulate fertility has had a strong impact on society - the major consequence being that couples can decide to postpone childbirth.

In the sixties, hormones (oestrogen derivatives) and gonadotrophins became available to stimulate fertility. This was followed by the development of Assisted Reproductive Technologies (ART) in the seventies, and in 1978, the world's first "test-tube" baby, Louise Brown, was born. Robert Edwards, who pioneered the development of human *In Vitro* Fertilization (IVF) therapy which led to the birth of baby Louise, was awarded a Nobel Prize in 2010 in recognition of his ground-breaking work in this field.

ART have since become widely implemented around the world and constant developments in every aspect of these technologies have led to their high level of efficacy in treating infertile couples.

Since the basis of any effective treatment is an accurate diagnosis, the exploration of infertility has greatly benefited from the refinement and automation of immunoassay techniques. All major hormones playing a role in menstrual cycle regulation, implantation and early pregnancy can be accurately and reliably measured using fully automated immunoanalyzers, which have become the cornerstone of clinics seeking to provide highly effective, rapid and safe treatments to their patients.

This guide is intended to be a useful support for biologists and clinicians in their endeavors to maximize success rates in their patients using modern infertility treatments.

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INTRODUCTION

The combination of high quality reagents and robust automation has enhanced the widespread availability of a broad range of essential parameters to the clinician. On-hand availability of a complete panel of hormones and infectious disease parameters is of primary importance, especially for infertility doctors practicing Assisted Reproduction Technologies (ART).

Individualized patient-oriented treatment is now possible through the use of highly qualitative tests with short turn-around times and clinically validated reference ranges. Good clinical practice dictates serological testing for infectious agents before treating the couple and banking their gametes and/or embryos, as well as for the prevention of infections during gestation. Data from literature indicates that careful monitoring of reproductive hormones significantly contributes to increased live birth rates (*Loumaye et al., 1997; Smitz et al., 2007; Fleming et al., 2008; Bosch et al., 2010*).

Making the right choice of immunoanalyzer to be used in infertility diagnosis and therapeutic hormonal monitoring has important consequences for efficacy, safety and consistency of treatment success.

Method validation using a substantial amount of clinical samples reflecting the reference population and the most important pathological conditions observed in the patient population enables differentiation of the commercialized immunoanalyzers (*Coucke et al., 2007*).



For easy reading and reference, look for the colored boxes highlighting the key points in each chapter.

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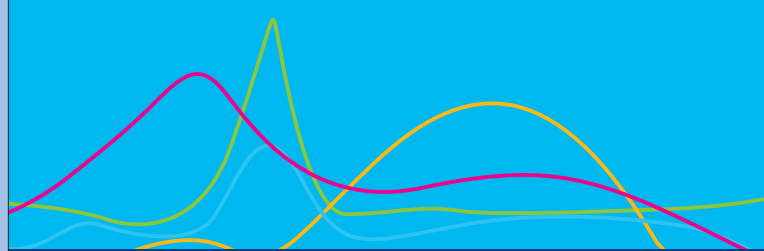
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■ PHOTO 1: Expanded oocyte-cumulus-complex (OCC)

■ PHOTO 2: Mature oocyte and ICSI

■ PHOTO 3: Fertilized oocyte with 2 pronuclei ■ PHOTO 4: 4-cell embryo
Photos: Center for Reproductive Medicine, Free University Brussels (VUB).



1 PREVALENCE OF INFERTILITY IN SOCIETY

In general, a couple is considered subfertile (infertile) if pregnancy has not occurred after 1 year of regular unprotected intercourse.

Prevalence of infertility differs widely between societies and regions. While in Western Europe and North America, the incidence is 10% of couples, this figure rises to 25% in North Africa, Southern Asia and South America. In sub-Saharan Africa, though only partially studied, the incidence is estimated to be 35% (Boivin *et al.*, 2007).

One of the clearly progressing trends in developed countries over the past few decades is the postponement of childbearing to later ages, due to the dissociation of sexuality and reproduction (Te Velde *et al.*, 1998).

Female fertility declines with age: the monthly pregnancy rate is halved between ages 30 and 35, and is reduced to 25% at age 38 (van Noord-Zaadstra *et al.*, 1991). With the progress of industrialization, this phenomenon is now observed worldwide. At age 41, most women are already infertile.

The age-related decline of fertility is largely linked to an increased risk of early miscarriage: 10% at age 25 and 50% at age 45 (O'Connor *et al.*, 1998; Sauer, 1998). This risk is due to a diminished oocyte quality with age, observed in natural cycles (Volarcik *et al.*, 1998). This trend is also observed in data from large *in vitro* fertilization (IVF) registries: while the success of IVF can be as high as 40% at age 20, it is disappointingly low (around 5%) between the age of 40 to 43.



The decline in quality and quantity of oocytes plays a pivotal role in reproductive aging, while the role of the endometrium is less influenced by age.

Age-related fertility decline is largely genetically determined, but lifestyle and environmental factors also have a significant influence.

The fetal origin hypothesis for adult infertility has also been considered for fertility decline. Severe fetal growth restriction can result from distorted entero-placental transfusion and, as a consequence, the follicle pool in the fetal ovaries may be reduced (Cresswell *et al.*, 1997). Other obvious reasons for reduction of the ovarian follicle pool include chemotherapy, ovarian surgery, radiotherapy, smoking, and free oxygen radicals (Gosden and Finch, 2000).

In developed countries, the etiology of female infertility is often due to hormonal dysregulation, whereas in the developing world, infection, unsafe abortions, post-partum complications and tuberculosis are the predominant causes (Ombelet *et al.*, 2007). Epidemiological studies on the evolution of male fertility in developed countries have identified toxicological reasons linked to industrialization as responsible for decreased sperm counts. However, most cases of reduced sperm quality are due to sexually transmitted diseases. Severe hormonal dysfunction that can be effectively treated is only the origin of infertility in a minority of male cases.

Etiology of infertility in couples

(approximate distribution)

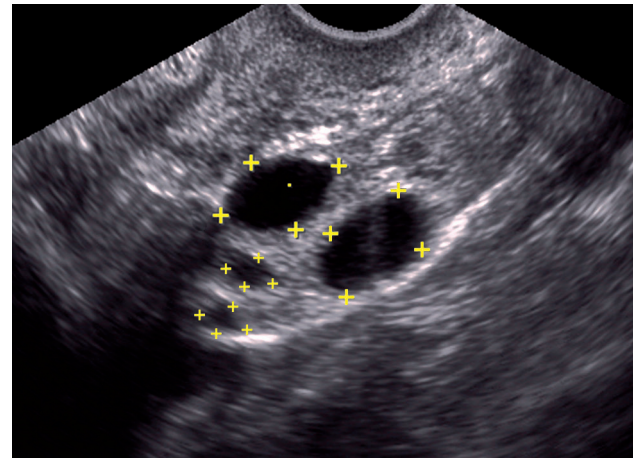
- 30% female factors
- 30% male factors
- 40% common factors (sub-fertile partners).



2 THE REVOLUTION OF INFERTILITY TREATMENTS THROUGH ASSISTED FERTILIZATION

Almost every fertility treatment (whether of female or male origin) will include hormonal stimulation of the ovaries. The aim is to increase the amount of developing ovarian follicles that contain the oocytes. The availability of more than 1 oocyte (the quantity present in a natural cycle) dramatically increases the chances of achieving a pregnancy.

Recent data show that aiming for 5 to 7 oocytes per retrieval session in IVF or ICSI is probably the best compromise (Baart *et al.*, 2009; Heijnen *et al.*, 2007), taking into consideration the risks for aneuploidy in oocytes, the reduced quality of the endometrium in the stimulated cycle and the potentially severe side effects induced by gonadotrophins and other fertility drugs (ovarian hyperstimulation syndrome - OHSS).



- Ultrasound image of stimulated ovary.
Day 7: 2 medium and 2 small follicles are visible in the ovary.



To maintain a good balance between efficacy and safety, **ultrasound techniques** and easily accessible, correctly **validated immunoassays**, used in combination, have become **essential tools** in the modern practice of the fertility specialist.

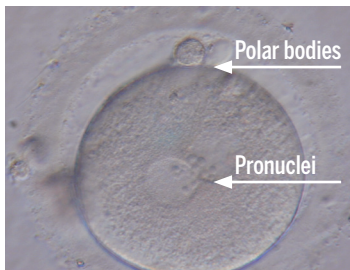
3 ASSISTED REPRODUCTIVE TECHNOLOGIES : IVF AND ICSI

In Vitro Fertilization (IVF)

A great step forward in the success of infertility treatment has been the laparoscopic retrieval of oocytes from the stimulated ovaries and their fertilization by selected sperm cells in the laboratory. IVF is the most effective treatment for infertility (ESHRE Capri Workshop Group, 2009).

While IVF was initially aimed to overcome tubal infertility, it is actually used for idiopathic infertility, several forms of endometriosis, resistant cases of polycystic ovary syndrome (PCOS) and for several forms of male infertility.

Male patients with a moderately reduced sperm quality can be treated by normal IVF, because the best fraction of their sperm prepared in the laboratory is brought in close contact with more oocytes obtained after stimulation of their partner.



■ Fertilized oocyte with 2 polar bodies and 2 pronuclei

Intra-Cytoplasmic Sperm Injection (ICSI)

The IVF technique has been shown to be successful in infertile males who have at least 5 million sperm in the ejaculate. However, males with only a few normal sperm cells still remained childless even with IVF. The practical application of ICSI in humans provides a solution for almost all severe cases of male infertility, by injecting a single sperm cell directly into the ooplasm (Van Steirteghem et al., 1993).



■ Microinjection of a single sperm cell into a mature oocyte

In Vitro Maturation (IVM) and Oocyte Capacitation

In vitro maturation of oocyte-cumulus complexes is a promising new technique by which immature oocytes from small follicles <10 mm are matured during 30 hours in a specific culture medium. Oocytes becoming metaphase 2 can be inseminated by ICSI (Sanchez et al., 2017).

Very often, oocyte-cumulus complexes originate from a 2 to 6 mm oocyte and, in this case, a prematuration or “capacitation” culture of 24 or 48 hours is needed before using the IVM culture medium (Sanchez et al., 2017).

Genetic diagnosis

In parallel to *in vitro* techniques for embryo culture, molecular methods for the genetic analysis of single cells have been developed. A few cells from a human blastocyst can be aspirated via a hole drilled by laser in the zona pellucida for genetic analysis. An innovative combination of techniques allows for pre-implantation genetic diagnosis e.g. in case of family predisposition for severe disease. The technique, known as PGS (preimplantation genetic screening), is currently being evaluated to detect embryos with a decreased potential for further normal development. This strategy could improve success rates per IVF attempt, as the chromosomal abnormality rate in human embryos from infertile patients aged over 37 years is nearly 70% (Franasiak et al., 2014). If screened embryos are chromosomally abnormal after PGS, these are not replaced, and avoid occurrence of miscarriage.

4 MAKING A CORRECT DIAGNOSIS

A CORRECT DIAGNOSIS IS A PREREQUISITE TO SUCCESSFUL TREATMENT

A wide range of diagnostic tools are available to the infertility specialist to determine the cause of infertility. These include ultrasound, computerized tomography scan (CT-scan), magnetic resonance imaging (MRI), hysteroscopy, hystero-salpingography, laparoscopy, blood karyotyping, histopathology of reproductive tissues, microbiology, serology and hormone analyses.

■ **In the case of suspicion of male infertility**, a sperm sample will be analyzed 2 or 3 times at weekly intervals, taking into account a 3-day period of abstinence before production of the sample. The sample is best produced in the biology laboratory in a sterile recipient, which can be handed over immediately to the andrology laboratory in order to reduce temperature variability.

The recently revised criteria for rating a sperm sample as normal (*Cooper et al., 2010*) are defined as:

- the ejaculate has a minimal volume of 1.5 ml,
- a sperm cell concentration of > 15 million sperm cells/ml,
- a progressive (A + B) motility of > 34%,
- and a morphology where normal forms are > 4 %.

Further (more specialized) analyses for sperm include:

- detection of anti-sperm antibodies,
- evaluation of the acrosome reaction,
- study of the structure of the flagellum.

A more objective method of analysis can be performed using Computerized Image Analysis (e.g. CASA) which takes into consideration several parameters to score the sperm. However, none of these current tests are able to predict with 100% certainty whether the sperm sample under consideration has the capability to fertilize. Therefore, in the presence of reduced sperm quality, the role of IVF / ICSI procedures will be as much diagnostic as therapeutic.

■ **For the investigation of female infertility**, the use of the above-mentioned non-invasive methods is guided by the patient's medical history and will be applied depending on whether the infertility is primary or secondary. Infertility is considered to be **primary** if the patient has never been pregnant. **Secondary infertility** affects patients who have had a pregnancy, but subsequently lose the capacity to achieve another pregnancy. Obviously the origins of the problem may be due to either of the partners or a combined problem.

Fertility is not only determined by adequate functioning of the pituitary and the reproductive tract and its associated glands. Other endocrine organs such as the thyroid, the adrenal gland, the liver and total body composition also contribute to a normal body condition that allows conception. The infertility specialist has to adopt a holistic approach, and make sure that several endocrine axes are functioning normally. Endocrine systems sometimes become dysregulated without clear phenotypical manifestations. These can be detected by an imbalance in the blood hormone parameters. Functional dysregulation of the reproductive axis might also be induced by stress as a result of long-term psychopathological diseases (e.g. depression).

As a general rule the gynecologist can categorize patients into different groups according to the World Health Organization (WHO) by measuring hormones in blood:

	LH	FSH	E2	P	T
WHO I	low	low	low	low	low
WHO II	normal to high	normal	normal	low	normal to high
WHO III	high	high	low	low	low
Hyperprolactinaemia	increased serum PRL level				



Easy access to highly sensitive and specific automated immunoassays with short turn-around times helps clinicians to make a differential diagnosis within a single consultation day.



Additional diagnostic tests can subsequently be planned so that most of the underlying causes of infertility can be determined within less than a week.

INVESTIGATION OF INFERTILITY

THE MOST FREQUENTLY OCCURRING CAUSES OF FEMALE INFERTILITY CAN BE DETERMINED BY HORMONE ANALYSIS.

Determine the reason for amenorrhoea and classify the anovulation in accordance with WHO criteria.

Between Day 2 and Day 5, measure:

- **First-line parameters:** hCG, FSH, LH, Prolactin, Estradiol (E₂), TSH
- **Second-line parameters:** Progesterone ('mid-luteal'), Testosterone, SHBG, 17OH Progesterone, DHEA-S

Is the patient pregnant?

- Despite menstruation, it occasionally happens that the patient is pregnant.
- Measure hCG and perform transvaginal ultrasound if hCG positive to confirm presence of a gestational sac at week 6 (last menstrual period).

Does the patient ovulate?

- Measure progesterone in blood.
- Perform timed vaginal ultrasound monitoring of the ovaries.

Is there another endocrine pathology interfering with the reproductive axis?

- Measure TSH as first-line parameter. Refine diagnosis with FT₄, FT₃, anti-TPO.
- Evaluate nyctohemeral rhythm of cortisol.
- Measure urinary free-cortisol in 24-hour urine collection.
- Measure DHEA-S, 17OH Progesterone, androstenedione, SHBG to exclude hyperandrogenism of adrenal origin or late-onset congenital adrenal hyperplasia.

Is there a CNS-related pathology? (e.g. a pituitary adenoma)

- Measure LH, FSH, PRL, ACTH, TSH, GH, IGF-1.
- Perform dynamic tests with releasing-hormones to detect paradoxical responses.
- Request a CT scan or MRI of the *sella turcica* and surrounding region.
- Check that the patient's visual field is intact.

Is there a corpus luteum insufficiency?

- Use ultrasound to monitor follicle growth pattern and measure maximal follicle diameter.
- Monitor LH for occurrence of the mid-cycle peak.
- Measure progesterone seven days after the LH peak.
- Calculate number of days between LH peak and onset of menses.

KEY POINTS

Is the endometrium receptive and healthy?

- Monitor LH and follicle growth by ultrasound.
- Measure Progesterone seven days after the LH peak.
- Take a Pipelle biopsy seven days after the LH peak.
- The pathologist will establish histological dating of the endometrium according to the Noyes criteria and also exclude chronic asymptomatic endometritis (*Noyes et al., 1975*).

What is the status of the patient's ovarian reserve?

- Measure serum AMH, or alternatively serum FSH on Days 3-5, combined with serum E₂. An FSH < 12 IU/L in the presence of an E₂ > 60 pg/mL is not predictive. Both AMH and antral follicle count by vaginal ultrasound have a good predictive value.

Can ovarian response to gonadotrophin treatment be predicted?

- The most recent studies indicate that indicate that both a single measurement of AMH in blood or ultrasound measurement of the follicles in the ovaries at the beginning of the cycle (i.e. "antral follicle count") have a good predictive value.

Can occurrence of ovarian hyperstimulation syndrome (OHSS) be predicted before treating a patient with gonadotrophins?

- Patients with either a high basal LH value, high AMH value, increased serum androgens (testosterone, androstenedione, DHEA-S) or reduced SHBG concentrations on cycle day 3 have a higher risk of over-reacting to gonadotrophin treatment (*Nelson et al., 2008*).
- These patients generally have long cycles or are anovulatory. Ultrasound scanning shows ovaries of an increased volume with multiple subcortical cysts.

Can an endometrial biopsy reveal whether the infertility is due to a dysregulation of the endometrium?

- It has been found that a high diagnostic sensitivity can be obtained only when the biopsy is taken on a precise day after the LH surge (generally 7 days after the LH peak value). It is therefore good practice to perform a monitored natural cycle with daily LH, E₂ and P measurements starting at cycle day 8 and to track the LH surge until P ≥ 2 ng/mL (ovulation).

See page 40 for list of abbreviations

OFFERING EVIDENCE-BASED SOLUTIONS TO PATIENTS' INFERTILITY

As soon as the diagnosis has been determined, a specific treatment program can be proposed, taking into account the female patient's basal endocrine profile, age, follicular reserve and expected response to gonadotrophin treatment.

➤ Current policy is to take several patient-specific elements into consideration in order to use the least invasive and most economical therapies first.

This treatment plan also takes into account the fertility status of the male partner and the patient's professional obligations.

To reduce anxiety and obtain good compliance to treatment, the therapeutic plan with its stepwise progression, as well as the possible alternatives, should be clearly explained to the couple. Good understanding and acceptance of the plan by the patient will greatly contribute to treatment compliance and help avoid unnecessary costly over-treatment.

IMPORTANT REMARK CONCERNING HORMONE IMMUNOASSAYS

Reference values cannot be extrapolated between different immunoassay methods. Therefore, the values provided in this booklet are only valid for the bioMérieux VIDAS® systems (Coucke et al., 2007). For all parameters, the reference values are available in the relevant package insert.



BEFORE STARTING AN INFERTILITY TREATMENT SCHEME and hormonal follow-up of ART, the following measurements should be performed to determine the patient's hormonal and serological profile.

BASAL HORMONAL SERUM MEASUREMENTS

FIRST-LINE TESTS (Day 2-5):



- hCG ■ LH ■ FSH ■ Prolactin ■ Estradiol
- TSH ■ AMH

SECOND-LINE TESTS (DAY 2-5):



- Progesterone ■ Testosterone ■ SHBG
- 17OH Progesterone ■ DHEA-S ■ FT4 ■ anti-TPO

SEROLOGY MEASUREMENTS

(essential for screening partners prior to starting treatment if safe storage of human gametes is intended)

OBLIGATORY TESTS (EUROPEAN DIRECTIVE 2004/23/EC):



- HIV
- Hepatitis B (HBV) ■ Hepatitis C (HCV)
- Syphilis (only if gamete donation outside the couple is considered)
- In case of history of exposure or travel: HTLV-1, Malaria, Chagas disease
- In case of sperm or egg donor banking: perform Nucleic Acid Testing (NAT tests) for HIV, HBV, HCV, Chlamydia for direct use of gametes. If no NAT test: maintain gametes frozen in quarantine for 180 days and check negativity of serology before using donor cells.

In seronegative patients (both partners) who prefer NOT to be vaccinated for HBV, a control should be made for each treatment cycle, before the potential storage of gametes and /or embryos.

GOOD CLINICAL PRACTICE RECOMMENDS PRE-CONCEPTION TESTING FOR:



- Toxoplasmosis ■ Rubella
- Cytomegalovirus (CMV)

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INFERTILITY TREATMENT SCHEMES AND HORMONAL FOLLOW-UP OF ART

Depending on the diagnosis, different levels of treatment complexity can be applied:

LEVEL 1: NATURAL CYCLE MONITORING WITH:

- timed intercourse
- intrauterine insemination (IUI)

LEVEL 2: MILD OVARIAN STIMULATION (for IUI)

This treatment is combined with timed natural intercourse, including intrauterine insemination. The aim is to avoid multiple follicle development, but ideally to induce mono-ovulation.

Hormonal products used:

- a/ Clomiphene citrate (CC)
- b/ Gonadotrophins: highly purified urinary or recombinant products. Use of FSH (uFSH, rFSH); HMG (uFSH + uLH + uhCG); LH (rLH); hCG (uhCG, rhCG).

LEVEL 3: SUPEROVULATION TREATMENT USING HIGH-DOSE GONADOTROPHINS (IVF OR ICSI)

The aim is to obtain multiple oocytes to fertilize *in vitro* and to replace 1 or 2 embryos in the same treatment cycle. The supernumerary embryos can be cryopreserved.

Standard protocols and follow-up

- a/ GnRH-agonists: used to suppress the patient's LH/FSH hormone production, prior to stimulation.
- b/ GnRH antagonists: used to prevent a spontaneous LH rise.
- c/ Gonadotrophins: highly purified urinary or recombinant products. Use of FSH (uFSH, rFSH); HMG (uFSH + uLH + uhCG); LH (rLH); hCG (uhCG, rhCG).

Note: Urinary (e.g. uFSH or HMG) and recombinant (e.g. rFSH) products are both commercially available and have equal efficacy as demonstrated in prospective trials and meta-analyses (Platteau *et al.*, 2008; Coomarasamy *et al.*, 2008)

PRECAUTIONS WHEN USING GONADOTROPHINS FOR OVULATION INDUCTION

It is good clinical practice to monitor steroid hormone levels and follicular growth to reduce risks of multiple gestation and ovarian hyperstimulation syndrome (OHSS).

Minimal Assisted Reproduction (Level 1 and Level 2):

In the case of a patient with a normal cycle and in the absence of cervico-uterine disease, bilaterally blocked Fallopian tubes and dramatically low sperm counts (<10 million/ml), a first approach is to help the couple optimize their chances for conception by timing intercourse with or without a light ovarian stimulation treatment (such as Clomiphene citrate or low-dose gonadotrophin injections).

The type of stimulation protocol to choose depends on the patient's anovulation type: WHO I, WHO II, WHO III, hyperprolactinaemia (see page 9).

Using cycle 'monitoring' means that the growth and ovulation of (a) follicle(s), the production of E₂ and progesterone and the start of the spontaneous LH-rise (LH > 15 IU/L) can be followed-up on a regular basis to help make decisions for timing of intercourse or artificial insemination.

The main goal is to ensure that sperm cells are deposited at the most fertile time, i.e. in close proximity in time and place to the ovulating oocyte(s).

THE DECISION-MAKING PROCESS FOR CHOOSING TREATMENT IS BASED ON CLINICAL EVIDENCE AND DISCUSSED WITH THE COUPLE

Indications to the couple for starting one of the 3 treatment levels depends on the preliminary detection of blocked Fallopian tubes, severe uterine disease conditions, severely altered sperm, high titer of sperm autoantibodies, poor ovarian follicle recruitment, advanced age (>35 years) of the female partner. If one of these conditions is present, IVF/ICSI will usually be prescribed upfront. ICSI is better indicated than IVF only in couples with severe male infertility.

If the sperm quality is acceptable - generally 5 to 10 million motile sperm cells in total in a potential insemination sample (ESHRE Capri Workshop Group, 2009), a policy can be discussed with the couple to begin with treatment levels 1 or 2, taking into consideration the effort, cost and success rates per treatment cycle. Recent literature on insemination practice enables the following statements to be made to help the couple decide:

1. in stimulated cycles (clomiphene citrate or low dose gonadotrophin therapy), the maximum pregnancy rate per cycle is approximately 10% for clomiphene and around 20% for gonadotrophins.
2. the risk for multiple pregnancy cannot be avoided and can even be as high as 30-40% (with gonadotrophins).
3. intra-uterine insemination is cheaper and less demanding on the couple than IVF/ICSI.

LEVEL 1

NATURAL CYCLE MONITORING WITH TIMED INTERCOURSE AND INSEMINATION

TREATMENT (see Figure 1)

On a first attempt, the gynecologist can decide to prescribe **regular timed intercourse** with the rationale that, if there is, for example, a slight male infertility factor involved, intercourse should be planned at the most fertile moment of the cycle. This most fertile period is when ovulation takes place. It can be easily tracked with regular blood samples (measure basal values on Day 2 and then more frequently from Day 8 when estrogens (E) start to rise). Blood samples are assayed for the hormones E₂ and LH. When LH rises over a value of 15 IU/L in the presence of increasing E₂ concentrations > 150 pg/mL and if a follicle larger than 17 mm diameter is seen on ultrasound, the couple is instructed to have intercourse. An optimal sperm sample can be expected after not ejaculating for 3 days.

If the sperm count is too low, the patient can be **inseminated** with a preselected fraction of her partner's sperm when her E₂ level is still high.

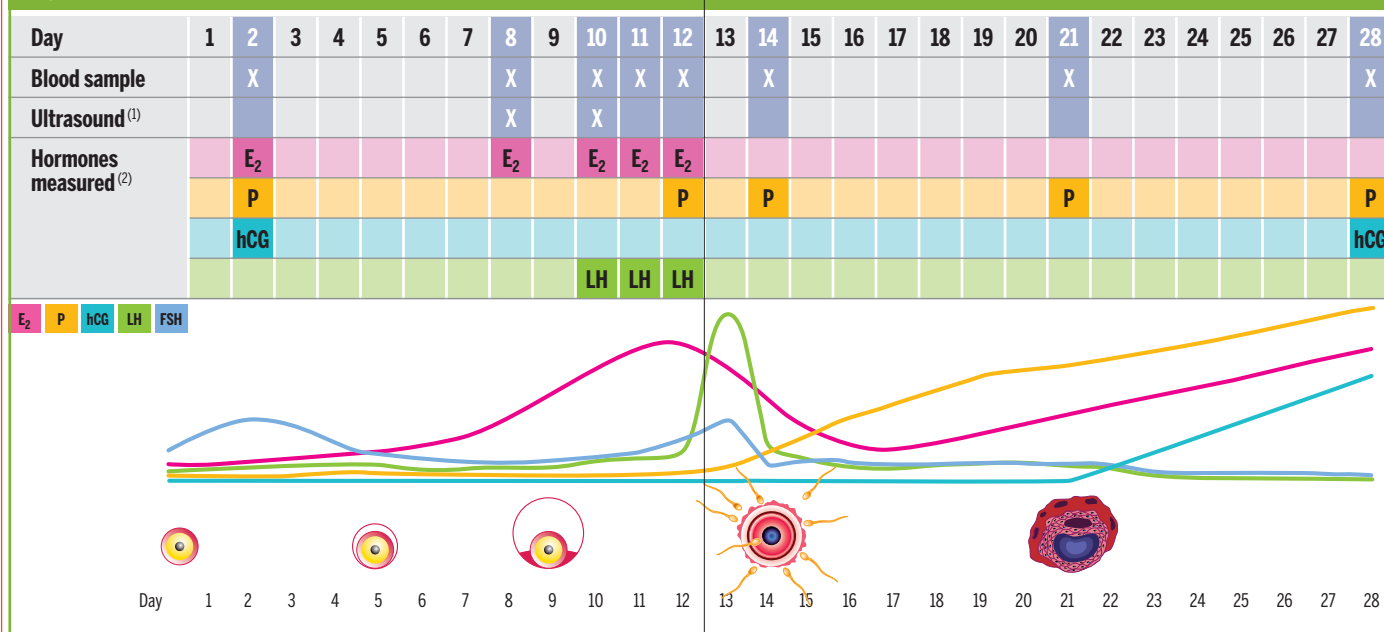
At the moment of insemination, a highly fluidic mucal secretion can be observed at the cervical ostium. This is a sign of good estrogen impregnation suitable for sperm migration. The progesterone level is still low (< 1.5 ng/mL) at this time. An increase in progesterone will make the patient's mucus less permeable for sperm cells. The correct monitoring of hormones can therefore contribute to more rapid success.

The day after a well-timed insemination, LH should still be high (generally ≥ 10 IU/L) and progesterone should be on the rise.

FOLLOW-UP (see Figure 1)

■ **Intra-uterine insemination** can be planned (only once) ± 30 hours after the first spontaneous LH surge (generally LH > 15 IU/L indicates the midcycle LH rise) or 32-36 h after hCG injection (ESHRE Capri Workshop Group, 2009).

Figure 1: Timed intercourse and insemination in a natural cycle



1) Ultrasound: emerging dominant follicle of 12 mm
 2) As soon as: E₂ ≥ 150 pg/mL, LH ≥ 15 IU/L, P < 1.0 ng/mL, advise the couple to have intercourse on same evening and on day after *

* With this kind of hormonal profile, hCG (5,000 IU) might be injected and progesterone administered intravaginally (in which case there is no need to measure progesterone in the luteal phase).

LEVEL 2

MILD OVARIAN STIMULATION (FOR IUI)

The main purpose of mild ovarian stimulation therapy is to obtain ovulation of a maximum of 2 to 3 fertilizable oocytes, but to avoid a multiple pregnancy.

The main indications for **LEVEL 2** treatments are the following:

- 1. Idiopathic infertility** (i.e. no clear origin found for the infertility)
- 2. Polycystic ovary syndrome** (enlarged ovaries, multiple cysts on the periphery, dense stroma)
- 3. Mild male infertility** There should be minimally 1 million hyperactive grade A motile sperm cells after washings for selection to have a fair chance of success by intra-uterine insemination (IUI) (*Ombet et al., 1995*).

LEVEL 2.A.

TREATMENT: CLOMIPHENE CITRATE (CC) (DAILY DOSES FROM 50 MG TO MAX. 150 MG/DAY ORALLY): FROM D2 - D7

A mild stimulation regimen can be given by using an estrogen receptor blocker (clomiphene citrate, CC). This treatment can be taken orally, and requires only minimal monitoring if the patient responds well (80%). Anti-estrogens are inexpensive and require few controls, but can only be effective if the pituitary gland is functioning normally (normal LHRH production, normal response of FSH/LH to LHRH). The medication for anovulation will be determined based on previously determined type of anovulation (see page 9).

Patients with severe hypogonadotropic hypogonadism (WHO Type I) cannot benefit from clomiphene citrate (CC) therapy and 20-30% of patients with gonadotrophic dysregulation (WHO Type II) are resistant to this drug.

Generally, the serum E₂ concentration will start to increase exponentially from Day 7 or 8, after an initial rise from Day 1 to Day 6, and may reach 500 to 1,200 pg/mL. For each follicle larger than 15 mm diameter, the E₂ value may vary from 150 to 300 pg/mL, and the smaller follicles between 8 and 14 mm also slightly contribute to the total E₂ serum content (*Anckaert et al., 2002*).

IMPORTANT REMARK

Basal LH secretion is enhanced by clomiphene and values between 12 and 20 IU/L during and after clomiphene intake are not uncommon. This increased basal LH value should not be interpreted as the start of an LH peak. Monitoring for clomiphene therapy is generally started on cycle day 10 and controlled every 2 days to estimate the number of growing follicles and their diameter. After completing clomiphene treatment, a spontaneous LH peak will almost always occur, but is quite often slightly delayed to Days 13-14.

Patient insemination should occur when:

LH is still on the rise, E₂ is high: > 500 pg/mL (200 pg/mL per follicle > 15 mm) and Progesterone is still low (≤ 1.5 ng/mL). It is not always easy to unite these criteria, but when successful, there are in general only < 10% twins and almost no occurrence of OHSS (Ovarian Hyperstimulation Syndrome). Treatment with artificial insemination after clomiphene medication holds success rates per cycle of nearly 7% (*ESHRE Capri Workshop Group, 2009*).

If there is still no pregnancy after 4 to 6 cycles of clomiphene treatment with monitoring, the decision should be made to change to ovarian stimulation treatment with gonadotrophins (see Level 2.B.).

FOLLOW-UP (see Figure 2)

With clomiphene citrate (CC), the LH concentration will be increased from Day 8 (this is a direct effect of CC on LHRH and the gonadotroph). Therefore, an increased LH value (between 10 to 20 IU/L) on Day 8 should not be misinterpreted as the start of an LH mid-cycle surge. Under CC treatment, a minimal level of LH ≥ 20 IU/L is necessary to indicate an LH peak.

Almost all cycles treated with CC that show rising E₂ concentrations on Day 10 will have a spontaneous LH peak, sometimes occurring only when the follicle has reached a fairly large diameter (≥ 20 mm).

Generally, CC-stimulated cycles develop 2-3 ovulatory follicles (and E₂ levels reach 600-1,200 pg/mL). As soon as an E₂ value of 500 pg/mL is measured, combined with at least 1 follicle of at least 17 mm in diameter, some practitioners prefer to inject 5,000 IU of hCG in order to reduce the risk of a multiple pregnancy as a result of the co-development of some smaller follicles which can co-ovulate.

LEVEL 2 MILD OVARIAN STIMULATION

Figure 2: Follicular phase monitoring scheme

Day	m	2	3	4	5	6	7	8	9	10	11	12
Blood sample		X								X	X	X
Ultrasound		X								X		
Hormones measured		E ₂								E ₂	E ₂	E ₂
		P								P	P	P
		hCG										
										LH	LH	LH

On Day 2, hormones should be basal.

On Day 10, P is measured to evaluate premature progesterone rises (P > 1.5 ng/mL), and LH to track the spontaneous LH surge.

LEVEL 2.B.

TREATMENT: "LOW DOSE" GONADOTROPHIN THERAPY

In general, **the diagnostic work-up** will give an indication of the type of treatment and the doses to administer:

- high FSH, low AMH: indicates a low follicular reserve (WHO type III)
- high AMH, high androgens, increased basal LH: indicates a polycystic ovary (PCO) (WHO type II)

If the patient is anovulatory:

Five days of progesterone substitution therapy (progesterone withdrawal test) will enable the gynecologist to determine whether the patient has either:

1. a hypogonadotropic hypogonadism (WHO I, rare): in this case, withdrawal bleeding at the end of progesterone administration will not occur,
2. a frequent PCO-like syndrome (WHO II): in this case, menses will start the day after stopping progesterone intake. These patients have often a gonadotrophin imbalance (basal LH > FSH).

The **progesterone withdrawal test** is a useful indicator for the starting dose:

- if there is no menstrual bleeding: it is considered that the patient has no estrogenic impregnation due to hypogonadotrophism. The treatment will then generally be started with 150 IU of HMG (administered once daily) or 100 IU of recombinant FSH (rFSH).
- if there are menses: the patient has some estrogen impregnation and an endogenous pituitary function. Generally, treatment is started cautiously with 75 IU of rFSH or HMG (administered once daily).

General principles are to start with treatment when the patient has a basal profile: hCG negative; E₂ ≤ 50 pg/mL; P < 1 ng/mL.

When the patient reports onset of menses, it is recommended to request a blood sample the next day to control whether hCG is unmeasurably low (no pregnancy) and P < 1 ng/mL (no rescued corpus luteum from previous cycle).

Serum hormonal monitoring and ultrasound scanning are performed as shown in Figure 2. For each follicle over 15 mm, an E₂ concentration of ±150-300 pg/mL can be considered as normal.

With gonadotrophin therapy, a **dose of 5,000 IU hCG is always needed to rupture the follicle at ovulation**. hCG is generally administered when the largest of the 3 follicles reaches a mean diameter of 17 mm.

USE OF GONADOTROPHINS

Gonadotrophins are very potent drugs with a direct impact on the ovaries. They induce follicle growth when the FSH concentration reaches the patient's threshold value. The longer the FSH concentration remains above the FSH threshold value, the more follicles will continue their final growth phase and be saved from atresia. Monitoring of serum hormones (E₂/LH) is required as up to 30% of the treated women will have premature LH surges (*Lambalk et al., 2006*).

Use of gonadotrophins requires strict blood monitoring and ultrasound scanning. Progressive adjustment of daily doses of gonadotrophin will avoid severe complications. In preparation for an insemination, a low-dose gonadotrophin regimen is generally recommended, which can increase the success rate by up to ±12% per cycle. However, 20% of the pregnancies will be twins and occasionally (less than 3% of the treatments), there will be a high-rank multiple pregnancy (*ESHRE Capri Workshop Group, 2009*).

LEVEL 2 MILD OVARIAN STIMULATION

FOLLOW-UP PROPOSED MONITORING OF OVULATION INDUCTION TREATMENT USING GONADOTROPHINS FOR INTRA- UTERINE INSEMINATION (see Figures 2 and 5)

The decision to inject hCG (5,000 IU) is based on follicle diameter measurement (ultrasound) and follicle numbers on both ovaries. A 'leading' follicle of 17 mm in diameter would justify hCG. The E₂ value should correspond to between 150 and 300 pg/mL per follicle larger than 15 mm diameter. Progesterone should still remain < 1.5 ng/mL.

Monitoring this kind of treatment is very challenging, since most high-rank multiple pregnancies have their origin in these types of stimulations (i.e. outside IVF/ICSI programs).

If LH is over 15 IU/L and E₂ exceeds 150-300 pg/mL per follicle > 15 mm diameter, and P is still < 1.5 ng/mL: insemination should be done the next day.

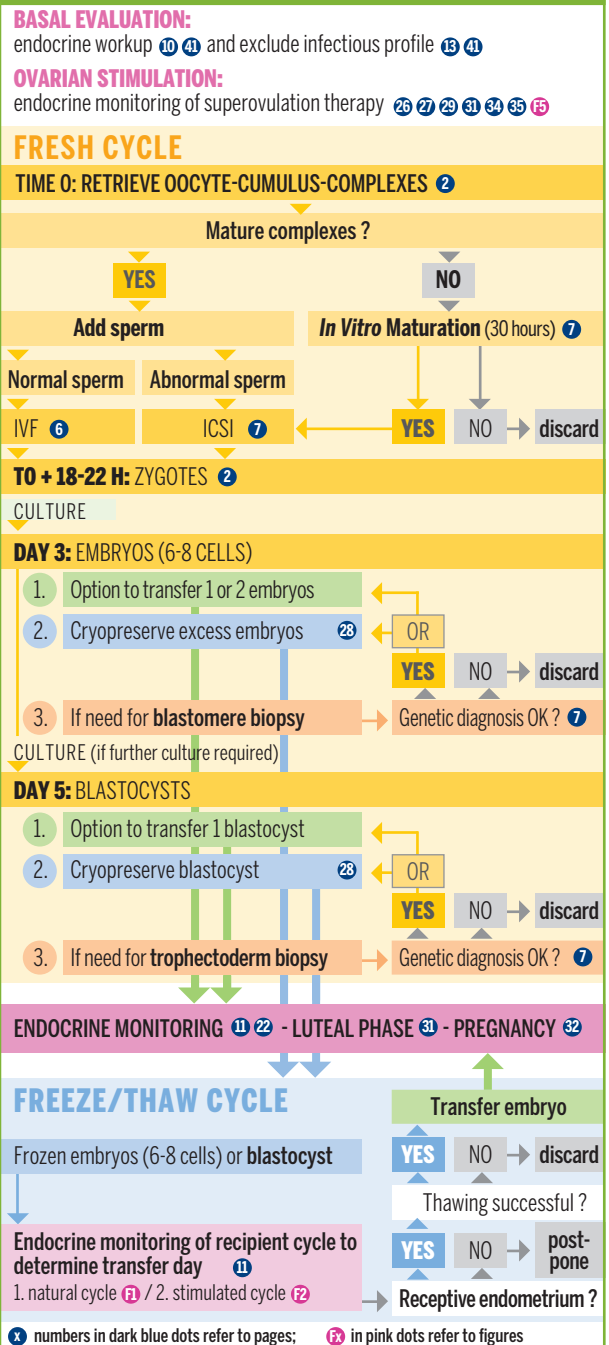
If LH is increased (> 15 IU/L), P ≥ 3 pg/mL and E₂ is decreasing: the LH peak has been missed and the patient has ovulated. There is therefore no interest in inseminating the patient in this cycle. For the next gonadotrophin treatment cycle, the patient should be called 2 days earlier to give a blood sample.

LUTEAL PHASE SUPPORT using natural micronized progesterone

When administering gonadotrophins, the endogenous secretion of the patients' gonadotrophins is suppressed. This leads to insufficient stimulation of the corpus luteum due to a lack of LH. As a consequence, progesterone levels can become too low at the mid-luteal phase and/or progesterone secretion will be prematurely arrested in the second half of the luteal phase, resulting in early menstrual bleeding. These conditions are luteal phase defects, which are partly iatrogenically induced by the high steroid levels caused by gonadotrophin treatment.

Studies have shown a significant increase in pregnancy rates from exogenous progesterone supplementation in gonadotrophin-stimulated patients (*Posaci et al., 2000; Tavaniotou et al., 2001; Fatemi et al., 2007*).

Figure 3: ART Treatment Process (illustrated example of typical process)



LEVEL 3

SUPEROVULATION TREATMENT AND IVF-ET OR ICSI

TREATMENT

Superovulation therapy to obtain multi-follicular growth for oocyte retrieval followed by single or double embryo transfer.

Ovarian stimulation for IVF and ICSI is today almost exclusively carried out using gonadotrophins. The bioactivity of these preparations is still expressed in International Units (IU) of bioactivity measured in a rat model. It is generally accepted that the units used for the different preparations are bioequivalent. Therefore, for reasons of clarity, the IU further mentioned in this brochure are valid for all commercially available preparations.

Pregnancy rates from IVF and ICSI in Europe range between 25 and 30% per treatment cycle.

Two types of strategies can be followed by fertility clinics to start stimulation:

1. stimulation with strict planning (in line with the fertility clinic's working days).
2. stimulation based on Day 1 of patient's cycle (in this case, the fertility clinic also operates on weekends).

Treatments can use GnRH agonists or antagonists; those using agonists are longer but less costly, whereas those using antagonists are shorter but more costly.

PLANNING (see Figure 4)

If planning is preferred, this can be achieved in 2 different ways:

1. put the patient on a contraceptive pill for a number of days/weeks. Stopping intake will cause menstrual bleeding = Day 1.
2. administer a GnRH agonist for at least 12 days (this is generally initiated on Day 21 of the cycle) so that the patient becomes downregulated. From then on, stimulation can be started on any preferred day.

If no planning is required, the patient can start gonadotrophin treatment on Day 2 of the cycle.

FOLLOW-UP

(see monitoring scheme p.28 and Figure 5)

LUTEAL PHASE SUPPORT

After oocyte retrieval, the luteal phase is preferentially supported by natural micronized progesterone for vaginal application (see page 22).

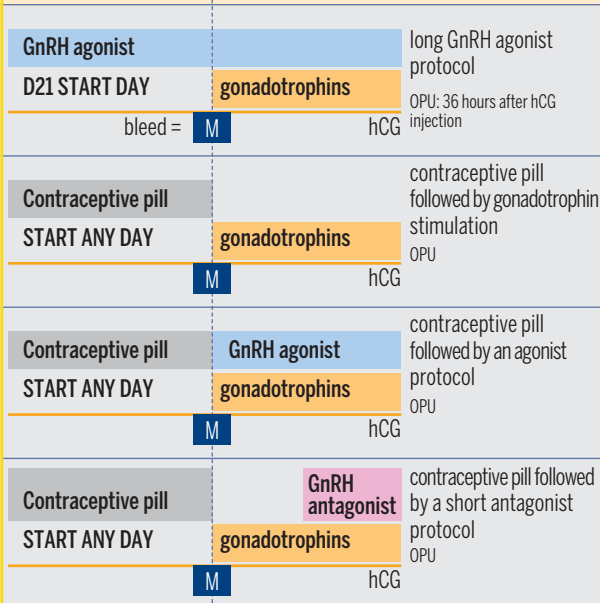
Figure 4: Overview of the most frequently used stimulation protocol schemes

I. WITH PLANNING:

treatment duration will generally exceed 3 weeks to reach oocyte pick-up (OPU).

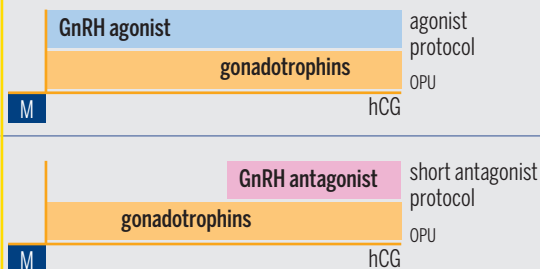
→ FLEXIBLE PRE-TREATMENT (to plan the START)

→ STIMULATION TREATMENT



II. WITHOUT PLANNING:

treatment will take less than 2 weeks to reach OPU.



LEVEL 3: SUPEROVULATION TREATMENT AND IVF-ET OR ICSI

FOLLOW-UP MONITORING SCHEME (HORMONES AND ULTRASOUND)

(see Figure 5)

Total hormonal control panel (hCG, LH, FSH, E₂, P):

- at start of treatment
- repeated on day before starting gonadotrophins: at this time results should be basal and hCG negative

Restricted hormonal control panel (hCG, E₂, P):

- repeated on Day 7 of gonadotrophin treatment to adjust the daily dose (E₂)
- repeated daily from Day 10 onwards (E₂, P)
- measure serum hCG on Day 13 to be sure it has been properly injected (6-12 hours after hCG injection)

Ultrasound measurements:

- Day 2 = day of start of gonadotrophins
- Day 7, Day 10 and after Day 10, every 2 days until follicles are > 17 mm diameter

CRITERIA FOR HCG OVULATORY DOSE ADMINISTRATION (5,000 IU or 10,000 IU)

- 1) 3 follicles ≥ 17 mm on both ovaries
- 2) E₂ value: 150-300 pg/mL x number of follicles ≥ 15 mm
- 3) At this point, the Progesterone value should still be ≤ 1.5 ng/mL

The crucial injection of the ovulatory dose hCG is controlled within 8 to 12 hours after injection by blood sample measurement as a matter of security. If no hCG is detected and serum LH is also still low, a new hCG injection is given and the pick-up is postponed by 36 hours. If LH is high (≥ 25 IU/L), the pick-up is planned 30 hours later. These measures reduce the occurrence of the so-called 'empty follicle syndrome'. Without an hCG or LH increase in the follicle, the oocyte cannot be released from the follicle wall by mucification of the cumulus cells.

Figure 5: Level 2B and Level 3 monitoring scheme

GONADOTROPHIN STARTING DOSES (given for first 5 days):				INCREASE DOSES EVERY 3 DAYS BY:				
<ul style="list-style-type: none"> • hypogonadism: 150 IU/day • normal patient: 150 IU/day • PCO-like patient*: 75 IU/day 				<ul style="list-style-type: none"> 75 IU 75 IU 37.5 IU* <p>* In PCO syndrome, dose adjustments are made only every 5 to 7 days with a max. increase of 37.5 IU daily per 5 to 7 days.</p>				
SERUM HORMONAL MONITORING								
Day (morning samples)	2	7	10	12	13	21	28	
	↓	↓	↓	↓	↓	↓	↓	
	E ₂ < 50 pg/mL P < 1.0 ng/mL hCG < 5 IU/L	E ₂ > 150 pg/mL	E ₂ P	E ₂ P	hCG LH	P	P hCG	
	give daily gonadotrophin dose for 5 days	if E ₂ < 150 pg/mL: add 1 ampoule to daily dose, but increase in steps of 3 days if E ₂ > 150 pg/mL: stay on same dose		E ₂ : count 150-300 pg/mL per follicle > 15 mm P: if P > 1.5 ng/mL LH: if LH > 15 IU/L hCG: measure serum level			P: if P > 5 ng/mL and hCG > 10 IU/L control every 3 days	
Ultrasound Monitoring	X		X	X				
	- Measure numbers of follicles/class (small - medium - large) - Measure endometrial thickness (≤ 5 mm)			- Measure endometrial thickness (> 8 mm)				
IMPORTANT DECISIONS	<p>→ START OF HORMONE ADMINISTRATION</p> <ul style="list-style-type: none"> • if E₂ > 50 pg/mL and P > 1 ng/mL: → wait, a residual corpus luteum is still active → there is a functional cyst to aspirate • if hCG > 5 IU/L: do not start treatment, but monitor further until negative value for hCG • early pregnancy may subsist and should be supplemented with micronized progesterone. 			<p>→ DECIDE ON INJECTING hCG (5,000 or 10,000 IU):</p> <ul style="list-style-type: none"> • if 2-3 follicles and a leading follicle of 17 mm • if P > 1.5 ng/mL: indicates the start of luteinization • if P > 3 ng/mL and if LH > 15 IU/L: LH peak has been initiated and OPU needs earlier scheduling (30 hours) 				<ul style="list-style-type: none"> • if hCG > 10 IU/L: follow up every 3 days • if P < 5 ng/mL: follow up every 3 days <p>Suspicion of early abortion or ectopic pregnancy</p>

6 SAFE STORAGE OF SPERMS, OOCYTES AND EMBRYOS

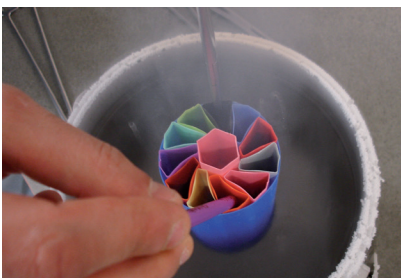
Safe storage implies that the serological status of each sero-negative patient is re-tested for each treatment cycle.

During an IVF or ICSI cycle, embryo storage is prescribed by most European Health Regulators to restrict the number of embryos to transfer to 1 or 2. The supernumerary embryos should be vitrified (frozen) for further use by the couple in subsequent embryo replacement cycles.

Current European regulations stipulate that before banking embryos (or gametes), the couple's serological profile for HIV and hepatitis B and C should be checked in order to avoid contamination of the bank. The European Directive mentions that similar precautions should be taken when banking sperms or oocytes.

Donations of gametes for heterologous use can only be performed if the Nucleic Acid Test (NAT) is negative for HIV, Hepatitis, Chlamydia and HTLV-1 (in case the donor originates from a risk area).

It is also Good Clinical Practice to evaluate the patient's serological profile for syphilis, toxoplasmosis, rubella and cytomegalovirus, as these congenital infections could irreversibly harm the fetus. Vaccination (if existing) can be proposed to sero-negative patients, or they can be instructed to take into account specific hygiene rules during pregnancy to avoid contact with the pathogen.



➡ Cryopreservation is performed either by computer-controlled freezing in an apparatus, or by vitrification.

7 ROLE OF HORMONE MEASUREMENTS IN PRACTICE

WHAT CAN HORMONE MEASUREMENTS TELL US?

General criteria for good ovarian stimulation regardless of the stimulation regimen

GONADOTROPHIN STIMULATION FOR IVF/ICSI SHOULD BE INITIATED ONLY IF ALL HORMONES ARE BASAL

If the patient is pretreated (GnRH agonist or contraceptive use):

FSH and LH ≤ 2 IU/L, $E_2 \leq 50$ pg/mL, $P \leq 1$ ng/mL

Any increased value means that the patient is:

- either not basal yet: if LH > 2 IU/L, the GnRH agonist dose was insufficient
- or has formed a cyst that is still producing steroids: $E_2 > 50$ pg/mL; $P > 1$ ng/mL

SOLUTION:

- Patient receives an adapted dose of GnRH agonist
- Patient requires cyst aspiration (ambulatory procedure)

Effectiveness of the corrective therapy is controlled by a new blood sample taken 3 days later to confirm basal values.

If the treatment is started on Day 1 or 2 of the patient's natural cycle and if $E_2 > 50$ pg/mL or $P \geq 1$ ng/mL, this means that the corpus luteum of the previous cycle has still not completely regressed.

SOLUTION: In this case, a repeat sample should be taken the next day.

If E_2 and/or P values remain persistently high, there might be an inadvertent pregnancy. The pregnancy is revealed by a positive hCG result.

SOLUTION: Follow-up the pregnancy by blood sampling every 3 days to evaluate hCG increase (should be exponential) and Progesterone increase.

ALERT: Some of these pregnancies may end in an extra-uterine pregnancy or early abortion.

The increase in E₂ should reflect the number of growing follicles

- Blood sampling after 5 to 6 days of continuous gonadotrophin use will reveal if the FSH threshold for follicle growth has been reached: the E₂ value should be at least ≥ 150 pg/mL. In this case, the patient can stay on the same dose of FSH. If not: add 75 IU daily of FSH or HMG and control blood 3 days later.
- After Day 8 of stimulation, E₂ values should double every 2 to 3 days.
- If E₂ doses do not increase over a 2-day interval, it means that luteinization of the largest follicles has started. This is then visible by the doubling of the P value over 24 hours.
- For a follicle larger than 15 mm diameter, an E₂ value reaching values of 150 to 300 pg/mL indicates a preovulatory follicle.

NOTE: The decision to inject hCG is taken on an E₂ value concordant with the follicle diameters measured by ultrasound (i.e. leading follicle > 17 mm/diameter).

THREE ALERT SITUATIONS INDICATED BY E₂

1. If, in the presence of growing follicles (evidenced by ultrasound), there is almost no increase in E₂ in blood, it means that the patient has insufficient LH activity at the ovarian level

SOLUTION: Give a gonadotrophin preparation containing LH bioactivity as a supplement.

2. If, after 6 to 7 days of stimulation, a decrease (instead of an expected increase) is suddenly observed in the E₂ level, concomitant with a rise in P, this may indicate the start of luteinisation.

SOLUTION: Stop FSH/hMG administrations and inject hCG, because no further follicle growth can be expected unless *in vitro* maturation (IVM) is initiated.

3. If E₂ has suddenly reached values > 3,000 pg/mL and a multitude of small and medium sized follicles are visible on ultrasound in both ovaries, this predicts ovarian hyperstimulation syndrome.

SOLUTION:

- 1) Stop FSH injections and keep patient under GnRH analogs until the follicles become atretic. This requires close patient monitoring (at least every 2 days) until E₂ values have returned to basal.
- 2) Administer a dose of GnRH agonists as an ovulatory trigger (instead of hCG) and vitrify all embryos (*Humaidan et al., 2011*)

Progesterone measurements in the follicular growth phase are important predictors of pregnancy

1. At the beginning of the cycle (Day 1-3)

Progesterone increases (≥ 1 ng/mL) in the early follicular phase indicate the rescue of the corpus luteum from the previous menstrual cycle.

SOLUTION:

- 1) Do not initiate treatment and wait for proper regression of the rescued corpus luteum.
- 2) Administer GnRH antagonist during 3 days and control if the corpus luteum has regressed (P and E₂ basal).

2. At the end of the Follicle Stimulation Phase

Progesterone increases at the end of the stimulation period to values ≥ 1.5 ng/mL can indicate:

- the start of luteinization in the largest follicle(s) (often this happens if there is not a well-synchronised cohort: a single large follicle of > 20 mm diameter with a cohort of smaller follicles).
- the stimulation has been too long and/or excessive

SOLUTION: the next treatment should use a gonadotrophin preparation containing LH bioactivity (HP-HMG or a lower FSH dose).

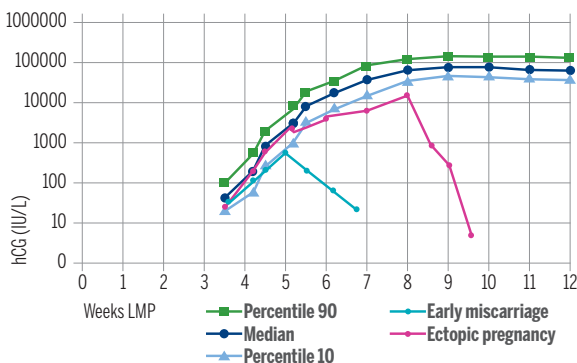
ALERT: Progesterone elevations before hCG injection have been associated with a 50% decrease in pregnancy rate due to negative effects on endometrial cells (*Smitz et al., 2007; Fleming et al., 2008; Bosch et al., 2010*).

➤ WHY IS THE TIMING FOR HCG TESTING IMPORTANT?

The measurement of hCG in serum remains the most sensitive and specific parameter to measure the implantation of one or more embryos.

Generally, patients are requested to provide a blood sample on Day 15 after the ovulatory hCG injection, even if they have already started menses. This sample is sufficiently distant from the pre-ovulatory hCG dose (10,000 IU) so as not to interfere with it.

Figure 6: Follow-up of outcome in IVF/ICSI



A normally implanted embryo will produce hCG values that will double every 2 days. This exponential profile of hCG is generally accompanied by progesterone values higher than 10 ng/mL (unstimulated cycle in weeks 4 and 5 LMP) or 30 ng/mL (stimulated cycle).

- Post-stimulation progesterone values are higher than in the normal cycle due to the multiple ovulation sites.

Deviations from normal, i.e. hCG values increasing slowly (under percentile 10 and/or deviating from the previous percentile value) and associated with values of Progesterone < 5 ng/mL, indicate an ectopic pregnancy or miscarriage.

- Successive blood samples, rather than a single sporadic sample will clearly indicate whether values continue to thrive or rather deviate from the normal curve.

If more than one embryo has been transferred, multiple gestation can be revealed by increased hCG values. For double implantations, the percentile 50 of hCG will be equal to the percentile 90 of the hCG profile of a single implantation.

It is recommended to follow-up pregnancies after ART using hCG, E₂ and P. Any deviation from normal progression of hCG requires control samples every 3 days until complete documentation by ultrasound (weeks 6 and 7). Ectopic pregnancy, molar pregnancy, early abortion should be monitored to avoid severe complications (Anckaert et al., 2005). Early diagnosis and precise monitoring allows to make the right choice for either a medical treatment (Methotrexate therapy) or a more invasive laparoscopic intervention.

WHY IS HORMONE MEASUREMENT DURING STIMULATION THERAPY FOR IVF/ICSI SO IMPORTANT?

In the natural cycle, ovulation of a single fertilizable egg is the result of very precise interaction between different hormones which synchronize the different crucial events in the fertile cycle: ovulation of a matured oocyte, luteinization of the corpus luteum, opening the receptivity window of the endometrium.

Using ovulation induction therapy, the fine balance is overridden by exogenous gonadotrophin use and this induces asynchrony into maturation between the different biological compartments (e.g. oocyte maturity and granulosa differentiation stage; endometrial differentiation stage and embryo development stage).

The fine-tuning of medication with other therapeutic decisions can only be evidence-based when monitoring is performed to measure the main reproductive hormones at predefined timepoints in an individualized patient-oriented way.

Literature provides clear evidence from large-scale studies that success rates can be predicted by hormone measurements at well-determined days of the cycle (Andersen et al., 2006; Smitz et al., 2007; Bosch et al., 2010; Fleming et al., 2010).

Unmonitored treatment administration followed by follicle puncture results more often in the collection of oocytes with a reduced maturation and development potential. Furthermore, there are as yet no good monitoring tools to predict the receptivity status of the endometrium, leading to the risk of transferring competent embryos into a non-receptive endometrium.

CASE STUDY: An example of the relationship between hormone values and outcome is given in the following pages.

CASE STUDY

IVF STIMULATION WITH GNRH AGONIST AND LOW-DOSE HMG

CURRENT CASE: A PCO-like patient (with an AMH value of 14 ng/mL) treated with a GnRH agonist-long protocol. Initially, desensitisation is attempted, which takes several weeks if done by intranasal spray. After 3 weeks (W3), hormones all show basal values, and stimulation can be started with 150 IU of HP-HMG daily, while maintaining the GnRH agonist treatment.

Figure 7: This chart shows values for the 3 hormones measured

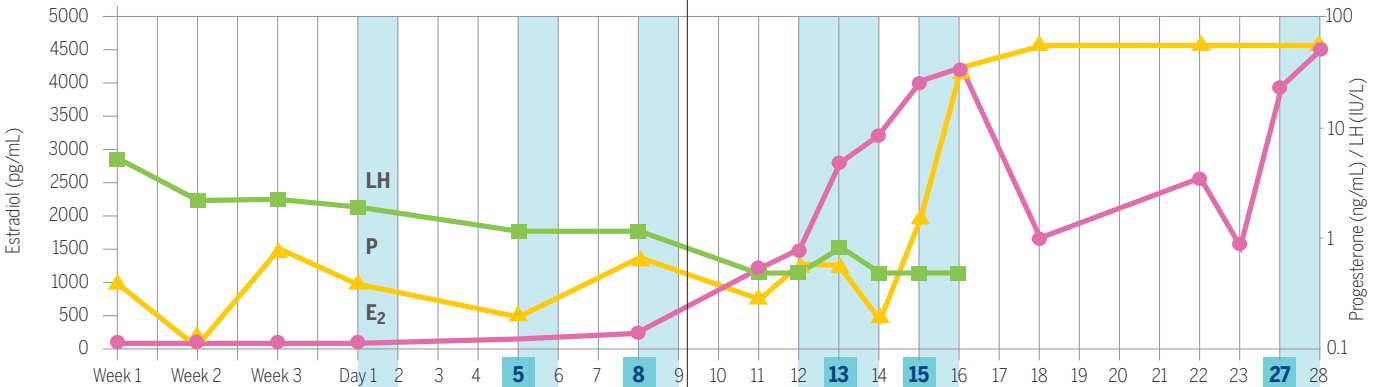


Table 1: Treatment protocol

GnRHa	x	x	x	x	x	x	x	x	x	x	x	x	x	x																																												
hMG							1.5	1.5	1.5	1.5	1.5	1.5	1.5	2	2	2	2	2	Micronized Progesterone 600 mg/day intravaginally																																							
hCG																		10,000 IU																																								

Table 2: Absolute values measured for the 5 hormones

hCG IU/L																																														<5	<5	68	102							
FSH IU/L	2.9	2.4	1.8	3.5				6.2	8.1		8.1	76	5.4	6.2	7.1	4.5																																								
P ng/mL	0.4	0.1	0.8	0.4				0.2	0.7		0.3	0.6	0.6	0.2	1.6	36																																	>60	>60	>60	>60				
E ₂ pg/mL	60	58	58	<50				146	242		1232	1531	2824	3288	4028	4315																																				1729	2598	1592	3989	4683
LH IU/L	5.3	2.2	2.2	1.9				1.2	1.2		0.5	0.5	0.8	0.5	0.5	0.5																																								
Day	W1	W2	W3	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	27	28																												

PANEL 1

Diam	L	R
< 10		
> 10		
N° cysts	0	0

PANEL 2

Diam	L	R
> 25		
20-25		
16-19	1	6
10-16	7	5
< 10	3	

PANEL 3

Number	
OOCYTES RETRIEVED	30
EMBRYOS TRANSFERRED	2
EMBRYOS VITRIFIED	10

Panels 1 and 2 summarize the number of follicles in left (L) and right (R) ovaries according to the specific diameter ranges measured by ultrasound.

Panel 3 indicates the harvest of the oocytes, the embryos transferred in this cycle and the cryopreserved embryos.

Day 5 and Day 8: hormones are measured and show that E₂ is rising slowly. Dose is increased to 2 ampoules.

Day 12: E₂ production reaches 1,531 pg/mL. Only a few large follicles (16-19 mm) are present, but there are many medium (10-16 mm) follicles.

Day 13: the control E₂ value reaches 2,824 pg/mL and it is decided to induce the final maturation with 10,000 IU of hCG.

Day 15: E₂ has risen to 4,028 pg/mL. This is caused by the multitude of follicles. At 36 hours

post-hCG, the follicle puncture results in an excessive number of 30 oocytes. After pick-up and fertilisation, 1 embryo is transferred and 10 others are cryopreserved.

Day 27: twelve days after OPU, the hCG value is 68 IU/L. The patient is pregnant.

The E₂ and Progesterone values are high and testify that the patient is developing a hyperstimulation syndrome, that will be treated conservatively (measure hemodynamic and hematological parameters) and will resume spontaneously.

The patient's pregnancy continued uneventfully. A healthy child was delivered at 39 weeks.

8 FREQUENTLY ASKED QUESTIONS

Q1: If, after 10 days of GnRH agonist pre-treatment, a patient's blood sample persistently shows E₂ to be around 200 pg/mL and P fluctuating around 2.5 ng/mL, what should be initially suspected and investigated?

Presence of hCG.

If positive, the early gestation should be supplemented with Progesterone and further followed by ultrasound.

If in follow-up, hCG increases within normal limits, the inadvertent pregnancy can be rescued by P supplementation. If hCG does not increase sufficiently, an early miscarriage or ectopic pregnancy can be suspected.

Q2: Why also measure progesterone before ovulation?

Progesterone induces a programme in the estrogenized endometrium that opens and closes the receptivity window. When P > 1 ng/mL on Day 2 of the cycle, it indicates that the corpus luteum of the previous cycle is still active.

When P > 1.5 ng/mL at the end of a gonadotrophin stimulation, it indicates an imbalance due to lack of LH bioactivity or an overstimulated ovary in the presence of exaggerated FSH administration. These patients have their chances for pregnancy reduced by 50% (*Platteau et al; 2008*). In some centers, all embryos are frozen in this case. For the next cycle, it is advised to use lower doses of FSH and to preferentially use a gonadotrophin preparation containing HCG or LH. The next cycle must be adapted therapeutically.

Q3: Why is testosterone in the blood of infertile women seeking treatment a useful parameter?

It can indicate hyperandrogenism due to PCOS. These patients are at risk of hyperstimulation and should be treated very cautiously with low gonadotrophin doses, and with a very slow dose increase.

Q4: Is the measurement of LH/FSH important during treatment?

If not downregulated by a GnRH analogue, the sudden increase of LH can cause ovulation. Therefore, LH is monitored together with E₂ (and P on Day 1 of the cycle and on the last day of gonadotrophin stimulation). Also, if after 10 days downregulation with a GnRH-agonist, LH and FSH remain unsuppressed, this may indicate poor treatment compliance by the patient.

Q5: Can the midcycle LH rise be precisely determined by taking only a single blood sample per day?

No, this is not precise enough, because from the moment that LH is sensed by the ovary, there is a set time of 30 to 36 hours after which the follicle will ovulate. So measuring only once per day for LH is not enough.

In an IVF/ICSI setting, 4-hourly blood sampling is able to precisely detect the time of the start of LH, so that oocyte retrieval can be planned 30 hours later (before spontaneous rupture of the follicle).

LIST OF ABBREVIATIONS

ACTH	Adrenocorticotrophic hormone
AMH	Anti-Mullerian hormone
Anti-TPO	Anti-thyroid peroxidase antibody
ART	Assisted reproduction technologies
CASA	Computer-assisted sperm analysis
CC	Clomiphene citrate
CNS	Central nervous system
CT-scan	Computerized tomography scan
DHEA-S	Dehydroepiandrosterone sulfate
E2	Estradiol
FSH	Follicle-stimulating hormone
FT3 / FT4	Free triiodothyronine / Free thyroxine (thyroid function tests)
GH	Growth hormone
GnRH	Gonadotrophin-releasing hormone
hCG	Human chorionic gonadotrophin
HMG	Human menopausal gonadotrophin
HP-HMG	Highly purified HMG
ICSI	Intra-cytoplasmic sperm injection

IGF-1	Insulin-like growth factor 1
IU	International units
IUI	Intra-uterine insemination
IVF	<i>In vitro</i> fertilization
IVF-ET	<i>In vitro</i> fertilization - embryo transfer
IVM	<i>In vitro</i> maturation
LH	Luteinizing hormone
LMP	Last menstrual period
M	Menses
MRI	Magnetic resonance imaging
NAT	Nucleic acid testing
OHSS	Ovarian hyperstimulation syndrome
OPU	Oocyte pick-up
P	Progesterone
PCO	Polycystic ovary
PCOS	Polycystic ovary syndrome
PGS	Preimplantation genetic screening
PRL	Prolactin
SHBG	Sex hormone binding globulin
T	Testosterone
TSH	Thyroid-stimulating hormone

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