Chapter 7
Ovarian Stimulation Prior to Elective Oocyte Cryopreservation

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Introduction

The demand of fertility preservation with no urgent medical indication or for social reasons has increased in recent years.

The main reason is that society has changed the traditional time for pregnancy for professional, personal or social reasons. Women decide to postpone their first pregnancy to a different time than when the biological fertile period is optimal.

Socio-cultural changes, longer life expectancy and professional activity have led to preserve fertility to guarantee the possibility of future pregnancy [1].

Gynecologists should perform the primary prevention of ovarian aging during gynecological reviews by measuring the ovarian follicle reserve and offering the oocyte freezing possibility.

Vitrification is an efficient method to preserve oocytes by quickly lowering temperature using liquid nitrogen (Chap. 8).

By this freezing method, cells are preserved and conserve the same characteristics and quality they had upon freezing [2] (Fig. 7.1).

Fertility preservation providers should inform women about their specific probabilities according to their age at vitrification, and emphasize the fact that egg freezing does not guarantee success, but increases the possibilities of having a biological child in the future [3].

Ovarian stimulation protocols should be as convenient, short and sure as possible to ease the procedure.

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	Vitrified	Fresh	P value
MII oocytes no. %	231 (87.2)	219 (89.7)	.363
MI oocytes no. %	19 (7.2)	11 (4.5)	.203
GV oocytes no. %	15 (5.7)	14 (5.7)	.974
Survival no. %	224/231 (96.9)	-	
No. Of injected oocytes	224	219	
Normal fertilization no. %	171 (76.3)	180 (82.2)	.128
Abnoraml fertilization no. %	9 (4%)	12 (5.4)	.469
Degenerated oocytes no. %	7 (3.1%)	6 (2.7)	.809

Fig. 7.1 Oocyte distribution, survival and fertilization (modified from Cobo A et al. Clinical outcome of oocyte vitrification. Fertil Steril. 2008)

Delaying the Desire of Pregnancy

Nowadays, more and more women contemplate the possibility of getting pregnant precisely when natural fertility is worse.

Various reasons justify society delaying the desire of pregnancy. In the last few years, economic problems have limited the possibility of emancipation and forming a family.

Women's professional activity makes it more difficult to deal with family conciliation issues and lack of time to attend its demands. Women in the western world are full-right city dwellers who adopt opposite attitudes to the traditional roles of domestic work and upbringing.

Longer life expectancy, facility of displacement, social activities and labor opportunities are other reasons to postpone first pregnancy [1].

This trend to leave first pregnancy until a later age and to a time when natural fertility is limited will diminish the possibility of getting pregnant.

Effect of Biological Aging on Ovarian Preservation

As time passes, the number of antral follicles begins to lower. This rhythm of progression is variable according to hereditary genetic determinants and environmental factors, like tobacco or exposure to environmental toxins and/or previous radio- or chemotherapy.

Initial publications by Menken, and more recently by Broekmans, have confirmed the progressive aging of ovarian reserve with time [4, 5] (Chap. 1).

This progressively slow deterioration begins at about the age of 30, and a more drastic quicker drop starts at around 35 years until ovarian reserve disappears in the first few years after reaching the age of 40.

This leads to higher oocyte aneuploidies, which reduce the quality of oocytes and, therefore, fertility.

Armstrong reported that advanced maternal age was related to meiotic incompetence, which makes fertilization rates rise by inducing anomalies in different embryonic development stages to induce more miscarriages.

Age-related abnormalities of oocytes include:

(a) meiotic incompetence or the inability to complete meiotic maturation, which results in oocytes being incapable of fertilization.
(b) errors in meiosis, which can be compatible with fertilization, but lead to genetic abnormalities that compromise embryo viability.
(c) cytoplasmic deficiencies, expressed in several development stages before or after fertilization [6]. Considering these effects of advanced age on oocyte quality, oocyte preservation should be performed as soon as possible to obtain more and better oocytes in women under the age of 35.

Evolution of Stimulation Protocols

The first IVF baby was born in a natural *in vitro* fertilization cycle without ovarian stimulation.

IVF success rates in natural cycles are low due to the limited number of oocytes retrieved per cycle. However, recent studies have shown that IVF in either a natural cycle or modified natural cycle might be a promising low-risk and low-cost alternative to standard stimulated IVF treatment since the available dominant follicle of each cycle is used [7].

Considering this limitation, ovarian stimulation initially using urinary gonadotropins significantly increases both the number of eggs retrieved and successful IVF rates.

In the natural cycle, follicular dominance is achieved by induced estradiol, which provides negative feedback to the pituitary gland which, in turn, lowers FSH levels.

In IVF stimulated cycles, addition of exogenous gonadotropins is used to achieve supra-threshold levels of gonadotropins in the follicular phase to induce multiple follicular recruitment.

However, various problems may occur in stimulated IVF cycles, like premature luteinization and failed synchronous follicular recruitment due to early dominant follicle selection, which implies lower success rates.

Another problem is spontaneous ovulation, which may occur at any time. So gonadotropin-releasing hormone agonists and antagonists (GnRHa and GnRHant) are used to induce pituitary desensitization in order to avoid spontaneous ovulation [8].

Pituitary desensitization using either GnRH agonists or GnRH antagonists eliminates possible interference by endogenous hormones, enables synchronous follicular development and prevents premature luteinization, which enhance the control oocyte retrieval of timing. Finally, the LH surge is substituted for exogenous hCG to induce oocyte retrieval [9].

Ovarian Stimulation Protocols for Oocyte Preservation

Different protocols have been proposed to hyperstimulate ovaries. Some protocols utilize GnRH agonists, while others use antagonists to achieve pituitary desensitization.

Protocols also vary as to the gonadotropins uemployed for ovarian stimulation, and recombinant or highly purified follicular stimulating hormone (rFSH or HP-FSH) are employed.

Addition of GnRH agonists to the luteal phase of the previous cycle in long protocols and in the early follicular phase in short protocols results in an initial flare-up effect, followed by pituitary desensitization.

In contrast, the GnRH antagonists (single or multiple doses) given in the mid-follicular phase, immediately prior to the rise in LH levels, results in rapid pituitary desensitization.

Long Protocol

This protocol starts in the mid-luteal phase of the previous cycle with GnRH agonists being administered daily for about 2 weeks or until down-regulation is completed.

Once down-regulation has been achieved, usually on the first days of menstruation, gonadotropins are administered subcutaneously to stimulate follicular growth with the GnRH agonist being continued at a lower dose.

The hMG/FSH dose is subsequently adjusted according to follicular growth, as monitored by serum E2 levels and transvaginal ultrasonography.

Human chorionic gonadotropin (hCG) is given once the follicular cohort consists of at least three follicles of over 18 mm in diameter to induce oocyte retrieval 36 h later.

This protocol provides excellent cycle control, but its longer treatment duration, higher gonadotropin consumption and more expensive cost are its main disadvantages.

There are different GnRH agonists, like buserelin, leuprorelin, nafarelin and triptorelin.

Nafarelin and buserelin can be administered as a nasal spray. They need to be given between twice and six times a day, and absorption fluctuates which results in an unpredictable response. However, buserelin, leuprorelin and triptorelin are administered as subcutaneous injections once a day.

Wong et al. published a meta-analyses of some trials by comparing different antagonist GnRH preparations. It found no difference in either pituitary suppression efficacy or IVF outcomes in terms of the number of oocytes collected and pregnancy rates. However, these authors reported that women treated with nafarelin required fewer ampoules of gonadotropins for ovarian stimulation, and fewer days of stimulation, compared to leuprorelin, triptorelin and buserelin.

The use of depot preparations of GnRH agonists has been associated with increased gonadotropin requirements and longer times for ovarian stimulation, even though IVF outcomes did not significantly differ [10].

Short Protocol Using GnRH Agonist

In this protocol, GnRH agonist administration starts in the early follicular phase and gonadotropins start the next day. Monitoring, hCG injection timing and oocyte retrieval are the same as with the long protocol.

This protocol in normally used for low-responder patients, or for those who presented a previous poor response in the long protocol, to obtain benefits from the initial flare-up of endogenous FSH release from the pituitary gland, which is induced on the first days of GnRH agonist administration [11].

Short Protocol Using GnRH Antagonist

GnRH antagonists are competitive inhibitors of endogenous GnRH given their receptor binding property, which rapidly inhibit gonadotropin secretion to reduce FSH and LH secretion within 8 h after administration, which is a potential advantage over GnRH agonists.

In this stimulation protocol, gonadotropins are administered on day 2 of the cycle and GnRH-ant is added in the mid-follicular phase to prevent premature LH surge.

Two different molecules, cetrorelix and ganirelix, are available, prove equally efficacious and can be used in two different protocols, the single and multiple dose protocols.

The multiple-dose GnRH-ant protocol involves daily subcutaneous injections of 0.25 mg of either cetrorelix or ganirelix from day 6 of stimulation (the fixed start) until the trigger administration of human chorionic gonadotropin (hCG) or the agonist trigger.

The single-dose protocol involves a single subcutaneous injection of 3 mg of GnRH-ant on day 7 or 8 of stimulation, which provides 4 days of pituitary suppression.

If the patient needs more days of stimulation, a daily dose of 0.25 mg of GnRH-ant injections is required until hCG trigger is performed. The monitoring, criteria for hCG administration and oocyte retrieval is similar to the agonist protocols.

In the flexible start protocol, addition of GnRH-ant begins when the diameter of the leading follicle is 14 mm or more.

In the multiple-dose GnRH-ant protocol daily subcutaneous injections of 0.25 mg of either cetrorelix or ganirelix are given until the trigger is performed.

However in the single-dose protocol, a single subcutaneous injection of 3 mg of GnRH-ant is injected when the diameter of the leading follicle is 14 mm or more to provide 4 days of pituitary suppression. If the patient needs more days of stimulation, daily 0.25-mg GnRH-ant injections are required until the trigger is performed. The monitoring criteria for hCG administration and oocyte retrieval are similar to the agonist protocols [12].

Fixed *Versus* flexible

Al-Inany et al. published a meta-analysis of randomized studies by comparing the fixed and flexible approaches. They concluded having found no statistically significant difference in pregnancy rates, despite finding a trend of the fixed protocol obtaining a higher pregnancy rate. However, the amount of the recombinant FSH and antagonist used with the flexible protocol significantly reduces [13].

Single *Versus* Multiple Dose GnRH Antagonist Protocol

The single dose GnRH antagonist protocol has the advantage of using fewer injections, with only 10% of cycles requiring additional daily doses of the GnRH antagonist.

The potential suppression of endogenous LH does not bring about any significant difference in pregnancy rates, as shown in a multicenter study that compared multiple and single dose protocols of cetrorelix [14].

Wilcox et al. published a prospective randomized trial, and found no significant difference in pregnancy rates between the ganirelix multiple dose and cetrorelix single dose protocols [15].

Advantages of the Antagonist Protocol

The antagonist protocol offers several advantages over the long one, which confirms the elective protocol in fertility preservation ovarian stimulation cycles. The advantages include: shorter treatment duration, fewer menopausal symptoms, less cyst formation due to the initial flare-up effect of GnRH, and fewer gonadotropin requirements.

	Mild protocol	GnRHant protocol	GnRH ag protocol	P value<0.05
Oocyte aspirations	166	1096	111	
FSH	6.4+-1.8	6.7+-2.1	6.9+-2	
Oocytes (per cycle)	1500(9+-5.2)	10249 (9.4+-5)	12004 (10.8+-5.6)	P=0.0001 (mild Vs antg P<0.0001(antg Vs ag)
MI oocytes %	240 (16)	1602 (15.6)	1687 (14.1)	P=0.0420 (mild Vs antg P<0.001(antg Vs ag)
2PN %	865 (57.7)	5440 (53.1)	6235 (51.9)	P=0.0009 (mild Vs ag P<0.0001(mild Vs ag)
Embryos%	845 (56.39)	5198 (50.7)	5968 (49.7)	P=0.0001 (mild Vs antg P<0.0001(mild Vs ag)
ET%	151 (91)	1017 (92.8)	1006 (90.5)	
Cycles with embryo freezing %	50 (30.1)	317 (28.9)	210 (18.9)	P=0.0008 (mild Vs ag P<0.0001(antg Vs ag)
OHHS	0	4	12	
Frozen embryos (per cylce)	0.8+-1.4	0.9+-1.8	0.5+-1.3	P=0.0243 (mild Vs ag P<0.0001(antg Vs ag)

Fig. 7.2 The COH outcome in terms of oocytes and embryos (modified from Stimpfel M et al. Comparison of GnRH agonist, GnRH antagonist, and GnRH antagonist mild protocol of controlled ovarian hyperstimulation in good prognosis patients. Int J Endocrinol. 2015)

Stimpfel et al. reported that the GnRH antagonist mild protocol of controlled ovarian stimulation could be the best method of choice in good prognosis patients due to significant differences in the average number of retrieved oocytes, immature oocytes, fertilized oocytes, embryos, transferred embryos, embryos frozen per cycle, and cycles with embryo freezing. However, this group did not identify any differences in live birth rates (LBR), miscarriages and ectopic pregnancies [12] (Figs. 7.2 and 7.3).

The most important advantage published in a Cochrane review is the significant reduction in the incidence of severe OHSS in antagonist cycles compared to agonist cycles ($p = 0.01$; OR = 0.60, 95%CI 0.40–0.88) [16].

Considering this information, in over-responders and polycystic ovarian syndrome patients, the GnRH-antagonist protocol lowered the incidence of OHSS in high responders.

The main advantage of this protocol is that the final oocyte maturation induced by the GnRH agonist can be used to prevent OHHS from developing [17] (Chap. 11).

	Mild protocol	Antagonist protocol	Agonist protocol	P value<0.05
Pregnancies	69	367	355	
P. Per cycle %	41.6	33.5	32	P=0.04 (mild Vs ang) P=0.01(mid Vs ag)
P per ET 23.2%22.1	45.7	36.1	35.3	P=0.02 (mild Vs antg) P=0.01(mid Vs ag)
Miscarriages %	23.2	22.1	19.4	
BQ preg	2	12	16	
LBR per cycle %	31.3	25.3	25.3	
LBR after FET	7	35	23	
Cumulative LBR per cycle %	35.6	28.5	27.3	P=0.02 (mild Vs antg)

Fig. 7.3 The outcome of COH in terms of pregnancies, miscarriages and deliveries (modified from Stimpfel M et al. Comparison of GnRH agonist, GnRH antagonist, and GnRH antagonist mild protocol of controlled ovarian hyperstimulation in good prognosis patients. Int J Endocrinol. 2015)

However, the antagonist cycle is less programmable than the agonist cycle, and such lack of flexibility poses a problem for some patients and IVF centers.

How to Optimize Ovarian Response

A normal response to ovarian stimulation is expected in patients aged under 40 with regular menstrual cycles (21–35 days), and a normal basal FSH (below 10), or normal AMH levels (2 ng/mL). This patient profile can be stimulated using both protocols to obtain similar results.

The response to stimulatory drugs in an IVF cycle depends on several factors: number of antral follicles, their sensitivity to FSH and bioavailability of FSH.

The expected response usually means the retrieval of 8–10 oocytes due to optimal ovarian stimulation.

Failure to recruit an adequate number of follicles and to retrieve 4–5 mature oocytes is termed a poor response, while the recruitment of 20 follicles or more in high responders increases the risk of OHSS.

To optimize ovarian response, personalization of protocols and selecting optimal gonadotropin doses should be considered.

The antagonist protocol should be offered to reduce the risk of ovarian hyperstimulation ovarian syndrome in patients with high ovarian reserves, like polycystic ovarian syndrome.

The flare-up protocol offers better success rates in women with low ovarian reserve as the long protocol leads to poor results in patients with poor ovarian reserve due to profound pituitary suppression.

Some models have been proposed to determine an optimal stimulation dose. Yet it is still difficult to obtain the desired response as ovarian response is affected by other factors that are yet to be determined [18].

Gonadotropin Dose Selection

Estimating the correct starting dose is extremely important to obtain optimal results.

A gonadotropin dose for standard patients varies between 100 and 250 IU/day.

However, ovarian response variability to the same gonadotropin is influenced by several factors that affect the response to controlled ovarian hyperstimulation, such as: patient's age, body mass index, smoking status, background of endometriosis, antral follicle count (AFC), ovarian volume, stromal blood flow, as well as endocrine parameters like basal FSH levels, inhibin B and Anti-Müllerian hormone serum levels.

The CONSORT study reported that the use of fixed dose regimens calculated by computerized dosing algorithms based on basal FSH, BMI, age and AFC resulted in adequate oocyte yield and good pregnancy rates (an overall of 34.2%) [19].

There are three gonadotropin dose regimes for ovarian stimulation:

- In the fixed dose regime, gonadotropin dose is kept constant throughout stimulation.
- In the step-down regime, a high starting dose of gonadotropins (300–450 IU) is used for the first 2 days, followed by a reduced dose (150–225 IU/day).
- This enables supra-physiological levels of gonadotropins to increase follicular recruitment in the early follicular phase and seems to result in greater follicle synchrony.
- In the step-up regime, the starting gonadotropin dose is low and is increased on cycle day 5, or later, depending on the response.

A variety of controlled ovarian stimulation protocols has been adopted with mixed success rates, but no single approach is appropriate for all patients in a given population. Treatment protocols should be adapted for individual patients to obtain as many oocytes as ovaries can produce. The more oocytes fertilized, the better chances of pregnancy patients will have (Fig. 7.4) [8].

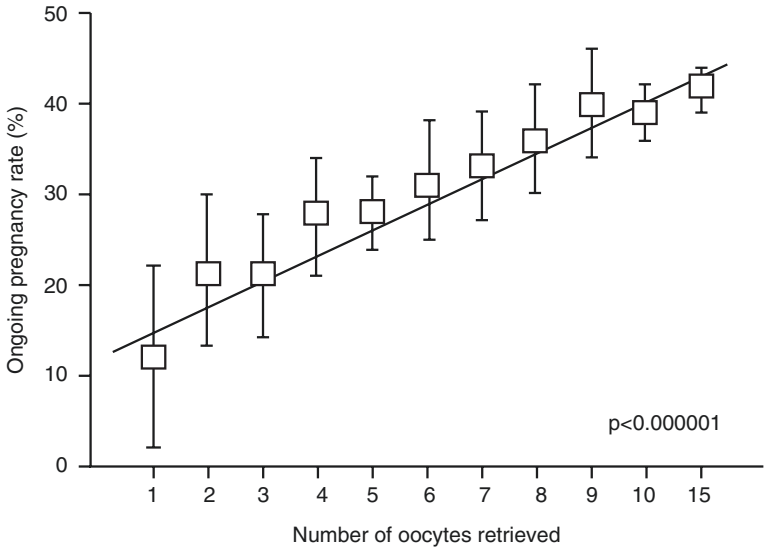

Fig. 7.4 (taken from Bosch E, Ezcurra D. Individualised controlled ovarian stimulation (iCOS): maximising success rates for assisted reproductive technology patients. Reprod Biol Endocrinol. 2011 Jun)

Ovarian Stimulation Cycle Control

Starting with spontaneous or induced menstruation, with previous birth control pill administration, an ultrasound scan should be done to confirm that both ovaries are inactive. Comparable outcomes can be obtained using an oral contraceptive pill that contains 0.030 mg of ethinyl estradiol and 0.15 mg of desogestrel to schedule the patients who undergo the antagonist protocol [20] (Fig. 7.5).

The ovarian stimulation period is variable with an average from 9 to 12 days of gonadotropin self-administration. Cycle monitoring is an essential part of any IVF protocol as it can indicate over- or under-response, which enables dose adjustments to optimize responses. This control is done by performing ultrasound scans every 2–3 days and measuring serum estradiol levels during stimulation.

One of the main aims of cycle monitoring is to prevent the ovarian over-response recognized during cycle monitoring. If the risk of OHSS seems very high, the final trigger is performed by an agonist trigger, and it also allows the next menstruation to start sooner due to the lutheolisis effect [21].

However, Kwan reported no significant difference in pregnancy rates and live births in the cycles monitored using ultrasound and serum estradiol, nor in those monitored by ultrasound alone [22].

The first monitoring visit is usually made on day 5 or 6 of stimulation when an ultrasound scan is done.

	OCP, n (%)	No OCP,n (%)	P value
BQ PR	61/115 (53)	67/113 (59.3)	.17
CPR	56/115 (48.7)	64/113 (56.6)	.12
OPR	55/115 (47.8)	61/113 (53.9)	.18
Multiple PR	15/56 (26.7)	18/64 (28.1)	.43
IR	75/207 (36.3)	80/204 (39.2)	.26
Miscarriage rate	5/56 (8.9)	11/64 (17)	.09
LBR	51/115 (44.3)	53/113 (47)	.35

Fig. 7.5 Cycle outcome (modified from García-Velasco et al. Cycle scheduling with OCPs. Fertil Steril. 2011)

The next scan control depends on ovarian response and is usually performed on day 8 or 9 of stimulation until follicles of 17 mm of diameter are measured and the final trigger is performed.

Random Start

Random start development in IVF cycles has taken huge strides to enable patients to vitrify oocytes when not much useful time is left.

In random-start protocols, the number of total and mature oocytes retrieved, oocyte maturity rate, mature oocyte yield and fertilization rates are similar to those in conventional (early follicular phase start) protocols.

When considering this approach, the random ovarian stimulation provides a main advantage as it shortens the total time for oocyte pick up, and ovarian stimulation in emergent settings can be started on a random cycle date for fertility preservation purposes without compromising oocyte yield and maturity [23].

Social Freezing Protocol at IVI Centers

The main stimulation protocol for social freezing that IVI centers use is the antagonist one with an agonist trigger.

This approach offers different advantages: fewer days of stimulation, patients do not need as many injections, and we can induce ovulation using a GnRHs agonist if a hyper-response of ovaries occurs. Finally, we can start a new stimulation sooner if the lutheal phase is shorter, if necessary.

We also consider administering long-acting FSH gonadotropins to avoid the daily pain of injections and to make stimulation easer for patients [24].

Considerations Before Treatment

It is important to inform patients properly about the procedure, and patients must deliver a signed informed consent with an explanation of the procedure, its risks and its success rates.

Patients should know that not all the antral follicles observed in the basal ultrasound scan will develop uniformly, not all follicles longer than 17 mm will have oocytes inside, and not all them will become mature oocytes that can be frozen.

Further relevant information that patients must receive is that the thawing rates of oocytes are higher than 90%, but they will depend on the patient's age and presence of some competing pathology that determines the quantity and quality of oocytes, such as the endometriosis, polycystic ovarian syndrome or previous ovarian surgery.

Patients should receive information about their chances of pregnancy depending on maternal age. Due to worse oocyte quality and a larger number of aneuploidies in patients older than 38, genetic preimplantation screening should be offered to select the euploid embryos to be transferred.

The aneuploid rate in patients aged between 38 and 42 years is 82–92%. The best practice in these patients is to reject aneuploid embryos and to select those with implantation possibilities.

Patients also should be informed about the commonest side effects during the procedure, like discomfort of injections in the place they are administered, morning sickness, headache and mood changes, the risks of anesthesia, injuries to pelvic organs, vaginal hemorrhage and post-surgery pain. These side effects are usually very mild and will have no long-term effects, regardless of the number of ovarian stimulation cycles [25].

One well-known complication is the risk of developing ovarian hyperstimulation syndrome. However, antagonists of GnRH stimulation protocols and the final trigger with GnRH agonist avoid this complication [2].

Patients should know that after oocyte pick up, menstruation will begin after 5–7 days. At this time, performing a scan of ovaries is recommended to determine their post-surgery status. If the number of retreived mature oocytes is lower than expected, a new ovarian stimulation can commence.

Key Messages

1. The election of the ovarian stimulation protocol as well as the gonadotropin doses based on AMH, BMI, age and AFC are crucial to optimize the ovarian response.
2. GnRH agonists or antagonists minimizes possible interferences by endogenous hormones, enables synchronous follicular development and prevents premature luteinization, which enhance the timing for oocyte retrieval.
3. The advantages of the antagonist protocol include shorter treatment duration and fewer gonadotropin requirements, fewer menopausal symptoms, lower cysts formation, and lower incidence of ovarian hyperstimulation syndrome.
4. Random start ovarian stimulation provides as main advantage the shortening of the interval from cycle initiation to oocyte pick up without compromising oocyte yield and maturity.
5. Long-acting FSH gonadotropins could be considered particularly in these patients to reduce the number of injections and to make stimulation protocols more convenient.

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