

Ovarian Stimulation to Collect Oocytes



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Introduction

The aim of ovarian stimulation is to obtain mature oocytes in order to preserve them in an unfertilised or fertilised state as a fertility reserve. In the *FertiPROTEKT* network, stimulation for the collection and cryopreservation of unfertilised or fertilised oocytes was carried out in approximately 40% of all fertility-preserving therapies performed [1].

The distinctive features of this form of fertility preservation are, on the one hand, that the treatment should be carried out as quickly as possible, so that necessary treatment for the underlying disease is not unnecessarily delayed. On the other hand, only one attempt is usually possible, which must be as efficient as possible. The maximum possible number of mature oocytes should be obtained without the risk of overstimulation.

It is also important to note that fertilised eggs can only be transferred to the woman with the consent of both partners. Since the cells can be stored for several years before a transfer, a possible separation of the couple must be considered during this time, which means that the woman would no longer have the option of retransferring of the fertilized cells. To avoid this risk, even in a stable partnership, it is recommended that all oocytes are frozen unfertilised or that splitting is carried out (50% fertilised, 50% unfertilised cryopreserved) to guarantee the woman's independence.

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Table 1 Advantages and disadvantages of ovarian stimulation

Advantage	Disadvantage
Established procedure	Time necessary approximately 2 weeks
Well-known chance of conception, dependent on female age and ovarian reserve	Success rate dependent on ovarian reserve (antral follicle count, AMH concentration)
Procedure possible even with low ovarian reserve	Hormone exposure (cave: Hormone dependent tumours)
Also possible with ovarian metastasis	Relatively high cost
	Vaginal sonography and transvaginal follicle aspiration required

Table 2 Average number of oocytes collected from 809 women according to the *FertiPROTEKT* register [1]

Age (years)	Average number of retrieved oocytes (<i>n</i>)
<30	11.7
31–35	12.8
36–40	8.4
>40	4.6

Ovarian stimulation which begins at the time of menstruation is a routine procedure. Measures to minimize the risk of ovarian hyperstimulation syndrome are also clinically established (Table 1).

Stimulation started after menstruation, e.g. in the luteal phase to limit the duration of stimulation to 2 weeks, has already been tested in several clinical studies [2–4]. Double stimulation [5–7] and stimulation immediately after removal of ovarian tissue have also been the subject of several studies [8, 9].

Effectiveness

The number of oocytes collected depends on the age of the patient and the underlying individual ovarian reserve. However, the underlying disease has very little effect on the number of oocytes obtained [10, 11]. According to the *FertiPROTEKT* register, the average number of oocytes obtained from 809 women was [1] (Table 2):

Exceptions [1] are women who have undergone a surgical intervention on the ovaries before stimulation (see chapter “Ovarian Tumors and Ovarian Cancer”) or with Hodgkin’s lymphoma (see chapter “Hodgkin’s Lymphoma”). Fewer oocytes may be obtained after ovarian stimulation in women with a BRCA mutation, presumably due to a lower ovarian reserve (see chapter “Breast Cancer”).

If vitrification is adequately performed as a freezing technique, the survival and fertilisation rates of cryopreserved oocytes are very good. Numerous studies comparing unfertilised and fertilised cryopreserved oocytes with eggs without cryopreservation showed no relevant differences in pregnancy rates [12–14].

Table 3 Theoretical birth rate per stimulation depending on the age of the woman, calculated based on 125 follicular aspirations [15]

Age (years)	Average number of retrieved oocytes (<i>n</i>)	Average number of fertilised oocytes (<i>n</i>)	Estimated live birth rate (%)
<26	13.5	8.6	~ 40
26–30	11.3	7.3	~ 35
31–35	11.0	6.1	~ 30
36–40	8.3	5.1	~ 20

The effectiveness of oocyte vitrification has also been confirmed in oncological patients. In 11 women whose oocytes were collected at an average age of 35.6 years (30–41) and thawed and fertilized after the disease, an oocyte survival rate of 92%, a fertilization rate of 77% and an implantation rate of 64% were found. Pregnancy occurred in 7 of the 11 women and 4 (36%) gave birth [15].

Based on the number of oocytes that were removed and successfully fertilized before cytotoxic therapy, a *FertiPROTEKT* [15] study calculated the theoretical birth rate depending on the age of the woman using 125 follicular aspirations (Table 3):

Therefore, after ovarian stimulation with cryopreservation of unfertilized or fertilized oocytes, the theoretical birth rates mentioned in Table 2 can be assumed if the specified number of oocytes is obtained.

Risks

Ovarian stimulation can lead to side effects from the medication, as well as complications during follicular puncture (www.deutsches-ivf-register.de). Women may experience temporary weight gain, mood swings and a feeling of abdominal pressure due to the increase in size of the ovaries. Clinically relevant bleeding from follicular puncture or inflammation is rare. During stimulation of patients in the *FertiPROTEKT* network, severe ovarian hyperstimulation syndrome occurred only once in 708 stimulations [4]. Chemotherapy has so far only had to be postponed once by one day.

In addition, Del Pup & Peccatori [16] were unable to demonstrate a worsening of prognosis in hormone receptor-positive breast cancer patients stimulated with letrozole compared to non-stimulated controls.

Practical Approach

General

The standard protocol for stimulation is the antagonist protocol with ovulation induction using a GnRH agonist (GnRH_a) (triptorelin 0.2 mg s.c.) to minimize the risk of the ovarian overstimulation syndrome [17]. In practice, a daily gonadotropin

dose which is approximately 50 IU higher than that of stimulation with an intended fresh transfer is often administered to increase the number of oocytes.

Random Start Stimulation

Ovarian stimulation can be started at any time during the menstrual cycle with the exclusive aim of collecting oocytes (without fresh embryo transfer) with the same number of oocytes and the same fertilisation rates [1–3] (“random start stimulation”). Pregnancy rates are equally high compared to standard stimulation protocols, when stimulation is started in the luteal phase [18] and the malformation rates are unaffected [19]. Identical results have also been demonstrated for egg donors and there is currently no disadvantage from “random start” stimulation [20]. According to previous studies, stimulation takes 1–2 days longer when stimulation is started in the luteal phase than when stimulation begins in the early follicular phase.

Stimulation can be performed as follows, depending on the cycle phase (Fig. 1):

- *Start of stimulation in the early and middle follicular phase:* conventional antagonist protocol with FSH or FSH/LH, addition of an GnRH antagonist (GnRHant) if dominant follicle >13 mm and GnRH agonist triggering with triptorelin 0.2 mg s.c. if 3 follicles \geq 17 mm. Stimulation dose approx. 50 IU higher than with an intended fresh transfer.

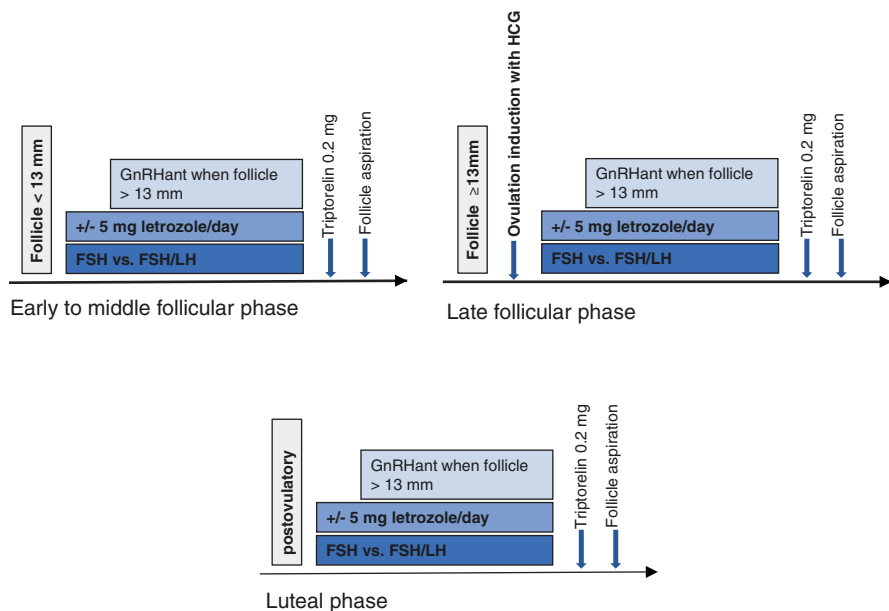


Fig. 1 Ovarian stimulation in different cycle phases

- *Stimulation start in the late follicular phase with a dominant follicle \geq approx. 13 mm:* ovulation induction with triptorelin 0.2 mg s.c., followed by luteal phase stimulation directly after ovulation.
- *Stimulation start in the luteal phase:* conventional antagonist protocol with FSH or FSH/LH, and GnRH agonist triggering with triptorelin 0.2 mg s.c. Stimulation dose approx. 75 IU higher than with an intended fresh transfer after a stimulation start in the early follicular phase. GnRH antagonist started when new dominant follicle $>$ approx. 13 mm.

Progesterone Primed Ovarian Stimulation

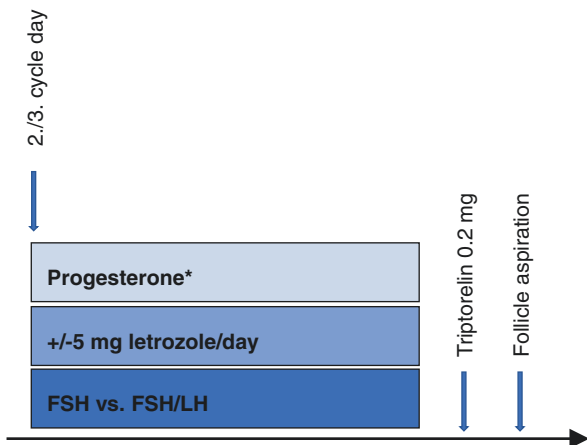
Progesterone-primed ovarian stimulation (PPOS) was derived from luteal phase stimulation, which showed that no LH increase occurs under the influence of progesterone. Since the function of the endometrium does not play a role in fertility preservation (freeze-all strategy), the negative effect of progesterone on the endometrium does not come into effect [21]. Efficient ovulation inhibition, a low rate of side effects and predominantly comparable oocyte counts, fertilisation, implantation and pregnancy rates were demonstrated for 4 and 10 mg medroxyprogesterone acetate (MPA), 10 and 20 mg dydrogesterone and 100 and 200 mg oral micronized progesterone.

The only exception was the latest study by Begueria et al. [22], which showed a lower efficiency of 10 mg MPA compared to the GnRH antagonist protocol in an egg donation programme. Furthermore, in the studies published to date (now >2600 women), there were no increased malformations among the children. In the protocols, the progestins were started on the third day of the cycle at the same time as the gonadotrophins (Fig. 2). It should be noted that an approx. 25 IE higher gonadotrophin dose/dose must be administered to obtain the same number of oocytes and the stimulation takes about 1 day longer (corresponding to luteal phase stimulation). When using MPA and dydrogesterone, the physiological production of progesterone in serum can be demonstrated, whereas this is not the case for oral micronized progesterone. The use of gestagens for ovulation suppression is also conceivable in principle with “random-start” protocols, but their efficiency has yet to be proven.

Double Stimulation

Double stimulation allows an increase in the number of oocytes obtained within approximately 4 weeks [5, 7, 23] (Fig. 3). Most double stimulations are “poor responders”, but they have also been described occasionally in the context of fertility preservation. The similar stimulation protocols all resulted in a higher number of mature oocytes the second stimulation with good developmental quality (summarised in [6, 18]).

Fig. 2 Progesterone-primed ovarian stimulation (PPOS)



- 4 mg or 10 mg MPA
- 10 mg or 20 mg dydrogesterone
- 100 mg or 200 mg micronised progesterone (oral)

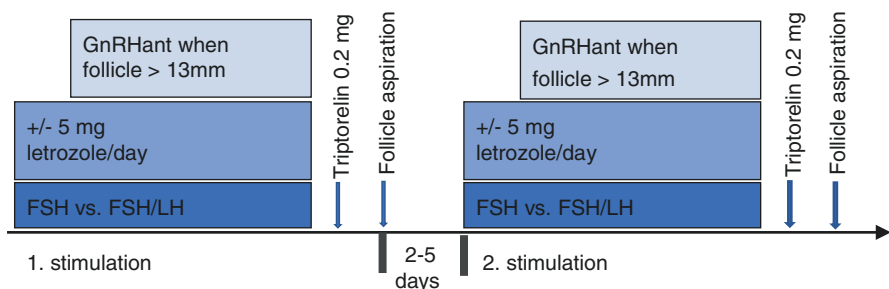


Fig. 3 Double stimulation

With this method, stimulation with a classical antagonist protocol and follicle aspiration is performed first after ovulation induction with a GnRH_a. It can be assumed that the first stimulation can also be started in every cycle phase (random start stimulation). A second stimulation is started 2–5 days after follicular puncture according to the luteal phase stimulation described above [23]. A prerequisite is that the ovary does not have too many large follicles (whether the administration of GnRH_{ant} for 2 days after follicle puncture accelerates luteolysis has not yet been proven). To rule out premature ovulation, GnRH_{ant} are administered additionally as

soon as the new dominant follicle exceeds a size of 13 mm [7] (Fig. 3). Double stimulation takes approximately 30 days.

Reduction of Estradiol Concentration in Hormone Receptor Positive Tumours

Hormone-receptor-positive tumours, in particular hormone receptor-positive breast cancer, represent a special case, since their growth could at least theoretically be increased by supraphysiological estrogen concentrations.

To reduce the increasing estrogen concentrations during ovarian stimulation, the addition of aromatase inhibitors, e.g. letrozole 5 mg (2.5 mg each morning and evening from the first day of stimulation), is recommended for ovarian stimulation in hormone receptor-positive patients [14]. Since letrozole is not approved for use in ovarian stimulation, treatment is off-label. Previous studies have not shown increased malformation rates in children after stimulation with letrozole [24, 25]. The number of oocytes [26] and the pregnancy rates described so far [14, 27] are also unaffected by the addition of letrozole. A more recent study of egg cell quality after letrozole stimulation and an ovulation trigger with GnRHa showed good egg cell quality based on an analysis of gene expression of granulosa cells and local estrogen concentration in the follicular fluid [28]. Oktay et al. [29] recommended that ovulation should only be triggered when the follicle size reaches 20 mm.

It should be noted that stimulation of a hormone receptor-positive patient should only take place after consultation with the responsible oncologists.

Combination of Ovarian Stimulation with the Removal of Ovarian Tissue

Ovarian stimulation can be combined with cryopreservation of ovarian tissue to increase the chance of success in treatments with high gonadotoxicity (Fig. 4) [7, 8]. Ovarian stimulation begins approximately 2 days after the laparoscopic removal of 50% of an ovary. In addition, a GnRHa depot injection could be given for fertility preservation on the day of follicular puncture. According to the studies carried out so far, there is no increased risk of complications. The number of oocytes obtained is not reduced after the removal of ovarian tissue. The time required for the combination of both therapies is approximately 2.5 weeks.

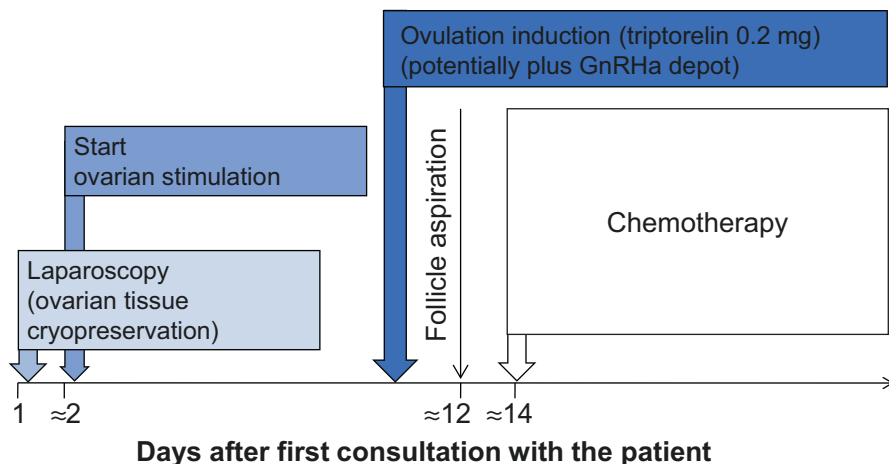


Fig. 4 Combination of ovarian stimulation with the removal of ovarian tissue and the administration of a GnRH α

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