

# Potential Benefit of Mild Stimulation Regimen on Embryo Quality in IVF-ICSI Cycles

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## Abstract

**Purpose:** To assess the potential benefit of mild stimulation regimen on embryo quality in a specific subgroup of women who had previously experienced poor embryo quality after conventional regimen.

**Methods:** In this study performed in a single University Hospital, patients with at least 2 consecutive In Vitro Fertilisation (IVF) or Intra Cytoplasmic Sperm Injection (ICSI) attempts leading to poor embryo quality following conventional regimens were enrolled. The main purpose was to compare the absolute and relative number of good and top quality embryos following mild stimulation and conventional regimen.

**Results:** Mild stimulation regimen allowed the retrieval of a lower number of oocytes as compared to conventional protocol but the mean number and the percentage of good and top quality embryos were significantly increased in patients receiving the mild stimulation regimen

**Conclusion:** In a specific subgroup of women with poor embryo quality following conventional IVF-ICSI protocols, a mild stimulation regimen seems to improve embryo quality.

**Keywords:** Mild stimulation; GnRH antagonist; Embryo quality; IVF-ICSI

## Introduction

Ovarian stimulation is actually one of the major determining factors for a successful outcome of in vitro fertilisation (IVF) techniques. However, the optimal stimulation regimen is not firmly established. Indeed, it is still uncertain whether conventional regimen is the most effective approach for getting high quality embryos. Indeed, the potential negative impact of gonadotropin therapy has been reported in *in-vivo* animal models where gonadotropins negatively impact on embryo quality [1-4]. In addition, stimulation regimens using high doses of gonadotropin in unselected population may be associated with an increased rate of hyperstimulation syndrome (OHSS) and with a deleterious effect on endometrial receptivity [5,6]. Consequently, a more friendly ovarian stimulation using lower doses of gonadotropin for a shorter period of time has been advocated [7,8]. The “so-called” mild stimulation regimen [9] which consists in gonadotropin administration started from mid follicular phase after spontaneous follicular recruitment is actually close to the physiological events of the natural cycle. This mild strategy has been associated with a higher percentage of good quality embryos in relation with a lower rate of aneuploidy and mosaicism [10]. These data suggest that the strategy aiming at less interference with ovarian physiology could be recommended in a specific subgroup of young normo-ovulatory women who displayed unexplained poor embryo quality following conventional regimen.

Therefore, the purpose of this study was to assess the beneficial effect of mild stimulation regimen on embryo quality in a subgroup of patients who had previously experienced two attempts with poor embryo quality.

## Materials and methods

### Patients

This analysis was performed from data collected during the last 4 years in a single university ART centre. Patients who had previously undergone two IVF-ICSI cycles using conventional protocols and

resulting in a low rate of good quality embryos were selected for this study. In this sub-group of patients, a mild stimulation protocol was proposed and then applied in order to assess whether embryo quality could be improved.

The inclusion criteria were as follows: women with regular, normal length and ovulatory cycles, aged < 43 years, BMI (Body Mass Index) between 19 and 35, two previous IVF – ICSI attempts using conventional GnRH analog protocol and resulting in a low (<20%) rate of good embryo quality.

The exclusion criteria were as follows: patients with no spontaneous follicular development, previous cycle cancellation for inadequate response to treatment, cycle without oocyte following ovarian pick-up, male indication related to non obstructive azoospermia.

### Conventional protocols

GnRH agonist or antagonist protocols could be used in the 2 previous cycles.

GnRH long luteal agonist protocol consisted in luteal subcutaneous administration of Triptorelin (Decapeptyl<sup>®</sup>, Ipsen, Boulogne, France) for at least 12 days. When pituitary down regulation was achieved (serum estradiol (E2) level < 50 pg/mL, progesterone < 1ng/mL and no follicle > 10 mm), a daily administration of recombinant FSH (Follitropin alpha GonalF<sup>®</sup> Merck Serono Lyon, France; Follitropin beta Puregon<sup>®</sup> Schering Plough Neuilly, France) was started at a dose determined according to age, BMI and ovarian reserve. FSH dose

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adjustment was performed on day 6, 8 and 11 according to the ovarian response monitored by pelvic ultrasound and hormonal determinations. Ovulation was triggered by injection of human Chorionic Gonadotropin (r.hCG, Ovitrel® Merck Serono Lyon, France) performed when at least 3 follicles reached a diameter of 17 mm. Oocyte retrieval was performed 36h later and collected oocytes were inseminated according to IVF or ICSI procedures.

GnRH Antagonist protocol was prescribed in patients who previously received a pre-treatment with either oral contraceptive (Lévonorgestrel 0.15 mg / Ethinylestradiol 0.03 mg Minidril®) for 3 weeks or with natural estrogens (Micronized estradiol, Provames® 2 mg p.o) started on day 22 of the precedent cycle and maintained for at least of 12 days. After a “wash out” period of respectively 5 and 3 days, a daily administration of r.FSH was started with a subsequent dose adjustment as previously reported for GnRH agonist protocol. GnRH antagonist (Cetrorelix Merck Serono Lyon, France; Ganirelix Schering Plough Neuilly, France) was administered in flexible way, when the largest follicle reached 14 mm or when serum E2 exceeded 500 pg/ml. Subsequent procedures were similar to those described for GnRH agonist protocols.

### Ovarian mild stimulation regimen

A daily dose of r.FSH (100 to 200 IU) determined according to BMI, age and prior response to treatment was started on day 5 of a spontaneous cycle and was subsequently adjusted on day 8 according to the ovarian response. Administration of GnRH antagonist was introduced in a flexible way as described above. Subsequent procedures were similar to those described for conventional protocols.

### Assessment of the embryo quality

Embryo quality was assessed on day 1, 2 and 3 following oocyte fertilization. Embryos were quoted according to the following criteria [11-15] :

“good quality” : < 20 % fragmentation rate and 3 – 5 cells on day 2 or 6 - 9 cells on day 3

“top quality” : < 20 % fragmentation rate and exactly 4 cells on day 2 or 8 cells on day 3.

### Statistical analysis

In order to improve the statistic relevance we compared the data of the last conventional cycle to those of the mild stimulation. Non parametric tests were used for statistical comparison between the two treatment groups. For data obtained on embryo transfer at day 2, a Wilcoxon test for paired analysis was used whereas, for data obtained on embryo transfer at day 3, we used a Mann Whitney test suitable for unpaired analysis. P values < .05 were considered statistically significant. Results are expressed as mean +/- SEM.

### Results

Among 2319 controlled ovarian stimulations performed in our centre for IVF-ICSI over 4 years, a retrospective analysis of our data allowed to identify 75 women who experienced 2 consecutive cycles with less than 20% of good quality embryos. Only 18 of them accepted to undergo a mild stimulation regimen.

The clinical characteristics of these patients are listed in Table 1: mean age: 34.2 ±1.25 yrs, BMI: 24.3 ± 0.82, average rank for IVF-ICSI attempt: 4 ± 0.24. Indications for ART were tubal infertility (9/18), pelvic endometriosis (3/18), male infertility (3/18), mixed factors (1/18) and unexplained infertility (2/18). As a low fertilization rate was previously observed in 6 couples, 9 patients underwent sperm microinjection following both conventional and mild regimens

A paired comparison between the last conventional and the mild stimulation protocol showed that the total amount of r.FSH dose was significantly lower in patients treated with the mild protocol (1137 ± 63 versus 1967 ± 189 IU p < .01) but serum E2 values on the day of hCG day were not different (1307 ± 113 versus 1425 ± 184 pg/ml). In addition, serum LH and progesterone values at the time of hCG administration did not significantly differ.

As shown in Table 2, mild stimulation regimen was associated with a significantly lower number of retrieved oocytes but the numbers of metaphase II, fertilized oocytes and cleaved embryos were not significantly different. More importantly, the fertilization rate and the cleavage rate assessed by the percentage of cleaved embryos per metaphase II oocyte were significantly higher in patients treated according to mild stimulation regimen as compared to conventional one.

Table 3 shows the number and the percentage of top or good quality embryos on day 2 and day 3 which were significantly higher after mild stimulation than after conventional regimen.

### Discussion

This retrospective analysis shows that mild stimulation regimen may be associated with an improved embryo quality in a subgroup of women who had previously experienced 2 IVF-ICSI failures related to poor quality embryos following conventional IVF protocols.

Age at (n-1) attempt (yrs)	33,6 (1.3)
Age at mild attempt (yrs)	34,2 (1.2)
Time between n-1 and mild attempt (month)	5,6 (0.8)
Rank of the mild attempt (n)	4,0 (0.2)
BMI (kg/m <sup>2</sup> )	24,3 (0.8)
Cause of infertility n (%)	
Tubal factor	9 (50%)
Severe endometriosis	3 (16.6%)
Male factor	3 (16.6%)
Mixed factors	1 (5.5%)
Unexplained	2 (11.1%)
IVF (n)	9 (50%)
ICSI (n)	9 (50%)
Severe male factor	3 (33.3%)
Fertilization failure	6 (66.7%)

Table 1: Patients' characteristics: mean (SEM) or n (%).

IVF regimen	Retrieved oocytes (n)	Metaphase II oocytes (n)	Fertilized oocytes (n)	Cleaved embryos (n)	Fertilization rate (%)	Cleavage rate (%)
Conventional stimulation	10.78 (1.05)	7.06 (0.91)	4.39 (0.70)	3.22 (0.56)	60,3 (7.15)	45.5 (6.05)
Mild stimulation	7.22 (0.71) <sup>a</sup>	5.17 (0.61)	3.89 (0.49)	3.39 (0.42)	74.1 (6.26) <sup>b</sup>	66.3 (5.8) <sup>b</sup>
P Value	<.01	.13	.62	.67	.03	.02

Results are expressed as mean (SEM)

<sup>a</sup> P < .01 for conventional vs. mild stimulation

<sup>b</sup> P < .05 for conventional vs. mild stimulation

Table 2: Comparison of oocyte et embryo quantity between stimulation regimens.

	Day 2		Day 3	
	Top quality	Good quality	Top quality	Good quality
<b>Conventional</b>				
n (± SEM)	0.39 (0.14)	0.44 (0.15)	0	0.25 (0.13)
% (± SEM)	5.2 (1.9)	6.2 (1.92)	0	4.2 (2.18)
<b>Mild</b>				
n (± SEM)	0.94 (0.24) <sup>c</sup>	1.1 (0.33) <sup>c</sup>	0.7 (0.33) <sup>b</sup>	1.6 (0.67) <sup>b</sup>
% (± SEM)	25.9 (6.6) <sup>a</sup>	28.2 (7.17) <sup>a</sup>	14.6 (7.17) <sup>b</sup>	34.6 (11.22) <sup>a</sup>

Results are expressed as mean (SEM)

<sup>a</sup> P < .01 for conventional vs. mild stimulation

<sup>b</sup> P < .02 for conventional vs. mild stimulation

<sup>c</sup> P < .05 for conventional vs. mild stimulation

**Table 3:** Comparison of embryo quality on day 2 and day 3 following conventional and mild regimens.

These data challenge the concept that the use of supra-physiological doses of gonadotropin which is commonly recommended for IVF-ICSI cycles in order to get a high number of oocytes and embryos is optimal for cycle outcome of every woman. This strategy has been justified by the correlation commonly reported between the number of retrieved oocytes and the pregnancy rate [16]. However, conventional ovarian stimulation not only exposes to the risk of life threatening situations such as ovarian hyperstimulation syndrome (OHSS) but is sometimes associated with low fertilization rate, specifically in a subgroup of patients exposed to hyper-response such as women with Polycystic Ovary Syndrome [17]. In addition, the optimal number of collected oocytes to get the best proportion of high quality embryos has not been clearly established in an unselected group of women.

Different approaches have been proposed with the objective to collect a minimal amount of oocytes providing high quality embryos. One of them, the so-called “mild stimulation”, consists in sustaining the development of spontaneously recruited follicles by providing FSH supplementation from the mid-follicular phase. Subsequent administration of GnRH antagonist in the late part of ovarian stimulation actually controls the risk of endogenous LH surge. The rationale behind the mild ovarian stimulation regimen is that a natural follicular recruitment could be associated with an improvement in oocyte and embryo quality.

In line with this concept, a previous study reported a significant improvement in fertilization rate [18]. The high efficacy of mild stimulation was later confirmed by extending the number of enrolled patients. Indeed, the 1-year cumulative term live-birth rate was actually similar in women younger than 38 years treated with a mild stimulation as compared to those treated with a standard protocol [19]. As recently reported [20], a similar fair embryo implantation rate was observed following retrieval of 5 oocytes after mild stimulation as compared to 10 oocytes after conventional stimulation. Our data are in line with this concept that oocyte quantity is not positively correlated to conceptus quality.

The most clinically relevant issue to be addressed is the potential negative impact of high dose gonadotropin on oocyte and embryo quality.

In vitro experiments performed in mice whose oocyte maturation was induced by high doses of FSH showed an increased rate of aneuploidy and abnormal meiotic spindle morphology [21,22]. In addition, in vivo studies, performed in mice and hamsters, similarly demonstrated the negative impact of ovarian stimulation on embryo quality assessed by an increased rate of aneuploidy [2], increased abnormalities in meiotic spindle formation [4], reduction in the rate

of development to blastocyst stage [1,5] and decreased viability embryo rate after transfer [3].

In human beings, data regarding the impact of high doses of gonadotrophins are still scarce and inconclusive. The main reason is that both oocyte and embryo quality are routinely assessed by morphological criteria which cannot exactly reflect their biological ability to provide a healthy conceptus.

Nevertheless, using morphological criteria, mild stimulation regimen has been associated with a reduced fragmentation rate of the embryo and with an improved developmental stage related to the woman's age [18]. Additional informative data have been provided by using pre-implantation genetic screening technologies [10]. Indeed, the genetic analysis of one biopsied embryonic cell demonstrated that the proportion of chromosomally abnormal embryos was significantly reduced following mild stimulation as compared to conventional regimens. Additionally, when two embryonic cells were biopsied, the percentage of abnormality (abnormal and mosaic embryos) also significantly decreased in patients treated with mild stimulation [10]. This study data showed for the first time that using a low amount of gonadotrophins may be associated with a decreased incidence of chromosomal embryonic mosaicism. However, these data deserved confirmation by extending the number of enrolled patients and by analyzing further which sub-group of patients could actually benefit from the strategy of mild stimulation.

Our study brings new insight into this issue. Indeed, we deliberately selected patients who experienced a low rate of high quality embryo in 2 previous consecutive cycles with conventional regimen. In our hand, the proportion of patients who fulfilled these criteria is actually low. This partly explains the limited number of women enrolled in this study. In addition, as mild stimulation was not universally advocated in this situation before the report [10], only a small number of patients were included in this protocol. Nevertheless, their clinical characteristics deserve further analysis. In our series, advanced age cannot explain the high incidence of poor quality embryos because, in the vast majority of patients, age was below 38 yrs. Similarly, a low ovarian reserve cannot account for the poor embryo quality as demonstrated by the range of retrieved oocytes following mild stimulation protocol. These data are in line with our previous report that, in young women without decreased ovarian reserve, cycle outcome remains fairly good even if the ovarian response to gonadotropins is lower than expected [23].

Regarding indications for IVF or ICSI, some of our patients underwent ICSI because of previous low fertilization rate following IVF. While our study was not focused on that issue, it is tempting to speculate that couples whose infertility is related to low fertilization rate could also benefit from mild stimulation regimens.

Therefore, our data suggest that a mild stimulation could be an interesting alternative to conventional regimen in a selected group of young women with normal ovarian reserve and whose the percentage of high quality embryo is repetitively low. This observation needs to be confirmed in additional trial with a larger number of enrolled patients.

In conclusion, while limiting the number of retrieved oocytes, a mild stimulation regimen may improve embryo quality in a subgroup of patients who had previously experienced IVF-ICSI failure due to a poor embryo quality.

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