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Review Article

Oocyte Quality In Mild Ovarian Stimulation

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ABSTRACT

All ART specialists work with objective of increasing the number of oocytes available for fertilization by use of exogenous hormones. Complex and demanding ovarian stimulation protocols are usually applied in order to compensate for the poor laboratory effectiveness to achieve this aim. This increases the complexity of treatment, amount of medication and cost.

Mild stimulation protocols aim to induce only a subtle interference in the physiological process of follicle domination, select the healthiest, homogenous cohort of good quality chromosomally normal oocytes and achieve a more physiological response. These protocols are less complex, with less requirement of medication, less time consuming and cheaper, making IVF more accessible for a broader patient population. There are reduced chances for complications, patient discomfort and drop-outs. The implementation of mild stimulation into standard clinical practice appears to be justified.

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INTRODUCTION

Since the early ages of human in-vitro fertilization (IVF) it turned out clearly that the effectiveness of the procedure, when performed on natural single-egg cycles, was very limited. An important step towards getting better results was represented by the availability of medications which could induce multiple ovulations. For several years, and until now, ovarian stimulation with exogenous hormones has been widely applied with the aim of increasing the number of oocytes available for fertilization (1).

For years, pharmaceutical companies have been competing in the market using as a tool, the potency of their respective drugs to get more oocytes. Cancelling cycles in which ovarian stimulation obtains a lower number of developing follicles has become a popular choice, especially in countries in which the fierce competition among IVF clinics is based on the pregnancy rate. More often, it is considered inconvenient to go on with cycles in which a poor oocyte yield is predictable. Furthermore, in countries where either the public health system or the private insurance system offer only a limited number of attempts for free or at very low costs, the yield of at least a dozen of oocytes is considered of great value by IVF specialists and, as a consequence, by patients. Furthermore, IVF clinics running an oocyte donation program are particularly satisfied when a patient produces enough eggs to be treated herself and to give surplus oocytes donation. 'More oocytes, more embryos and more pregnancies better IVF program' is the most widely accepted principle all over the IVF world.

However, it is out of discussion that the need of getting a rather high number of oocytes arises from the overall inefficiency of IVF laboratory procedure: several oocytes are needed to finally get just a few embryos and much less born babies. It is easy to calculate that the ratio of live birth rate to inseminated oocytes is extremely low in human IVF, averaging around 2-4%. Thus the complex and demanding ovarian stimulation protocols are usually applied in order to compensate for the poor laboratory effectiveness. The IVF lab has indeed improved significantly in the past three decades: new media and new equipment for embryo culture have been made available and new scientific knowledge has been obtained. As a result, the overall efficiency of IVF procedure has markedly improved from the 80's until now. Is it still necessary to work on a high number of oocytes to get a baby?

Ovarian Physiology.

The complete follicular development in humans takes about 220 days. The includes three distinct phase according to the developmental stage and to the dependence from pituitary gonadotrophins:

(a) Initial recruitment of resting primordial follicles.

(b) Development of preantral and early antral follicles.

(c) Cyclic recruitment of a limited cohort of antral follicles followed by the selection a single dominant follicle (2).

When follicle-stimulating hormone (FSH) circulating levels rise and increase over a threshold a cohort of small antral follicles is recruited to grow (3). The beginning of follicular growth is characterized by morphological changes including the proliferation and change in shape of granulosa cells, enlargement of the oocyte and the formation of the zona pellucida. The early theca is acquired at the end of the primary follicle stage, whereas the external theca forms as the follicle grows and compresses the surrounding stroma (3). During the early preantral follicle development, FSH, estrogen and androgen receptors appear on the granulosa cell surface. However, at this stage, the follicle is still unaffected by the lack of gonadotrophins. After the initial rise, FSH blood levels plateau during the early follicular phase and finally decrease during the mid-to late follicular phase as a consequence of the negative feedback exerted by inhibin B and estrogens on the hypothalamic-pituitary axis (2).

FSH window .

The presence of FSH is an absolute requirement for the development of larger antral follicles. Around the mid-follicular phase, the most mature follicle (the one with the highest number of FSH receptors on granulosa cells) gains dominance over the others; despite progressively decreasing FSH blood levels, the dominant follicle continues to grow and acquires responsiveness to LH. The remaining follicles from the recruited cohort undergo atresia and programmed cell death. The time during which FSH blood concentration is above a given threshold appears to be essential for a single dominant follicle selection (1).

Conventional "long" protocols

In the conventional "long" protocol, gonadotrophins (FSH, HMG or FSH+LH) are given to induce multiple follicular development and a GnRH analogue is given to prevent the premature LH surge, that would compromise the chance of retrieving oocytes (5). It took approximately 15 years of experience with GnRH agonists to identify that the best results were obtained starting their administration in the mid-luteal phase of preceding cycle (6, 7). Thus in the "long" protocol, GnRH agonist is started in the luteal phase of the run-in-cycle and continued until the administration of HCG (ovulation trigger). An initial flare of gonadotrophin release (about 5 days) takes place before the receptors are down-regulated and GnRH action on the pituitary is blocked (6). Long protocol, probably the most widely used throughout the world with the following advantages:

- quite good predictability of the work in IVF units
- getting a relatively high number of pre-ovulatory follicles, retrieved oocytes and as a consequence, embryos available for transfer

These properties help leading to a satisfactory pregnancy rate (8). However, the "long" protocol is associated with some problems:

- Ovarian Hyper-stimulation Syndrome (OHSS) (9): a life-threatening, high cost complication of human IVF.
- High rate of drop-out : the complexity of the "long" protocol, entailing weeks of daily injections and/or intra-nasal spraying, several blood samples and frequent ovarian ultrasound scans for monitoring, can impact on a woman' s life(8).

"Mild" Stimulation Protocol

As proposed by International Society for Mild Approaches in Assisted Reproduction (ISMAAR) a 'mild' IVF cycle is defined as

- A stimulation regimen in which gonadotrophins are administered at a lower-than-usual dose and/or for a shorter duration throughout a cycle in which GnRH antagonist is given as co-treatment, or
- A stimulation in which oral compounds (e.g. anti-estrogens or aromatase inhibitors) are used either alone or in combination with gonadotrophins and GnRH- antagonists (10).

The aim of such a protocol is to limit the number of oocytes obtained to less than eight. Table 1 shows the comparative concepts of various types of IVF cycles in terms of their methodology and oocytes retrieved. The "mild" stimulation approach for IVF treatment is aimed to develop more patient-friendly protocols in which the risks are minimized and the results are still acceptable (8).

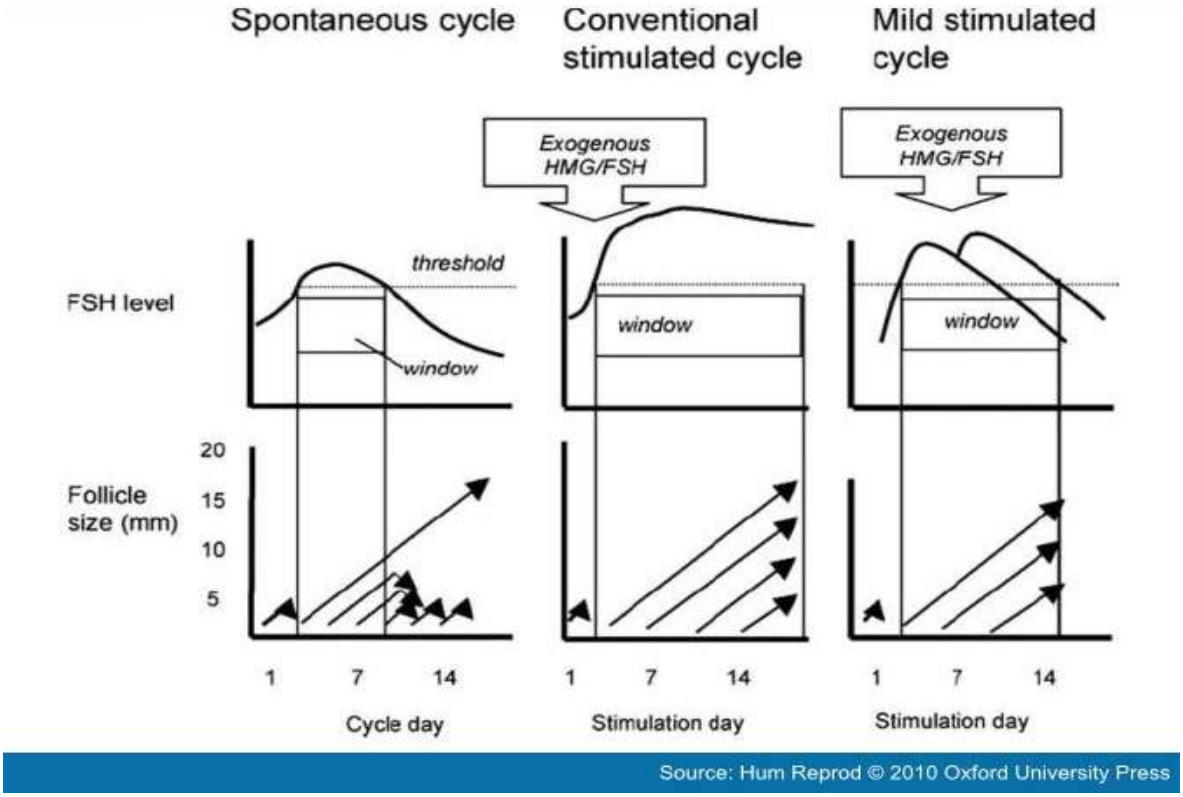
Table 1: Concepts of various IVF cycles

Type of IVF Cycle	Methodology	No. of Oocytes expected
Natural cycle	No medication	Single
Modified Natural Cycle	hCG only GnRH antagonist and FSH/ HMG add-back	Single
Conventional IVF	GnRH agonist or antagonist conventional FSH/HMG	≥ 8 Oocytes
Mild IVF	Low dose FSH/HMG, oral compounds and GnRH Antagonist	2-7 Oocytes

Theoretical concept of the "Mild" Stimulation.

A moderate but continued elevation of FSH during the mid-to-late follicular phase is able to extend the FSH window and overcome the single dominant follicle selection, leading to the growth of several follicles (2). In the "mild" ovarian stimulation, a low-dose gonadotrophins administration is delayed until the mid-follicular phase (day 2-to-5 of the cycle). The aim is to allow initial follicle recruitment by endogenous FSH, then prevent the decrease of FSH levels overcoming dominance and inducing multi-follicular development (1). In women with a normal ovarian follicular reserve, multiple follicle development can be induced when the initiation of FSH is postponed until cycle day 7, although there is a tendency toward a higher percentage of mono-follicular responses compared with patients starting on cycle day 2 to day 5. A fixed daily dose of 150 IU FSH is usually enough to induce multiple follicular growth when ovarian stimulation is initiated on cycle day 5 (4). Figure 1 shows the comparison of timing of medication in various IVF cycles.

Figure 1: conceptual difference in natural, conventional and mild stimulated cycle



The Key Role of GnRH Antagonists In "mild" Stimulation Regimens

Although the use of GnRH antagonists is probably not absolutely required for "mild" ovarian stimulation (11), their introduction in the clinical practice has represented the key event to start using "mild" protocols in IVF. The action of GnRH antagonists is characterized by an immediate suppression of the pituitary release of gonadotrophins and rapid recovery of normal gonadotrophin secretion when the drug is withdrawn (6). The mid-cycle LH surge requires the secretion of native GnRH and can therefore be effectively prevented by GnRH antagonist administration. The immediate action of GnRH antagonists allows blocking the pituitary just when the circulating estradiol rise approaches the threshold level at which LH surge is generated by the positive feedback loop on the pituitary. At the beginning of stimulation cycle ovarian stimulation can be initiated by endogenous gonadotrophins with a normal early follicular phase recruitment of a cohort of follicles, without any pituitary block (6). Later GnRH antagonists can be added as per various regimes given in table 2

Table 2 : Approaches for the GnRH antagonist co-treatment in IVF.

(a) single large dose subcutaneously on approximately the eighth day of stimulation with gonadotrophins.

(b) daily injections of small doses initiated on a fixed day of stimulation (**fixed protocol**)

(c) daily injections of small doses initiated depending on the size of the dominant follicle or on Estradiol levels (**flexible protocol**) (6).

Comparison of "mild" Stimulation Protocol vs. Classic "long" Protocol.

1. **IVF Results**

At present, the "long" GnRH-agonist regimen with relative high doses of exogenous gonadotrophins is probably the most frequently used stimulation protocol. In the last years, the availability of GnRH antagonists has allowed the clinical development of "mild" ovarian stimulation protocols involving minor interference with single dominant follicle selection. Some studies have compared the success rate of "mild" vs. standard ovarian stimulation regimens, either in women with normal ovarian reserve or with poor ovarian reserve. There have been three prospective, randomized controlled trials (RCT) comparing the effectiveness of "mild" stimulation regimen with the conventional "long" GnRH agonist protocol with an early follicular phase FSH start.

The single centre RCT by Hohmann et al. [18] included 142 patients with good reproductive prognosis who were randomly distributed into three groups: (A) (n= 45) treated with the GnRH-agonist Triptorelin and, after down regulation, with a fixed daily dose of 150 IU rFSH; (B) (n= 48) and (C) (n= 49) treated with rFSH initiated on cycle day 2 (group B) or 5 (group C) and with the GnRH antagonist Cetrorelix starting when the largest follicle reached 14 mm diameter. The results of this study suggest that a low number of retrieved oocytes after "mild" ovarian stimulation could have a different meaning from a low number of retrieved oocytes after a conventional stimulation. It was shown that the ideal number of oocytes after a conventional "long" protocol is 13 and that when the number of retrieved eggs is much lower (or even much higher), the pregnancy rate is compromised [19]. A low overall dose of exogenous FSH probably stimulates only the most mature follicles having

optimal receptor endowment, and allows a sort of "quality selection" among follicles (and finally among oocytes) avoiding to force poor quality follicles to grow anyway (20).

Another RCT by Heijnen et al. (21) included 404 regularly cycling, normal BMI patients and almost 800 consecutive IVF cycles. In this study, one group was given "mild" ovarian stimulation with GnRH antagonist co-treatment combined with selected single embryo transfer (SSET), whereas the other received a standard ovarian stimulation with the GnRH agonist "long" protocol combined with the transfer of two embryos. According to this study, reduced chances of birth per cycle in the "mild" regimen might be compensated by the increased number of IVF attempts in a set time. In fact, the overall discomfort to patients, evaluated with the hospital anxiety scale, the depression scale, the somatic Hopkins' subscale and the subjective sleep quality scale, were lower in the group assigned to the "mild" stimulation. Interestingly, also the economical costs of the treatment were significantly lower with the mild stimulation/SSET compared with a standard treatment involving conventional stimulation. It must be remarked, however, that the money saving was mainly due to the almost complete absence of twin pregnancies in the "mild" group rather then to the cost of IVF procedure itself (21). Interestingly, a prediction model aimed to estimate the chance of ongoing pregnancy after "mild" stimulation and SSET has been prepared using multivariate logistic regression analysis; this model is claimed to be helpful to optimize results identifying patients for which the "mild" stimulation plus SSET strategy can be appropriate (22).

The third RCT was performed by Baart et al [23] on 111 patients who were randomized into two groups, one (n = 67) receiving rFSH 150 IU/day from day five of the cycle plus GnRH-antagonist in a flexible schedule, the other (n = 44) receiving rFSH 225 IU/day after two weeks of pituitary down-regulation by GnRH agonist. This study suggests that the reduced pharmacological interference with ovarian physiology could generate genetically better oocytes quality wise. Indeed, some data in the mouse model shows that exogenous disturbances in the signals regulating folliculogenesis may alter the late stage of oocyte growth, increasing the risk of altered chromosome segregation in subsequent meiotic divisions. In fact, an increased incidence of chromosomal abnormalities was observed in oocytes after exposure to high doses of gonadotrophins during in vitro maturation of mouse oocytes (23, 24).

Taken together, these three RCTs included 592 first IVF attempts, among which 313 were performed with the "mild" stimulation protocol and 279 with the classical "long" regimen. Although individually these trials found comparable results in terms of IVF effectiveness, pooling data together the ongoing pregnancy rate per started cycle sorts out to be 15% in the "mild" group and 29% in the classical group, a difference that suggests a well definite trend toward a lower IVF effectiveness when the "mild" strategy is applied. This suggestion is further reinforced by the fact that the three RCTs did not consider freeze-thaw cycles, and the chance of obtaining surplus embryos/oocytes to freeze is obviously much lower in "mildly" stimulated cycles than in the classical ovarian stimulation cycles. Having frozen embryos/oocytes to transfer in a subsequent cycle can probably increase the overall IVF pregnancy chance per oocyte pick-up by approximately 10-15%. Thus, the gap between the two competitors could probably be wider considering freeze/thawing cycles, and could possibly reach statistical significance.

A factor limiting the effectiveness of "mild" strategy in terms of pregnancy rate per cycle is likely to be the relatively high rate of cycle cancellation due to mono- or bi-follicular response (around 15-20%) when gonadotrophins are started on day 5 of the cycle. When such a response is observed, a valuable option is to stop stimulation and start it again the next month starting earlier with medications (on day 2-4). Ovarian aging and high BMI have been identified as relevant variables to predict the risk of insufficient

response to "mild" stimulation, and a predictive model have been developed in order to minimize the need of cancelling the cycle (25).

2. Effect on endometrium in "mild" vs classic "long" protocols

Classical ovarian stimulation is claimed to be a factor able to impair endometrial maturation and consequently embryo implantation chance. Some studies indeed showed that the gene expression profiling of the endometrium in conventionally stimulated cycles is extremely different from the one that can be observed during a natural cycle (26, 2). Interestingly, the endometrial gene expression pattern is more similar to the natural one in cycles with GnRH antagonists than in cycles with GnRH-agonists (28).

Furthermore, classical ovarian stimulation regimens are associated with about ten-folds supra physiological circulating estradiol levels that have a well documented negative impact on the developmental and implantation potential of human embryos (24,29). Some data suggests that the best endometrial receptivity to embryo implantation may be found in natural cycles, but mild stimulation have lower impact on endometrial quality than classical regimens (30, 31). This is explained by the fact that "mild" stimulation regime is associated with significantly lower peak estradiol levels with a soft impact on the endometrium than classical regimens. Thus the "endometrial factor" can probably represent one scored point in favor of "mild" stimulation.

3. **Women with poor ovarian reserve**.

Ovarian stimulation for women with a poor ovarian reserve is probably one of the most frustrating aspects of IVF procedure. Most of the treatments proposed to enhance oocyte yield and pregnancy rates in "poor" responders have failed to show any convincing evidence (32). The standard approach to women estimated to be poor responders is based on starting the "long" protocol with a daily dose of approximately 300 FSH IU / day (33); the starting FSH dose used in any subsequent cycle is then adjusted (up to 600 FSH IU/day) according to the individual response in the first cycle (34). The strategy of performing an upward dose adjustment in women with poor ovarian reserve, however, has not shown any consistent benefit. In fact, in previous poor responders the IVF outcome of those assigned to a starting dose of 225 FSH IU/ day vs. those receiving 450 IU/day was shown to be similar, despite the later resulting in retrieval of more oocytes (35).

Also, other studies showed that predicted poor responders had no benefit from increasing the FSH starting dose (36,37). High gonadotrophin doses may indeed lower the cycle cancellation rate, but have been observed to reduce the likelihood of clinical pregnancy and live birth rate and to increase the risk of spontaneous miscarriage (38). A negative effect to high dose regimens on the endometrial quality has been claimed to be responsible for the poor outcome of this regimen (39), although probably even factors linked to embryo quality itself play a relevant role. High dose of FSH recruit "resistant" follicles rescuing them from atresia, but the oocytes that they host are of poor quality and usually do not result in the generation of good quality embryos (40, 41). Since the cost of gonadotrophins is one of the major expenditures in IVF treatment, the huge increase in drug costs linked to high-dose gonadotrophin regimens appears to unjustified if not paralleled by a significant improvement in clinical outcome.

A combination of Clomiphene citrate (CC) plus gonadotrophins and GnRH antagonists has been proposed as a "mild" stimulation alternative for poor responders. CC is known to act as an anti-estrogen on the central nervous system, increasing the pulse frequency of endogenous FSH and LH and giving a moderate gonadotrophin stimulus to the ovary (42). CC has been used for over thirty-five years to induce ovulation in WHO type II anovulatory women, and is still appreciated for its oral administration and low price. The combination with gonadotrophins may counterbalance its undesired anti-estrogenic effect on the

endometrium and at the same time may reduce the amount of gonadotrophins required, owing to the combined synergistic effect on the ovary. The level of evidence supporting the use of the "mild" stimulation protocol with CC/GnRH antagonist in patients with poor ovarian reserve is rather poor, as properly designed studies on an appropriate number of patients are still unavailable. The first report describing the use of CC/Gn/GnRH antagonists in poor responders included only 18 patients; compared to their response in a previous standard GnRH-agonist cycle, light improvements in cycle cancellation rate, oocyte yield and gonadotrophin requirement were observed (43). Unfortunately, neither the number of patients, nor the study design allowed getting an acceptable level of evidence.

Takahashi et al. (44) studied 40 poor responders with history of multiple IVF failures with the "long" protocol. Treating them with CC/FSH/GnRH antagonist resulted in an ovarian response comparable to the previous ones, but a significantly higher blastocyst development rate and a very good (41.2%) ongoing pregnancy rate. Again the study design was not very informative and the patient number was too little. D' Amato et al (45) compared the combination of CC/FSH/GnRH antagonist to a long GnRH agonist protocol enrolling 145 women with a prior poor response. They observed that significantly lower cancellation rate, higher peak oestradiol level, more retrieved oocytes and higher pregnancy and implantation rates were achieved with the antagonist protocol. In this study, however, high FSH amounts and not "mild" gonadotrophin stimulation were used. The observations, once again, are only indicative of the possibility of using this kind of stimulation at lower doses in poor responders.

Some other information may be deduced from studies that compared the outcome of CC/Gn/GnRH antagonist treatment with a standard "long" protocol in patients with normal ovarian reserve. Pregnancy rates comparable to the standard stimulation regimens were obtained by the "mild" strategy, with a significant reduction in the total dose of gonadotrophin needed and at economical costs (46-48). These results appear to be encouraging, although it remains to be proven that they can be replicated even with patients with poor ovarian reserve.

Interestingly, it was shown that in CC/Gn/ GnRH antagonist cycles, when the circulating level of LH is less than one-third at the time of HCG than it was at the beginning of stimulation, both the pregnancy and implantation rates are significantly reduced (49). This observation suggests to choose medications containing LH or HCG rather than FSH alone to be associated with CC in this kind of protocol.

Overall, the published results suggest that in patients with poor ovarian reserve the choice of a "mild" stimulation protocol instead of a classical, high dose regimen could be particularly indicated. Although these patients have a very low risk of OHSS even using high doses, the quality of both their oocytes and their endometrium would likely be better when a smoother stimulation approach is used. Further research, anyway, is needed to add scientific evidence to this hypothesis.

4. **Risk of Ovarian Hyperstimulation Syndrome (OHSS)**.

It is well known that IVF treatment exposes women to the risk of short-term complications, among which the severe form of OHSS is the most important. It is a serious and potentially life-threatening complication with a mean incidence of 1-3% in IVF programs involving standard ovarian stimulation regimens (50). Some patients are definitely considered at high risk. Young, lean women with polycystic ovaries (PCO) are a typical example with a risk of 6-9% for developing severs OHSS after a conventional ovarian stimulation for IVF.

The incidence of severe OHSS is significantly lower when GnRH antagonists are used instead of agonists (16, 17) probably due to the smaller cohort of recruited follicles and lower circulating estradiol levels during ovarian stimulation. The meta-analysis of Kolibianakis

found that the incidence of hospital admission for OHSS is significantly lower in GnRH antagonist cycles than in agonist cycles (OR 0.46, 95% CL 0.26-0.82' P= 0.1) (16). Also the Cochrane review reported that the incidence of severe OHSS is significantly lower in protocols with GnRH antagonists than in protocols with GnRH agonist (RR 0.46'95% CL 0.21-0.93; P 0.03) (17). Further, the risk of severe OHSS is reduced until around zero if ovulation trigger is elicited using a single dose of GnRH agonist instead on HCG (51).

There is significant reduction in severe OHSS risk using "mild" stimulation regimens. Heijnen (21) reported an incidence of 1.4% for OHSS with the mild protocol and 3.7% with the long protocol. In another study, Karimzadeh et al. (46) observed a zero incidence of OHSS in the group treated with "mild" stimulation vs. 6% in the group treated with conventional stimulation.

5. **Risk Of Long Term Health Problems**.

The discussion about long-term health consequences of ovarian stimulation for IVF, especially concerning the association between hormones and cancer, is far from being concluded. The epidemiological studies published so far in humans remain inconclusive, due to a huge amount of confounders and to a relatively short follow up period of time (52, 53).

Although gonadotrophin treatment is not considered oncogenic it appears to be safer to use the lowest possible hormone doses, especially in cases of repeated IVF attempts.

6. **Emotional Stress**.

Emotional stress represents well known negative side effects associated with IVF treatment. In fact, the psychological burden of treatment is one of the most frequent causes of drop-out, and a significantly lower drop-out rate was observed in more patient-friendly "mild" stimulation programs (22, 54). Some studies suggest that women who receive milder approaches in ovarian stimulation could be more prone to face a new treatment attempt compared with women receiving a standard protocol. Mild stimulation has a lower daily impact on the quality of life and lower incidence of the so-called minor symptoms (abdominal pain, nausea, etc.) that can be associated with conventional ovarian stimulation protocols is probably a factor that increases the patients' attitude to repeat IVF. A better predisposition to repeat the treatment after a failed attempt may obviously have a positive impact on the cumulative treatment success rate and could eventually compensate for a lower pregnancy rate per cycle following "mild" stimulation.

On the other side, however, the lower pregnancy chance per attempt with the "mild" approach can be itself a reason of emotional stress. Treatment failure, in fact, is associated with a deterioration of emotional well being (55), subclinical depression (56) and/or anxiety (57). Furthermore, one of the most stressing events in an IVF cycle is oocyte retrieval, either preformed under general or local anesthesia. So, if a stimulation strategy gets lower" per cycle "results, patients will be more frequently forced to repeat oocyte retrieval, with a possible increase of emotional stress. Some studies comparing the conventional ovarian stimulation with the "mild" regimen failed to observe a difference in the anxiety or depression levels, or on sleep quality (21, 58).

Overall, the evidence about a possible psychological benefit of "mild" protocols is still inconclusive. Even when looking at the patient's emotional well-being, a careful balance between the daily physical problems of stimulation and the overall effectiveness of IVF treatment must be carefully considered, discussing with each single couple pro and contra of the choice of a specific protocol.

7. **Economic burden**

A milder ovarian stimulation undoubtedly associated with lower medication consumption and with a lower cost for purchasing drugs. A couple of studies showed the superiority of the "mild" stimulation strategy over the standard approach in terms of economical costs (21, 59),

especially when the "mild" strategy includes SSET (21). Once again, however, the balance between lower costs and lower "per cycle" results must be carefully considered. If IVF effectiveness is under a critical threshold, the frequent need to repeat treatment (even several times) leads to new economical costs for the patient. This could represent a concrete problem particularly for people living in those countries where patients pay for their own treatment or the public health/private insurance systems provide only a limited number of free / low cost attempts.

The convenience of adopting the "mild" stimulation strategy in developing or third world countries has not yet been convincingly demonstrated. The lower effectiveness of IVF procedure can also become a problem for IVF clinics choosing the "mild" strategy, for competing in the market with clinics following classical stimulation concepts. If the clinic loses patients for the lower "per cycle" effectiveness of its IVF program, it could be forced to go back to classical stimulations or, alternatively, to increase prices, finally weighting on patients' budget.

8. Quality of Oocytes

The optimal number of oocytes retrieved in IVF is dependent on the stimulation regimen used. The retrieval of a modest number of oocytes following mild ovarian stimulation is associated with the optimal chance of achieving a pregnancy (60). This is in striking contrast to the well-established relationship between a poor ovarian response and poor clinical outcome related to ovarian ageing (61). The fear of obtaining low numbers of oocytes, which drives current practice in ovarian stimulation, is unjustified. Additionally, it is seen that in both stimulation protocols, a moderate ovarian response leads to a higher chance of pregnancy than a hyper response as in both protocols the number of pregnancies following the retrieval of 18 oocytes or more was very low (60).

The observed relationship between low oocytes numbers and a high chance of achieving an ongoing pregnancy following mild stimulation suggests that when few oocytes are obtained, they are likely to represent a homogenous group of good quality oocytes. This could be the result of the minimal deviation in the natural selection of good quality oocytes or the minimized exposure of growing follicles to the potentially negative effects of ovarian stimulation. In contrast to the conventional stimulation protocol with GnRH agonist co-treatment resulting in pituitary desensitization, ovarian stimulation with GnRH antagonist co-treatment enables the endogenous inter cycle FSH rise to occur (62). Therefore, cyclic follicle recruitment and initial stages of gonadotrophin-dependent growth of the recruited cohort of follicles can proceed undisturbed (63; 64). By postponing the initiation of ovarian stimulation to the mid-follicular phase, exogenous FSH may only stimulate the most mature follicles to ongoing development up to the Graafian follicle stage giving rise to the best quality oocytes (65).

Supportive evidence regarding the potentially negative effects of ovarian stimulation comes from several human and animal studies reporting detrimental effects of ovarian stimulation on oocyte and embryo quality. Increased incidences of morphological and chromosomal abnormalities have been observed in oocytes after exposure to high doses of gonadotrophins during in vitro maturation of oocytes (66; 67; 68). Ovarian stimulation and concurrent high estradiol levels were shown to have a negative impact on the developmental and implantation potential (69; 70; 71) as well as the chromosomal constitution of embryos (72). Moreover, ovarian stimulation might disrupt mechanisms involved in maintaining accurate chromosome segregation (73; 74). A randomized trial concerning the chromosomal constitution of human embryos following mild ovarian stimulation for IVF showed a significantly higher proportion of euploid embryos compared with conventional stimulation, suggesting that through maximal stimulation the surplus of obtained oocytes and embryos are of lower quality (75).

A low number of oocytes following conventional stimulation are related to ovarian ageing caused by the depletion of the primordial follicle pool, leading to a decreased number of developing follicles and diminished oocyte quality (61; 76). Few reported studies have addressed the issue of the optimal number of oocytes in a conventional stimulation protocol (77; 78). Indeed, most previous studies on this subject report a steady increase in pregnancy rates with increasing numbers of oocytes which eventually levels off (79; 80).This confirms the concept that in conventional stimulation, increasing oocyte numbers enhances the ability to select the best embryo(s) for transfer. Beyond a certain point however, pregnancy rates decrease due to the aforementioned detrimental effects of the development of large quantities of follicles and concomitant supraphysiological hormone levels on oocyte and embryo quality (81; 82; 83) as well as endometrial receptivity (84; 85). In the mild stimulation group, this effect was clearly demonstrated when more than nine oocytes were obtained.

CONCLUSION

Mild stimulation protocols aim to induce only a subtle interference in the physiological process of follicle domination assuming that these regimens will select the healthiest, homogenous cohort of good quality chromosomally normal oocytes and achieve a more physiological response. A poor oocyte yield after classical ovarian stimulation likely reflects a poor ovarian responsiveness to FSH, that is associated with poor IVF outcome, whereas a low number of oocytes after “mild” stimulation probably represents a normal response, a smoother selection of follicles (and oocytes) more likely to finally result in high quality embryos and in a pregnancy.

The additional benefits of such regimes are less complex, less requirement of medication, less time consuming and cheaper treatment protocol making IVF more accessible for a broader patient population. There are reduced chances for complications, patient discomfort and drop-outs.

Still, mild stimulation would not guarantee a complete selection against chromosomally abnormal embryos, since a moderate incidence of aneuploidy has still been reported in IVF embryos from mild stimulation protocols. This hypothesis that mild stimulation approaches, aiming at a more physiological response, might be able to improve the genetic quality of oocyte and embryos, needs to be validated by further trials including a higher number of patients and embryos, and possibly using techniques (e.g. CGH) able to study the whole set of chromosomes on single blastomeres. Newer RCTs including freeze-thaw cycles in the comparison between “mild” and classical stimulation regimens are definitely needed to get a higher level of evidence about the respective effectiveness of the two strategies.

The implementation of mild stimulation into standard clinical practice appears to be justified, although more studies are needed to further elaborate the various mild stimulation approaches.

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