Should mild stimulation be the order of the day?

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ABSTRACT

Mild stimulation protocols aim to reduce the physical, financial and emotional burden associated with the conventional IVF protocol without compromising the pregnancy rate. Such protocols help to decrease the complications and the discomfort related to the prolonged administration of agonist and large doses of gonadotrophins, by limiting the number of oocytes recruited to no more than eight. The per cycle pregnancy rates are lower though the cumulative pregnancy rate in a year is equivalent. This CPR comes by going through earlier repeat cycles. Whether this reduces the physical, emotional or financial burden remains a matter of debate. There is need to standardize these protocol and do more trials to compare the two effectively. Till such time there is a clear benefit above the conventional protocol it will not be the protocol of choice with most physicians.

KEY WORDS: Gonadotrophins, infertility, *in vitro* fertilization, mild stimulation protocol

INTRODUCTION

The advent of *in vitro* fertilization (IVF) saw oocyte retrieval from a single follicle in a natural cycle. The disadvantages of having only one oocyte to work with, lead to the introduction of ovarian stimulation (OS) for IVF. More oocytes meant more embryos, which offered the possibility of embryo selection, this in turn helped to improve pregnancy rates-assisted reproductive technology (ART) had finally taken a step forward.

Three seminal events changed the course of IVF. Introduction of gonadotrophins, which increased oocyte yield, gonadotropin-releasing hormone (GnRH) agonist to prevent the premature luteinizing hormone (LH) surge and availability of cryopreservation to freeze supernumery embryos. Availability of cryopreservation initiated a trend to maximize the number of oocytes through hyper stimulation of the ovaries. Unfortunately, this lead to the ovarian hyperstimulation syndrome (OHSS), which increased patient morbidity and mortality. Cycle programming to ease out the work schedule of physicians and embryologists added to the physical burden of treatment. Contraceptive pills given in the previous cycle and agonist injections continued until the timing is convenient for the clinic, leads to increased requirement of gonadotrophins and probably compromises the reproductive performance.[1]

Today the pendulum has started swinging back. Problems associated with OHSS, complex and expensive protocols, weeks of daily injections and the resultant high drop-out rate forced physicians to rethink their stand on OS protocols. In addition improved laboratory conditions and culture media have reduced the need for a large number of oocytes. Edwards *et al*.[2] in 1996 were the first to advocate milder stimulation for IVF. Advent of Gonadotrophin releasing hormone antagonist (GnRH antagonist) paved the way for development of more patient friendly protocols, which involved mild stimulation. The aim of mild protocols is to reduce treatment burden without compromising the pregnancy rate.

Introduction of a new concept does not gain immediate acceptance - there always are proponents and opponents. This article aims to examine the pros and cons of the process. The decision to adopt either rests with the treating physician based on the evidence presented.

DEFINITION

Over the years there have been no well-defined criteria to define mild stimulation; in fact

even the terminology has been varied with terms such as soft, gentle, minimal, mild stimulation being used when a deviation was made from the standard stimulation protocol. This lead to the formation of "ISMAAR" (International Society for Mild Approaches in Assisted Reproduction), which aimed to clearly define the various non-conventional stimulation protocols.

Mild IVF stimulation is defined by ISMAAR as the administration of:[3]

1. Low doses or fewer days of exogenous gonadotrophins in GnRH antagonist co-treated cycles or
2. Use of oral compounds (such as anti-estrogens, or aromatase inhibitors) with or without gonadotrophins with antagonist co-treatment for OS.

Aim of mild stimulation is to limit the number of oocytes obtained to less than eight.

STIMULATION PROTOCOL

The concept of "follicle-stimulating hormone (FSH) threshold" and "FSH window" forms the basis of all our stimulation protocols. FSH threshold being the level of FSH required to initiate follicular growth and FSH window is the time frame for which this FSH level plateau's to obtain cohort recruitment. Drop in FSH levels following the rise in estradiol level is responsible for selection of the dominant follicle. It follows then that the wider the FSH window the more the follicles recruited.[4] Thus in conventional IVF multifollicular recruitment is achieved by administering a high dose of gonadotrophins for a longer duration thus keeping the FSH window open for a longer time. In mild IVF a moderate elevation is sought since the aim is to have a small cohort of follicles hence the gonadotrophin dose is reduced. In the conventional protocol follicular recruitment is totally dependent on exogenous FSH since the pituitary is down-regulated. In the mild stimulation protocol on the other hand initial recruitment is by the endogenous FSH rise in the late luteal phase. Exogenous FSH added subsequently (CD2-5) prevents decrease of FSH levels inducing multi-follicular development by preventing follicular dominance.[5]

DRUGS USED FOR OS IN "MILD STIMULATION PROTOCOL"

Both oral and injectable OS agents can be used since the pituitary is not down-regulated. The drugs used are:

1. Gonadotrophins - recombinant FSH (rFSH), urinary FSH (uFSH), urinary HMG (uHMG)
2. Anti-estrogens - Clomophene citrate
3. Aromataze inhibitor (AI) - Letrozole.

Gonadotrophins

Gonadotrophins with or without oral ovulogens still remain the mainstay of OS. The choice between recombinant and urinary drugs is a question of availability and economics since it has been shown unequivocally that there is no difference in outcome (Cochrane data base 2011). Van Wely *et al*.[6]

Dose of gonadotrophin

The dose of gonadotrophin is kept at 100-150 IU starting from day 2 to 5 of the cycle. A fixed daily dose of 150 IU rFSH compared with 100 IU/day was found to be more effective in inducing multifollicular growth when OS was started on CD5.[7] The starting dose of gonadotrophin in the conventional protocol varies between 225 and 300 IU though it may be lower in polycystic ovarian syndrome patients.

Day of starting stimulation

Gonadotrophin administration can be initiated from cycle day (CD) 2-5 in the mild protocol while it starts from CD2 in the conventional protocol. A cancellation rate of almost 15-20% is observed when starting from CD5 because of mono or bi-follicular response. Starting on CD2 allows for more follicles to be recruited de Jong *et al*.[7] 2000 suggested that OS could be initiated as late as CD7, however the number of women showing multifollicular development with this protocol was lower than with those starting stimulation on CD2-5[8] and it never became popular.

Clomiphen citrate

CC is an anti-estrogen and has been used very successfully in ovulation induction for many decades Trounson *et al*.[9] were the first to use CC for OS in IVF in 1981. Once gonadotrophins were introduced they replaced CC as they were far more effective in getting a multifollicular response. Introduction of GnRH agonist for pituitary down-regulation in IVF protocols spelt a death knell for CC since CC needs an intact hypothalamo-pituitary-ovarian axis for its action. The reintroduction of this antiestrogen came about with the use of antagonist in IVF. Addition of CC reduces the dose of gonadotrophin required for stimulation[10] thereby reducing the cost of the IVF cycle. The dose used is 100 mg for 5 days from cycle day 2 along with 150 IU of gonadotrophin. The dose of CC has not been standardized. CC can be used by itself as well, however the number of follicles recruited is lower and its anti-estrogenic effects can be detrimental to implantation.

AIs

AI's inhibit the aromatization of androgens to estrogens thereby providing a negative feedback to the pituitary. In addition, the increased intra-ovarian androgens increase the sensitivity of the antral follicles to FSH[11] and may increase the number of pre-antral and antral follicles.[12] Advantage over clomiphene is thus two fold- no anti-estrogenic effect on the endometrium/no depletion of E2 receptors[13]

and improvement of antral follicle sensitivity to FSH thus improving recruitment. The dose varies from 2.5 to 5 mg daily for 5 days starting from cycle day 2 and is administered orally.

AI have been used with gonadotrophins extensively in poor responders and patients requiring fertility preservation as it keeps the E2 levels low. At present letrozole is an off label drug and its use as an ovulation induction agent is banned in India due to concerns about teratogenecity.

PREVENTING THE PREMATURE LH SURGE

GnRH analogs are used in IVF to prevent the premature LH surge. The agonist has been in use for more than 20 years and after many years of experience it has been established that the long down regulation regime gives the best results in IVF. The antagonist was introduced in 2000 and after some initial hiccups is slowly gaining ground.

GnRH antagonist

GnRH antagonists prevent the premature LH rise by competitive binding to the pituitary GnRH receptor. This leads to immediate suppression of gonadotrophin secretion. Unlike the GnRH agonists they do not cause an initial flare of FSH and LH and there is rapid recovery of pituitary action once the effect wears off in 24 hours,

GnRH antagonist is typically started as a daily injection of 0.25 mg administered s/c, in a fixed protocol from CD6 or a flexible protocol when the follicle size is between 12 to 14 mm and E2 > 200 pgm/ml. This allows the use of endogenous FSH action for initial follicular growth and helps to reduce the dose of gonadotrophins. It is also given as a single dose of 3 mg s/c but this is not available in India.

The advent of GnRH with its rapid and reversible action brought to fore a surge of protocols using oral and a combination of oral and injectable OS agents. These protocols helped to reduce the physical and financial burden of ART treatment. The introduction of antagonist protocols was met with a lot of skepticism since they were reported to give lower pregnancy rates, Al Innany *et al*., 2006.[14] The ease of administration and reduced medication used in patient especially one's with poor ovarian reserve overrode these concerns and as experience grew with the drug, claims of lower pregnancy rates were nullified.[15] Antagonist protocols using gonadotrophin only and a combination of clomiphene citrate/letrozole with gonadotrophins are popular for mild stimulation IVF.

GnRH agonist

Long down-regulation protocol involves starting GnRH agonist in the luteal phase of the previous cycle. This protocol is the most favored IVF protocols. Deep suppression of the pituitary necessitates the use of heavy doses of gonadotrophins for OS hence mild stimulation protocols cannot be used effectively with agonist suppression. GnRH acts by receptor depletion and hence there is an initial gonadotrophin flare from the pituitary. Protocols using this action of the agonist are called agonist flare protocols'.

Two major problems associated with agonist suppression are the need for higher doses of gonadotrophin with a consequent increase in the chances of hyperstimulation and almost 21 days of agonist injection.

IMPLICATIONS OF MILD STIMULATION IVF

Acceptance of any IVF protocol is intimately connected to the pregnancy rate and live birth rate achieved. This in turn would depend on the oocyte and embryo quality and alterations in endometrial receptivity. Physical and emotional burden of a regime also play an important role.

van der Gaast *et al*.[16] 2006 have shown that the ideal number of oocytes after a conventional long protocol is 13. When the number is lower or higher the pregnancy rate is compromised. In this context aiming for a lower number of oocytes would seem both contradictory and counterproductive. The reduction in complications, reduced physical and emotional burden and the reasonable pregnancy rates achieved with mild stimulation have obligated physicians to consider this approach to improve patient experience. Reduced oocyte numbers obtained through mild stimulation appear to differ from reduced numbers obtained in the conventional regime. It appears that poor oocyte yield after classical OS probably reflects a poor ovarian response to FSH and that is associated with poor IVF outcome. However low number of oocytes after mild stimulation probably represents a "quality selection" i.e. stimulation of only the most mature follicles which result in high quality embryos and in a pregnancy.[5]

COMPARISON OF PREGNANCY RATES

Studies have compared the success rate of mild versus standard OS in women with normal and poor ovarian reserve. In fact the advantage of mild stimulation was first recognized in poor responders.

PR in women with normal ovarian reserve

Three randomized controlled trials (RCT's) compared mild with the classical stimulation regimen. Pooled data showed an on-going pregnancy rate per started cycle of 15% in the mild group and 29% in the conventional group showing that mild stimulation is not as effective as the conventional

strategy.[17] Freeze-thaw cycles were not included in these studies. Inclusion of freeze-thaw cycles would improve the cumulative pregnancy rate (CPR) in the conventional group as cycles with mild stimulation may not generate supernumerary embryos.

Of the three RCT's the first by Hohmann *et al*.[18] 2003 included 142 normal responders who were divided into three groups. Group A-long down regulation protocol, group B and C-antagonist protocol. In group B stimulation was started on CD2 and in group C it was started on CD5. Gonadotrophin dose was 150 IU. There were no differences in PR between the 3 groups though women in Group C had a higher cancellation rate because of insufficient response.

Baart *et al*.[19] 2007 compared mild protocol with the conventional long down regulation protocol in 111 patients. A dose of 150 IU of rFSH was started from CD 5 in the mild group and 225 IU in the long protocol. The on-going pregnancy rate per started cycle was 21% in the "mild" group and 18% in the control group, which was not statistically significant. PGS was performed on these embryos and there were fewer numbers of aneuploid embryos in the mild stimulation group.

The largest RCT by Heijnen *et al*.[20] 2007 included 404 women who had approximately 800 cycles. In this study, the group with mild stimulation had a selected single embryo transfer while the conventional group had 2 embryos transferred. The number of oocytes retrieved was lower and the pregnancy rate per cycle was significantly lower in the "mild" stimulation group (17.6% vs. 28.6%, $P < 0.0001$). Patients however tolerated this protocol better and the rate of discontinuation of treatment was lower. The cumulative live birth rate after 1 year of IVF treatments was comparable in the two groups (43.4% with mild protocol, 44.7% with the conventional regimen), the twinning rate was also significantly lower in the "mild" stimulation-SSET (selective single embryo transfer) transfer group (0.5% vs. 13.1%, $P < 0.0001$). According to the authors, reduced chances of birth per cycle in the "mild" regimen might be compensated by the increased number of IVF attempts in a fixed time.

Ovarian aging, ovarian reserve and high BMI predict the risk of insufficient response to "mild" stimulation and a predictive model has been developed in order to minimize the need of cancelling (Verberg *et al*. 2007).[21]

Women with poor ovarian reserve

OS of women with poor ovarian reserve is beset with problems and frustration. Despite high doses of gonadotrophins oocyte yield remains poor and cancellations are high. It has been the trend to use doses as high as 600 IU to achieve good follicular recruitment. Unfortunately, such strategies have not proven very useful[22] primarily because you cannot force out of a bank what it does not have. The poor pregnancy rates cannot justify the greatly increased cost of medicine hence there has been a shift toward mild stimulation.

Land *et al*.[23] 1996 observed that the IVF outcome of patients given a starting dose of 225 FSH UI/day versus those receiving 450 UI/day was similar, even though more oocytes were obtained with the higher dose. High gonadotrophin dosage may prevent cycle cancellation, but provides no advantage in terms of pregnancy rate, live birth rate or miscarriage rate. It is believed that high doses 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.[24]

Cc/gonadotrphin/antagonist regimes

Reduce the cost and physical burden of treatment. In most studies, gonadotrophins 150/225 IU are combined with CC in a dose of 100 mg/day for 5 days from cycle day 2. During the early follicular phase. Unfortunately there is a high rate of heterogeneity in studies.

Two randomized trials that compared CC/HMG antagonist protocol to conventional agonist protocol came up with contradictory results. In the study by Dhont *et al*.[25] 1995 there was a significantly higher cycle cancellation rates and lower pregnancy rates per cycle ($P = 0.002$). The study by Lin *et al*.[26] 2006 concluded that Pregnancy Rates were similar in the two protocols, gonadotrophin used and number of stimulation days and number of oocytes retrieved were lower in the CC group. A similar outcome was achieved by other authors in retrospective studies.

AIs

AIs are administered orally and help to reduce the cost of treatment by reducing the requirement of gonadotrophins, especially in patients with poor ovarian reserve Grabia *et al*.[27] 2006 observed a PR of 27% in good prognosis patients. Most studies have used letrozole with the standard dose of gonadotrophins in antagonist protocols. Verpoest *et al*.[28] 2006 randomized 20 good prognosis patients for the use of 150 IU rFSH from CD2 with or without the addition of 2.5 mg letrozole. GnRH antagonist co-treatment was started from CD6. Use of aromatase inhibitors resulted in higher numbers of oocytes and a tendency toward higher clinical pregnancy rates per started cycle in the letrozole group.

In conclusion, oral ovulogens in combination with gonadotrophins have a place in cost-effective mild OS treatments especially in poor responders. More RCT's however are needed to assess the true benefit of these protocols.

COMPARISON OF EMBRYO QUALITY

High estradiol levels have a negative impact on the developmental and implantation potential of embryos.[29] An increase in aneuploid embryos has also been reported.[30] It has been hypothesized that OS might disrupt mechanisms involved in maintaining accurate chromosome segregation.[31] Baart *et al*.[19] 2007 found a higher number of aneuploid embryos in the conventional protocol suggesting that more oocytes do not necessarily mean more good quality/more chromosomally normal oocytes. These findings imply that mild stimulation selects less oocytes, but with a better quality that lead to the production of euploid embryos.

COMPARISON OF ENDOMETRIAL RECEPTIVITY

Supra physiological levels of estradiol negatively impact endometrial receptivity (Simón *et al*. 1995)[32] and are responsible for implantation failure. This point has been amply proved by the higher pregnancy rates in oocyte donation cycles where the endometrium is not subject to high steroids. Global gene profiling of the endometrium has revealed that there are alterations in the endometrial gene profiles during the phase of receptivity, in patients who have undergone stimulation.[33] The comparisons of gene expression from the same patients between natural and stimulated cycles revealed that endometrial profiles showed moderately altered receptivity in most cases (86%) and a strongly altered receptivity in 14% during COS.[34] Mild stimulation protocols aim at a more physiological response and hence would improve implantation rates.[35] Between agonist and antagonist the endometrial gene expression pattern is closer to the natural cycle in the GnRH-antagonists protocols.[36]

PSYCHOLOGICAL ASPECTS

Couples faced with infertility are under immense emotional stress, which is compounded by the stress related to treatment. Patients are on an emotional roller coaster oscillating between hope, anxiety and bitter disappointment cycle after cycle. With respect to treatment failure patients have symptoms of depression, anger and guilt, psychological stress is the most important reason for patients to discontinue treatment.[37]

Mild stimulation protocols have fewer symptoms of depression after IVF failure, the drop-out rate is lower and patients go for repeat cycles earlier thus improving their CPR.[38-40] However, lower per cycle pregnancy rates and repeated IVF attempts by themselves would increase stress. Devroey *et al*.[41] 2009 failed to observe a difference in anxiety levels or depression between patients in the mild and conventional protocol. So far there is inconclusive evidence to confirm a psychological benefit with mild protocols.

COST COMPARISON

Cost per cycle is lower in the mild stimulation protocol, but since more fresh attempts are require to achieve pregnancy the cost evens out. Cost of antagonist is still much higher than agonist. There is however an overall reduced cost until deliver because of the reduction in multiple pregnancies.[42] Low cycle cost may provide accessibility to patients in the lower socio-economic strata giving them an opportunity to have at least one cycle.

PHYSICAL BURDEN

Reduction in the days and number of injections, reduced visits for monitoring, reduced blood tests and finally a reduction in OHSS[20] dramatically alleviate the physical burden of treatment in mild protocols. Long-term health risks related to excessive OS need to be kept in mind though so far studies on this front have been reassuring.

Tabulated below are the advantages and disadvantages of "mild stimulation IVF."

Advantages

1. Decreased dose of gonadotropins
2. Decreased days of injections
3. Decreased chances of OHSS
4. No difference in CPR
5. Decreased aneuploidy rate
6. Decreased alteration of endometrial receptivity
7. Lower rate of twins
8. Lower per cycle cost because of lower drug cost and delivery of singletons.

Disadvantages

1. Decreased number of oocytes recovered - 35% reduction
2. No or few embryos available for cryopreservation. an overall increase in PR of 10-15% with availability of frozen embryos for transfer
3. Ultimately cost and physical burden may go up because of repeat fresh cycles
4. No decrease in emotional burden
5. Optimization of OS protocols still awaited.

CONCLUSION

IVF is an ever evolving technology. There has been a sea change in technique both in the clinic and the laboratory and

an improvement in drug quality and mode of administration. Despite these changes there is still an immense physical and emotional burden attached to treatment. The treatment involves daily injections, frequent ultrasounds and blood tests and anaesthesia general or local, for oocyte retrieval. Among the complications the most terrifying one is OHSS which can be life threatening.

Mild stimulation protocols resulted from a desire to make the procedure more safe and simple. The fact that there has been an immense improvement in the IVF laboratory gave courage to the physician to aim for fewer eggs reducing the dose of gonadotrophins required and consequently the cost and complications. Unfortunately the change was not universally accepted because the per cycle pregnancy rates are lower and the CPR though projected to be similar takes many more cycles of stimulation since there are less embryos available for cryopreservation. The cost too though, low per cycle ultimately levels out.

The social scenario is also changing with more and more older women coming for IVF. Studies comparing the two protocols specifically in women over 38 years are not available and more are required even in the younger age group. The contention that the emotional distress is lower has also been challenged. Hence the question should mild stimulation be the order of the day? Remains unanswered. One can only surmise that currently the decision has to be based on physician discretion and patient acceptance after full information of the pros and cons the future may well be different.

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