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Minimal and Natural Stimulations for IVF

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1. Introduction

In vitro fertilization-embryo transfer (IVF-ET) procedures have widely been used in most reproductive centers for many years. The protocol aim is to create a maximum number of oocytes to allow selection of the best embryos and provide extra embryos for future embryo transfers without undergoing ovarian hyperstimulation. So far, most IVF centers enjoy very good pregnancy rates using these conventional stimulation protocols. However, the conventional stimulation requires higher dosages of FSH injections, which are very expensive. Sometimes, the process of ovarian hyperstimulation creates health risks especially the dreadful ovarian hyperstimulation syndrome (OHSS). There has been a recent interest in using a much lower dosage of FSH to use for controlled ovarian hyperstimulation (COH) protocols for IVF. The multiple variations of IVF lower dosage include starting on day 5 instead of day 3 with FSH dosages 50% lower so called minimal (min) stimulation IVF, even lower dosages of FSH starting the gonadotropins even later allowing apoptosis of "less quality" follicles with dosage of FSH 1/4 to 1/3 of conventional dosages (micro IVF) or natural cycle IVF which can be completely natural or used with a gonadotropin releasing hormone antagonist and a mild dosage of FSH to allow better timing of oocyte retrieval. Other options – mild stimulation can also utilize other drugs that either block estrogen receptors on the pituitary or inhibit estradiol production by inhibiting the aromatase enzyme that recruits less follicles, e.g., clomiphene citrate or letrazole either alone or followed by low dose FSH stimulation. In some instances dosages of FSH above conventional levels are used especially women with diminished oocyte reserve in an effect to stimulate more follicles. This is referenced to as high dosage FSH stimulation.

For years the attitude of IVF centers has been "the more eggs the merrier." This chapter will discuss the benefits and risks of these various ovarian stimulation protocols. Also there will be a description as to the advantages and disadvantages of conventional vs. mild stimulation vs. high dosage FSH stimulation according to the degree of ovarian oocyte reserve.

2. Basic theory of ovarian stimulation

2.1 Oogenesis and hormone function on ovarian

A necessary factor for the development of antral follicles into dominant follicles is a hormone called the follicle stimulating hormone (FSH). In those normal ovulating women, a complex interaction occurs between the FSH and granulosa theca cells of these follicles which are

associated with up and down regulation of FSH receptors on these granulosa-theca cells. This process of FSH receptor up and down regulation is possibly related to the pulsatility of the gonadotropin releasing hormone (GnRH) which causes pulsatile release of FSH and luteinizing hormone (LH), leading to the progressive increase in estradiol (E2). The rise in E2, in turn, suppresses FSH release from gonadotropin cells leading to the usual recruitment and the development of only one dominant follicle each cycle from the multiple antral follicles. Though over simplified, basically the follicle developing the most FSH receptors in the granulosa cells is the one that can continue to develop into a dominant follicle despite the progressive drop in serum FSH from the early follicular phase to mid-cycle. Theoretically, but not proven, this process leads to the selection of at least one of the best quality antral follicles in the group to develop one mature oocyte each month. Follicles that have not developed adequate FSH receptors will undergo atresia in the presence of decreasing serum FSH (1).

With the advent of follicle maturing drugs, e.g., clomiphene citrate or gonadotropins, it was realized that raising serum FSH by using drugs that cause endogenous or using exogenous gonadotropins can allow the recruitment and development of multiple antral follicles to the dominant follicle stage. Follicles with less development of FSH receptors can respond to a higher FSH stimulus.

Because of multiple follicles the rising serum E2 levels can sometimes induce the luteinizing hormone (LH) surge before any one follicle has attained full maturity with a metaphase II oocyte. Thus most of these conventional IVF COH protocols using 225 to 300 units of FSH from day 2 or 3 of the menstrual cycle will also add either a gonadotropin releasing hormone (GnRH) agonist from mid-luteal phase until the human chorionic gonadotropin trigger in the late follicular phase of the next cycle or a GnRH agonist from early follicular phase or a GnRH antagonist from the mid to late follicular phase to prevent premature luteinization and cancellation of the oocyte retrieval.

2.2 Types of ovarian reserve and serum FSH and LH pattern

One of the ways to determine the oocyte reserve is to measure the number of antral sized follicles in the early follicular phase which is known as the antral follicle count. Two main hormones suppress the secretion of FSH by the pituitary – E2 and inhibin B. Since antral follicles make very little estrogen but do secrete inhibin B, women with less antral follicles will generally have an elevated serum FSH on day 2 or 3 because less inhibin B is secreted from less follicles (1).

Women with normal oocyte reserve will generally demonstrate on day 3 a serum FSH greater than LH but the FSH will be ≤11 mIU/mL. Women with supra-normal antral follicles, produce an increased amount of total estrogens related to conversion of androstenedione to estrogen. The positive feedback effect of estrogen on LH release from the pituitary but negative effect on the FSH secretion, frequently is manifested with an LH/FSH ratio greater than 1.8 to 1.

In the natural cycle the endogenous FSH advances the antral follicles and with the rise in serum E2, serum FSH gradually declines allowing monofollicular ovulation from the one dominant follicle that acquired the most FSH receptors. The challenge for natural oocyte retrieval is to retrieve the oocyte at the appropriate time interval from the LH surge to allow advancement of the oocyte to the meta-phase II stage.

So far although the germinal vesicle stage or metaphase I oocyte may be in vitro cultured to the metaphase II stage and further fertilized and cryopreserved for the subsequent embryo transfer, live deliveries have been reported with a lower expected pregnancy rate (2).

3. Types of FSH stimulation for follicular development

3.1 Mild stimulation protocols

There are a spectrum of mild stimulation protocols varying from no exogenous FSH at all to 150 units FSH from days 3-5 with a possible increase to 225 U of FSH if a GnRH antagonist is added or the serum E2 fails to rise sufficiently.

It seems logical and there is some supporting evidence that it is not a coincidence which of the antral follicles develops into the dominant follicle, and thus it may be the best follicle with the "best" oocyte. It seems reasonable that the first follicles to undergo atresia have the least quality oocytes. The ones progressing past the mid-follicular phase may have better quality related to better FSH receptors in the granulosa theca cells. If one does not intervene at this point by a small dosage of exogenous FSH the continued drop in FSH from rising serum E2 will cause atresia of these "better follicles" also except the one dominant follicle.

The problem with a completely natural cycle is that one cannot predict when the spontaneous LH surge will occur. Thus, we may face the risk that the oocyte could release before oocyte retrieval. Even though a bolus injection of human chorionic gonadotropin (hCG) is used before the spontaneous LH rise, it must be done without compromising the maturity of the follicle and the oocyte within.

In order to overcome this problem, some IVF centers trying to attain the one best dominant follicle will wait until the dominant follicle approaches a 14mm size and boost with 75 IU FSH with or without a GnRH antagonist. A natural cycle with a boost of FSH protocol can also be used with a mild GnRH agonist protocol to prevent premature luteinization. One method is to use a GnRH agonist for only 3 days, e.g., day 2-4 to prevent a premature LH surge in the late follicular phase (3,4). Actually, the GnRH agonist mildly stimulates the follicles and this stimulation is maintained by a low dosage of FSH starting around day 5 or later. Another method is to use a diluted dosage of the GnRH agonist and a low dosage FSH from the early follicular phase known as the microdose flare (5).

A mild stimulation protocol sometimes uses an anti-estrogen drug which recruits less of the antral follicles followed by a low dosage of FSH (or LH and FSH combined). For example, 100mg clomiphene citrate may be given from days 3-7 or 5-9 with 75-150 IU of FSH started on the last day of clomiphene (6-8). Another selective estrogen modulator, e.g., tamoxifen or an aromatase inhibitor, e.g., letrozole can be substituted for the clomiphene (9,10). Mild stimulation could employ 75-150 IU FSH or human menopausal gonadotropin from days 3-5 of the menstrual cycle. This can be used by any of the GnRH antagonist or agonist regimens that were previously mentioned. It should be noted that frequently when starting a GnRH antagonist, e.g., cetrorelix or ganirelix, one raises the FSH dosage by 75 IU.

3.2 Conventional stimulation protocols

There are several variations of conventional COH regimens. They usually either employ a GnRH agonist from mid luteal phase or sometimes the GnRH agonist from day 2, so called

short flare protocol trying to take advantage of the initial "agonistic" effects of GnRH agonist before the negative effect on gonadotropin release occurs later in the follicular phase. Some cases use a GnRH antagonist from the late follicular phase sometimes when the leading follicle reaches 14mm. Most conventional COH protocols start with 225-300 IU FSH frequently, but not always, with the addition of 75-150 IU LH. Many IVF centers will try to induce multiple follicles with 225-300 IU FSH, then decrease by 75-150 IU in an effort to continue the stimulation of the advancing follicles but not stimulate much smaller follicles. Usually, hCG is given when the two leading follicles reach 18-20mm. Sometimes a GnRH agonist is used in 1 or 2 injections to stimulate endogenous gonadotropin release instead of hCG to reduce the risk of OHSS (11).

3.3 High dose FSH protocols

The high dosage FSH protocols are those that start with greater than 300 U of FSH. They are frequently used by IVF-ET centers to try to increase the follicular response in previous poor responders.

4. Theoretical advantages of various stimulation schemes – Normal oocyte reserve

4.1 Conventional FSH stimulation over mild stimulation

Conventional COH produces more oocytes and thus more embryos. Theoretically this procedure will obtain more top quality embryos for transfer, especially considering a blastocyst transfer. With more embryos there will be a greater opportunity for subsequent frozen embryo transfer. A frozen embryo transfer does not create a risk of OHSS and is usually much less expensive than fresh IVF cycle. Furthermore there is no cost for expensive gonadotropins and GnRH agonists or antagonists and no charge for anesthesia. The most important aim of IVF program is to obtain a live delivered pregnancy from a given oocyte harvest whatever a fresh or frozen embryo transfer is performed (12). Thus, the more embryos obtained, the greater the chance of achieving a pregnancy per oocyte harvest (12).

4.2 Mild dosage FSH stimulation over conventional stimulation

One main advantage of mild FSH stimulation is low cost of medication. Also, the price of the IVF-ET cycle can be greatly reduced because of less work in the embryology laboratory. Our IVF center has reduced the price by 50% when the mild stimulation method is used. Also, using less FSH markedly reduces the risk of OHSS.

Interestingly, one of the arguments in favor of conventional stimulation is that the more embryos developed the better chance of chromosomally normal embryos. Proponents of mild stimulation consider that oocytes with meiotic errors identified in the natural ovulatory process are more likely to undergo apoptosis and can not advance to a dominant follicle stage. A randomized controlled trial comparison of mild vs. conventional COH on rates of aneuploidy found that both regimens created the same number of chromosomally normal embryos, i.e., an average of 1.8 per cycle (13). Thus no higher number of chromosomally normal embryos is produced by conventional higher FSH dosage regimens than mild stimulation according to this study (13).

Also, some IVF programs favor transferring chromosomally normal embryos by pre-implantation genetic diagnosis (PGD). Completing this procedure requires more oocytes and embryos. Current PGD fluorescent in situ hybridization (FISH) technique has been replaced by the competitive genomic hybridization or microarray analysis which can evaluate all chromosomes. The trophectoderm biopsies of blastocyst embryos may significantly reduce embryo harm than day 3 embryo biopsy (14). However, these procedures add extra expense and need for higher FSH dosage stimulation. The mild stimulation could allow natural selection of the best oocytes. Thus the best embryo may be obtained at a much lower price.

4.3 Relationship of stimulation scheme with embryo cryopreservation

Another way to avoid severe OHSS is to freeze all embryos and defer transfer, but this places the burden on an IVF center of having a good success rate with their frozen embryo transfers. One advantage of mild stimulation is if the cryopreservation program is not superb they do not have to fear a lower chance of pregnancy if fresh embryos are transferred. In fact, when evaluating a given center's pregnancy rate per transfer, one should not ignore the concept of pregnancy rate per oocyte harvest. Pregnancy should be evaluated based on fresh or frozen embryo transfer together or at a minimum the pregnancy rate of the first transfer irrespective if it is fresh or frozen (12).

One theoretical advantage of mild stimulation is that it allows "mother nature" to recruit the best follicles. It is possible that all multiple embryos produced by conventional stimulation have morphologically similar quality, but they may have poor likelihood of implantation. The oocytes with chromosome abnormalities are more likely to undergo atresia. If there is a good cryopreservation program, all embryos will eventually be transferred. However, those IVF centers that do not excel in embryo freezing programs may not transfer the "best ones" on fresh transfer but the odds of transferring the better embryos fresh may be greater with mild stimulation.

5. Controlled ovarian stimulation – Effects on the post-ovulatory endometrium

By comparing pregnancy rates from infertile oocyte donors sharing half their oocytes with recipients, a very significant adverse effect of COH has been suggested based on a much higher pregnancy rate in recipients vs. donors (15). However it became clear that a good portion of the differential was related to the failure to realize that salpingectomy should be performed for hydrosalpinges (16-18). There still does appear to be a mild adverse effect of conventional COH on embryo implantation in some women as evidenced by comparing pregnancy rates in infertile donors and their recipients in the era of salpingectomy for hydrosalpinges (19).

Sometimes one case can vividly establish an interesting concept that controlled studies can not so firmly establish. One woman with amenorrhea from polycystic ovarian syndrome was promoted to ovulate every cycle with clomiphene citrate or gonadotropins plus progesterone in the luteal phase for 6 years. All known infertility factors were corrected but she failed to conceive. This woman had 10 IVF-ET cycles with 92 embryos for fresh transfer in three top IVF centers without pregnancy, but in her 11th IVF cycle, all embryos were

purposely cryopreserved. Finally she conceived and delivered a healthy baby on her first frozen embryo transfer (20). After that, this woman started naturally to ovulate and spontaneously conceived by natural intercourse and finally a healthy baby was born with luteal phase progesterone supplementation (21).

Kerin et al showed that the aspiration of only preovulatory graafian follicle for purpose of IVF-ET following spontaneous ovulation did not cause a luteal phase defect (22). Yet as far back as 1980, Edwards, Steptoe and Purdy suggested that the luteal phase of all stimulated cycles is abnormal (23). When Edwards et al published their data, the use of GnRH agonists and antagonists were not used as part of the COH protocol. Thus the luteal phase defects had to be related to the use of follicle stimulating drugs (23). With the advent of GnRH agonists various theories developed suggesting that they were responsible for luteal phase defects related to a delay in pituitary recovery from suppression by the GnRH agonists. However a subsequent study showed that despite rapid recovery of pituitary function when GnRH antagonists were used luteal phase deficiency still persists and pregnancy rates greatly suffer unless supplemental progesterone or hCG injections are given (24).

Thus the prevalent theory today for the etiology of luteal phase deficiency following COH and IVF-ET is related to the supra-physiological concentration of steroids secreted by multiple corpora lutea during the early luteal phase which directly inhibit LH release by negative feedback to the pituitary and hypothalamus.

Bourgain and Devroey summarized the adverse effects of FSH stimulation on the post-ovulatory endometrium (25). Compared to natural cycle, FSH stimulation cycles showed 1) premature secretory changes in the post-ovulatory and early luteal phase of IVF cycles followed by a large population of dyssynchronous glandular and stromal differentiation in the mid-luteal phase; 2) a modified endometrial steroid receptor regulation; 3) a profound anti-proliferative effect in IVF cycles and 4) support was provided for the theory of the implantation window with premature expression of various endometrial products including pinopodes, integrins and leukemia inhibitory factor (25). Some studies demonstrated that an immunomodulatory protein known as the progesterone induced blocking factor (PIBF) may be much earlier detected in the early luteal phase following COH. The PIBF is expressed by gamma/delta T cells at the maternal fetal interface which in turn inhibits local natural killer cell activity. This factor supports premature trophoblast invasion as a cause of failure of embryo implantation in some circumstances since the production of PIBF requires trophoblastic invasion to allow this allogeneic stimulus to induce P receptors on gamma/delta T cells (26). These data suggest premature trophoblast invasion may account for failure for successful implantation (26). It is clear that periovulatory maturation exceeding 3 days results in extremely poor (possibly zero) pregnancy rates (25).

It is suspicious that the aforementioned woman who experienced 6 years of ovulation induction and 10 IVF-ET cycles with 92 embryos for transfer and finally got pregnancy with frozen ET cycle and a natural cycle conception might have the advancement of the periovulatory window and premature trophoblast invasion to explain these findings (20, 21). However, some evidence indicates that luteal phase inadequacy can be corrected by adding supplemental progesterone or hCG in the luteal phase so as to increase pregnancy rates per transfer in the modern IVF era (27-33), but some studies thought that the luteal phase support does not increase the delivery rate (34).

6. Author's experience with conventional vs. mild FSH stimulation

The ideal study to determine the proper therapeutic recommendation could be based on a large prospective randomized controlled trial (RCT), but very few studies have been conducted. Meta-analysis of prospective studies can increase the power but frequently there are journal reviewer and author biases in the publication of multiple studies. Clinically important conclusions can be reached from large retrospective studies comparing two therapeutic options if there are no apparent biases or inadvertent confounding variables. It is impossible to compare conventional vs. mild FSH stimulation with a large prospective RCT since there is little motivation for a pharmaceutical company to fund such a study.

When comparing conventional vs. mild COH protocols it is essential that the concept of pregnancy rate per harvest is taken into consideration. Thus a credible large retrospective study must come from an IVF center with a good pregnancy rate following frozen embryo transfer. Our IVF center developed a modified slow-cool embryo cryopreservation technique that allows equal pregnancy rates with the transfer of fresh or frozen thawed embryos (35-37). Thus our center data would qualify to evaluate pregnancy rate per first transfer, i.e., fresh or frozen, in case all embryos needed to be cryopreserved because of the risk of OHSS. Similarly our center could evaluate the pregnancy rate per harvest before requiring the need for another COH IVF-ET cycle with consideration of transfer of all frozen embryos (12).

We summarize data on the decision for using conventional vs. mild FSH stimulation in women with normal ovarian reserve from a large retrospective study over a 10 year time period (data was presented at the 2011 World Congress of IVF in Tokyo, Japan). These data were based strictly on financial reasons with 50% less charge for IVF-ET plus reduction on at least 50% of the cost of FSH drugs. No significant differences were found in two stimulation schemes (Table 1 and 2). If one looks for a trend for higher pregnancy rate it would favor mild FSH stimulation for first transfers irrespective of fresh or frozen embryos.

	High stim cycle			Low stim cycle		
Age at retrieval	Totals	≤35	36-39	Totals	≤35	36-39
# of Retrievals	859	536	323	396	265	131
# of Transfers	678	418	260	288	194	94
% Clinical pregnancy/transfer	44.5	50.2	35.4	43.8	51.0	28.7
% Ongoing/transfer	39.8	46.4	29.2	41.3	47.9	27.7
% Delivered/transfer	36.1	41.9	26.9	38.5	44.8	25.5
Implantation rate (%)	27.0	32.1	19.7	30.0	34.6	20.1

Table 1. Pregnancy rates of the first retrieval with fresh embryo transfer cycles

	High stim cycle			Low stim cycle		
Age at retrieval	Totals	≤35	36-39	Totals	≤35	36-39
# of Transfers	790	498	292	342	238	104
% Clinical pregnancy/transfer	43.5	49.2	33.9	44.4	49.6	32.7
% Ongoing/transfer	39.4	45.8	28.4	41.8	46.6	30.8
% Delivered/transfer	35.7	41.2	26.4	39.2	43.7	28.8
Implantation rate (%)	26.0	31.0	18.6	29.8	33.0	22.2

Table 2. Pregnancy rates for the first transfer – fresh or frozen Ets

Also, no significant differences were found in pregnancy rate per oocyte harvest (Table 3) in the younger groups, a higher pregnancy rate trend with conventional stimulation was observed. The only significant difference was that women aged 36-39 had a higher pregnancy rate with conventional stimulation than mild stimulation (32.5% vs. 26.7%, $p<0.05$).

	High stim cycle			Low stim cycle		
Age at retrieval	Totals	≤35	36-39	Totals	≤35	36-39
% Clinical pregnancy/transfer	55.9	64.4	41.8	48.2	57.0	30.5
% Ongoing/transfer	49.2	58.0	34.7	44.4	52.5	28.2
% Delivered/transfer	45.3	53.0	32.5	41.9	49.4	26.7

Table 3. Pregnancy rates per oocyte oocyte harvest

7. Diminished oocyte reserve and infertility

It is well known that as age advances, the antral follicles in the early follicular phase become less and less (38). With the less antral follicles, the less inhibin B is secreted, which leads to a higher day 3 FSH level as long as it is not being falsely lowered by a higher serum E2 level from a more advanced follicle. The oocytes of women with advanced reproductive age are much more prone to meiosis errors which result in a very high percentage of embryos with aneuploidy. Even if they have normal serum FSH, the women over age 45 rarely achieve pregnancies (39).

One explanation to the phenomena associated with poor pregnancy rates and high miscarriage rates is that the oocytes with the best mitochondria are more likely to advance to a secondary oocyte and eventually develop into antral follicles because there is a natural selection of the best follicles with oocytes with the best mitochondria. By natural selection, older women have "de-selected" follicles. Less than adequate mitochondria lead to a greater risk of meiosis errors which cause poor pregnancy rates and higher miscarriage rates.

Another alternate hypothesis is that the selection of follicles is simply positional but age itself leads to aging of the mitochondria in the follicles and further leads to meiosis errors. Several 1980s studies found very poor pregnancy rates even in younger women with diminished oocyte reserve as manifested by elevated day 3 serum FSH levels (40-43). Even in the modern IVF era some of the top IVF centers still claim extremely poor (or even zero) live delivery rate in younger women despite the transfer of several normal morphologic embryos especially if day 3 FSH exceeded 15 mIU/mL (44,45). Based on these data the conclusion favored by many reproductive endocrinologists (but not this author) is that the poor pregnancy rates are related to poor quality oocytes allegedly with quality more akin to women of advanced reproductive age (46).

8. Author's experience with diminished oocyte reserve

If remaining oocytes in women with marked diminished oocyte reserve were of the same poor quality as their 52 year old "FSH" peers where pregnancy rate is almost zero, it is difficult to explain how a group of women with hypergonadotropic amenorrhea and estrogen deficiency for a minimum of one year achieved a pregnancy rate of 28% (19/68)

in those who ovulated and a live rate of 11.7% per ovulation cycle without any assisted reproductive procedure (47). The techniques used to induce ovulation involve gonadotropin suppression with ethinyl estradiol plus restoration of down-regulated FSH receptors followed by low dose gonadtoropin therapy in some but not all cases (47). A study of euestrogenic women age ≤39 with a mean serum 18.9 mIU/mL FSH and without assisted reproductive technology achieved a clinical and ongoing 6 month pregnancy rate of 46.1% and 34.6%, respectively (48). Successful pregnancies could be achieved without ART not only in menstruating women with serum FSH levels >100 pg/mL (49), but also in a woman in apparent menopause with serum FSH levels of 164 mIU/mL (50), and even women in apparent menopause with ovaries appearing as streaked gonads (51,52). A successful pregnancy was even achieved by merely lowering the elevated FSH and restoring sensitivity to endogenous FSH in a 40 year old woman in apparent menopause with several years of amenorrhea and estrogen deficiency with a documented serum FSH of 124 mIU/mL (but a claimed level of 180 mIU/mL) who failed to conceive despite 4 previous transfers of fresh embryos derived from donor oocytes (53). At the 2011 American Society for Reproductive Medicine we presented data on natural cycle conception in women aged ≤37 with day 3 serum FSH >15 mIU/mL using natural cycles or mild FSH stimulation plus progesterone support in the luteal phase. The clinical and live delivered pregnancy rates after 3 treatment cycles were 41.6% (n=24) and 33.3% respectively vs. 70.8% and 62.5% respectively for matched controlled women with normal (≤8 mIU/mL) day 3 serum FSH.

Successful pregnancies have been recorded in apparent menopausal women with tubal factor by ovulation induction following restoring sensitivity of some of the few remaining follicles and by lowering the elevated serum FSH levels (54, 55). One menstruating woman with an elevated day 3 serum FSH achieved 3 live deliveries out of 4 IVF-ET cycles with ICSI over an 8 year time span (56). Roberts et al.'s study showed that any age women who ever once had a serum FSH more than 15mIU/mL can not achieve a live pregnancy even if they stimulate adequately and have morphologically normal embryos (45). Their hypothesis suggested that high serum FSH results in a loss of best oocytes and the remaining ones have poor quality similar to ≥45 year old woman (45), but the fact that live delivered babies have been achieved despite the extreme of oocyte depletion suggests Roberts et al's hypothesis is incorrect (45,46,57).

Recently, a study evaluated the relative effect of blastomere number and fragmentation indices of day 3 embryos on pregnancy and implantation rates by undergoing IVF women with a markedly decreased egg reserve and >15 mIU/mL serum FSH levels (57). The study consisted of only women having a single embryo transfer. Transferring embryos with over 6 blastomeres (which represented 65% of the transfers) showed 40% clinical pregnancy rate per transfer and 31.7% live birth rate, while transferring only 4 and 5 cell embryos had just 3.8% and 9.5% pregnancy rates (57).

Many controlled ovarian hyperstimulation regimens for women with normal egg reserve begin on day 2 or 3 with at least 225mIU/mL FSH and frequently 300mIU/mL. When attempting to stimulate a woman with diminished egg reserve, most IVF centers will increase the starting dosage of FSH hoping to get more follicles. Women with the least egg reserve will usually fail to respond to high dosage gonadotrophins, thus, their cycles are cancelled. However, the reports are generally only in those women with greater egg reserve

and response sufficient to obtain possibly a minimum of 5 oocytes and very poor pregnancy rates when conventional or high FSH COH protocols are used (40-45).

9. Hypothesis to explain the discrepancy in results with the aforementioned studies with negative outcome vs. the author's positive experience

The principal of trying to establish ovulation in an apparent menopausal woman is based on the assumption that some antral follicles are still present but they have acquired a resistance to exogenous and endogenous gonadotropins because the chronically high level of serum FSH causes down regulation of the FSH receptor (58). The theory implies that lowering the serum FSH by exogenous estrogen can allow restoration of the down-regulated FSH receptors leading to the development of a dominant follicle by stimulation with endogenous and/or exogenous gonadotropins (59). One could argue that the estrogen may directly improve the sensitivity of the follicles to FSH without the need to suppress endogenous FSH. However, the fact against this theory is that ovulation induction in hypergonadotropic amenorrhea can also be achieved by lowering the serum FSH with either gonadotropin releasing hormone (GnRH) agonists or antagonists (47, 59, 60).

At the cellular level an adverse effect of an excessive exposure to hormonal stimulation is frequently regulated by receptor down regulation. This would explain the frequent observation of a high dose FSH failing to stimulate any or just a few follicles whereas mild dosage FSH allows a better response. A vivid example was an iatrogenic menopausal woman caused by raising her endogenous FSH levels with clomiphene citrate. By simply stopping the clomiphene therapy, she was able to restore ovulation by stimulating 3 dominant follicles with a serum E2 >800pg/mL in a natural cycle (61). Thus the probable explanation for such diverse success results is the use of mild vs. conventional or supra-conventional dosages of exogenous FSH to try to stimulate more dominant follicles. Otherwise there were no differences in methodology used for IVF in this population.

Though it is not known for sure why high dosage FSH stimulation results in such poor outcome in these women, the adverse effect seems to affect the embryo rather than the endometrium (author's unpublished experience on cryopreservation). Deferring fresh transfer does not overcome the adverse effect in women with diminished oocyte reserve. Two possible mechanisms of the high dosage FSH leading to poor pregnancy rates could be that the increase of FSH in the follicular phase causes higher meiosis errors which results in aneuploidal embryos or the high FSH down-regulates the receptors leading to the production of implantation factors attached to the embryo itself.

10. The author's mild stimulation protocol for women with diminished oocyte reserve

The basic principle of using mild FSH stimulation for women with diminished oocyte reserve is try to avoid adding exogenous FSH while the endogenous serum FSH is already elevated. It is important to restore FSH receptors in granulosa-theca cells by lowering the serum FSH in women who appear to be in menopause (62, 63). The author's preference is to use compounded ethinyl estradiol to lower the high serum FSH since it is inexpensive and helpful to create adequate endometrial thickness and good cervical mucus so that in case the oocyte is released before retrieval, it is possible for conception with intercourse. In contrast

to other estrogen preparations, such as serum 17-beta estradiol assay, ethinyl estradiol allows detection of a recruited follicle and determination of maturity (64). Also, ethinyl estradiol allows the lengthening of the follicular phase to give more exposure time of the endometrium to estrogen so that endometrial progesterone receptors obtain proper development (65, 66).

Sometimes in this scenario of reversing menopause the FSH remains elevated while a single follicle grows and the serum E2 rises. In this case the better way may be to allow completely natural development of the follicle without the addition of exogenous FSH (55). If the FSH is only mildly elevated but the follicular maturation is not rapidly progressing enough, a boost of low dosage (75-150 IU) FSH may be used at that point. Similarly, if there are only 1-2 antral follicles and the high serum FSH decreased to the normal range, the low dosage gonadotropins (FSH or LH/FSH combination) may be used at this point.

In the aforementioned study of women with very high FSH and single embryo transfer there were 92 initiated cycles in women doing completely natural cycles (57). Sixty of them lead to oocyte retrieval. The data are analyzed according to cycles initiated but excluding some cancellation cases because of very little expense for medication, the cancellation for not reaching a mature follicle or release of oocyte before retrieval does not have the same negative impact as cancellation of stimulation with conventional or high dosage FSH. Only 19 of the 60 (33%) retrievals led to an embryo transfer and 21% clinical pregnancy and 16% live delivery.

The group "good enough" to allow a boost of gonadotropins had a somewhat better outcome in that about 70% (80/116) proceeded to oocyte retrieval leading to about a 75% transfer rate. The clinical and live delivered rate per transfer for this group was 29% and 24% respectively (57). With just diminished ovarian reserve as evidenced by a 3 day serum FSH >12 mIU/mL but where frequently (but not always) the woman is euestrogenic, one common technique is to allow natural follicular maturation to proceed until the endogenously rising E2 decreases the serum FSH in the follicular phase when either 75-150 IU FSH is added. This group will frequently have more than one embryo to transfer.

If the day 3 FSH is only mildly elevated or top normal mild stimulation consists of 75-150 IU FSH initially around day 5, and a GnRH antagonist is added later. This less severe group can perform a lot better. Almost all of these initiated cycles go to retrieval and most retrievals lead to embryo transfers. Our data showed that miscarriage rates are directly proportional to age but not the FSH level (presented at the 2011 Pacific Coast Reproductive Society). Comparing relative pregnancy rates based on age and rising FSH levels in younger women ≤age 39 was listed in Table 4 and older women aged 40-44 in Table 5. It is clear that until age 43 the FSH level does not negatively affect the live delivered rates when the mild stimulation are used. Actually since these data were obtained to evaluate aneuploidy as evidenced by miscarriage rates, all IVF cycles were included. Thus there may have been some bias with the normal FSH group since our IVF center attracts some difficult cases who failed several previous IVF cycles in other centers, while the high FSH group may never have any previous IVF cycles being rejected because of their high day 3 FSH levels.

Age at time of retrieval	≤35				36-39			
Baseline FSH levels (mIU/mL)	≤11	12-14	15-17	>17	≤11	12-14	15-17	>17
# transfers	2120	111	37	88	1313	120	47	93
% live delivered preg/transfer	45.1	42.3	48.6	45.5	33.4	35.0	29.8	36.6
% SAB/clin. pregnancy	11.9	13.9	13.3	12.8	17.2	11.4	7.1	22.9

Table 4. Pregnancy rates by age and FSH levels – younger group

Age at time of retrieval	40-42				43-44			
Baseline FSH levels (mIU/mL)	≤11	12-14	15-17	>17	≤11	12-14	15-17	>17
# transfers	737	103	30	65	121	30	18	25
% live delivered preg/transfer	23.1	20.4	30.0	27.7	24.0	10.0	0.0	8.0
% SAB/clin. pregnancy	27.3	32.3	36.4	30.4	34.4	75.0	100.0	75.0

Table 5. Pregnancy rates by age and FSH levels – older group

11. Other studies using mild stimulation for diminished oocyte reserve

Not all studies agree that mild stimulation is the key for achieving a reasonable pregnancy rate in "poor responders". Kolibianakis et al did not achieve any live pregnancies in 78 modified natural cycles although they started 100 unit FSH and ganirelix when the follicle reached ≥16mm but no hormonal studies were obtained (44). Possibly they did not wait long enough for full maturation of the follicle before administering hCG injection (44). Kim et al found a 13.5% live delivered pregnancy rate with low dose FSH but not higher than the 16.7% live birth rate from a multidose FSH dosage (67). Thus this study does not support the idea of poor outcome in other studies related to the high dose FSH regimen. However, it should be noted that the dosage of 225 IU FSH daily is a lower dosage than most centers treat women with diminished oocyte reserve where frequently higher dose FSH regimens are used.

Another retrospective study compared the implantation rates according to natural vs. various types of regimens using conventional and high dosage FSH IVF dosages in women whose response was so poor that only one embryo to transfer. Authors reported a 20% rate (6/30) with natural vs. 8.3% (23/274) with high dose FSH (68). Though these studies used "lower dosage" FSH stimulation, they did not adhere to the tenets of the author's specific regimen. These differences in protocol could explain somewhat lower pregnancy rates in this other study (69).

12. Conclusions

Women with normal oocyte reserve seem to have a similar chance of live deliveries following IVF-ET whether they use mild or conventional FSH stimulation protocols. Considering the risk of OHSS and the increased cost from conventional FSH stimulation, it is logical to use milder FSH stimulation for women with normal oocyte reserve. Perhaps for women of advancing reproductive age, i.e., >age 35, it is better to choose the conventional FSH dosage stimulation. If the cryopreservation techniques are used, frozen extra embryos

for women at this age will provide the hope for infertile couples to have another child in the future.

A lot of data supports the use of low dose FSH protocols for women with diminished oocyte reserve. It seems logical that the very poor pregnancy rate in women with diminished oocyte reserve recorded by some of the finest IVF-ET centers was not related to poor quality oocytes, rather than a direct adverse effect of the conventional or high dosage of FSH used. The main principle for those women is not to further increase FSH level but to wait for endogenous or exogenous estrogen to lower the FSH closer to normal levels before instituting any FSH stimulation.

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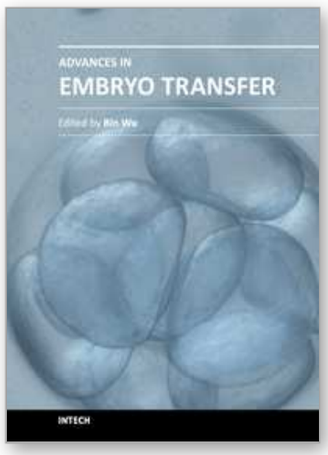

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Embryo transfer has become one of the prominent high businesses worldwide. This book updates and reviews some new developed theories and technologies in the human embryo transfer and mainly focus on discussing some encountered problems during embryo transfer, which gives some examples how to improve pregnancy rate by innovated techniques so that readers, especially embryologists and physicians for human IVF programs, may acquire some new and usable information as well as some key practice techniques. Major contents include the optimal stimulation scheme for ovaries, advance in insemination technology, improved embryo transfer technology and endometrial receptivity and embryo implantation mechanism. Thus, this book will greatly add new information for readers to improve human embryo transfer pregnancy rate.

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