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Premature ovarian insufficiency - Fertility challenge

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Premature ovarian insufficiency, defined as amenorrhea with estrogen deficiency in a woman younger than 40 associated with a serum follicle stimulating hormone (FSH) >35 mIU/mL, can be temporarily reversed with ovulation achieved resulting in live delivered pregnancies. Though this may occur spontaneously the frequency of ovulation can be considerably increased by various techniques of lowering the elevated serum FSH level and thus up-regulate down-regulated FSH receptors in the granulosa-theca cells. This can be accomplished by either suppressing FSH release from the pituitary by negative feedback through high dose estrogen or by suppressing FSH production by inhibiting the gonadotropin releasing hormone (GnRH) by either using GnRH agonists or antagonists. The estrogen method is the technique of choice because it is much less expensive than GnRH analogues, and helps stimulate cervical mucus and endometrial development. Ethinyl estradiol is the preferred estrogen because it does not contribute to the measurement of serum estradiol and thus allows proper monitoring of follicular maturation. Sometimes exogenous gonadotropins are needed as a boost but the dosage should be low so as not to down-regulate FSH receptors again. The technique is referred to as the FSH receptor restoration technique. Progesterone should be supplemented in the luteal phase. Physicians should be cognizant of trying to help prevent premature ovarian insufficiency by judiciously choosing less gonadotoxic cancer treatment alternatives that are equally efficacious. Also surgery for ovarian endometriomas should be performed only when absolutely necessary.

KEY WORDS: Amenorrhea - Primary ovarian insufficiency - Menopause, premature - Endometriosis.

This review will focus on fertility options for women with premature ovarian insufficiency or the potential for this condition. Before proceeding it is important to better define the subject group. In a review of the subject for Obstetrics – Gynecology Clinical Expert series in 2009 Robert Rebar referred this group as premature ovarian failure.[1] He writes: "the term is more widely used than ever before as more young women (typically aged younger than 40 years) are found to have elevated basal levels of follicle-stimulating hormone (FSH) and decreased circulating levels of estrogens when they are present with absent or irregular menses or infertility". Apparently he takes

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this definition from a previous publication in 1982 where he was the lead author.[2] The subjects for the present topics will be a much different group than defined by Rebar because the subjects he includes might be better termed as having somewhat diminished oocyte reserve.[1, 2]

In a 2010 review in Lancet DeVos, Devroey, and Fauser refer to the condition as primary ovarian insufficiency.[3] They state: "we and others regard ovarian insufficiency as more accurate than ovarian failure because ovarian insufficiency can be used to describe a wide range of impaired ovarian function". The others in this quotation were publications by Armstrong *et al.* (2007), Popat *et al.* (2008), and Welt (2008).[4-6] The title of Welt's article is "Primary ovarian insufficiency a more accurate term for premature ovarian failure".[6] The reason for changing the term is that failure implies finality whereas DeVos *et al.* state that: "the term also suggests that ovarian follicular activity might intermittently recover, even years after the diagnosis and lead to pregnancy in some women".[3]

A view was published in the Annals of New York Academy of Sciences by Kalantaridou and Wilson entitled "Premature ovarian failure is not premature menopause".[7] The title of the present manuscript is premature ovarian insufficiency used in this present review not primary ovarian insufficiency. The word primary helps determine that the etiology of the amenorrhea and estrogen deficiency stems from a paucity of ovarian follicles as opposed to pituitary insufficiency. However, the term ovarian insufficiency implies the ovary as the source of the problem and the term primary ovarian insufficiency does not distinguish this pathological state from women undergoing natural menopause. Thus the term premature ovarian insufficiency helps to determine that the condition occurred at an earlier age and takes into account the occasional intermittent ovulation and pregnancies that have occurred. However, though not included in the title these cases will all have hypergonadotropism not hypogonadotropism.

DeVos *et al.* do seem to favor the definition employed by Coulam *et al.* in 1986 in which they attempted to determine the incidence of premature ovarian insufficiency (called failure by Coulam *et al.*[8]). They state primary ovarian insufficiency is characterized by the triad of amenorrhea for at least 4 months, sex steroid deficiency, and two recordings of serum concentrations of FSH more than 40 IU/L at least one month apart in a woman aged less than 40.[3, 8] A large percentage of articles clearly dealing with premature ovarian insufficiency use the definition used by Coulam *et al.*[7, 8]

The importance of establishing a definition for the purpose of consideration of treatment options to achieve follicular maturation, ovulation and possible subsequent pregnancy is to be able to determine if a proposed therapy achieves ovulation and pregnancy any better than the spontaneous ovulations that have been recorded either with or without hormonal replacement or while on oral contraceptives.[9-12] In the review article by DeVos *et al.* they quote some studies suggesting that: "pregnancies can arise in up to 10% of women as a result of intermittent ovulation in the early stages of the disorder".[3, 13, 14]

It is not clear how the arbitrary 4 month duration of amenorrhea was determined by Coulam *et al.*[8] Since the largest series of treating premature ovarian insufficiency involved 100 patients and had more austere criteria for selection including amenorrhea for greater than one year, the criteria used for that study will be used throughout this manuscript to define the group with "premature ovarian insufficiency".[15] Thus for this discussion premature ovarian insufficiency is considered in a woman who is less than 40 with more than 1 year of amenorrhea whose serum estradiol (E2) is less than 25 pg/mL and whose serum FSH is >35 mIU/mL. This group was considered to have hypergonadotropic amenorrhea with estrogen deficiency[15]. This term should be included when performing literature searches for premature or primary ovarian failure or insufficiency. For that study all women failed to have menses following 10 mg x 10 days of

medroxyprogesterone acetate and failed to generate a rise in serum E2 despite 150 IU of gonadotropin for at least 5 days.[15] Failing to respond to gonadotropins is important to cover the rare circumstances of secondary amenorrhea and estrogen deficiency related to the replacement of the normal gonadotropin producing cells of the pituitary with a gonadotropinoma secreting increased immunoreactive FSH that was not biologically active.[16]

A thorough search of the literature failed to identify any reports of primary amenorrhea with ovarian insufficiency and sexual infantilism with either spontaneous ovulation or pregnancies. Thus when discussing ovulation induction and pregnancies in women with premature ovarian insufficiency this should imply exclusion of true premature ovarian failure with sexual infantilism (assuming no hormonal replacement). Inclusion of this group with sexual infantilism would only dilute the determination of the efficacy of treatment. Thus conditions such as congenital adrenal hyperplasia with a 17 alpha hydroxylase deficiency, pure gonadal dysgenesis, aromase deficiency, and Turner's syndrome with sexual infantilism would be excluded. However some causes of Turner's syndrome, especially a Turner variant where sexual development without hormone replacement occurred with perhaps some menstrual cycles would be included.

Pharmacological induction of ovulation despite premature ovarian insufficiency

In the 2009 review by Rebar he states that: "as noted the spontaneous pregnancy rate is only 5-10%. Thus, *in vitro* fertilization using donor oocytes may be appropriate for women with premature ovarian failure during pregnancy".[1] Thus, even though the group included by Rebar based on his definition may not be as nearly as depleted as the group chosen for this review, he implied that there are no other treatment options but donor oocytes.[1]

In the review of Kalantaridou and Nelson they also use the less strict criteria of Rebar *et al.* to define premature ovarian insufficiency.[7, 17] They state that: "there is no treatment to restore fertility in young patients with premature ovarian failure that has proven safe and effective in prospective controlled studies".[7] They go on to state: "theoretically these unproved therapies might even prevent one of these spontaneous pregnancies from occurring".[7] These authors discounted anecdotal reports claiming ovulation induction and pregnancies with clomiphene citrate,[18] human menopause gonadotropin,[19] and gonadotropin releasing hormone analog with purified urinary FSH [20] because they claim these reports resulted in no greater chance of ovulation than those seen with untreated patients referring to a study published by Rebar *et al.* in 1992.[21] Thus, they state that: "for women with premature ovarian failure desiring fertility, oocyte donation is an option".[7]

In the review by DeVos *et al.* they state: "In cases of primary ovarian insufficiency couples typically have the options of adoption or egg donation". However, they did at least allude to the therapy rendered to the aforementioned series of 100 consecutive women with hypergonadotropic amenorrhea with estrogen deficiency.[15] DeVos *et al.* state: "Evidence suggests that pregnancies might occur in women with primary ovarian insufficiency when high FSH concentrations are suppressed by ethinyl estradiol or gonadotropin-releasing hormone analogues, followed by ovulation induction using low dose gonadotropins. However, there are no published prospective trials of sufficient power to support the hypothesis that reduction of FSH concentrations is more likely to allow development of follicles than is chance intermittent change in ovarian function".[3] One of the main goals of this present review is to describe in detail this aforementioned technique and present the data from some of the non-randomized series and case reports of treating women with premature ovarian insufficiency to allow the reader to determine if treating these women with this technique seems reasona-

ble or should they heed the advice of some of the top experts in the field of premature ovarian insufficiency who either suggest totally expectant therapy or perhaps at least normal hormonal replacement and hope for chance, or advise adoption or donor egg programs.

The FSH receptor restoration technique

Basic concept

The objective of this section is to provide evidence that the aforementioned technique alluded to by DeVos *et al.* that involved suppression of FSH by ethinyl estradiol or GnRH analogues possibly followed by low dose gonadotropins can increase the chance of ovulation above that of spontaneous chance alone.[3] The theoretical principal of the technique and specific details will be provided.

The main concept of this technique is that antral follicles are developed in women with marked diminished oocyte reserve much more frequently than evidenced by the frequency of spontaneous ovulation. The reason why they do not typically advance to a dominant graafian follicle is that the chronically high levels of serum FSH down-regulates FSH receptors on the granulosa-theca cells. Follicle stimulating hormone does not have a direct effect but must interact with its receptor to produce the various enzymes involved in the folliculogenesis process. The tenet of this procedure is if one lowers serum FSH by inhibiting release from the pituitary by negative feedback with high dosage estrogen (preferably ethinyl estradiol for reasons that will be shortly explained) or by the use of gonadotropin releasing hormone (GnRH) agonists or antagonists, sometimes the FSH remains high enough to stimulate progression to a dominant follicle without exogenous gonadotropin.[22] Sometimes a small boost of exogenous FSH is needed to complete the maturation process.[22] Frequently there is a sufficient endogenous luteinizing hormone (LH) surge to allow oocyte release and luteinization but sometimes exogenous human chorionic gonadotropin is needed. Only mild FSH stimulation is used because high dosage will raise the serum FSH and thus down-regulate receptors again and thwart the follicular progression.[22] Evidence to support the concept that raising serum FSH too high can down-regulate FSH receptors was provided by a case report of a 42-year-old woman with secondary infertility. She was menstruating regularly though with only 23 day intervals. Treating her with clomiphene citrate and raising the day 3 serum FSH from 26 mIU/mL to 76 mIU/mL put her in apparent menopause. She was advised that donor oocytes were her only option. However, a second opinion considered that the hypergonadotropic amenorrhea with estrogen deficiency was iatrogenic. Her FSH was lowered by ethinyl estradiol, which was stopped, allowing endogenous estradiol to rise. She formed 3 dominant follicles with a serum E2 of 868 pg/mL without any exogenous gonadotropins.[23] To propose a shortened title for this technique hereafter in this manuscript the procedure will be referred to as the FSH receptor restoration technique.

Evidence that high dose FSH may cause meiosis errors and thus aneuploidy in women with diminished oocyte reserve

When one studies molecular genetic analysis of spontaneous miscarriages related to aneuploidy it has been determined that most are of maternal origin and most trisomies are related to errors in the first meiotic division involving non-disjunction of whole chromosomes or sister chromatids.[24, 25] A study by Handyside *et al.* found that the high FSH dosage used for *in vitro* fertilization especially women who averaged 40 years of age not only increases the quantity of embryos with aneuploidy but also causes marked changes in the cause and type of chromosomal abnormalities.[26] One difference is that over half of the aneuplodies following controlled ovarian hyperstimulation (COH) are from errors in the

second meiotic division.[26] When there were meiotic 1 errors the majority were related to malsegregation of single chromatids rather than whole chromosomes.[26] Finally most abnormal zygotes had multiple aneuploidies whereas natural miscarriages are usually related to single chromosome abnormalities.[26]

The question arises as to whether advancing age with deteriorating mitochondria was responsible for the susceptibility of oocytes to meiosis errors following COH or was it the generally higher levels of baseline serum FSH that occurs with age that made them further susceptible to the adverse effects of increasing levels of serum FSH. It has been hypothesized that even younger women with diminished oocyte reserve but still not in apparent premature menopause do poorly with conventional or high dosage FSH COH protocols because a key molecule or enzyme needed for the proper events of meiosis gets down regulated leading to meiosis errors even if the FSH receptors have not been suppressed sufficiently to inhibit follicular maturation.[27] This would explain why many excellent IVF centers report very low pregnancy rates despite the transfer of normal appearing embryos in younger women with increased FSH following conventional stimulation,[28-30] *vs.* those claiming good results as long as mild FSH stimulation is used.[31-33]

With mild stimulation for women aged <35 with diminished ovarian reserve *vs.* conventional FSH COH for age peers with normal reserve the live delivered pregnancy rate was 33.5% (over 300 transfers) *vs.* 42.2% (over 1000 transfers).[34] The margin widened slightly in the next age bracket of 36-39 (22.2% *vs.* 33%) but the margin was the greatest at age 40-42 (9.1% *vs.* 20.9%).[34] Thus the oocytes from younger women with diminished oocyte reserve seem to be slightly inferior to age peers with normal reserve undergoing IVF-ET. However, the best explanation to explain why some excellent IVF centers report very poor live delivered pregnancy rates following embryo transfer may be their use of COH regimens using a high dosage of FSH *vs.* only low dosage FSH used in the above study.[28-30, 34] Thus it would seem that high dosage FSH regimens for women who have elevated FSH may create the abnormal meiosis patterns previously mentioned in the embryos leading to aneuploidy, and thus very low live delivered pregnancy rates. Therefore an important part of the regimen for treating women with premature ovarian insufficiency is to try not to add any exogenous FSH when the FSH is already elevated. If one wants to provide a boost of FSH when the follicle is progressing as evidenced by increased follicular growth and rising serum E2 levels then one should use only very small dosages of FSH, *e.g.*, 75 IU and preferably when the serum FSH is close to normal or is normal.

Despite the marked adverse effect of high dosage FSH on oocytes when oocyte reserve is diminished, with normal reserve there is an advantage of creating more embryos. If one counts all transfers from a given oocyte retrieval whether fresh or frozen for women with diminished oocyte reserve using mild stimulation there was for the 3 age groups an average of 0.47, 0.27, and 0.10 live babies born *vs.* 0.91, 0.55, and 0.28 with normal reserve using conventional stimulation.[34]

Women with diminished oocyte reserve and those with premature ovarian insufficiency who are made to ovulate tend to have the need for increased progesterone supplementation in the luteal phase.[35] In a study of infertile women aged <37 with serum FSH >15 mIU/mL on day 3 where treatment was exclusively luteal phase progesterone support if the follicle was mature in natural cycles or at most a boost of low dose FSH from late follicular phase if the follicle was not mature, the live delivered pregnancy rate in a maximum of 5 cycles was 33.5% *vs.* 62.5% in matched infertile controls with normal oocyte reserve treated in a similar fashion.[36] The difference in pregnancy rates could be accounted for by the probability that oocytes from women with diminished oocyte reserve are somewhat more prone to meiosis errors even without the adverse effect of high dosage

FSH stimulation. Other confounding factors would include a high rate of premature luteinization in the group with low reserve (41.6%) compared to those with normal reserve.[37, 38] Another adverse factor is a greater frequency of the short follicular phase with the duration of estrogen exposure during the follicular phase being of great importance for developing progesterone receptors in the endometrium.[35, 39-41] Women with low oocyte reserve are less likely to achieve adequate follicular maturation compared to women with normal reserve.[36]

Description of the FSH restoration technique

The original description of the FSH receptor restoration technique was published in 1984.[42] Four of the five patients were treated with higher dosages of conjugated estrogen (2.5 to usually 5 mg per day) to lower the gonadotropins, whereas one was treated with ethinyl estradiol.[42] Case 3 had only three spontaneous menstrual cycles in her life since her menarche at age 16. She did menstruate to progesterone withdrawal at age 20, and then subsequently progesterone withdrawal was no longer able to induce menstruation. Before starting therapy at age 32 (which at this time the technique was to first suppress the FSH into the normal range and then add gonadotropins) her serum FSH was 96 mIU/mL and serum E2 was 18 pg/mL with predominantly parabasal cells on vaginal cytology. Though she failed to ovulate the first cycle, she did ovulate in the next 4 consecutive cycles. She was successful in that 5th cycle of ovulation and delivered a live baby. Progesterone was supplemented in the luteal phase but only at a dosage of 25 mg twice daily.[42] The dosage of human menopausal gonadotropin in that cycle totaled 3000 IU (40 ampules – mostly 150 IU per day which increased to 225 IU for a few days).

Patient A had 6 months of amenorrhea and estrogen deficiency with a serum FSH of 39.2. She ovulated 2 consecutive times but did not conceive following suppression of FSH with conjugated estrogen. She stopped this because she could not afford gonadotropins. Both of these women had failed to ovulate to gonadotropins alone previously (patient 2 used 4500 IU and patient 1 3000 IU) without any evidence of follicular maturation. Three of the 5 patients responded to therapy and 2 delivered live babies.[42]

It seemed clear from these results and the long interval from apparent premature ovarian insufficiency to treatment with the follicle restoration technique (case 3 who did not conceive was diagnosed at age 19 with hypergonadotropic amenorrhea and estrogen deficiency with a serum FSH drawn on several occasions averaging 120 mIU/mL. She ovulated the first two times following estrogen to lower FSH then gonadotropins). Thus, this response suggests that the FSH receptor technique was much more likely to be responsible for the ovulation than spontaneous occurrence. Because the conjugated estrogen used in 4 of the 5 cycles precluded the use of the serum E2 assay the decision to use hCG injection was based solely on ultrasound. Ethinyl estradiol was tried in one patient hoping it would not raise the E2 level and it was found that it did not.[42] From that time on ethinyl estradiol was frequently used in lieu of other estrogens.[43] Since 2 of the 5 women did not ovulate and the gonadotropins were expensive the technique was modified to try to cut costs.

When faced with premature ovarian insufficiency the possibility exists in a given individual there may be a few antral follicles present (or some may reach antral size in a short time) that could have responded to endogenous FSH were it not for down regulation of FSH receptors. At the other extreme there may not be any primary follicles left or primary follicles that will never develop to the stage of FSH sensitivity.[44] There may be those in between these extremes where a follicle advances to FSH sensitivity every three months or so.

Thus to be cost effective ethinyl estradiol is generally started in women with hypergonadotropic amenorrhea with estrogen deficiency at a dosage of 20 μg. One should be patient with the dosage since some women

will eventually show a rise in their serum E2 levels despite serum FSH that has been lowered but remains elevated (>15 mIU/mL). If the E2 does not rise and the FSH is still elevated the dose of ethinyl estradiol can be doubled and the serum E2 carefully monitored. One can wait to do ultrasound to check follicle size until some rise in serum E2 occurs.[22, 43]

Sometimes the combination of exogenous ethinyl estradiol and endogenous E2 can suppress the FSH below the level needed to allow progression of follicular development, so the ethinyl estradiol is decreased from 40 to 20 µg or 20 µg every other day. Ethinyl estradiol is one of the most commonly used estrogens in the world since it is the estrogen component of most oral contraceptives. However, though the oral contraceptive may be used to induce ovulation, it generally will preclude pregnancy by causing an abnormal endometrium (desidualization) and by adversely effecting cervical mucus. Unfortunately, ethinyl estradiol is no longer commercially available. The raw product must be purchased (Schering Germany is one company) and compounded by a pharmacy.

Generally speaking once a rise of serum E2 above 40 pg/mL occurs ultrasound to measure follicular maturation should be added to the monitoring of serum E2 and FSH. After a level of 80 pg/mL adding serum LH and even progesterone can be helpful to detect premature luteinization. If the LH and progesterone level seem to be climbing somewhat a gonadotropin releasing hormone (GnRH) antagonist, *e.g.*, ganirelix or cetrorelix, can be given. If premature luteinization cannot be avoided generally a GnRH agonist (leuprolide acetate) 0.5mg is started in mid-luteal phase and continued along with the ethinyl estradiol.

If there is failure to generate an endogenous rise in serum E2 despite the lowering of FSH to the normal range sometimes the ethinyl estradiol is stopped hoping that FSH receptors have been restored and the ensuing rise in FSH will stimulate folliculogenesis. If there is a prescription plan sometimes gonadotropins are added while ethinyl estradiol is maintained especially if an antral size follicle is seen on ultrasound.

Following oocyte release documented by ultrasound usually vaginal progesterone (90 mg or 8% progesterone gel, Endometrin 100 mg three times daily, or 200 mg progesterone vaginal suppositories twice daily are prescribed (this dosage is a lot higher than had been used originally 30 years ago when the technique was originally described).

If the FSH is not sufficiently lowered by just 20 µg of ethinyl estradiol but the woman cannot tolerate from a side effect standpoint a higher dosage of ethinyl estradiol, a GnRH agonist can be added. Though one can also use a GnRH antagonist, the former is less expensive.

Of course if even 20 µg of ethinyl estradiol is not tolerated or the use of estrogen is contraindicated, *e.g.*, a woman with a previous history of thrombosis or breast cancer follicular recruitment can be attempted by lowering FSH with either a GnRH agonist or antagonist exclusively.[45, 46] Similar to ethinyl estradiol low dose gonadotropins can be added at the appropriate times.

In general, the use of ethinyl estradiol has several advantages over GnRH agonists or antagonists including less cost, generation of better cervical mucus allowing natural intercourse, creating better endometrial thickness, and relief of vasomotor symptoms.[43]

Evidence that the FSH receptor restoration technique stimulates ovulation much more frequently than the rate of spontaneous fortuitous ovulation

In 1990 the results of a series using the FSH receptor restoration technique in 100 consecutive women with hypergonadotropic amenorrhea and estrogen deficiency was published.[15] Since the study included women up to age 47, the term premature ovarian failure or premature ovarian insufficiency was not used. Unfortunately one cannot determine how many of the 100 women were over 40 just that the age ranged from 19 to 47 with an average of 34 years. Thus, the results presented, especially the pregnancy rate, would probably be higher if only

women <aged 39 were included since age rather than degree of diminished ovarian oocyte reserve has the most negative influence on pregnancy rates.[47] Exceptions do occur however and there is even a case of ovulation induction in both cycles of treatment in a 45-year-old woman using the FSH receptor restoration technique who went into natural menopause. The second ovulation resulted in a live healthy baby.[48]

In the 1990 series of 100, the requirements for selection was a minimum of one year of amenorrhea, serum FSH >35 mIU/mL with a serum E2 <25 pg/mL and failure to have menses following 10 days of 10 mg/day of medroxyprogesterone acetate.[15] A further requirement was a minimum of four treatment cycles and a maximum of 8. The first 20 patients used the technique described in 1984 where estrogen was used for approximately two weeks to suppress serum FSH into the normal range then gonadotropins were started. Sixty-five used the modified technique where gonadotropins were only used if the ethinyl estradiol initiated an endogenous rise in serum E2 and the FSH had dropped below 15 mIU/mL. If the E2 was rising but the FSH was still elevated exogenous FSH was not supplemented. Nine women used leuprolide acetate to lower FSH and similarly hMG may or may not have been supplemented in small dosages.[15] The average total amount of gonadotropins used when it was given was 1050 IU per cycle.[15]

The average length of amenorrhea before initiated therapy with the FSH restoration technique was not provided. However, the average length of amenorrhea was 2.2 years for those conceiving *vs.* 4.8 years for those not conceiving. Since only 37% of those treated ovulated, it would seem reasonable that the average length of amenorrhea in those ovulating was over three years. It does not seem likely that 37 women with three years of amenorrhea would fortuitously spontaneously ovulate 68 times during the study period (4-9 cycles). It seems more likely that the estrogen receptor restoration technique was responsible for the 68 ovulations in 349 attempts (20%) rather than chance in these women with long term amenorrhea.[15]

This study also suggests that it is the lowering of the FSH rather than some other effect of estrogen responsible for follicular maturation for several reasons. Though only nine women were treated with leuprolide in lieu of estrogen, the results were comparable with leuprolide. There was ovulation in 7 of 43 initiated cycles (16.3%) *vs.* 61/311 (19.6%) with ethinyl estradiol and 3 of 9 women (33.1%) ovulated at least once with leuprolide *vs.* 34/91 (37.3%) with ethinyl estradiol.[15] It should be noted that 19 of 91 women (20.8%) conceived with the estrogen-hMG technique and 19 of 34 women (56%) who were made to ovulate.[15] The 50% miscarriage rate could be partially attributed to insufficient dosage of progesterone in the luteal phase and throughout the first trimester in the 1980s as compared to higher dosages used today.[15] Of course the pregnancy rate would have been higher if the study had been restricted to women with premature ovarian insufficiency (age <40) instead of including women even up to age 47 with hypergonadotropic amenorrhea with E2 deficiency.

To further prove that lowering the serum FSH is needed, with the likelihood of up-regulation of down-regulated FSH receptors, it should be noted that 27 of the 37 women who ovulated by lowering the serum FSH with possible use of low dose gonadotropins had previously failed to ovulate with hMG without lowering FSH in 46 cycles and used an average of 3400 IU of FSH in those failed cycles.[15] The average baseline serum FSH was 70.3 mIU/mL in those who ovulated *vs.* 66.5 mIU/mL in those who did not ovulate.[15] All women who ovulated had serum E2 levels <20 pg/mL.[15]

Besides this large series some case reports also strongly suggest that the FSH receptor restoration technique markedly improves the odds of ovulation as opposed to mere spontaneous ovulation or ovulation with just replacement estrogen therapy. First, one of the cases in the series of 100 despite 3.4 years of amenorrhea and failure to produce a rise of serum E2 above 20 with high

dose gonadotropins alone in 4 cycles was able to ovulate in 7 of 7 leuprolide-low dosage hMG treatment cycles.[15] One 25-year-old with two years of amenorrhea, estrogen deficiency and 3 serum FSH levels of 144.9, 145.6, and 164.2 mIU/mL was made to ovulate in 6 of 10 treatment cycles with ethinyl estradiol alone without any gonadotropin boost. She had a chemical pregnancy in cycle 9 and her ovulation in cycle 10 resulted in a live delivery of a healthy baby.[49]

Another woman stopped oral contraceptives at the age of 36 and developed oligomenorrhea and vasomotor symptoms. After 50 days of amenorrhea a serum E2 was 20 pg/mL and a serum FSH was 120 mIU/mL. She was advised by her reproductive endocrinologist her only option was to use donor oocytes and she was diagnosed with premature ovarian insufficiency. She began the ethinyl estradiol after 66 days of amenorrhea (her FSH was now 123 mIU/mL with a serum E2 <10 pg/mL) and ovulated in two consecutive cycles (no gonadotropins were added) but she failed to conceive. She did not make a mature follicle in cycle 3. She elected to return to her previous infertility specialist but failed to conceive in four fresh donor oocyte cycles. At age 40, after 2 years and a half of hypergonadotropic amenorrhea and estrogen deficiency she was helped to spontaneously ovulate again by lowering her very elevated serum FSH without any exogenous gonadotropins and she conceived and delivered a healthy baby.[50]

Resistance of the follicle to FSH versus oocyte depletion?

One could hypothesize that those women who ovulate to FSH suppression with or without exogenous gonadotropin may not actually have oocyte depletion as much as a gonadotropin resistant gonad.[10] Carrying this hypothesis further one could argue that the chemically elevated FSH needed to overcome an FSH receptor defect could eventually down-regulate the FSH receptor. Thus there is the theoretical possibility that only those women who have a normal number of remaining primary follicles as their age peers but with FSH receptor resistance related to FSH receptor down-regulation respond to this therapy but egg quality in those with primary follicular depletion do not respond, or even if they do ovulate, they do not become pregnant.

Theoretically one could find elevated FSH related to a less biologically active FSH. The beta subunit of FSH rather than the alpha subunit confers the unique biological property to FSH. However mutations of the FSH beta gene are rare and they usually present with sexual infantilism and prim ry amenorrhea rather than secondary amenorrhea (the type of premature ovarian insufficiency more amendable to improve fertility by treatment).[51, 52] Similarly, missense mutations in the FSH receptor gene are also very rare and present with primary amenorrhea and sexual infantilism when homozygous.[53]

When one finds a woman with apparent premature ovarian insufficiency with estrogen deficiency who responds to gonadotropins without suppression of elevated serum FSH the treating physician should consider the diagnosis of an FSH secreting gonadotropinoma making a biologically inactive FSH but with damage to the normal FSH producing cells of the pituitary.[16] This is probably the best use for measuring serum anti-mullerian hormone (AMH), since the level will be normal rather than markedly decreased. Since some women with non-detected or very low serum AMH still have regular menses this assay is not that helpful in determining management of women with premature ovarian insufficiency.[54]

Ovulation induction was achieved in two cases with "streaked gonads" with successful pregnancies by the FSH restoration technique despite previous failure to respond to high dose FSH stimulation.[55, 56] Thus since there is not any good evidence of FSH receptor abnormalities and therefore, FSH resistance despite normal numbers of primary follicles leading to premature ovarian insufficiency, and there is evidence that younger women have achieved pregnancies with such ovarian depletion that they would be comparable by degree of follicle depletion to 53-year-old women, supports the argu-

ment that age rather than degree of primary follicle depletion is responsible for poor oocyte quality. Thus it seems worthwhile to try to induce follicular maturation in younger women with hypergonadotropic amenorrhea with estrogen deficiency. The very poor pregnancy rates seen with very advanced reproductive age is probably more related to mitochondrial aging.[57] The evidence suggests that the resistance to both endogenous and exogenous gonadotropins is related to reversible down-regulation of FSH receptors.[57]

In vitro *fertilization and premature ovarian insufficiency*

In vitro fertilization is expensive. The FSH receptor restoration technique is not very expensive especially with the modified technique which allows predominantly follicular growth from endogenous FSH with only a small boost of expensive exogenous gonadotropins. The question arises as to whether the success rate is high enough to use IVF-ET when it is necessary, *e.g.*, for bilateral blockage of the fallopian tubes or a severe male factor. There are precedents for successful follicular maturation and pregnancies in women with blocked fallopian tubes and hypergonadotropic amenorrhea with estrogen deficiency even with the transfer of only one embryo.[58, 59]

At the 2013 American Society for Reproductive Medicine meeting the outcome of IVF-ET performed in women with premature ovarian insufficiency who needed IVF-ET because of tubal or male factor problems was presented.[60] Five consecutive cases of women with hypergonadotropic amenorrhea over one year duration with a serum E2 <20 pg/mL and a serum FSH >20 mIU/mL who needed IVF-ET to achieve a pregnancy were presented. They were aged <42. The live delivered pregnancy rate per transfer was 20% (4/20). The live delivered pregnancy rate per retrieval was 9.5% (4/43). The live delivered pregnancy rate per initiated cycle was 6.1% (4/65). Three of the 5 women conceived and delivered a live baby. Only 1 of these 5 women never matured a follicle (2 cycles attempted). One woman had 2 IVF-ET cycles transferring a single embryo but failed to conceive.[60]

It is not known if IVF-ET would increase the odds of better pregnancy rates in women with premature ovarian insufficiency *vs.* natural cycles in women with normal fallopian tubes and male partners with normal sperm. There are data showing higher pregnancy rates with IVF *vs.* natural in women with diminished oocyte reserve but where there is a better chance of obtaining more than one embryo.[61] Using the FSH restoration technique Tartagni *et al.* published a case of success following IVF for premature ovarian insufficiency where there was no clear cut need for IVF.[62]

Conclusions concerning the FSH receptor restoration technique

In the expert review by DeVos *et al.* their view on the FSH receptor restoration technique is that "there are no published prospective trials of sufficient power to support the hypothesis that reduction of FSH concentration is more likely to allow development of follicles than a chance intermittent change in ovarian function".[3] In fact, there was one randomized controlled trial that showed no benefit of estrogen pretreatment in ovulation rates in hypergonadotropic women.[63] However, the tenets of the FSH receptor restoration technique were not followed adequately by Taylor *et al.*[63]

On the other hand, a different recent randomized controlled trial did in fact reach the conclusion that women with premature ovarian insufficiency do have improvement in ovulation and pregnancy rates when estrogen pre-treatment is used to up-regulate down-regulated FSH receptors from prior exposure to high serum levels of FSH.[64] In making a clinical decision the reader must consider the summary of non-randomized series and unique case reports and the one randomized controlled trial supporting the efficacy of the FSH receptor restoration technique, then weigh the cost and side effects and potential benefits of this therapy to

decide if faced with a woman who desires a baby with her own oocyte and genetic make-up to try this FSH receptor restoration technique or not. The alternative, is in lieu of a prospective trial that convinces experts, *e.g.*, Michael DeVos, Paul Devroey, Bart Fausen and even Robert Rebar to try this technique on a woman wanting to conceive with their own oocytes but instead suggest the following advice "In cases of primary ovarian insufficiency couples typically have the options of adoption or egg donation".[3]

The frequency of premature ovarian failure may depend on the exact definition used and the population. By combining several studies Aimen and Smentels estimated a frequency of 0.3% of women of reproductive age.[65] This percentage would be even lower if one eliminates primary amenorrhea and sexual infantilism, which is generally not treatable and one eliminates those women in premature ovarian insufficiency who have already had children and are no longer interested in having any more. If one considers a 20% rate of ovulation for those treated with the FSH receptor restoration technique *vs.* 1% for placebo control and spontaneous ovulation, it is unlikely that any one study would have sufficient power to show significance in a randomized control study. One may also question as to whether it ethical to avoid treatment by using a placebo control in a woman who because of a delay may become oocyte depleted and not able to be helped.

As an example the study of 100 consecutive women showed a rate of ovulation of approximately 20% per attempted cycle.[15] A placebo-controlled randomized trial combining pituitary suppression with gonadotropin stimulation in women with premature ovarian insufficiency was performed in 1995 by van Kasteren *et al.*[64] They found that 3 of 15 women in the GnRH agonist group ovulated (20%) but none of the 15 controls did.[64] However, because of insufficient power, the difference was not statistically significant.[64] Reproductive endocrinologists favoring the FSH receptor restoration technique would use this as an example of evidence that the 20% frequency of ovulation in 354 cycles in the 100 consecutive cases was not merely fortuitous but was supported by a placebo controlled randomized trial.[15, 64] Those pushing donor oocytes without attempting ovulation induction with this technique could state that a randomized placebo controlled trial was performed and showed no significant difference with this FSH receptor restoration technique.[3]

If the woman prefers not to undergo intensive monitoring with blood tests and ultrasound an alternate approach based on the FSH receptor restoration technique principles would be for the woman to be placed on a slightly higher estrogen replacement dosage, *e.g.*, 4 mg/day estradiol. She could then do an old fashioned basal body temperature chart and supplement vaginal progesterone on the third day of the temperature rise. If no ovulation in 60 days menses could be induced with 13 days of medroxyprogesterone acetate and the cycle repeated.

Dehydroepiandrosterone (DHEA) for ovulation in premature ovarian insufficiency

The first report of using DHEA for primary ovarian insufficiency involved 5 women and 4 of the 5 ovulated and conceived.[66] Mamas and Mamas extended the treatment to 14 more women aged 36-40 with serum FSH varying from 62 to 98 mIU/mL. Eight of the 14 women (57.1%) conceived within 3-7 months of therapy.[67]

In the first study of 5 the serum FSH dropped to <20 mIU/mL in all 5 cases.[66] There is a strong possibility that the DHEA could have been converted in the body to higher levels of serum E2 generated by aromatase enzymes leading to negative feedback for FSH release from the pituitary.

The original concept of benefit from DHEA by Casson *et al.* was that the DHEA could be working by increasing the follicular androgen pool.[68] The possibility exists that the intrafollicular androgens may help pre-antral follicles progress to antral follicles that are now FSH sensitive. Thus it is pos-

sible that DHEA can not only restore sensitivity of follicles to FSH by up-regulating down-regulated FSH receptors in granulosa theca cells, but also increase the number of antral follicles.[68]

At first glance, the pregnancy rates with DHEA do seem superior to estrogen or GnRH analogue FSH suppression. However, the length of amenorrhea of 6-9 months was much less than in the 100 cases treated with the FSH receptor restoration cases. Furthermore, since the publication of Mamas and Mamas in 2009 this author has tried to use DHEA for those failing to ovulate with the ethinyl estradiol with possible low dosage FSH technique and so far there has not been one ovulation.[32]

Of more concern, one study evaluated pregnancy rates in women with decreased egg reserve undergoing IVF according to serum DHEA levels. Instead of attaining more oocytes and better pregnancy rates with higher serum DHEA sulfate levels, the opposite was found, *i.e.*, a trend for higher number of oocytes retrieved and higher pregnancy rates the lower the serum DHEA level.[69]

In addition, another study showed that follicular fluid DHEA concentrations showed significant negative correlation with the total number of oocytes retrieved, the number of metaphase II oocytes retrieved, the number of normally fertilized oocytes, the number of visible cleavage-stage embryos, and the number of viable blastocysts.[70] DHEA can also produce androgen side effects which also needs to be considered when deciding treatment for premature ovarian insufficiency.

Immunosuppression

DeVos, Devroey and Fauser state in their review that the main mechanism that leads to the disorder of premature ovarian insufficiency remains unknown.[3] Nevertheless, they do present one of the most complete tables listing in Panel 1 various disorders leading to ovarian insufficiency and in Panel 2 listing genes associated with primary ovarian insufficiency. Ultimately, all conditions leading to ovarian failure will have some genetic basis and the chromosomal defect could lead to inflammatory damage, *i.e.*, autoimmune etiologies.

The treatment with the FSH receptor restoration technique can induce folliculogenesis irrespective of the mechanism leading to the state of ovarian insufficiency with a few exceptions where biochemical abnormalities preclude the production of sex steroid, *e.g.*, the very rare 17 alpha hydroxylase deficiency or FSH receptor mutation condition.

In their table they list autoimmune lymphocytic oophoritis and polyglandular auto-immune syndrome.[3] Rebar, in his review states that "autoimmune disturbances are not uncommon in women with premature ovarian failure. In excess of 20% of affected women present with an autoimmune disease and this may begin before ovarian dysfunction becomes apparent. Most common is some form of thyroiditis".[1] This percentage is probably highly inflated because the assumption is made that if women have hypothyroidism or have thyroid peroxidase antibodies or anti-thryoglobulin antibodies present, one must assume that these antibodies could have been associated with antiovarian antibodies and subsequent ovarian damage. However, this is not a valid assumption since other studies show the same frequency of low thyroid levels and thyroid antibodies in women with normal oocyte reserve *vs.* low oocyte reserve.[71] In contrast to thyroid hypofunction there probably is an association with premature ovarian insufficiency and Addison's disease, but Addison's disease is extremely uncommon and the combination with premature ovarian insufficiency is even more rare.[72]

There have been cases where ovulation has resumed following treatment with immunosuppressive agents, *e.g.*, glucocorticoids and azathioprine.[73, 74] However, though Kalantunidou *et al.* report a successful case following treatment with glucocorticoids they present a second case who developed Cushing's syndrome. Thus, they illustrate the risks with immunosuppression.[75] In fact, one cannot be sure that the ovulation was not spontaneous and unrelated to the dangerous therapy. Also, those who have seem-

ingly responded could possibly have also responded to far less risky treatment with FSH receptor restoration. Thus, there is little reason to consider immunosuppressive therapy for premature ovarian insufficiency. In performing an evaluation, measuring a morning serum cortisol is only needed if there are symptoms suggesting adrenal insufficiency. Obviously if Addison's disease is detected then glucocorticoids should be given, but only in replacement dosages.

In Part 1 the review centered on inducing ovulation in women already suffering from premature ovarian insufficiency. Part 2 will discuss strategies that could provide a woman the best chance of preserving fertility potential despite circumstances that could lead to a high risk of premature ovarian insufficiency. These circumstances could include treatment for cancer with potential surgical damage to the ovaries, chemotherapy, and radiation therapy. Other risks could include endometriosis and surgery for endometriosis, and familial or genetic risk.

Chemotherapy and radiotherapy

In general the risk of developing premature ovarian insufficiency immediately following chemotherapy depends on certain factors including age (the younger the woman the lower the risk), type of chemotherapeutic agent (alkylating agents create the highest risk), and the existing number of primordial follicles (known as the ovarian reserve) at the time of chemotherapy.[75, 76]

Alkylating agents are especially toxic to rapidly proliferating cells with high turnover and thus growing ovarian follicles are one of the potential targets for direct damage by alkylating agents.[77, 78]

The alkylating agents, *e.g.*, cyclophosphamide result in intra-strand and inter-strand cross-linking of DNA which interferes with cell division.[79] They also have a mitochondrial effect causing a reduction in mitochondrial transmembrane potential and an accumulation of cytochrome c in the cytosol leading to activation of the caspase family with subsequent apoptosis.[80]

The adverse effect of certain chemotherapeutic agents can cause temporary hypergonadotropic amenorrhea with estrogen deficiency with subsequent recovery at some point after completion of therapy. This is related to the loss of the growing follicles which have rapidly proliferating cells.[79] However, subsequently the population of growing follicles will be replenished by those primordial follicles that were spared progressing to the growing stage leading to a normal estrogen state with either spontaneous menses including ovulatory cycles or at least the potential to induce menses with progesterone withdrawal and the potential to induce ovulation with standard therapy in anovulatory women.[79]

The loss of growing follicles may contribute to the loss of some of the not actively growing primordial follicles.[75] Growing follicles produce factors, *e.g.*, antimullerian hormone (AMH), which inhibit movement of primordial follicles into the growing follicle pool.[81] Thus with a second round of chemotherapy more growing follicles will have been recruited following the first round of chemotherapy and now these growing follicles are at risk from a second course of chemotherapy. The manuscript by Morgan *et al.* provides mechanisms of how other chemotherapeutic agents, *e.g.*, doxorubicin, cisplatin, irinotecan and etoposide, work against cancer cells and their potential adverse effect on oocytes.[79]

Chemotherapeutic agents can be classified as high risk, medium risk and low risk for gonadotoxicity.[75, 77] Table I lists various chemotherapy drugs according to their gonadotoxicity. Alkylating agents induce the most significant risk for premature ovarian insufficiency with an odds ratio of 4 *vs.* platinol derivatives with an odds ratio of 1.77. Other drug families do not increase the risk of ovarian failure very much and thus the observed increase does not reach statistical significance.[82]

Improved treatments, and therefore long-term survivors of cancer, allow a better perspective of the true impact of chemotherapeutic agents in causing premature ovarian insufficiency. It should be recalled

TABLE I.—*Gonadotoxicity of chemotherapeutic agents.*

High risk (mostly alkylating agents)	Medium risk (mostly platinum agents) and anthracycline antibiotics and taxoids	Low risk (vinca plant agents) anti-metabolites and some anthracycline antibiotics
Cyclophosphamide	Cisplatin	Vincristine
Busulfan	Carboplatin	Vinblastine
Chlorambucil	Adriamycin	Methotrexate
Procarbazine	Doxorubicin	5 fluorouracil
Melphalan	Docetaxel	6 mercaptopurine
Ifosfamide	Paclitaxel	Bleomycin
Chlormethine		

that by definition the development of hypergonadotropic amenorrhea with estrogen deficiency in a woman <40 is considered premature ovarian insufficiency. However, a young woman who develops ovarian insufficiency by age 35 could still have conceived and delivered healthy babies prior to that time. Thus in consideration of risks and potential therapeutic strategies one must consider what is the chance of developing overt permanent ovarian failure soon after the cessation of chemotherapy or at least within the window where it is considered safe to now achieve a pregnancy (related low risk of needing more chemotherapy during the 266 days of pregnancy).

One such study was recently published from the European Organization for Research and Treatment of Cancer Lymphoma Group and Groupe d'Etude de Lymphomas de l'Adult Cohort Study.[76] They confirmed that nonalkylating chemotherapy carries little risk of premature ovarian insufficiency.[76] However, even non-alkylating agents increase the risk of premature ovarian insufficiency if the woman's age when treated was >32 where the risk was 9% *vs.* 0.9% in the general population.[76]

Some chemotherapy regimens tried to spare gonadotroxicity and tried to avoid alkylating agents. One such regimen includes doxorubicin, bleomycin, vinblastine and dacarbazine (ARVD).[83] Another regimen included the following non-alkylating agents – adriamycin, epirubicin, bleomycin, vinblastine and prednisone (EBVP).[84] Regimens including alkylating agents could be MOPP (mechlorethamine, vincristine, procarbazine and prednisone) and BEACOPP (bleomycin, etoposide, doxorubicin, *i.e.*, anthracycline, cyclophosphamide, oncovin (*i.e.*, vinblastine), procarbazine and prednisone.[85] With a median follow-up of 16 years about 20% develop hypergonadotropic amenorrhea and estrogen deficiency compared to 0.9% of controls in females treated for lymphoma.[83]

If one looks at specific treatments, 23% were treated with radiotherapy alone with only 1 receiving iliac radiotherapy, 33% with combined radiotherapy and non-alkylating agents and 44% with combined radiotherapy and chemotherapy. There were 34% who developed premature ovarian insufficiency when treated with alkylating agents but only 3% when alkylating agents were not used.[83] The highest rate of premature ovarian insufficiency was found in women receiving iliac radiotherapy (41%).[83]

Escalated BEACOPP, which contains twice the dosage of cyclophosphamide increases the risk from 32% with the regular BEAMOPP protocol to 67%.[86] Thus the cancer patient who is interested in future children should discuss the chemotherapy/radiotherapy regimen with the treating oncologist and determine as to whether the oncologist can choose a protocol that provides the least gonadotoxic damage without jeopardizing their success of curing the cancer.

Besides choosing a less gonadotoxic regimen, some studies, *e.g.*, Blumenfeld *et al.*, found that women treated with escalated BEACOPP showed resumption of menses in 20 of 22 women when treated with a GnRH agonist *vs.* 9 of 14 without GnRH agonist.[87] Indeed a large randomized multicenter clinical trial found a significant reduction in chemotherapy induced hypergonadotropic

amenorrhea with estrogen deficiency when triptorelin was used concomitant to chemotherapy in women treated for breast cancer, which thus included an older group more susceptible to developing premature ovarian insufficiency with less primordial follicles to start compared to younger females.[88] It is not clear, however, why another large prospective study in women with breast cancer did not reach the same conclusions.[89]

The role of assisted reproductive technology in preserving fertility potential in women at risk for iatrogenic premature ovarian insufficiency

Post-pubertal females have the option of undergoing one round of controlled ovarian hyperstimulation (COH) and oocyte retrieval with cryopreservation of embryos if they are married or if they have a male partner, they can freeze embryos fertilized by donor sperm, or today the option of oocyte freezing (with marked improvement of attaining subsequent pregnancies by performing intracytoplasmic sperm injection on oocytes cryopreserved by vitrification).[90, 91] Some COH regimens use tamoxifen or letrozole plus low dosage gonadotropins to minimize the exposure to estrogen in some cancers such as estrogen receptor breast cancer.[92] However, considering the expense of the procedure one could still choose a traditional COH regimen aimed at maximizing the number of gametes or embryos cryopreserved arguing that such a short exposure to higher levels of estrogen is not likely to increase the risk of failure with cancer treatment failures especially since the estrogen levels can be markedly decreased quickly by using GnRH antagonists.

For prepubertal females, one can perform an oophoropexy to attempt to bring the ovaries out of the peak damaging field for radiotherapy. One could laparoscopically remove ovarian tissue and cryopreserve the tissue for auto-transplantation in the future. There have been at least 13 live births from this procedure.[93] However, one must consider the risk of re-introducing metastatic tumor cells back into a cured patient.

Another possibility is the use of *in vitro* maturation of cryopreserved ovarian tissue.[94] One has to consider the extra physical and emotional trauma of adding an extra surgical procedure to a pre-pubertal child. Furthermore since the younger the female the less chance of inducing premature ovarian failure before she enters her reproductive years despite chemotherapy and radiotherapy, one has to consider whether the biopsy or removal of an entire ovary, as is sometimes done, will decrease her chances of spontaneous ovulation or ovulation with mild ovarian stimulation.

Some of these children may resume menses but develop premature ovarian insufficiency before they are old enough for marriage and children. Thus one could consider oocyte cryopreservation after completion of chemotherapy once menses resumes. This same strategy can be applied to women who would consider pregnancy now but are advised to wait a certain number of years to be sure they are cured. Those with male partners could freeze embryos and those without freeze oocytes.

One argument could be that even if they resume a normal state there may be a terrible prognosis with IVF if they have diminished oocyte reserve as manifested by an elevated day 3 serum FSH. However, especially young women should be advised that the pregnancy rates are almost comparable to those with normal reserve as long as mild COH protocols are used.[32, 47]

Endometriosis

A very good review on endometriosis and its effect on oocyte reserve was written by Ruiz-Flores and Garcia-Velasco.[95] They conclude that just the presence of endometriosis as well as surgery for the removal of endometriosis (which, magnifies the problem even more) can cause a decrease in ovarian reserve and thus an increased risk of premature ovarian insufficiency.[95] Thus one strategy to minimize the risk for prema-

ture ovarian insufficiency is to avoid surgery for pelvic pain and/or "to improve fertility potential" by laparoscopic excision of laser vaporization of endometriotic implants.[95]

However, what alternative is there for pelvic pain if oral contraceptives are not effective? Impeded androgen and GnRH analogues have unpleasant side effects and high costs.[96] There is evidence that the sympathetic nervous system controls cellular permeability. Studies support that a large variety of pain syndromes including headaches, arthritis, complex regional pain syndrome, inflammatory bowel disease, interstitial cystitis, and various types of pelvic pain may be caused by sympathetic nervous system hypofunction which allow the absorption of unwanted chemicals and toxic material into these tissues which leads to inflammation and pain.[97] Despite long-term chronicity and resistance to standard conventional therapy, these disorders respond quickly and with a high rate of amelioration of the pain following therapy with the sympathomimetic amine dextroamphetamine sulfate.[97]

A recent presentation at the 2013 American Society for Reproductive Medicine meeting was given entitled "The use of sympathomimetic amines for severe pelvic pain may help defer potentially oocyte damaging laparoscopic surgery".[98] In a series of 15 women with severe dysmenorrhea (and also other types of pelvic pain) that failed to gain more than transient relief following previous laparoscopic surgery were treated with a low dosage of dextroamphetamine sulfate (started at 15 mg extended release capsules with possible increase to 30 mg). The results showed that 46.6% reported marked relief within 3 months of therapy and 53.3% by 6 months. Also 80% reported at least moderate relief of pain by 3 months and 93.3% by 6 months.[98] No woman failed to have any relief. Only 1 of 15 reported the relief was only mild, and even she was comfortable now just adding non-steroidal anti-inflammatory medication when the dysmenorrhea began.[98]

Besides preventing further damage of ovarian tissue from repeat laparoscopy the possibility exists that the cause of the pelvic pain was from inflammation. Thus the possibility exists that the association of endometriosis and decreased oocyte reserve could be related to chronic inflammation of the ovaries. Thus dextroamphetamine sulfate may while it is being used to treat pelvic pain, also may help to prevent ovarian primordial follicle depletion.

There has been an ongoing debate as to whether excisional surgical techniques are superior to laser vaporization technique, implying that the cause of pain is the return of endometriosis from inadequate removal. A study by Yeung *et al.* is very interesting. Using the excisional technique on teenagers not one demonstrated any endometriotic implants 2 years later on repeat laparoscopy.[99] However, interestingly, 50% had a return of the dysmenorrhea.[99] This suggests that possibly endometriosis occurs from the increased permeability state of the pelvic tissues, however inflammation from absorbed toxic substances related to increased permeability is the cause of the pain.

Conclusions and future considerations

Though not a new technique, it is hoped that though editorials and reviews more reproductive endocrinologists will try to induce ovulation with various FSH receptor restoration techniques in women with premature ovarian insufficiency to provide the opportunity for women to have a baby with their own genetic material in lieu of only being provided options of donor gametes, donor embryos, or adoption. The key points of the technique is to allow natural follicular development by lowering elevated serum FSH levels and providing at best a small boost of gonadotropins if necessary. The technique emphasizes being sure of an adequate length to the follicular phase to allow development of progesterone receptors in the endometrium and to provide luteal phase support with progesterone supplementation.

As far as participating in the treatment regimen for women undergoing poten-

tial gonadotoxic therapy, it is important to work with the oncologist to emphasize the women's desire to have children and thus to choose the ideal protocol to help cure the cancer with the least risk of ovarian damage.

Cost and need for no treatment delays could determine whether oocyte or embryo freezing is an option before treatment. Based on age and type of chemotherapy, the female being treated or her parents should be provided statistics on the likelihood of permanent damage *vs.* return of gonadal function so they can make the best choice of delaying treatment, and invasive and expensive procedures. They should be advised that oocyte freezing could be performed after completion of gonadotoxic therapy but before the time that the woman desires to have a baby to cover the possibility of developing premature ovarian insufficiency before they are ready to have a baby. Of course, even if premature ovarian insufficiency occurs, there would still be the chance of inducing ovulation by FSH receptor restoration techniques. However the sooner this is attempted the greater the chance there will still be some oocytes left.

Though not all studies are conclusive, there is a suggestion that GnRH agonists when used during chemotherapy can help reduce the gonadotoxic effect. One question that remains would be would the use of long-term GnRH therapy help to inhibit progressive atresia. The long-term side effects and costs without any supporting documentation would make long term GnRH agonists not something to consider today, but possibly for the future if some research studies support the efficacy. One has to consider that it could backfire in that it would inhibit the development of growing follicles. The lack of AMH at a certain stage of follicular development may be associated with accelerated apoptosis and atresia of the remaining primordial follicles.

If GnRH agonists are too costly or cause side effects one could consider oral contraceptives as co-treatment with chemotherapy to reduce potential gonadotoxicity. However, there are too few studies showing evidence of their gonadotoxic sparing effect to recommend them as first line therapy.[78]

In counseling the couple about ovulation induction despite premature ovarian insufficiency, the reproductive endocrinologist and/or geneticist should counsel the couple of what risks could occur to the baby on a genetic basis. Thus it is generally recommended to perform a chromosome analysis looking for X chromosome abnormalities and to screen for the X linked gene known as the fragile X mental retardation 1 (FMR1) gene mutation which could be associated with a reduction of mental capacity.[1, 5]

Dextroamphetamine sulfate has been used for years for children with attention deficit hyperactivity syndrome with few side effects and a very good long term safety record. It seems reasonable to evaluate its efficacy in preventing oocyte depletion in females with endometriosis especially if it alleviates pain at the same time. Perhaps it could be extended to females undergoing chemotherapy both during and possibly after to see if it can reduce the risk of premature ovarian insufficiency.

About 25 years ago, one of the world's leading gynecologists Robert Kistner (the author of Kistner's Textbood of Gynecology) stated at a plenary session at the American Society of Reproductive Medicine meeting that it should be considered malpractice if a teenager had severe pelvic pain and a laparoscopy was not performed to treat probable endometriosis from damaging her pelvic structures. Based on present data, if a young lady had a laparoscopy for pelvic pain and subsequently developed premature ovarian insufficiency the possibility of litigation exists because of performing a laparoscopy with known potential damage to ovarian tissue without a great likelihood of anything more than transient relief of pain. Thus though there will always be exceptions, the gynecologist should be cautious about possibly damaging a woman's future fertility potential by removing endometriosis.

For the very distant future neo-oogenesis in the adult human ovary may be possible.[100] Alternatively a recent study has shown that

female germline stem cells might be present in ovarian surface epithelium in mice and humans. When sampled in GFP transgenic mice, cultured for a long period and transplanted into ovaries of sterilized mice, these cells underwent oogeneis. Live births from these mice were produced. Thus the future could add to the restoration of fertility potential in women with premature ovarian insufficiency the development of new oocytes by this technique.[100]

Riassunto

Insufficienza ovarica prematura – La sfida della fertilità

L'insufficienza ovarica prematura, definita come amenorrea con carenza di estrogeni in una donna di età inferiore ai 40 anni, associata all'ormone follicolo stimolante (*follicle stimulating hormone*, FSH) >35 mIU/mL, può essere temporaneamente invertita con l'ovulazione ottenuta con conseguente gravidanza con parto. Sebbene ciò possa avvenire spontaneamente, la frequenza dell'ovulazione può essere notevolmente aumentata mediante varie tecniche di abbassamento del livello elevato di FSH sierico e quindi con una regolazione dei recettori FSH nelle cellule della granulosa e della teca. Ciò si può realizzare mediante soppressione del rilascio dell'FSH da feedback negativo attraverso l'alta dose di estrogeni, oppure mediante soppressione della produzione di FSH inibendo l'ormone di rilascio delle gonadotropine (*gonadotropin releasing hormone*, GnRH), utilizzando agonisti o antagonisti del GnRH. Il metodo con estrogeni è la tecnica di scelta perché è molto meno costoso degli analoghi del GnRH e contribuisce a stimolare il muco cervicale e sviluppo dell'endometrio. L'etinilestradiolo è l'estrogeno preferito perché non contribuisce alla misurazione di estradiolo sierico e permette quindi un corretto monitoraggio della maturazione follicolare. Talvolta le gonadotropine esogene sono necessarie come impulso, ma il dosaggio deve essere basso, in modo da non dover nuovamente ridurre i recettori FSH. La tecnica viene definita come la tecnica di ripristino del recettore FSH. Il progesterone dovrebbe essere somministrato nella fase luteale. I medici dovrebbero cercare di aiutare a prevenire l'insufficienza ovarica prematura scegliendo scrupolosamente alternative meno gonadotossiche di trattamento del cancro con farmaci altrettanto efficaci. Anche un intervento chirurgico per endometrioma ovarico deve essere effettuato solo quando assolutamente necessario.

PAROLE CHIAVE: Amenorrea - Insufficienza ovarica primaria - Menopausa prematura - Endometriosi.

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