Editorial Article

Ovulation disorders: Part II
Anovulation associated with normal estrogen

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Summary

Purpose: To present various types of anovulatory states associated with normal estrogen and various treatment options.

Methods: Evaluation and treatment of various conditions including polycystic ovarian syndrome, hyperprolactinemia, congenital adrenal hyperplasia are discussed as are methods to prevent certain complications of these therapies. *Results:* Clomiphene citrate seems equally effective to gonadotropins at least for the first three cycles but has a frequent complication of adversely affecting the cervical mucus so intrauterine insemination is frequently needed. Glucocorticoid therapy and insulin receptor drugs can exert a primary or more commonly an ancillary benefit when used in combination with other follicle maturing drugs. Complications, e.g., adverse cervical mucus, luteinized unruptured follicle (LUF) syndrome, premature luteinization, luteal phase deficiency and treatment options are presented. *Conclusions:* Vaginal progesterone can correct luteal phase problems, human chorionic gonadotropin (hCG) and follicle stimulation hormone (FSH) and gonadotropin releasing hormone (GnRH) agonists can help LUF syndrome and GnRH agonists and antagonists can help the complication of premature luteinization.

Key words: Serum FSH; Normal estrogen; Anovulation; Premature luteinization; Luteal phase defects.

Anovulation with normal estrogen

What is meant by normal estrogen does not necessarily mean attaining a normal mid-cycle serum estradiol (E2) level of 200 pg/ml. In fact, in general, with anovulation with normal estrogen the serum E2 does not reach 200 pg/ml. What is meant is that this woman has sufficient estrogen to menstruate spontaneously or menses can at least be induced by progesterone withdrawal. Of course sometimes the patient is somewhat in between estrogen deficiency and normal estrogen in that it may take several weeks of a lower dose of endogenous E2 exposure to build up enough endometrium to shed sufficiently with progesterone withdrawal to lead to menses. Sometimes these individuals can be hyperestrous with the presence of mid-cycle quality cervical mucus all the time, increased endometrial thickness, and even occasionally endometrial hyperplasia.

When women have amenorrhea and are estrogen deficient they do not respond to clomiphene citrate. Women with anovulation who are estrogen replete usually can be made to ovulate by clomiphene citrate. Clomiphene citrate is a selective estrogen receptor modulator and can occupy certain estrogen receptors but with much less or no estrogen effect. During the follicular phase estrogen exerts a negative feedback effect on the release of FSH from the anterior pituitary gland. The use of an inert (from the pituitary standpoint) competitive antagonist, e.g., clomiphene citrate, causes an increased release of FSH from the pituitary with a consequent increased serum FSH which leads to follicular maturation.

The thin cervical mucus found at mid-cycle is estrogen dependent. Originally clomiphene was not developed as a drug to induce ovulation but was designed to be a drug to prevent pregnancy by causing hostile cervical mucus. In fact even in the first treatment cycle we found that 69% of women had poor postcoital tests after taking clomiphene citrate [1]. Adding ethinyl estradiol from the day after clomiphene to time of ovulation improved the post-coital test in those with poor ones in cycle 1 to 50% in cycle 2 vs only 16.7% in those not receiving ethinyl estradiol [1]. The ethinyl estradiol for the few days after stopping clomiphene until the day of ovulation may counteract the anti-estrogen effect on the cervical mucus. Ethinyl estradiol rather than other estrogen preparations is used for similar reasons as in women with hypergonadotropism so that the response as measured by the serum estradiol can be monitored. In addition to ethinyl estradiol we usually prescribe 600 mg guaifenesin 2x/day from day 2 of cycle to ovulation [2].

Another anti-estrogen drug similar to clomiphene citrate in its mechanism of action my group has been using to induce ovulation is tamoxifen [3]. Usually, we start at 50 mg clomiphene citrate from day 3 to 5 for five days and use 20 mg tamoxifen in a similar manner. Failure to develop a mature follicle by ten days after stopping either drug will result in doubling the dosage. This increase in dosage can be given with or without first inducing menses with prog-

Revised manuscript accepted for publication December 18, 2006

esterone withdrawal. Generally we do not prescribe more than 150 mg clomiphene citrate for up to eight days. Our experience has found that clomiphene citrate is more apt to induce follicular maturation and ovulation than tamoxifen but the latter is less likely to create hostile cervical mucus.

Lowering the estrogen level can be achieved through another mechanism by inhibiting the conversion of the 19 carbon androgens to the 18 carbon estrogens by inhibiting the aromatase enzyme. These aromatase enzyme inhibitors, similar to tamoxifen, have been used for breast cancer. There are several studies, especially from Canada, suggesting that these drugs, e.g., letrazole can be effective in stimulating follicular maturation in some anovulatory women [4]. Another aromatase enzyme inhibitor anastrazole is being evaluated by a major fertility pharmaceutical company in the United States in hopes of gaining approval as a drug for ovulation induction and is currently used in Europe. These drugs have a much less adverse effect on the endometrial thickness [4]. They probably are, however, less effective than clomiphene in inducing ovulation.

Another way to raise serum FSH levels is to treat with FSH. Unfortunately there is no oral form so FSH must be given by injection. There are two formulations – one containing equal amounts of LH and FSH and the other just FSH alone. The original gonadotropin containing FSH and LH called human menopausal gonadotropin (hMG) and was marketed by the Serono company and called Pergonal. As the name implies, it was derived from the urine of menopausal women where there is a high level of LH and FSH excreted. Today there are only two human menopausal gonadotropin preparations on the market – Repronex and Menopur (Ferring Inc.) and similar to the original Pergonal, they are equal mixtures of LH and FSH (75 IU each). Repronex, similar to Pergonal, is best given intramuscularly but Menopur has less contaminants and can be given subcutaneously which allows self injection by the patient herself. Usually, but not always, especially with a hyper-response to gonadotropins with suppression of endogenous LH, advancement of meiosis and release of the oocyte from the dominant follicle requires the injection of human chorionic gonadotropin (hCG) which has the same biological effect of LH. Thus giving hCG provides the mid-cycle LH surge needed to advance the process of ovulation.

Originally all gonadotropins consisted of LH and FSH. In polycystic ovarian syndrome there is typically an increased LH:FSH ratio of ≥ 1.8:1. The thought was that the risk of the ovarian hyperstimulation syndrome (consisting of pain, marked enlargement of the ovaries with ascites to total body anasarca associated with a loss of intravascular fluid to a third space leading to the risk of renal hypoperfusion with potential subsequent hyperkalemia and acute tubular necrosis, a hypercoagulable state, with possible thromboemboli complications, and the risk of ovarian torsion) may be related to the high LH. It was considered that when treating with FSH alone there would be sufficient LH on board to allow proper follicular maturation and by almost eliminating LH there would be a considerable reduction in the risk of ovarian hyperstimulation syndrome (OHSS). Unfortunately, the risk for OHSS was found to be related to the FSH and not LH and in fact adding LH to the FSH treatment regimen might even reduce the risk of OHSS [5].

Originally the hMG or urinary FSH preparation had to be given intramuscularly. With improved purity, urinary derived production products were able to be given subcutaneously which allows self administration. The product by Ferring Inc. called Bravelle is the only one still available in the United States market. Eventually the purity would be improved even more by production of recombinant DNA technology bypassing the need for urinary products. Though initial claims were that recombinant FSH products were superior to urinary products there is no consistent data to support these contentions. Some women, but not all, do better when there is some LH in the preparation.

Sometimes, even with a low dose (75 IU or less of gonadotropins) some women, especially those with polycystic ovarian syndrome, will develop an excessive number of mature and immature follicles which markedly increases their risk for OHSS and multiple births. Once an antral follicle approaches a mature dominant follicle (around 14 mm) it can continue to grow in the presence of LH even without FSH. Thus the smaller follicles can be eliminated by only using recombinant LH (Luveris from Serono Inc.) or by using diluted hCG (100 IU/day) since hCG has the same biological action as LH while allowing fewer dominant follicles to proceed. We find this method superior to "coasting" where the gonadotropins are stopped completely and it is hoped that some follicles continue on their own or can then be restimulated by a small dose of gonadotropin after stopping for a period of time. Since release of the oocytes stimulated by controlled ovarian hyperstimulation can only occur if an hCG injection is given, withholding an hCG injection can prevent complications (but then there has been a waste of expensive gonadotropins). Sometimes a cycle with too many follicles is converted to an in vitro fertilization-embryo transfer cycle since draining follicular fluid from each follicle helps decrease the risk of OHSS and multiple births can be reduced by limiting the number of embryos transferred.

In 50% of cases polycystic ovaries are associated with insulin resistance (definite insulin resistance if the fasting serum glucose level divided by the insulin levels is < 4.5 and probable if the ratio is < 6.0). Insulin resistance plays a role in the anovulatory process by occupying insulin-like growth factor-I and II (IGF1 and IGF2) receptors causing an increased production of androgen by pre-antral follicles leading to higher serum estrogen and testosterone levels which have a negative effect on the release of FSH from the pituitary gland by gonadotropin releasing hormone (GnRH) that is secreted from the hypothalamus. Using a drug like metformin (Glucophage) or rosiglitazone (Avandia) or pioglitazone (Actos) restores down-regulated insulin receptors thus allowing insulin to bind to its own receptor instead of its IGF cousins. In fact, using these drugs can restore ovulation without the use of clomiphene citrate or other anti-estrogen drugs or gonadotropins, although it generally takes several months. Sometimes if a couple cannot afford

gonadotropins but is unresponsive to clomiphene citrate, 150 mg for ten days, follicular maturation can be achieved by combining drugs like metformin or rosiglitazine with clomiphene citrate.

The mechanism of action as to why drugs that restore down-regulated insulin receptor can improve response to clomiphene-resistant patients is because sometimes FSH is not raised sufficiently by the clomiphene itself to allow follicular maturation. While the suppressive effect of estrogen on FSH release by the pituitary is negated by the clomiphene alone, the suppressive effect of testosterone is not, and sometimes testosterone levels are increased by clomiphene citrate. These insulin-receptor restoring drugs, by restoring down-regulated insulin receptors, allow a reduction in testosterone levels thus removing the negative feedback effect on FSH release by the pituitary. Sometimes a low dose of glucocorticoids taken at bedtime can lower ovarian androgen in women with PCO not to mention lowering the contribution to the total serum level contributed by the adrenal gland, and responsiveness to clomiphene citrate can be improved [6]. Glucocorticoids are even more helpful when anovulation is accompanied by the adult onset type of congenital adrenal hyperplasia (especially 21-hydroxylase deficiency) and is diagnosed by an elevated serum 17 hydroxyprogesterone level (and sometimes an increased dehydroepiandrosterone sulfate (DHEA) level). Congenital adrenal hyperplasia can cause a PCO state.

Metformin has a lot of associated gastrointestinal side-effects whereas glitazones do not. However, because metformin can be taken throughout the first trimester and there have been some claims of it helping to reduce miscarriage rates, this drug is the one that I prefer to try first. Also for the obese population it tends to cause weight loss whereas the glitazones may increase weight. However, if side-effects preclude the use of metformin, I do not hesitate to use rosiglitazone or pioglitazone until pregnancy is achieved.

I do not believe that the ovulation rates are sufficiently high and the waiting time is too long to use these drugs first and only using follicle maturing drugs if they fail. Instead if I find an abnormal glucose:insulin ratio, I frequently will start metformin but concomitantly use the follicle maturing drugs since the infertile population are generally very frustrated and any further delays just frustrates them more. Since glitazones cannot be taken during pregnancy, I usually do not switch from metformin to this class of drugs if the woman is responsive to follicle maturing drugs and cannot tolerate metformin. However, if a woman is resistant to clomiphene citrate but gonadotropins induce too many follicles, I will frequently try adding one of the glitazones to see if ovulation can be achieved when combined with clomiphene citrate.

Theoretically, by lowering androgens most preantral follicles should be inhibited from getting to the antral stage in polycystic ovarian syndrome. However, I have been disappointed in not seeing this combination work well in inhibiting multiple egg recruitment to gonadotropins. To be fair, however, I may not have pretreated long enough with metformin before initiating gonadotropin therapy.

Estrogen, though exerting a negative feedback effect on release of FSH from the pituitary, actually exerts a positive effect on LH release as the estradiol approaches 200 pg/ml, the level found in the serum. As a dominant follicle reaches maturity, the LH begins to rise allowing advancement of meiosis of the oocyte and events get in motion to allow the oocyte to detach from the oocyte wall and eventually the egg is released from the follicle. With recruitment of many follicles the serum E2 rises way above the 200 pg/ml mark without any one follicle reaching a mature size. The rise in E2 can cause a premature release of LH which can cause premature luteinization of the follicle, i.e., early secretion of progesterone by a follicle that is not fully mature. The egg from the follicle making the progesterone becomes atretic but the other eggs from follicles that have not luteinized are still able to be fertilized and make normal embryos. However, pregnancy rates are poor because the early rise in progesterone makes the endometrium no longer a suitable environment for implantation. The evidence to support these contentions was based on evaluating the pregnancy outcome in egg donors who luteinized and who were giving half of their retrieved oocytes to a donor egg recipient. The pregnancy rate was much higher in the recipients than the donors but the pregnancy rates were no different from recipients receiving eggs from donors who did not have premature luteinization [7]. Infertile donors with premature luteinization had much lower pregnancy rates than infertile donors without premature luteinization [7].

As previously mentioned synthesis of LH and FSH from the pituitary and release of these hormones are under the influence of a 10-amino-acid decapeptide called the gonadotropin releasing hormone (GnRH). To achieve follicular development and ovulation FSH and LH need to be secreted at certain pulsatile intervals and varying amounts. Thus GnRH is secreted from the hypothalamus in pulses.

By substituting two aminoacids in the GnRH decapeptide a long-acting more potent GnRH can be created. Initially, this will cause an increase in the release of FSH and LH by the pituitary. However continued use will eventually suppress FSH and LH by ablating the pulsatility of these hormones. Depending on which two amino acids are used to substitute for the normal amino acids in these two positions a GnRH agonist as described above can be achieved. In the United States the two most commonly used are leuprolide acetate given subcutaneously or intramuscularly in a depo form or nafarelin which can be administered by nasal inhalation.

Leuprolide acetate and nafarelin can be used to take advantage of the initial potent stimulating effect (later I will discuss their role in effecting egg release from the follicle), but they are mostly used for their eventual suppressive effect on the release of LH and FSH with continued use. The suppressive effect of these GnRH agonists became popular as part of controlled ovarian hyperstimulation (COH) regimens used for IVF-ET. Since the purpose of the COH

regimen with IVF is to develop multiple follicles, achieved by using a high dosage of FSH from the early follicular phase, high serum E2 levels are achieved way before any follicles are dominant. This predisposes to a premature LH surge necessitating canceling of the cycle because of premature luteinization. By starting the GnRH agonists from the mid-luteal phase of the proceeding cycle and by continuing it until the injection of hCG the cancellation of IVF cycles for premature luteinization was markedly reduced [8]. It was subsequently found that even using an ultrashort course of leuprolide acetate on days 2-4 can block premature LH surges with a less adverse effect in response to gonadotropins [9].

Even before the agonists were developed, substitution of two other amino acid positions of the GnRH decapeptide with other amino acids produced a product that was a pure GnRH antagonist and could quickly suppress LH and FSH. However, they came out later on the market than GnRH agonists because of toxicity. Even when less toxic versions were developed there appeared to be a negative effect on embryo implantation. The "learning curve" with their continued use was to use the lowest dosage for the shortest time and with these modifications similar implantation rates and live delivery rates can be achieved as with the use of GnRH agonists but in a more convenient manner. Since GnRH agonists are best started in the mid-luteal phase they are not as convenient for cycle to cycle use since they will suppress corpus luteum function and interfere with a pregnancy from the cycle in which they are used. Supplementing the luteal phase with both estrogen and progesterone could negate this adverse effect to some degree but by using GnRH antagonists this concern is obviated.

Gonadotropin releasing hormone agonists can sometimes improve ovulatory response to gonadotropins and sometimes suppress response to exogenous FSH [10]. Whenever GnRH antagonists are used, e.g., ganirelix or cetrorelix, the dosage of gonadotropins is usually increased by 75 IU. Typically we start the GnRH antagonists with the establishment of a 14 mm follicle or sometimes we only use them if we observe the initiation of a LH rise before the follicle is sufficiently mature.

Sometimes patients may prefer to start with clomiphene citrate rather than gonadotropin because it is much less expensive. However, they would be willing to undergo treatment with gonadotropins even despite the increased cost if the success rates with the latter were higher. I usually advise them that the results of a study we performed found no difference in pregnancy rates comparing the two drugs for the first three months of therapy [11]. Of course the therapy with clomiphene citrate more frequently necessitates performing intrauterine insemination [11].

Hyperprolactinemia and treatment with bromocryptine or cabergoline for anovulation and estrogen deficiency has been described in part I. It should be noted that sometimes hyperprolactinemia can be associated with anovulation and normal estrogen levels but the same drugs can be used in this circumstance. However in a normal estrogen state they will normally also respond to clomiphene or gonadotropins. The advantage of bromocryptine or cabergoline over clomiphene is no adverse effect on cervical mucus or the endometrium.

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