Editorial

Cryptic infertility and therapeutic options

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Summary

Too often infertile patients are given a "herd type" fertility investigation which ultimately leads to expensive, time consuming, and risky in vitro fertilization. However, attention to certain simple details available by non-invasive methods, e.g., checking for premature luteinization, luteinized unruptured follicle syndrome, or performing the post-coital test at the appropriate interval, can lead to a quick solution of the infertility problem. Caution about persistent infertility related to iatrogenic factors, e.g., development of poor post-coital tests or excessively thin endometrium from clomiphene citrate, or development of luteinized unruptured follicle syndrome or premature luteinization by taking follicle maturing drugs, or creating a hostile environment from taking follicle maturing drugs when the woman already made a mature follicle (and would have had a higher success rate with luteal phase support with progesterone) will help achieve pregnancies without necessarily proceeding to the most expensive and invasive procedure of in vitro fertilization. Finally, many wasted cycles of treatment could be avoided by including the simple but very important hypo-osmotic swelling test and measurement of sperm autoantibodies with the first initial semen analysis.

Definition

Cryptic infertility exists if a couple has had an adequate number of cycles of sexual exposure but has not conceived and yet no etiology is found. Even more commonly, some infertility factor is diagnosed but the female partner has not conceived despite an adequate number of corrected cycles. So often the solution is to proceed to in vitro fertilization (IVF)-embryo transfer (ET) as a "catch-all" solution. Sometimes this strategy is successful. Sometimes the patient fails to conceive despite IVF-ET. Frequently the couple cannot afford IVF-ET.

Cryptic ovulatory problems - luteal phase defects

Some women have clear problems with anovulation. Yet despite many cycles of apparent normal ovulation, conception does not occur. One possible etiologic factor is that luteal phase defects have been found in increased frequency in anovulatory women treated with ovulation inducing drugs [1, 2]. Frequently physicians fail to perform endometrial biopsies to determine if there is adequate tissue effect of progesterone (P). Though mid-cycle serum P levels may be increased, so are other contra-progesterone hormones. Some studies have found no differences in the serum P levels in women with in-phase or out-of-phase endometria [3-5]. Also, even if the biopsy appears to be in-phase, the biopsy may be an archaic test and the future may evaluate luteal function by determining the effect of P on certain biochemical functions, e.g., the stimulation of immunomodulatory proteins, e.g., the progesterone induced blocking factor (PIBF) [6, 7]. Thus we have presented data suggesting that P support in the luteal phase should be given prophylactically whenever ovulation-inducing drugs are used [8].

Some women with fairly regular cycles are found to have luteal phase defects. Many are treated with follicle maturing drugs but repeat biopsies are not performed. However, there is evidence that the majority of women with luteal phase defects do attain a mature follicle [9]. For these patients the use of vaginal P support in the luteal phase was found to be far more effective in achieving successful pregnancies than the

use of follicle maturing drugs [9]. In fact, P vaginal suppositories were found to be very effective even in those failing to conceive with follicle maturing drugs [9].

Cryptic ovulatory problems - premature luteinization

Premature luteinization, where the luteinizing hormone (LH) and the serum P levels rise before the follicle is mature, may be a cause of cryptic infertility even in cycles without the use of follicle maturing drugs [10]. Premature luteinization may lead to atresia of oocytes [11] or reduced embryo viability [12]. Serum P and endometrial biopsies can be normal in this setting so it is important to monitor follicular maturation by determining follicle size by ultrasound and at the same time measure serum estradiol (E2), P, and LH [10]. This condition is even more common in women taking ovulation inducing drugs [13-15]. There is evidence that if this problem occurs in the first investigation cycle, it has a 93% chance of existing in subsequent cycles [10]. Treatment usually requires the use of a gonadotropin releasing hormone (GnRH) agonist which can be used for a longer course started in mid-luteal phase [16] or as little as three days starting in the early follicular phase [17]. Obviously, today one can also treat with GnRH antagonists.

Cryptic ovulatory problems - luteinized unruptured follicle syndrome

Pelvic sonography has been used to evaluate the failure to release oocytes from follicles [18-20]. A global review of the world literature on the luteinized unruptured follicle (LUF) concluded that LUF does exist as a clinical phenomenon, but from a clinical standpoint, it probably does not constitute a clinical syndrome [21]. The conclusion from Killick *et al.* was that failure to release oocytes from follicles is seen in 10% of menstrual cycles of normal fertile women [22].

However, we have data to support LUF as a syndrome that can occur in the majority of menstrual cycles in some infertile women [23]. We evaluated 220 first cycles of human menopausal gonadotropin (hMG) therapy [23]. There were 148 cycles where it was clear by ultrasound that oocyte release occurred and 20 became pregnant (13.5%). In contrast there were 56 cycles where release was indeterminate and only three (5.4%) conceived. There were 16 with clear-cut non-release and none (0%) conceived. For cycle 2 the pregnancy rates (PRs) in 197 patients were 15.7%, 4.0%, and 0%, respectively. These data at least showed that ultrasound diagnosis of LUF does correlate with PRs [23].

But is it a syndrome? We evaluated oocyte release in cycle 2 according to release in cycle 1; 91% of 128 women who released in cycle 1 released in cycle 2 (consistent with Killick and Elstein's conclusion that LUF can occur in 10% of cycles of fertile women [22]) vs 30% of 53 patients with indeterminate release vs 6% of 16 patients who failed to release in the first cycle [23].

These data show that the use of 10,000 units of human chorionic gonadotropin (hCG) does not necessarily guarantee oocyte release [23]. We have achieved oocyte release when adding follicle stimulating hormone (FSH) to the hCG [24] or using GnRH analogues, e.g., leuprolide acetate when merely using 10,000 μ hCG has failed [25].

Cryptic ovulatory problems - hostile uterine environment resulting from follicle maturing drugs

There is evidence that controlled ovarian hyperstimulation (COH) used for IVF may create a uterine environment that is less receptive to subsequent conception. Some of the early studies found superior PRs in recipients receiving oocytes from donors compared to women having IVF themselves who were matched to the oocyte donors [26, 27]. This was even more convincingly demonstrated by a study finding a significantly higher PR (twice as high) in recipients receiving equal quality oocytes from oocyte donors who were using the other half of the oocytes to be fertilized from sperm with their own male partner with subsequent ET [28]. This finding could be consistent with either an intrinsic uterine environment problem inhibiting implantation or an adverse effect of COH [28]. To help evaluate the etiology for lower PRs following COH we evaluated the outcome of the first frozen ET. An equal PR following frozen ET would suggest that the COH drugs were responsible. Though there was no longer a significant difference between donor and recipient there still was a trend for high PRs in recipients following frozen ET [28]. This left the hypothesis that possibly both the drugs used for COH and an intrinsic uterine environment problem led to reduced uterine receptivity in donors [28].

The aforementioned study was performed before the demonstration that the presence of hydrosalpinges could impair embryo implantation [29-34]. Since many donors for the shared oocyte program had tubal disease, we considered that hydrosalpinges could have accounted for some of the findings. Thus the study was repeated only this time using donors who either did not have a hydrosalpinx or where it had been present but removed [35-41]. The data continued to show a significantly improved PR in recipients following fresh ET but no longer showed a difference following frozen ET [42]. These data thus demonstrated that follicle maturing drugs when used to stimulate multiple oocytes adversely affect uterine receptivity.

An anovulatory woman with ten years of primary infertility who failed to conceive after ten IVF-ET cycles where 92 fresh embryos had been transferred but who conceived after her first frozen ET is a vivid example of how significantly these follicle maturing drugs can adversely affect uterine receptivity [43]. This same woman subsequently showed that follicle maturing drugs can inhibit embryos from implanting even when used in milder dosages aimed at creating one or at best a few mature follicles [44]. Following delivery her cycles became regular but she did not conceive after nine cycles. However, at the age of 40, in her next cycle she conceived when luteal phase support with P was used [44]. There have been data presented that suggest that these follicle maturing drugs allow premature trophoblast invasion rather than implantation failure as the mechanism for adverse affect on conception [45].

Cryptic implantation factors - hydrosalpinx

It has already been mentioned that the presence of hydrosalpinges can reduce pregnancy and implantation rates following IVF-ET [29-34] and that salpingectomy can improve conception outcome [35-41]. One has to be aware that even a unilateral hydrosalpinx can prevent conception and that following salpingectomy successful conception can occur quickly even in women with long-term infertility [46].

Cryptic implantation factors - male factor problems

The hypo-osmotic swelling test (HOST) is a measurement of the function of integrity of the sperm membrane. It is a simple and inexpensive test to perform [47]. When less than 50% of the sperm tails demonstrated swelling, one in vivo study found no live pregnancies in the female partners [48]. Interestingly, several studies found that sperm with low HOST scores result in normal oocyte fertilization, embryo cleavage, and embryo morphology following IVF-ET [49-54]. However, a matched prospective study found that sperm with abnormally low HOST scores create embryos that do not implant [55]. This conclusion was vividly supported by the demonstration of a 50% PR following ET in either oocyte donors or recipients when the HOST score was ≥50%, but was zero % in those donors or recipients who got the other half of the shared oocyte pool and where the oocytes were fertilized with sperm with HOST scores <50% [56]. This marked difference was seen even though there was no difference in the number or quality of embryos transferred [56].

Some preliminary data suggested that treatment of the sperm with the protein digestive enzyme chymotrypsin before sperm washing could improve HOST scores above 50% and allow some pregnancies to ensue [57]. However, IVF with intracytoplasmic sperm injection (ICSI) has been found to be the far more effective method to achieve successful pregnancy [58]. The high success with ICSI, but not conventional IVF, suggests that the defect associated with low HOST scores is related to a transfer of some toxic factor that was adversely affecting the functional integrity of the sperm membrane to the oocyte membrane by the supernumerary sperm that attach to the zona pellucida, and this subsequently per-sists and adversely affects the embryo membrane which, in turn, prevents implantation [59].

Another sperm test which is more time consuming to perform than the HOST is known as the sperm stress test; it may also predict sperm that will cause implantation disorders but is correctable with ICSI [60].

Once a male demonstrates a low HOST score it remains stable over time, in contrast to concentration and motility which may be quite variable [61]. However, despite the plethora of data supporting the importance of this simple inexpensive test, the large majority of clinicians fail to order this test either initially or even after subsequent persistent cryptic infertility. What is appalling is that the treating physician may now recommend IVF-ET for unexplained infertility and the patient may undergo many expensive, time consuming and risky IVF cycles without success because the adverse effect on implantation is not apparent to physicians or embryologists. This test should be routinely performed when evaluating the semen analysis initially.

Cryptic implantation disorders - immunologic

It certainly seems logical that persistent refractory infertility in some women may be due to immune rejection of the fetal semi-allograft. To date there are no very convincing studies demonstrating that immunological problems are related to persistent infertility. Some physicians claim success following various therapies such as lymphocyte immunotherapy, intravenous immunoglobulin treatment, selective serotonin re-uptake inhibitors, etc. Part of the problem is the lack of appropriate methodology to detect abnormalities so the proper patients can be selected for study and response evaluated to immune treatments.

If we can borrow from the highly debatable issue of whether immunotherapy helps to prevent spontaneous abortions, I present an important caveat. Fraser *et al.* provided a meta-analysis of studies of lymphocyte immunotherapy trials for recurrent spontaneous abortion and concluded that the current evidence did not support any benefit to this therapy [62]. However, most studies have been conducted in a way to test lymphocyte immunotherapy exclusively. Nevertheless, if it works in conjunction with another hormone its efficacy may be markedly reduced by this exclusion. When we compared P therapy alone to P therapy and lymphocyte immunotherapy to a group of women with recurrent spontaneous abortion the combined therapy proved more efficacious [63].

One immunologic theory suggests that the fetal semi-allograft escapes cytotoxic T-cell surveillance by the absence of major histocompatibility (MHC) type 1 antigens in the placenta. The MHC type 1 antigens are needed for processing the antigen for cytotoxic T-cell response. Natural killer (NK) cell activity does not require MHC 1 antigen. There are data suggesting that differences in HLA-E between fetus and mother induce P receptors in gamma/delta T-cells [64, 65]. The interaction of high concentrations of P, especially those generated at the maternal-fetal interface, will then lead to the production of a 34 kDa protein known as PIBF that inhibits NK cell cytolysis [66-68]. This hypothesis would allow selective inhibition of NK cell activity in the area of the fetus but not elsewhere in the body.

Progesterone induced blocking factor has been detected in the late luteal phase and has a positive correlation with conception outcome [7, 69]. Interestingly, lymphocyte immunotherapy has been found to increase PIBF levels [70].

Data has been presented that 90% of pathological pregnancies had low PIBF levels [71].

This would be related to either inability to stimulate adequate P receptors in gamma/delta T-cells (which may respond to immune therapy, e.g., lymphocyte immunotherapy or intravenous immunoglobulin) or inadequate P (which would be treated by P). Subsequent data suggested that if PIBF levels are low in untreated pregnant women it is most likely related to inadequate P rather than inadequate P receptors in gamma/delta T-cells [72]. More accurate tests for PIBF and more knowledge of the immune factors needed to prevent immune rejection are needed before the role of immunotherapy can be determined for prevention of spontaneous abortion let alone the role of the immune system in infertility.

Cryptic semen abnormalities

There are a great deal of studies presently investigating sperm function and biochemistry [73]. For this article we will deal only with sperm autoantibodies because they may be a cryptic cause of infertility. Frequently physicians are unaware that, in general, sperm motility with the semen analysis is not adversely affected by the presence of sperm autoantibodies [74]. The final immobilization of the sperm coated with autoantibodies is dependent on complement. Most semen samples are devoid of complement except rarely when injury allows complement leakage.

Theoretically suspicion of the presence of sperm autoantibodies would be present with the demonstration of a poor post-coital test despite the presence of normal quality cervical mucus. Interestingly when sperm autoantibodies are present and the post-coital test performed at least eight hours after intercourse is normal, the PRs are good [75]. However, a poor post-coital test should prompt testing for sperm autoantibodies. Unfortunately many clinicians perform the test with a much shorter interval, e.g., two hours which is insufficient time for complement to interact with the sperm coated with autoantibody.

Another caveat is that some clinicians when faced with a poor post-coital test instead of searching for the etiology merely believe they are obviating the problem by performing intrauterine insemination (IUI). However, if the diagnosis of sperm autoantibodies is made, then a more effective IUI could be obtained by first treating the sperm with chymotrypsin [76]. Ultimately, the most effective therapy is IVF with ICSI [77-81]. We actually test for sperm autoantibodies on the initial semen analysis.

Conclusions

If the treating physician of couples with infertility considers in the diagnosis and management the caveats listed above, many patients will conceive quicker and will be prevented from undergoing other therapies that are expensive or invasive and have potential risks, e.g., IVF-ET. In fact a woman may also be saved from undergoing procedures with surgical risks, e.g., laparoscopy. Nevertheless, there is a role for laparoscopy even in women who have had normal hysterosalpingograms to look for possible contributing factors, e.g., fimbrial adhesions and endometriosis. Though controversial, it is out opinion that mild endometriosis can be a cause of cryptic infertility and laparoscopic removal can significantly improve PRs [82]. However, it is also our opinion that in the majority of patients who have endometriosis, as long as luteal phase deficiency and LUF syndrome are corrected (which may occur with increased frequency with endometriosis), this entity contributes to persistent refractory infertility in only a minority of cases. Thus even if this condition is suspected by examination, symptoms, ultrasound, or increased CA-125 levels [83] we do not encourage the patient to have a laparoscopy as part of the initial therapeutic intervention [84].

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