FROM *IN VITRO* FERTILIZATION TO FAMILY

A JOURNEY WITH SHER FERTILITY SOLUTIONS (SFS)

Sher Fertility Solutions

THE · GIFT · OF · LIFE

GEOFFREY SHER, M.D.
SHERFERTILITYSOLUTIONS.COM

DREW TORTORIELLO, M.D.
SHERFERTILITYSOLUTIONS.COM

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https://www.sherfertilitysolutions.com

GEOFFREY SHER, M.D.

SHER@SHERIVF.COM

702-533-2691

&

DREW TORTORIELLO, M.D.

DREW.TORTORIELLO@SHERINSTITUTE.COM

646-792-7476

DEDICATION

This book is dedicated to the many thousands of infertile couples who have chosen Sher Fertility Solutions (SFS) as their point of embarkation for the journey from infertility to family. We thank them for ensuring our emergence as one of the most preferred venues for advanced reproductive services in the United States. We also recognize and appreciate the total commitment and compassion on the part of our wonderful staff, which in large part has been responsible for SFS' reputation as one of the most caring ART centers in the nation.

FOREWORD

This book is provided as a guide for patients seeking help in conceiving a family through Sher Fertility Solutions (SFS), located in New York, NY. The world of infertility can be a maze full of complicated and unfamiliar terminology. This book is intended to help patients make sense of this rapidly developing field. Its purpose is to introduce you to our unique philosophy regarding the indications, techniques, and methodology for the delivery of Advanced Reproductive Technologies (ART) at SFS.

Consumer advocacy has always been a part of SFS' commitment to provide affordable, quality medical care. Our philosophy is to provide patients with as much relevant information as possible, empowering them to make informed decisions as to their treatment. We attempt to give couples back the control over their lives that is often the first casualty of an

infertility problem. We also believe that the chance for a family is a basic human right and not a luxury available only to the wealthy. Unfortunately, infertility treatment is expensive and not often covered by insurance in this country. In addition, despite the high success rates offered by centers of excellence, many couples need more than one attempt to succeed.

Infertility treatment encompasses a wide range of possibilities, of which IVF is only one option. It is not the treatment of choice for everyone. However, while in the past, IVF was often considered the last common pathway, today, due to a myriad of factors, IVF is regarded as one of the mainstays for helping couples achieve the goal of a healthy family. In the final analysis, it is our individualized approach to treatment and our emphasis on putting patients at the forefront that has distinguished SFS from the rest of the IVF community. We recognize and encourage patients to undergo a thorough evaluation prior to beginning IVF treatment to identify and mitigate any detrimental factors that could adversely affect treatment outcomes.

SFS physicians have helped fashion the entire field of IVF:

- We were the first in the world to introduce complete chromosome embryo karyotyping (Preimplantation Genetic Screening /testing for Aneuploidy -PGS/PGT-A) to identify those embryos that are most likely to propagate viable pregnancies (embryo "competency") and in so doing vastly improve IVF efficiency and outcomes, especially in women who are older, possess diminished ovarian reserve (DOR), "unexplained" IVF failure, or recurrent pregnancy loss (RPL).

- We were first to identify a link between the ultrasound-determined quality and thickness of the endometrial lining and IVF outcome and how a poor endometrial

lining may be improved through the administration of medication (vaginal sildenafil aka Viagra).

- SFS doctors were also among the first to recognize and treat the immunologic implantation dysfunction (IID) that may underlie recurrent pregnancy loss (RPL) or recurrent failed implantation.

- We introduced a procedure known as "prolonged coasting" to prevent the risk (in certain patients) of developing the life-threatening condition known as severe ovarian hyperstimulation syndrome (OHSS).

- We believe immunomodulation of the endometrial lining in the presence of immunologic problems contributes to our higher success rates in many difficult cases, often where other programs have failed. In some instances, we have had patients with numerous (as many as 20 previous IVF failures) who succeeded through IVF with our doctors.

Medicine is often an art as well as a science. We do not presume to imply that the approach presented in this book is the only formula to achieve the goal of a healthy child. There are many superb infertility specialists who may approach infertility with different techniques, protocols, or emphases. We merely wish to make available to patients our many years of experience in helping couples to go from infertility to family. We hold that it is not the role of the physician to tell patients what they must do. Rather, it is our responsibility to provide enough information so couples can make informed choices and given those choices, to offer safe passage on a difficult journey.

If you think you may need infertility treatment, we invite you to access our web site where you can access an enormous amount of written and audio-visual information. Also, through this site you can communicate anonymously and directly with SFS as well as with other couples in a similar predicament to you. Good luck on your "journey to family", and may it be a short, easy, and successful one.

Geoffrey Sher, M.D.

Drew Tortoriello, MD

https://www.sherfertilitysolutions.com

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SETTING UP A MEDICAL CONSULTATION

Any individual or couple who might need fertility evaluation or treatment can schedule an in-person or online consultation with an SFS physician by contacting either Dr. Sher or Dr. Tortoriello (see contact information, above). Each couple will be asked to complete and submit a questionnaire.

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PROVIDING IVF SERVICES TO PATIENTS LOCAL AND FROM AFAR

INTRODUCTION

Many couples seek access to Sher Fertility Solutions (SFS) from afar. Traditionally, about fifty percent of our patients travel from out of state or from abroad to gain access to an SFS physician. What attracts patients from out of state and from >50 countries to SFS? It is our physicians' unique and individualized problem-solving approach, which targets those factors that adversely affect IVF outcome.

HOW WE PROVIDE OPTIMAL TREATMENT, EVEN AT A DISTANCE

Setting up a consultation

Couples from afar usually access an SFS physician to set up an in-person or online (Zoom/Teams) consultation. In such cases, a questionnaire (that can be downloaded from our website or emailed/faxed to the patients directly) needs to be completed in advance of the scheduled consultation. In addition, all

available relevant medical records will need to be forwarded in preparation for the scheduled consultation.

The initial physician consultation

Your SFS Physician will review all available medical records in preparation for the   consultation. During the consultation, he/she will review your history, ask additional relevant questions, and offer a preliminary opinion which is usually contingent upon supportive clinical and laboratory testing which he might recommend. You will thereupon receive a letter, along with supportive informational materials within five to ten working days.

The ART consultation with a Nurse Manager and Financial Advisor

At this consultation, the physician letter and recommended approach to evaluation will be reviewed with you and you will be familiarized with the logistics, time constraints, structure and process involved in an IVF cycle at SFS. You will be informed as to how this will enable you to plan the date(s) of your treatment with precision, even months in advance. You will learn that it rarely requires that the patient(s) spend more than two (2) weeks away from home to complete a full cycle of IVF at SFS. A Clinical Nurse Coordinator will be assigned to chaperone the patient (s) through the IVF cycle. She will also interact with both the patient and/or her primary care OB/GYN on a regular and ongoing basis.

Making the decision of whether to proceed

After completion of the above consultations, patients will be asked to contact us with a firm decision as to whether they wish to undergo IVF treatment at SFS. Thereupon, the

commitment will be sealed with a modest deposit to secure a place on our IVF calendar at a designated and mutually agreed upon time. This deposit is fully deductible from the fees or refundable (whichever may apply) unless the patient defaults from this arrangement without medical justification. At this point, we will provide a detailed calendar and a schedule to follow to ensure that all the necessary tests are performed appropriately and in a timely manner to ensure that your cycle of IVF can be conducted as scheduled.

On-going interaction and follow-up

Our staff at SFS will maintain regular contact with patients through both scheduled and unscheduled written, emailed, or online communications. However, patients and their physicians will have ready access to our staff.

How to get to SFS and where to stay when you get there

SFS staff will gladly advise and assist in obtaining the most affordable transportation and accommodations. Couples who elect to undergo IVF will find that accommodations and airfare (especially if scheduled well in advance) will likely be very affordable. In fact, we have planned with a few hotels in Manhattan for the provision of discounted rates to our patients.

Getting ready to go…conveniently and on time.

We work with patients to get all required tests done through our office or their local doctor's office. Remember, the primary OB/GYN is often quite capable of performing or facilitating the necessary tests and procedures you may need, in your hometown environment.

Starting the treatment process

Once the designated SFS physician has reviewed all test results, a customized protocol of treatment will be developed.

The assigned Clinical Coordinator will provide patients with a detailed calendar and will review it in person or by telephone, prior to initiating the IVF cycle of treatment. The cycle is often initiated with a birth control pill (long Lupron or agonist/antagonist conversion protocols) or estrogen pills (estrogen priming protocols). Depending upon the individual's scheduled date for IVF, she will continue taking the BCP/estrogen for several days, The provided calendar we send will also direct you as to when you should start taking prescribed medications. Once a period ensues, (menstruation will ensue within a week or so of stopping the BCP or estrogen therapy) a transvaginal ovarian sonogram and hormone levels (estradiol, LH, progesterone) will be measured at the onset of menstruation. An estradiol level of less than 70 pg/ml and a "quiet" ultrasound examination signal that the patient is ready to begin gonadotropin injections. The provided calendar dictates when to start the injections and when to arrive at SFS for monitoring several days later. Please note that patients will also be taking other medications during their cycle of treatment, as directed/authorized by the designated SFS physician.

Once arriving at SFS

Regular blood estradiol levels will begin along with ultrasound monitoring of your ovarian response and development of your uterine lining. Typically, the most important procedures, such as the egg retrieval and/or embryo transfer, will take place three to eight (3-8) days after your arrival. Many patients will defer` having embryos transferred to the uterus until a few months after the egg retrieval (ER). The embryos are frozen (vitrified) and held in this state until the frozen embryo transfer is done. This is usually required to allow for PGT-A to be conducted.

Blood pregnancy tests and subsequent ultrasound confirmation of pregnancy

This can readily be performed through a primary OB/GYN approximately two (2) weeks later.

INTRAUTERINE INSEMINATION (IUI):

Intrauterine insemination (IUI), the injection of sperm into the uterus by means of a catheter directed through the cervix, has been practiced for many years. The premise of this procedure is that sperm can reach and fertilize the egg more easily if placed directly into the uterine cavity.

In the early '60s, physicians were injecting small quantities of raw, untreated semen (sperm plus the seminal plasma) directly into the uterus at the time of expected ovulation. However, when more than 0.2 ml of semen was injected into the uterus, serious and sometimes life endangering shock-like reactions often occurred. It was subsequently identified that the reason for such reactions related to the presence of prostaglandins within the seminal plasma. This led to the practice of injecting small amounts (less than 0.2 ml) of raw semen. However, the pregnancy rates were dismal and side effects, such as severe cramping and infection were rampant.

Soon after establishing the Northern Nevada Fertility Center in Reno in 1982, the nation's first private IVF program, Dr. Sher began to recognize the potential advantage of washing and centrifuging raw semen to separate sperm from the seminal fluid, and thereby remove prostaglandins that cause most of the problems. He subsequently introduced and, thereupon, became

the first to publish, on IUI in the prestigious journal, Fertility and Sterility (April 1984).

INDICATIONS FOR IUI

Artificial insemination with cryopreserved donor (non-partner) sperm:

The recognition of HIV infection as a sexually transmitted disease, coupled with the fact that the virus is present in semen months before it can be detected in the blood, mandates that all donors have their semen cryopreserved (frozen) and stored for at least six months, whereupon they will be re-tested for HIV infection. Only upon confirmation of a negative test should the cryopreserved semen specimen be thawed and used for insemination. Since cryopreservation inevitably reduces sperm motility and function, it is not adequate to simply thaw the frozen specimen and then inseminate the raw semen into the vagina. Rather, the semen specimen should be processed for IUI. Provided that the recipient is ovulating normally, there is no specific need to administer fertility drugs.

Artificial insemination with husband's sperm:

In cases of sexual dysfunction (impotence, retrograde ejaculation, etc.) or timing issues, a husband's sperm may need to be collected and processed in preparation for IUI.

Cervical mucus insufficiency:

Sometimes the cervical mucus acts as a barrier to the activation and passage of sperm as it passes through the cervical canal. Such hostility may be due to poor physical qualities of the

mucus, cervical infection, or the presence of anti-sperm antibodies. In all but the latter case, IUI can readily be performed during natural cycles, unless the woman has ovulation dysfunction. However, when infertility results from the presence of antibodies in the cervical mucus, IUI will be ineffectual and should be replaced by IVF.

Abnormal Ovulation:

In some cases where the woman requires the use of fertility drugs to induce normal ovulation, the concomitant performance of IUI can optimize pregnancy rates.

SELECTING THE OPTIMAL CONTROLLED OVARIAN STIMULATION (COS) PROTOCOL FOR IUI

ORAL FERTILITY DRUGS:

Oral fertility drugs such as clomiphene and letrozole are gentle ovarian stimulants that are in general best used in younger women who have normal egg reserve but who suffer from either ovulatory dysfunction, mild sperm issues, or unexplained infertility. In cases of unexplained infertility, the American Society for Reproductive Medicine (ASRM) recommends starting with 3-4 cycles of ovarian stimulation and intrauterine insemination (IUI) with clomiphene or letrozole. Clomiphene or letrozole alone, timed intercourse alone, or IUI alone do not significantly increase the per monthly chance of conceiving with unexplained infertility. It is the combination of these oral stimulants plus IUI that can start to increase the pregnancy rate.

Clomiphene is by far the most widely prescribed agent for the induction of human ovulation for women who do not ovulate, those with dysfunctional ovulation and women with "unexplained" infertility. When used in young women (who have

adequate ovarian reserve) with these problems the viable pregnancy rate is reported as being between 6% and 10% per cycle of treatment. Aside from conventional ovulation induction, clomiphene has been used in preparing women for intrauterine insemination and even for IVF.

The main reasons for clomiphene's popularity is its low cost, simplicity of use, and the low risk of dangerous complications such as severe ovarian hyperstimulation syndrome (OHSS).Clomiphene treatment can be initiated at a dose of 50 mg (orally) daily for 5 days but it can be increased to as much as 200mg per day, starting on cycle day 2, 3, 4, or 5. A spontaneous LH surge will usually follow within about 8-9 days of the last 50mg dosage. In some cases, 10,000U of hCG can be given as a trigger when there is at least one ovarian follicle of 18-20 mm in size.

Clomiphene works by inducing ovulation through its "antiestrogen effect" which, by blocking estrogen receptors in an area of the brain known as the hypothalamus, tricks the brain into "thinking" that estrogen levels are low. In response, the hypothalamus prompts the pituitary gland to release an exaggerated amount of follicle-stimulating hormone (FSH), which in turn stimulates the growth and development of ovarian follicles, ultimately resulting in a surge in the release of pituitary LH. About 38-42 hours later, ovulation occurs from one or more of the larger follicles. As the follicles grow, they release more and more estrogen into the bloodstream, thus closing the feedback circle that the hypothalamus initiated in response to the anti-estrogen properties of Clomiphene.

Letrozole is an oral non-steroidal aromatase inhibitor that has been used (off-label) for ovulation induction as part of the IVF process since 2001.When Letrozole blocks aromatase activity, there is a drop in E2 levels and a concomitant increase in FSH (and LH) secretion that stimulates growth of ovarian follicles

and with it, estrogen production/release. Unlike clomiphene, Letrozole is not an anti-estrogen at the cellular level and therefore does not cause the cervical mucus to dry up or the endometrium to become less responsive to estrogen (and hence thinner). However, like clomiphene, Letrozole can cause an increase in pituitary LH production/release that can cause increased ovarian androgen (testosterone) production. As previously stated, while small amounts of testosterone are required to promote estradiol production with follicle growth and egg development, an excessive amount of ovarian testosterone inhibits egg and follicle development, thereby increasing the potential for egg aneuploidy ("incompetence). It therefore may be unwise to use letrozole or clomid in IVF cycles, especially in older women and those with DOR who in general tend to have increased LH biological activity. Letrozole can be used in patients with absent or dysfunctional ovulation that is NOT related to DOR. It can also be used as an alternative to clomiphene citrate which, because of its ant-estrogenic effect, sometimes causes poor endometrial linings.

The usual starting dose of Letrozole is 2.5 mg orally daily for 5 days starting on day 2, 3, 4 or 5 of the menstrual cycle. The daily dosage can be increased to 5 or 7.5 mg if required. Interestingly, some well-done studies suggest that letrozole is a more effective means of treating infertility than clomiphene for women with polycystic ovary syndrome, with evidence of higher rates of ovulation and rates of major congenital anomalies that were not significantly different.

Side effects for both clomid and letrozole are uncommon but similar and include hot flashes, sweating, nausea, tiredness, diarrhea, and joint pain.

There are several factors that need to be considered carefully before deciding to prescribe clomiphene to any woman:

- Clomiphene citrate therapy is less effective than gonadotropin therapy and its efficacy declines with advancing age. Ideally the use of clomiphene should in our opinion be restricted to younger women (under 35 years) who have normal "ovarian reserve" (as assessed by basal blood FSH, and anti-Mullerian hormone (AMH) levels). These are the women who are most likely to respond by producing multiple follicles. It is desirable that at least 2 sizeable follicles (>15mm) develop on clomiphene treatment, to override the "anti-estrogenic" effects of this drug and so insure adequate cervical mucus production as well as the development of a receptive endometrium.

- Clomiphene should usually not be administered for more than 3 consecutive (back-to- back) cycles: If used back-to-back for more than 3 consecutive cycles, clomiphene is not only ineffective, but starts to function as a "relative" contraceptive! This is often a shocking revelation to many women. Clomiphene's anti-estrogenic effect is not confined to the hypothalamus. Any cells that have a high concentration of estrogen receptors will also be so affected. The cervical glands (that produce estrogenic mucus to facilitate sperm transport and the endometrial lining (endometrium) that thickens under the effect of estrogen are also highly vulnerable to a buildup of antiestrogen effects over successive back-to back cycles of clomiphene therapy. Therefore, with >3 consecutive back-to back clomiphene cycles cervical mucus tends to thicken and dry up and the endometrium will thin, seriously reducing the likelihood of success. These anti-estrogenic manifestations require that following 3 back-to back clomiphene cycles of stimulation there be at least one resting (non-clomiphene treated) cycle, before doing a 4th cycle.

- Clomiphene should not be used in older women or in women who have diminished ovarian reserve (DOR): With clomiphene stimulation, the release of pituitary FSH is always accompanied by the concomitant release of Luteinizing Hormone (LH). LH causes the ovary to produce male hormone (androgens) and testosterone. The production by the ovaries of a modest amount of testosterone would not present a problem. However, an excessive production of ovarian testosterone prejudices egg development and thus ultimately compromises embryo competency. Older women and women with DOR are the most vulnerable because they tend to have overgrowth (hyperplasia)of ovarian connective tissue (stroma/theca) which is the site where androgens are produced. "Trapped" ovulation (LUF-Syndrome): About 20% of clomiphene cycles are associated with "trapped" ovulation (Luteinized Unruptured Follicle or LUF Syndrome). This means that despite hormone changes suggesting that ovulation has occurred, the egg remains trapped in the follicle. Obviously, this is not conducive to the establishment of a successful pregnancy.

- Women with long gaps between menstruation are often not ideal candidates for clomiphene: Women who consistently have >45 days between their periods will not respond well to clomiphene induction of ovulation and are likely better off going directly to injectable gonadotropins.

- Multiple pregnancy: The incidence of multiple pregnancies with clomiphene induction of ovulation is about 5-10%. This is much lower than the 25% rate encountered when gonadotropins are given to women with absent or dysfunctional ovulation.

Clomiphene therapy is often used as a first line approach to inducing ovulation in women with irregular or absent ovulation such as in women with polycystic ovarian syndrome (PCOS). Its use in our opinion is best confined to women who menstruate/ovulate irregularly (but who bleed at least every 45 days), younger women, women who do not have tubal disease or endometriosis, women under 40 years of age (preferably <35Y), and women who do not have DOR. It should also be avoided when there is co-existing moderate to severe male factor infertility. If pregnancy fails to occur after 3 consecutive cycles of clomiphene therapy, then, it is probably time to consider IVF.

Women with absent or abnormal ovulation that require fertility drugs in preparation for IUI should consider gonadotropins (Menopur, Gonal F, Follistim). Granted, these agents are expensive, but they have no antiestrogenic properties and, in the hands of the experienced physician, their pregnancy rates are 30% greater than with the oral agents.

IUI SUCCESS RATES

Success rates with IUI are contingent upon the following: (1) procedure is being performed for the correct indications, (2) avoiding the performance of IUI when contraindications exist (see below), (3) whether the woman is ovulating normally and (4) the age of the woman. Birth rates per cycle of IUI performed for the correct indications are reported to be about 15% for women under 30 years of age, 12% for women 30-35 years, 7-8% for women 35-39 years, and less than <3% for women over 40 years.

RELATIVE CONTRAINDICATIONS TO INTRAUTERINE INSEMINATION

- Age: Women over the age of 40 years have less than a 3% chance of conceiving through IUI .

- Tubal disease: Women with evidence of bilaterally blocked fallopian tubes by virtue of hysterosalpingogram x-ray require IVF. Since infection and inflammation damage the intricate inner lining of the fallopian tubes, there is no surgery to the outside of the tube(s) that will remedy damage done to the inner lining. Women with one patent or open fallopian tubes can try an IUI approach, but it is often the case that the same insulting factor that has closed one tube has partially damaged the other, and this may lower success. Moreover, the incidence of ectopic pregnancy is about 1 in 6. Bypassing the "damaged plumbing" with IVF is the only rational treatment in such cases.

- Male Factor Infertility: The performance of IUI in cases of severely abnormal semen parameters (< 10 million total moving sperm per IUI) does not significantly improve success rates over regular and well-timed intercourse alone. IVF with intracytoplasmic sperm injection (IVF/ICSI) is the best if not the only method to address severe male infertility.

- Endometriosis: While the exact cause of endometriosis remains an enigma, it is now apparent that immunologic dysfunction is a feature of this disease, and that a toxic environment exists in the pelvis (surrounding the tubes and ovaries) in patients with this condition. Consequently, ovulation, whether spontaneous or induced by fertility drugs, commits the egg to pass through a toxic pelvic environment to reach the sperm waiting in the fallopian tube. This significantly reduces the egg's fertilization potential. Furthermore, once the fertilized egg reaches the uterus, immunologic factors associated with endometriosis increase the risk of the embryo being rejected before pregnancy can be

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diagnosed. Such women may experience repeated "mini-miscarriages." Despite these anti-fertility influences, many women with mild endometriosis do conceive on their own or following ovarian stimulation with fertility drugs. However, for reasons already referred to, the chances of conception are significantly reduced, and if they are ovulating normally on their own, the addition of fertility drugs will afford no additional benefit. Simply put, women in their late 20's to early 30's, who have the time to wait, can anticipate about a 40% chance of conceiving on their own within two or three years, contingent upon their ovulating normally and having fertile male partners. The occurrence of pregnancy in the latter cases occurs despite, rather than due to, such treatment. Unless their quality of life is lowered by painful symptoms, women with endometriosis could therefore consider deferring invasive treatments in favor of a "wait-and-see" approach. Conversely, for women over the age of thirty-five whose egg quality is inevitably on the decline, IVF offers the rational approach.

IUI's RISK FOR MULTIPLE BIRTHS

Normally ovulating women usually develop several follicles (cysts containing oocytes and the cells that nurture them) and produce estrogen during the menstrual cycle. All but one (and sometimes two) of the follicles fail to develop to the point of ovulation. The process is known as "selection". In all normally ovulating women, the one or two follicles selected to ovulate will inevitably be larger than the remaining follicles. Simply put, one or two follicles will always show enhanced development over the others, and as soon as these selected follicles ovulate, all the remaining follicles are rendered incapable of following suit. As a result, normally ovulating women do not have a significantly greater incidence of high order multiple

pregnancies. In contrast, women who do not ovulate at all, and those who ovulate dysfunctionally, following the administration of fertility drugs for ovarian stimulation, may have numerous follicles develop at the same rate and several eggs can be ovulated simultaneously. This translates into a greater chance of pregnancy, but also into a greater chance of multiple pregnancies. It is interesting that almost all reported cases of high order multiple pregnancies (greater than twins) following the use of fertility drugs have occurred in women who do not ovulate normally on their own.

It follows that only those women with absent or dysfunctional ovulation are at risk for high order multiple pregnancies. They, therefore, need to be counseled regarding the consequences of premature birth and the availability of selective pregnancy reduction towards the end of the third month of pregnancy. Another alternative is to avoid the issue completely by choosing   IVF, where the number of embryos transferred to the uterus can limit the number of potential babies.

Intrauterine insemination (IUI), like any other form of fertility treatment, can be of great value if used appropriately and selectively for the correct indication. The use of fertility drugs should not be regarded as a necessary adjunct in all cases of IUI, which in turn should not be considered as a required preliminary to IVF.

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THE SFS APPROACH TO IN VITRO FERTILIZATION (IVF)

WHO SHOULD UNDERGO IVF/ET?

● Male infertility: IVF was always superior to IUI as the treatment for moderate to severe male factor infertility, but it was not until the late 90's with the introduction of ICSI that IVF for male factor infertility has become virtually as successful as when applied for female related causes. ICSI is a procedure where fertilization is achieved through the direct injection of one sperm into each egg.

● Tubal disease due to pelvic inflammatory disease (PID) and/or adhesions: In the early 90's, IVF birth rates began to improve to the point that tubal surgery for the treatment of infertility due to damaged or blocked fallopian tubes rapidly became obsolete. IVF performed in an optimum setting offers more than double the birth rate following a single month of treatment than can be achieved within two to three years following surgery.

● Endometriosis: Endometriosis is associated with the presence of "toxins" in the pelvic secretions that surround the fallopian tubes (where the sperm lie waiting to fertilize the egg). These toxins infiltrate the envelopment of the egg (zona pellucida) and reduce the ability of sperm to bind to receptors on the egg surface. This reduces their fertilization potential. As a result, the monthly conception rate (even in young women with fertile male partners) is about 5%, down from 20%. Accordingly, women who have endometriosis (especially if they are >35y of age or have diminished ovarian reserve (DOR) require IVF, preferentially. Regardless of whether fertility drugs are used, or whether IUI is

performed, the egg(s) will inevitably become exposed to "toxic" pelvic secretions as they enter the fallopian tube(s). Accordingly, such options are ineffective in the treatment of endometriosis-related infertility. Only IVF, where eggs are extracted from the ovaries before they contact pelvic secretions, bypasses this issue. In addition, about one third of patients with endometriosis can have an immunologic implantation dysfunction (IID) linked to uterine natural killer cell activation (Nka) and increased antiphospholipid antibodies (APA). In such cases, IVF success might be improved through the addition of selective immunotherapy.

- Age-related infertility: Older women (>39y), especially those with diminished ovarian reserve (DOR) need to be proactive. The birth rate per egg retrieval in women between 40-43y, who use their own eggs, is about 10%-20%. For older women who are often unable to produce enough "competent" eggs, a very individualized protocol for controlled ovarian stimulation (COS) is required to optimize egg yield and "competency". In addition, PGT blastocyst testing for aneuploidy will help identify blastocysts that are chromosomally normal (euploid) and are thus the ones that are more likely to be "competent" to propagate a viable pregnancy. For women >43Y and for those who regardless of age have depleted or near-depleted ovarian reserve, IVF using donated eggs offers a birth-rate of >60% per embryo transfer and thus represents a preferred course of action.

- Unexplained infertility and/or repeated IVF failures: When there is no apparent cause for infertility and the woman is over 35 years of age has failed to respond to other types of treatment, in vitro fertilization is the treatment of choice.

- Immunologic implantation Dysfunction (IID): This is most encountered in cases of a personal or family history of autoimmune conditions, unexplained infertility, failed IVF, and recurrent pregnancy loss (RPL): About 15% of women undergoing IVF, whose infertility is unrelated to a male factor and >50% of women with RPL or unexplained recurrent IVF failures, will be found to have serologic findings suggesting IID. Most such cases will be linked to an underlying immunologic implantation dysfunction (IID) which in 85%-90% of cases will be due to an autoimmune predisposition and is usually readily amenable to selective immunotherapy. However, in 10-15% of such cases the cause is "alloimmune" in nature and more difficult to treat (see later). Notwithstanding however, IVF is still the most efficacious form of treatment if simply because it optimizes literally every other facet of conception requirements.

- Preimplantation Genetic Testing (PGT): In many cases, access to DNA derived from the embryo is required to identify those that should be selectively transferred. This mandates the incorporation of IVF.

PREPARING FOR THE IVF CYCLE

All couples undergoing IVF should be screened for infectious diseases, genetic diseases, and other things such as blood type, blood count, TSH, prolactin, and a serum metabolic profile. In addition, the woman's blood should be assessed on the 1st, 2nd, 3rd or 4th day of a menstrual cycle for FSH, Estradiol (E2) and anti-Mullerian hormone (AMH). The results of these blood tests, coupled with the woman's history of response to fertility medications in prior cycle(s) of stimulation, assist us in

deciding upon the ideal dosage and regimen of fertility drugs for the upcoming IVF cycle at SFS. In addition, in cases where IID is suspected, selective immunologic testing may be considered. In cases of recurrent miscarriage, chromosomal (karyotyping) testing is recommended for both partners to assess for translocations.

The semen or cervical secretions will on occasion be cultured for Ureaplasma/Mycoplasma. The uterine cavity will be assessed (HSG, saline sonogram, or hysteroscopy) performed within cycle days 5 to 10, or a saline ultrasound or hysteroscopy may also be done at the time of an egg retrieval procedure when the woman is under conscious sedation.

The female partner should undergo a full physical exam by her OB/GYN or primary care physician. She must also provide evidence of a recent normal pap smear. Women over 40 years of age must have a recent normal mammogram and normal blood metabolic profile. The male partner may also have his blood selectively tested for anti-sperm antibodies and in select cases, will be referred for a thorough urologic evaluation. In some cases, where hypogonadotropic sperm dysfunction is suspected, the male partner's blood is tested for FSH/LH/testosterone, and prolactin.

Given the emotional impact of infertility and infertility treatments, couples undergoing IVF might also benefit from a consultation with a counselor.

FACTORS CENTRAL TO IVF SUCCESS

- The female partner must be sufficiently healthy to undertake a pregnancy.
- Optimal ovarian dosage of stimulation: As stated above, this requires a blood measurement of AMH/FSH/LH/E2) concentration on the 1st -4th of spontaneous or induced menstruation. An E2 concentration of greater than 70 pg/ml (or >200pmol/L), an FSH of >9MIU/ml and an AMH of < 1.0 ng/ml suggests that the woman might have significantly diminished ovarian reserve (DOR) and be relatively resistant to stimulation with fertility drugs.
- A normal internal uterine environment and appropriately thick endometrium (uterine lining) healthy uterine environment: We evaluate the uterine lining by ultrasound around the time of spontaneous ovulation or during peak stimulation with fertility drugs (a measurement of < 8mm at the peak of estrogen induced endometrial development) is suggestive of an anatomical implantation dysfunction.
- Absence of immunologic dysfunction: At SFS, some doctors will request that in cases associated with "unexplained" infertility, recurrent unexplained IVF failure, or RPL, a simple immunologic evaluation be undertaken. This includes (but is not necessarily limited to) measurement of antiphospholipid antibodies (APA), anti-thyroid antibodies (ATA), reproductive immunophenotype (RIP) and Natural Killer Cell Activity (NKa). In addition, (in certain cases of RPL) we may request that the recipient and the sperm provider have their genotypes assessed for DQ alpha and HLA matching. Patients who test positive for one or more of these parameters are considered for selective immunomodulation therapy.

- Selecting the ideal protocol**:** IVF success rates are dependent upon embryo "competence". This is more a function of the number and "competency" of mature eggs retrieved rather than the sperm parameters. When it comes to stimulation protocol selection, there is no such thing as a "recipe" or a "one size fits all" approach. This is most important when it comes to women who are older and those that have diminished ovarian reserve. Additionally, different doctors may favor one protocol over another for the same purposes, and in so favoring become so adept with the nuances of a particular protocol that they will invariably possess a performance execution with said protocol that surpasses others. Ideally, an intelligently designed protocol is managed under the care of a physician who is well-versed with its intricacies. Against this background, the importance of the IVF stimulation protocol cannot be overstated. Our experience is that the use of customized COS protocols can improve IVF outcome. While no one can influence underlying genetics or turn back the clock on a woman's age, any competent IVF specialist should be able to tailor the protocol to meet the individual needs of the patient.

CONTROLLED OVARIAN STIMULATION -COS

Selecting the ideal protocol for an individual woman is based upon numerous important considerations that include (but are not necessarily limited) to the following:

- Whether the woman has undergone prior cycles of controlled ovarian stimulation (COS): This will provide a reference value about previous response, thereby improving the ability to select an ideal protocol.

- Whether the woman is ovulating normally: Women who have irregular menstrual cycles and/or dysfunctional ovulation (commonly seen in conditions such as PCOS or hypothalamic ovulatory dysfunction) often hyper-respond to COS by generating large numbers of follicles and very high blood E2 levels, placing them at risk of developing severe ovarian hyperstimulation syndrome (OHSS) and poor-quality eggs.
- Age: The older the woman, the poorer the quality of her eggs, regardless of egg number.
- Low ovarian reserve is often accompanied by enhanced resistance to stimulation with COS. Such women often propagate fewer and poorer quality eggs/embryos.
- The type of medications prescribed previously. Drugs such as Clomiphene and Letrozole often result in increased ovarian production of male hormones such as testosterone, which, if present in excess, can compromise egg and embryo "competency." Also, gonadotropins containing much LH/hCG (e.g., Menopur) may exert the same effect.
- Prior response to protocols used for ovarian stimulation protocols: The number and sizes of follicles propagated as well as the peak blood estradiol level and egg maturity percentage is important.
- The types and dosages of medications prescribed for the "trigger shot.

Gonadotropins (LH and FSH), whether produced by the pituitary gland or administered by way of fertility drugs, have different "targeted" sites of action in the ovary. FSH targets granulosa cells which line the inner wall of the follicle and surround the oocyte (cumulus cells that bind the egg to the inner surface of the follicle). Granulosa cells are responsible for estrogen production. LH, on the other hand, targets the ovarian connective tissue (stroma/theca) that surrounds ovarian follicles

resulting in the production of "male" hormones such as testosterone, androstenedione, and DHEA. These androgens are then transported to the granulosa. There FSH converts these androgens to estradiol, causing granulosa cells to proliferate and produce more estradiol, allowing follicles to grow and eggs to mature. It follows that ovarian androgens (mainly testosterone) are indispensable to follicle/ egg growth and development. However, the emphasis is on a "normal" amount of testosterone. Over-exposure of the follicle to testosterone might compromise egg development and lead to an increased likelihood of chromosomal irregularities (aneuploidy) following LH/hCG-induced egg maturational division (meiosis) and compromise embryo "competency/quality. (Excessive production or administration of ovarian androgens may also compromise response of the endometrium to estrogens, thereby compromising endometrial growth and development. This comes into play when one aims to do a fresh embryo transfer.)

A significant percentage of older women and those who have DOR have increased LH activity. Such women either over-produce LH and/or the LH produced is more biologically active. Chronically increased LH activity leads to overgrowth of ovarian connective tissue (stroma/theca). This condition, which is often referred to as stromal hyperplasia or hyperthecosis can result in excessive ovarian androgen production and poorer egg-embryo quality. Similarly, women with polycystic ovarian syndrome (PCOS), who characteristically have stromal hyperplasia/hyperthecosis due to chronically increased LH activity, also often manifest increased ovarian androgen production.

The over-administration of LH-containing menotropins such as Menopur, which is comprised of roughly equal amounts of FSH and hCG (which acts similar to LH), to older women, to women with DOR, and to those who have PCOS, may also lead to

reduced egg/embryo "competency". Similarly, as stated above, drugs such as clomiphene or Letrozole that cause the pituitary gland to release excessive amounts of LH, are also potentially harmful to egg development and in my opinion, are best omitted from IVF protocols. This becomes especially relevant when it comes to older women and those with DOR, who we believe, should preferably be stimulated using FSH-dominant products such as Follistim and Gonal-F.

Gonadotropin releasing hormone agonists (GnRHa): GnRHa such as Lupron are often used to launch ovarian stimulation cycles. They act by causing an initial outpouring followed by a depletion of pituitary gonadotropins. This results in LH levels first rising but after a few days falling to low concentrations, thereby establishing a relatively low LH-environment. When GnRHa is administered for about 7 days prior to initiating gonadotropin stimulation ("long pituitary down-regulation"), the relative LH depletion that will exist when COS is initiated 6-8 days later, will usually be protective of subsequent egg development. In contrast, when the GnRHa administration commences along with the initiation of gonadotropin therapy (the "short" or "Flare" protocol), there will be a rapid increase in pituitary LH output with the potential to increase ovarian testosterone to egg-compromising levels, from the very outset of COS. This could be harmful, especially when undertaken in older women and those who have DOR who, to start with, often already have increased LH activity.

GnRH-antagonists such as Ganirelix and Cetrotide, on the other hand, act very rapidly (within hours) to block pituitary LH release. The purpose in using GnRH antagonists is to prevent the release of LH during COS. GnRH antagonists are traditionally given, starting after 5th -7th day of gonadotropin stimulation. However, when this is done in older women and those (regardless of age) who have DOR, LH-suppression might be

reached too late to prevent the deleterious effect of excessive ovarian androgen production on egg development in the early stage of ovarian stimulation. Therefore, it is often better to administer GnRH-antagonists starting closer to the initiation of gonadotropin administration.

PROTOCOL OPTIONS FOR COS

1.   "**Long" GnRHa (Lupron) Pituitary Down-regulation Protocol**: The most commonly prescribed protocol for GnRHa/gonadotropin administration is the so-called "long down-regulation (DR) protocol". Here, GnRHa is given, starting a week or so prior to menstruation. This results in an initial rise in FSH and LH, which is rapidly followed by a precipitous fall to near zero. It is followed by a withdrawal bleed (menstruation), whereupon gonadotropin treatment should commence, while daily Lupron injections continue, to ensure a "low LH" environment. This protocol is ideal for women with normal ovarian reserve.

2.   **Short ("Flare") GnRHa Protocol:** This approach involves commencing gonadotropin therapy commensurate with initiation of gonadotropin administration. The supposed objective is to deliberately allow the GnRHa to elicit an initial surge ("flare") in pituitary FSH release to augment FSH administration by increased FSH production. Unfortunately, this "springboard effect" often constitutes a "double-edged sword". While it indeed increases the release of FSH, it at the same time causes a surge in LH. As stated, we believe that the latter can evoke excessive ovarian stromal/thecal androgen production which could potentially compromise egg quality, especially when it comes to older women and women with DOR.

3.   **Conventional GnRH antagonist protocol**: Here, starting about 5-6 days after the initiation of gonadotropin therapy, GnRH-antagonist therapy commences and is continued until the hCG/Ovidrel trigger. In many such cases we would supplement with human growth hormone (HGH) to enhance egg mitochondrial activity to enhance egg development.

4.   **Agonist/Antagonist Conversion Protocol (A/ACP):** This is a modification to the "conventional" GnRH-antagonist protocol and is ideal for women with moderate or severe DOR. With the A/ACP upon the onset of a GnRHa-induced bleed, the GnRHa is supplanted by a GnRH antagonist (Ganirelix/Cetrotide) and this is continued until the hCG trigger. In many such cases we supplement with HGH. This approach is often augmented by using preimplantation genetic testing/screening (PGT-A/PGS) of all embryos that reach the expanded blastocyst stage of development by day 5-6 post-fertilization. We also selectively recommend blastocyst banking to many such patients who have severe DOR or are older (mid-40's).

5.   **Modified Estrogen Priming Protocol**: Estrogen priming has succeeded in enhancing ovarian response to gonadotropins in many women who are very poor responders to gonadotropin therapy. It can theoretically be incorporated into any protocol and is best used antecedent to stimulation medications to optimize follicular receptivity. We often start priming with estradiol pills, 2mg twice daily, on cycle day 21. Often HGH is started simultaneously. When menses starts about 1 week later, the estrogen stops, the HGH continues, and gonadotropins are started. A GnRH antagonist is started once follicles reach a size of 14 mm or larger. The gonadotropins continue until the day of the hCG "trigger". This approach is often augmented by PGT-A of all embryos that reach the expanded blastocyst stage of development by day 5-6 post-fertilization.

6.   **Low-stimulation (aka mini-IVF) or natural cycle IVF**: Low stimulation IVF is a procedure that usually involves ovarian stimulation with oral fertility drugs such as clomiphene or letrozole to promote the development of follicles for egg extraction. Its supporters tout that mini-IVF is a gentler, more natural approach, which will cut costs by reducing the need for medications and intensive monitoring, without compromising success. Natural IVF is an extension of the same concept. It relies on the development of follicles during natural ovulatory cycles, sufficient to permit extraction of one egg while completely avoiding the need for any stimulatory fertility drugs. Regarding the argument that low stimulation or natural IVF will yield comparable success rates to conventional IVF, please consider the following counterarguments:

- Use of fewer drugs may not translate into lower cost because success rates with low stimulation IVF per treatment cycle are much lower than with conventional ovarian stimulation. More important is the fact that the cost of IVF should be expressed in terms of "the cost of having a baby" rather than "cost per cycle of treatment." When this is considered, the cost associated with low-stimulation IVF will be significantly higher than conventional IVF. There is the additional emotional cost associated with a much higher IVF failure rate with low-stimulation IVF.

- "Absent or milder stimulation using oral agents such as clomiphene, letrozole reduces stress on the ovaries and overall risk associated with IVF". This argument, while perhaps having some merit when applied to younger women who usually have normal ovarian reserve, is less valid when it comes to older, DOR patients. Furthermore, there is evidence to show that both clomiphene and letrozole increase the release of LH by the pituitary gland which so increases ovarian testosterone as to potentially

further compromise egg development and maturation. This is especially detrimental when it comes to older women and/or those who have DOR. Unfortunately, this is often the very group who are targeted for low-stimulation IVF.

- That women with DOR will respond better to "milder/gentler stimulation" and have better egg quality is a flawed assertion. It is like saying that applying less force to lifting a heavy object will increase the likelihood of moving it. That is simply not how FSH stimulates follicle development.
- "Low stimulation IVF is less technology driven, less stressful and easier to execute.": There is some merit to this assertion, although all IVF cycles require careful monitoring and the same involvement of the embryology laboratory.

7.   **EZ-IVF**: This is, a low-cost, minimal risk, and potentially successful alternative to low stimulation or natural IVF. EZ-IVF is well suited to women under 40Y of age who have adequate ovarian reserve. The process involves the use of low dosage gonadotropin stimulation (administered every other day), thereby virtually eliminating the risk of complications. It does all this at the same low cost as low-stimulation IVF while offering a higher potential for success and a greater likelihood that there will be left-over embryos for cryopreservation with a view to later use.

USE OF THE BIRTH CONTROL PILL IN IVF:

In natural (unstimulated) as well as in cycles stimulated with fertility drugs, the ability of follicles to properly respond to FSH stimulation is dependent on their having developed FSH-

responsive receptors. Pre-antral follicles (PAF) do not have such primed FSH receptors and thus cannot respond properly to FSH stimulation with gonadotropins. FSH receptor responsivity requires that the pre-antral follicles be exposed to FSH, for several days (5-7) during which time they attain "FSH-responsivity" and become fluid-filled small antral follicles (AF). In regular menstrual cycles, the rising FSH output from the pituitary gland ensures that PAFs convert to AF's. The BCP (as well as prolonged administration of estrogen/progesterone) suppresses FSH. This suppression needs to be countered by artificially causing blood FSH levels to rise to cause PAF to AF conversion prior to COS commencing. Absent this, pre-antral to antral follicle conversion will not take place in an orderly fashion, the duration of ovarian stimulation will be prolonged, and both follicle and egg development may be compromised. GnRH agonists cause an immediate surge in release of FSH by the pituitary gland thus causing conversion from PAF to SAF. (In contrast, the BCPs will blunt any concomitant flare in LH, a potentially beneficial effect.) The agonist causes FSH to be released by the pituitary gland and if overlapped with the BCP for several days, this will (within 2-5 days) facilitate PAF to AF conversion, in time to start COS with the onset of menstruation. Initiating ovarian stimulation in women taking a BCP without doing this is suboptimal.

ADJUNCT THERAPIES

Numerous treatments have emerged that are intended to enhance egg quality and/or embryo implantation.

- Use of Human Growth Hormone or HGH (Saizen/Omnitrope): Older women and those (regardless of age) with DOR have greater difficulty in conceiving

naturally or through assisted reproductive technology (ART). This is due to an inevitable increase in egg aneuploidy (numerical chromosome irregularity). However, although less significant than the rising increase in egg aneuploidy, advancing age and DOR are both also thought to be associated with non-chromosomal egg deterioration involving a decline in mitochondrial activity as well as a progressive reduction in the ability of the granulosa cells to respond to FSH stimulation. Getting older women and those with DOR to respond optimally to ovarian stimulation often represents a serious challenge. Many will fail to respond to standard ovarian stimulation regimens, requiring any individualized and strategic approach to ovarian stimulation…. one that regulates and limits exposure of ovarian follicles and eggs to LH-induced local androgens. This, in our view, is addressed by using a modified long pituitary down regulation protocol with a GnRHa coming off a birth control pill. Thereupon, as soon as the period starts, the agonist is supplanted by a GnRH antagonist and stimulation with recombinant FSH (FSHr) along with a small amount of menotropin (e.g., Menopur) until optimal follicle development prompts initiation of the hCG trigger. Recently, researchers have shown that the administration of human growth hormone (HGH), as an adjunct to ovarian stimulation, might enhance follicle response in older women and those with DOR. Two basic mechanisms have been proposed: a) enhanced response to FSH by up regulating the FSH receptors on follicular granulosa cells and b) through a direct effect of HGH on the egg itself whereby mitochondrial activity is enhanced. Human eggs do have receptors to HGH, but eggs retrieved from older women show decreased expression of such receptors (as well as a reduced

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number of functional mitochondria. It was recently observed that some women treated with HGH, show an increase in egg functional mitochondria along with improved egg quality.

- Endometrial Receptivity Array (ERA): The blastocyst and the endometrium are in a constant state of crosstalk. For successful implantation to take place, the blastocyst must be at the appropriate stage of development and needs to signal a well synchronized endometrium to "accept it." This dialogue between embryo and endometrium involves growth factors, cytokines, immunologic accommodations, cell adhesion molecules, and transcription factors. These are all mostly genetically driven but are also heavily influenced by numerous physiologic, pathophysiologic, hormonal, and molecular mechanisms capable of profoundly affecting the receptivity of the secretory endometrium to the embryo. Embryo implantation takes place 6-9 days after ovulation. This period is commonly referred to as the "window of implantation (WOI)." In the past it was believed that if the embryo reached the uterus in this 4-day time frame, its chance of implanting would not be affected. In 2013, after evaluating 238 genes in the secretory endometrium and applying bioinformatics, Ruiz-Alonzo, et al. introduced the Endometrial Receptivity Array (ERA). Using this test, they categorized mid-secretory endometria into four categories: "a) proliferative, b) pre-receptive, c) receptive or d) post-receptive". They claimed that women with pre-receptive or post-receptive endometria were more likely to experience failed implantation post-embryo transfer (ET). It was in large part this research which suggested that the concept of a relatively "wide" (4 day) WOI, was flawed, that an optimal WOI is much narrower and could

be a critical factor in determining the success or failure of implantation post-ET. Ruiz-Alonzo also reported that about 25% of women with recurrent IVF failure (RIF), have prior post-receptive endometria. They presented data suggesting that viable IVF pregnancy rates could be enhanced by deferring FET by about 24 hours in women who had pre-receptive endometria and bringing ET forward by the same amount of time, in women with post-receptive endometria. There is no doubt that ERA testing has opened the door to an intriguing arena for research. Data subsequently published by separate authors has found conflicting results. Given this, as well as its large out of pocket cost and need to perform time-consuming "mock" FET cycles to allow a well-timed referential endometrial biopsy, we believe that ERA testing should presently be considered as one additional diagnostic tool offered mainly to women with "unexplained" recurrent implantation failure, especially with PGT normal embryos.

- Platelet Rich Plasma (PRP); Some have advocated intraovarian and/or intrauterine injection of a patient's-own platelet rich plasma. The former has been proposed as a method by which to "rejuvenate" ovarian egg production and quality in older women and those with DOR, while the latter has been recommended to try and enhance embryo implantation in patients with unexplained repeated implantation failure and/or RPL. The data is limited at this point, but these procedures hold promise. Therefore, if patients elect to try ovarian or uterine PRP, as a last resort, it can be attempted following detailed counseling and full disclosure as to the potential value of such treatment.

- Intrauterine hCG infusion: Some have proposed infusing an hCG solution into the uterine cavity prior to ET, in the hope of enhancing implantation. Here again, proof of efficacy is lacking.

- Uterine "scratch": This involves intrauterine insertion of an instrument (such as biopsy catheter) to create minor trauma to the uterine lining prior to transfer. It has been suggested that this will create a local endometrial reaction that could enhance implantation. Why such trauma, which precedes a menstrual flow, would facilitate implantation in the following month seems mysterious at best, and the most recent large, pooled data collections seem to argue against its efficacy.

- Embryo "glue": It was first suggested >30 years ago that the introduction of a fibrinoid substance into the uterine cavity prior to ET might help the embryo attach: Proof of efficacy is lacking.

SEVERE OVARIAN HYPERSTIMULATION SYNDROME (OHSS)

OHSS can be a life-endangering complication of ovarian stimulation with gonadotropins. The risk of OHSS begins with the hCG "trigger". The complication occurs in high responders to gonadotropin stimulation. Women with PCOS, irregular cycles, and very high AMH levels are at the greatest risk of developing OHSS. It has been demonstrated that if more than 25 follicles develop following stimulation with fertility drugs and the woman's plasma estradiol at its highest level exceeds 6,000 pg/ml, a large amount of fluid is exuded into the abdominal cavity (ascites). This can cause the abdomen to distend severely and may even compromise breathing. In very severe cases, the

woman may become so dehydrated that she will develop organ failure and deep venous blood clots. OHSS all too often results from inappropriate use of gonadotropins, but due to its unpredictable nature can sometimes occur even in cycles with superb management. The risk and severity of OHSS is linked to the exposure to hCG. Therefore, it only emerges after the hCG trigger. If pregnancy does not ensue, it will rapidly subside on its own, within 2 weeks of the hCG "trigger". On the other hand, if the woman conceives in the same cycle, ever increasing production of hCG exposure will exacerbate the risk and severity of complications. This will likely continue until the 8-9th week of pregnancy. It follows that severe OHSS is avoidable in patients at risk: Canceling the cycle prior to the initiation of the hCG "trigger" will, regardless of the level of ovarian stimulation, eliminate the risk of OHSS, completely. Also, the severity of OHSS can be reduced by triggering with a GnRH agonist (e.g., Lupron), rather than hCG.   Another strategy that reduces the severity of OHSS is by freezing all embryos and deferring their transfer for a month or two by which time the risk associated with OHSS would no longer be present. Finally, the risk and severity of OHSS can be mitigated by "prolonged coasting".

In "coasted" patients, as soon as 50% of all follicles reach 14mm and the estradiol exceeds 2,500pg/ml, gonadotropin stimulation is abruptly stopped while daily agonist injections continue ("coasting"). Daily blood estradiol level is tracked, (without necessarily continuing serial ultrasound follicle measurements). The estradiol will almost invariably continue to rise for a few days whereupon it will begin to drop.   As soon as it drops below 2,500 pg/ml, a "trigger" shot of hCG is administered and an egg retrieval is performed 35 hours later. At this point, all mature (MII) eggs are either cryobanked (vitrified) or (as is far more commonly the case), are fertilized by ICSI and are then cultured for 5-6 days to the blastocyst stage whereupon they are vitrified, either with or without a preceding biopsy for PGT.   The

outcome of coasting depends on the precise timing of the initiation and conclusion of "prolonged coasting". If you start too early, follicle growth will arrest, and the cycle will be lost. Conversely, if you start too late, you will encounter too many post-mature/cystic follicles (>22mm) that usually harbor abnormally developed eggs. Use of "coasting" avoids severe OHSS and minimizes the risk of poor egg/embryo quality in a group of women who otherwise would be at severe risk of life-endangering complications and prone to producing a high percentage of "incompetent" eggs/embryos.

TRIGGERING" EGG MATURATION

Triggering with hCG With ovulation induction using fertility drugs, the administration of 10,000 hCG (Pregnyl; Profasi, Novarel) or 250mcg hCGr (Ovidrel) "trigger") sends the eggs into maturational division (meiosis). This process is designed to halve the chromosome number, resulting in mature eggs (M2) that will have 23 chromosomes rather than   the 46 chromosomes they had prior to the "trigger".   Chromosomally normal (euploid) mature (MII) eggs, upon being fertilized, will (hopefully) propagate euploid embryos that have 46 chromosomes and will be "competent" to propagate viable pregnancies. Unless the risk of OHSS seems untenable, it is preferred to "trigger" with 10,000U of hCGu. Depending on the woman's BMI, a lesser dosage may reduce the efficiency of meiosis and increase the risk of the eggs being aneuploid.

Triggering with GnRHa: The reason for using a GnRH agonist (Lupron) "trigger" is that by inducing meiosis through compelling a surge in the release of LH by the pituitary gland, it reduces the risk of OHSS. LH has a short half-life, unlike hCG, and it can foster meiotic change without thereafter stimulating the

prolonged vascular leakiness that is the hallmark of OHSS. This usually works well, but there can be a downside if the agonist fails to engender a sufficiently high LH spike. For this reason, women with hypothalamic amenorrhea, who at baseline already manifest poor hypothalamic gonadotropin release, will not respond well to the agonist and as such should use hCG triggers if possible. Another caveat is that Lupron will not work as a trigger in any patients using GnRHa down-regulation as part of their IVF protocol.

SPERM PROCESSING

Sperm is usually obtained from a masturbation specimen derived from the male partner. On some occasions however, physical, medical and/or religious constraints demand that sperm be obtained through condom collection following intercourse, or extracting sperm directly from the testicle, using aspiration or biopsy. (Testicular Sperm Extraction (TESE) or Testicular sperm aspiration (TESA). TESE/TESA are procedures of choice in cases where there is blockage of the sperm ducts (as occurs following vasectomy or following severe injury or infection), where the man is born without sperm ducts (congenital absence of the vas deferens) and in testicular insufficiency cases where no sperm reach the ejaculate. Sometimes, in cases of retrograde ejaculation, sperm can be collected from the man's bladder. Infrequently, in men with spinal cord injuries, ejaculation is facilitated by electrical stimulation (electroejaculation). Donor sperm, obtained from a sperm bank, can be used when indicated. At SFS, we routinely use a selection process called ZYMOT, to identify the best sperm to inject into the egg with ICSI. However, there are some cases where sperm quality is so poor that the ZYMOT procedure is not

sufficient. In such cases, we often consider a procedure called PICSI to select sperm for ICSI.

Sperm must undergo a biochemical and structural change known as capacitation before eggs can be fertilized. Capacitation (which under normal circumstances takes place in the woman's reproductive tract) must be accomplished in the embryology laboratory prior to insemination of the eggs. Motile sperm are processed and activated in specialized culture media and sophisticated techniques are used to enhance poorly mobile sperm.

THE EGG RETRIEVAL

Egg retrieval involves a non-surgical procedure where under direct ultrasound guidance, a needle is passed along the side of a vaginal ultrasound probe through the top of the vagina into follicles (small fluid filled spaces that each contain an egg), within the ovary (ies). The follicular fluid is aspirated and collected in a test tube, which is promptly delivered to the embryologist for analysis and processing. The procedure itself is done under conscious sedation and is hence painless, however patients commonly experience some residual postoperative abdominal discomfort and/or cramping that rarely persists for more than a few hours. Postoperatively, all patients are given detailed instructions and are discharged within an hour or two with a prescription for analgesics (pain killers) and other medications as indicated.

EGG FERTILIZATION

In vitro fertilization literally means "fertilization in glass". Fluid aspirated from ovarian follicles is examined in the embryology laboratory. The eggs are identified and extracted and are placed in a specialized culture medium. Several hours later, approximately 50,000 -100,000 processed sperm are placed around each of the eggs. The eggs and sperm are allowed to incubate together in a carefully controlled environment. Approximately 16-24 hours later, the eggs are inspected microscopically for fertilization as evidenced by the presence of two nuclear bodies. These binuclear unicellular bodies are referred to as "pro-nuclear embryos".

ICSI has literally revolutionized the treatment of male infertility. The procedure involves the direct injection of a single sperm into each egg under direct microscopic vision. The successful performance of ICSI requires a high level of technical expertise. In centers of excellence, when ICSI is employed, the IVF birth rate is unaffected by the presence and severity of male infertility. In fact, even when the absence of sperm in the ejaculate requires that ICSI be performed on sperm obtained through TESE/TESA, the birth rate is no different than when IVF is performed for indications other than male infertility.

The introduction of ICSI has made it possible to fertilize eggs with sperm derived from men with the severest degrees of male infertility and in the process to achieve pregnancy rates as high, if not higher than that which can be achieved through conventional IVF performed in cases of non-male factor related infertility. The indications for ICSI have broadened dramatically, with the process now being used for a variety of indications other than male factor infertility. For example, at SFS we now use ICSI

to assist in the fertilization of eggs that are believed to have a hardened or thickened outer shell (zona pellucida). This is frequently found in association with polycystic ovarian syndrome (PCOS), thawed eggs, and in eggs derived from older women (over 40 years). ICSI is also frequently recommended in cases of "unexplained infertility" and where there is a history of poor fertilization during one or more prior IVF attempts.

EMBRYO GRADING (MICROSCOPIC VERSUS PGS/PGT-A)

In the past, embryos were graded microscopically (morphologically). The higher the grade, the greater the likelihood that the embryo was "competent" (able to propagate a viable conceptus). The higher the grade of a day 3 (cleaved) embryo, the greater was the likelihood of it developing into a blastocyst by day 5-6 post fertilization and of that blastocyst being "competent". But such morphologic/microscopic grading systems, while helpful, were   flawed. For example, regardless of its grade, a cleaved, day 3 embryo that failed to progress to blastocyst by day 6 post-fertilization almost invariably was "incompetent". Moreover, cleaved (day 3) embryos that developed into blastocysts while representing the ones that were the more likely to be "competent", often times also turned out to "incompetent".

It was not until 2005 that SFS physicians became the first to report reliably on the fact that it is the numerical chromosomal integrity (karyotype/ploidy) of an embryo that is the most important (but not the only) factor that determines its "competency". Using a method called preimplantation genetic screening known as PGS /PGT-A (preimplantation genetic screening/testing for aneuploidy) , where advanced embryos are

biopsied to remove one or more of their cells (blastomeres) for chromosomal karyotyping (using Comparative Genomic Hybridization-CGH or Next generation gene sequencing- NGS) to identify euploid embryos for selective transfer , our SFS team was able to show and report on the observation that by selectively identifying and freezing storing (vitribank) these for dispensation to the uterus in a subsequent frozen embryo transfer (FET) cycle, we could dramatically improve IVF outcome. This was especially the case in older women and women with DOR who tend to propagate a greater percentage of "incompetent" eggs/embryo and in women with RPL and those who experience recurrent IVF failures.

Furthermore, by using PGS/PGT-A, we were able to limit the number of embryos/blastocysts transferred to one, or at most two at a time, thereby dramatically improving the "baby rate" per embryo transferred, reducing the incidence of high order multiple pregnancies (triplets or greater), aneuploidy-related miscarriages and birth defects such as Down syndrome. Finally we corroborated the pre-PGS/PGT-A assertion that those embryos that failed to reach the advanced (pre-implantation) phase of development (blastocyst) by the 6th day post-fertilization were almost invariably aneuploid and "incompetent" and that transferring them earlier would NOT have improved their ability to propagate viable pregnancies.

ASSISTED HATCHING (AH)

In selected cases where it is felt that the zona pellucida (the envelopment of the embryo/blastocyst) is unusually tough or thickened, a process known as Assisted Hatching (AH) may be employed. The process involves deliberately weakening of the wall of the embryo mechanically (using laser) or chemically (using

Acid Tyrode's Solution), to promote hatching (rupturing) and thereby facilitate implantation. It remains controversial as to whether AH improves pregnancy rates. AH also in some studies has been associated with a slight increase in the rate of monozygotic (identical twins).

EMBRYO TRANSFER (ET)

The Process: When the woman is in the proper position, and her bladder is adequately filled, the physician first inserts a speculum into the vagina to expose and rigorously clean the outer cervix with a sterile, isotonic saline solution to remove any mucus or other secretions, followed by a gentle lavage of the outer cervical canal with sterile normal saline or culture media.   An abdominal ultrasound transducer is placed suprapubically on the lower abdomen to allow clear visualization of the uterus. Thereupon, using sterile technique, I introduce a sonically activated embryo transfer cannula (with an empty internal catheter) through the entire length of the cervical canal until the sonically activated tip reaches the junction of the cervical canal and uterine cavity. The laboratory is then notified to load a catheter with the embryo (s) to be transferred and deliver them to me in the procedure room. At this point the empty catheter is removed from the positioned cannula and the embryo-loaded catheter is passed via the perfectly positioned cannula, to within approximately one (1) centimeter of the top of the uterine cavity, whereupon the embryologist is directed to slowly inject the embryo(s) into the uterus. The passage of the embryos into the uterine cavity can be tracked by ultrasound visualization. A period of about 30 seconds is allowed to elapse, whereupon the catheter and cannula are simultaneously withdrawn slowly. Thereupon, the catheter is immediately returned to the laboratory where it is examined under the microscope to make sure that all the embryos have

been released. Any residual embryos would be promptly re-transferred using the same technique.

FROZEN EMBRYO TRANSFER (FET)

Available evidence suggests that FET (of previously cryopreserved embryos) is at least as successful as is the transfer of "fresh" embryos and might even have the edge. The probable explanation is certainly unlikely to have anything to do with the freezing process itself. The reason likely has to do with being able to better prepare the uterus for embryo implantation by using targeted hormone replacement therapy that may render the lining more receptive than when a "fresh" transfer is performed immediately following ovarian stimulation with fertility drugs.

POST-ET MANAGEMENT

Post- transfer, we have the patient recline in place for no more than 10 minutes given that recent data suggests that prolonged immobilization post-transfer is, if anything, counter-productive to conception. Immediately prior to being discharged following the embryo transfer procedure, an exit interview is conducted whereby the patient/couple is/are given directions. Hormonal supplementation usually involves the administration of intramuscular injections of progesterone and/or vaginal suppositories (comprising estradiol valerate and micronized progesterone) until a blood pregnancy test is performed approximately eight days later (the chemical diagnosis of pregnancy). In selected cases, such progesterone treatment can be replaced with Crinone 8% vaginal applications, once or twice daily. If the pregnancy test is negative or the plasma hCG levels

fail to rise appropriately in the ensuing days, all hormonal support is discontinued. A positive pregnancy test followed by an appropriate rise in the plasma hCG concentration is an indication to continue hormonal support until the 11th week of pregnancy. An ultrasound examination is performed approximately two to three weeks after the chemical diagnosis of pregnancy.

EMBRYO CRYOPRESERVATION

There have been dramatic advances in the technology of freezing and storing human embryos for future use. Today we use an ultrarapid freezing process known as vitrification which freezes the embryo so rapidly as to avoid damage to intracellular structures. The result is that vitrified/banked (vitribanked) embryos survive the freeze-thaw process in virtually the same condition as when they were prior to being vitrified.

While most embryos are vitrified and stored as blastocysts, we can also cryopreserve embryos 3 days after fertilization, and sometimes, one day after fertilization (the pronucleate stage), dependent upon patient-specific indications and choices. Regardless of when the freezing process is done, at SFS most embryos are transferred in the blastocyst stage. This means that pronucleate or day-3 embryos are thawed and cultured for an additional few day. Those that attain the blastocyst stage of development are eligible for transfer to the uterus. Frozen blastocysts are thawed and transferred a few hours later. Recent technological advances have enhanced embryo/blastocyst freeze-thaw survival rates resulting in a significant improvement in pregnancy rates following Frozen Embryo Transfers (FETs). At SFS we currently report better pregnancy rates following FETs than with the transfer of fresh

embryos. Obviously, this cannot be attributed to the freezing process enhancing embryo "competency". Rather, it is likely due to one or both of the following factors:

1.   Improved endometrial receptivity through hormonally medicated preparation for FET

2.   A large percentage of FET's are conducted using PGS/PGT-A selected euploid blastocysts.

Cryopreservation technology will continue to advance and will likely contribute to an ever-greater extent to IVF treatments. However, the future lies in the successful cryopreservation of human eggs.

EGG CRYOPRESERVATION/BANKING

Since the birth of the first "frozen egg baby" in the mid 1980's, fewer than 10,000 births resulting from the fertilization of thawed eggs have been reported, worldwide. Compare this to >5 million IVF babies born worldwide in the same time period, and > 3 million babies resulting from the transfer of frozen embryos. Harvesting eggs for freezing typically involves giving a woman fertility drugs to stimulate her ovaries to produce multiple eggs, and then harvesting those eggs from her ovaries using ultrasound guided needle aspiration. In average cases (where the mean age of the woman is <36y), it takes about one cycle of fertility drug administration to harvest 10 to 15 eggs. Presently, in cases where embryos derived from the eggs of women under 35 years are frozen, survive the thaw and are transferred to the uterus, the birth rate per embryo transfer is about 30-35%. In those cases where the eggs were derived from women between 35y and 40y of age, the birth rate is about 25-

30% per embryo transfer (ET) procedure. For women of >40y the comparable birth rate per ET is about 5-10%.

While on the face of it, this sounds like a reasonable outcome (especially when it comes to younger women), it should be borne in mind that many eggs do not survive the freeze/thaw and a significant number of those that do survive, fail to fertilize. Moreover, of those that do fertilize, a significant percentage fail to progress to the expanded blastocyst stage of development (regarded as being the ideal stage for ET). That is why depending on their age, women who elect to bank their eggs for fertility preservation (FP) are encouraged to undergo as many egg retrieval procedures as needed to bank 15 -20 eggs to have some degree of confidence of ultimately being rewarded with a live birth. Since the percentage of eggs that are chromosomally normal (euploid) declines with advancing age, the older the woman becomes, the greater will be the number of eggs (and egg retrieval procedures) needed.

The ability to accurately identify eggs that are numerically chromosomally normal ("euploid") and are thus the ones most likely, upon being fertilized and transferred to the uterus, to propagate a live birth is of relevance. Women in their twenties manifest a 60-70% euploid egg rate that by their mid-forties has dropped to <10%. It follows that potential for a successful outcome using frozen eggs largely hinges on their chromosomal integrity ("competency").

In October 2008, we became the first to report ("Reproductive Biomedicine Online") on a process that allows for a several fold improvements in the baby-rate per (frozen) egg. It relies upon the selective storage and dispensation of chromosomally normal eggs. The process we reported involved removing the 1st polar body from mature (MII) eggs for the performance of preimplantation genetic screening (PGS/PGT-A followed by the

selective storage of only those found to have all 23 chromosomes (i.e. euploid) intact. This resulted in a baby rate of 27% per frozen egg (a 3-fold improvement), an 85% freeze/thaw survival rate, an 80% successful fertilization rate and > 60% birth rate following the transfer of one or two blastocysts. While likely highly effective, this complex process is presently cost-prohibitive.

FEMALE FERTILITY PRESERVATION (FP)

This refers to the process whereby a woman's eggs are frozen (cryopreserved) and banked for future use. It has been estimated that the potential demand for FP using frozen eggs exceeds that for conventional IVF by a factor of 4-6 times. The need addresses:

- o Women who face a looming prospect of losing their ovarian function – either because of impending menopause, pending surgical removal of their ovaries, and/or exposure to radiation therapy and/or chemotherapy.

- o Women with basically normal egg reserve but who anticipate delaying or deferring childbearing due to lifestyle choices or circumstances

- o Women/couples undergoing in vitro fertilization who are opposed to embryo freezing on moral, ethical, or religious grounds.

While the demand for FP is growing rapidly, a word of caution is appropriate here: Women need to be encouraged to bank their eggs at a younger age (<35y) where their chance of the eggs frozen being euploid ("competent") is greatest. In addition, older women should be cautioned that their ability to propagate viable

(usable) eggs diminishes with advancing age. Regardless of age, all should be made aware of the fact that it could take several egg retrieval procedures to generate enough frozen eggs to provide a reasonable chance of subsequently having a baby. Finally, women of >40y (especially those with diminished ovarian reserve) should be counseled about the low return for investment with egg banking in their case due to the high incidence of egg aneuploidy. For such women, the banking of PGS/PGT-A-tested, euploid embryos is, in our opinion, preferable (even if this would require the use of donor sperm).

COMMERCIAL DONOR EGG BANKING

In this scenario, viable eggs derived from young egg donors are stored and subsequently made commercially available for IVF and embryo transfer to women for whom egg donor-IVF provides the only means by which they can go from infertility to family.

It has been estimated that presently in the United States alone, about 20,000 IVF procedures involving the transfer to the uterus of embryos derived from donor eggs are performed annually. This comprises 10- 15% of IVF treatment cycles in the United States. In addition, a growing number of IVF/egg donation-seeking couples travel abroad in search of lower cost treatment. In the last few years, numerous frozen egg banks have sprung up, offering access to non-genetically tested cryobanked eggs. As a result, more and more egg donor agencies are offering commercially available banked, donor eggs as an alternative to the use of fresh eggs derived through a designated and dedicated egg donor.

Despite the convenience associated with the use of donor eggs available through commercial egg banks, aside from convenience, there is not as much financial benefit in using such banks as would initially be expected. Moreover, for reasons cited above, frozen eggs are less likely than fresh eggs to generate viable embryos. Finally, blastocysts derived through the fertilization of fresh (non-frozen) eggs, whether transferred directly to the recipient's uterus or first vitribanked and then transferred to the uterus at a later date yield an overall success rate that is about 20% higher than when embryos derived from cryobanked eggs are used. Thus presently, aside from the convenience factor and a marginal cost differential, using fresh eggs obtained through an agency-derived egg donor has considerable benefits. However, as technology improves, the pendulum could well shift in the opposite direction.

It is as well to recognize that the "cost" of IVF should not come down to the financial outlay associated with doing a procedure. Rather, it should be viewed as the "cost" of having a baby. And the cost is both financial and emotional, with the latter often being far more depleting.

We are very hopeful that access to egg PGS/PGT-A and the ultimate adoption of selectively banked PGS-tested, euploid eggs could pave the way to improved egg banking.

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GONADOTROPINS: FERTILITY HORMONE INJECTIONS

Gonadotropins are hormones produced by the pituitary gland and which stimulate sex hormone production as well as

gamete (sperm and egg) production in the man/woman. They are also responsible for the expression of secondary sexual characteristics such as hair growth, muscular development, voice changes and breast development. There are two gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH). The gonadotropins are excreted in the urine. Two types are available pharmaceutically. The first, human menopausal gonadotropin (e.g., Menopur) is derived from the urine of menopausal women, and the second is recombinant FSH (FSHr), e.g., Follistim or Gonal-F. Because gonadotropins cannot be absorbed through the stomach into the bloodstream, they must be administered by injection rather than in pill form. While urinary gonadotropins must be injected intramuscularly, FSHr can be administered subcutaneously, thereby rendering the injections easier to administer and far less painful. The usual injection schedule is from day 2 or 3 through day 12 to 14   of the menstrual cycle.

MENOTROPINS (HUMAN MENOPAUSAL GONADOTROPIN (HMG)

The urine of menopausal women is a good source of both FSH and LH. This is because a menopausal woman's pituitary gland, in response to a feedback message that her ovaries are no longer producing enough estrogen, greatly increases the output of FSH and LH to stimulate the failing ovaries to ovulate. The excess FSH and LH are excreted in the urine. Urine used for gonadotropins is distilled, filtered, and purified by an expensive process. One (1) ampule or vial of HMG (75 IU) costs about $60-$80 in the United States, and the average woman might require 25 or more ampules per treatment cycle. In the United States, the most prescribed menotropin is Menopur. It has equal amounts of both FSH and LH/hCG. It has been postulated that

over-exposure to the LH/hCG component of gonadotropins can cause the tissue surrounding the ovarian follicles (ovarian stroma/theca) to over-produce testosterone. While testosterone is an essential hormonal precursor   which augments follicle and egg development as well as estrogen production, too much testosterone   can have a detrimental effect. This is especially likely in older women, women who have DOR and those with polycystic ovarian syndrome (PCOS) who often over- produce pituitary LH. It is also believed that over-production of androgens will often inhibit the proper development of the endometrial lining and result in dysfunctional embryo implantation. In this way, excessive ovarian androgen production induced by LH could have a deleterious effect on egg and embryo quality as well as on the potential for healthy implantation in the endometrium.

RECOMBINANT HUMAN FSH (FSHr)

Urinary gonadotropins have, in recent times, largely been supplanted by purified FSH derived by way of genetic engineering that makes it possible to induce bacteria to produce the product. Known as recombinant FSH (FSHr), it appears to be more bioactive than urinary derived FSH products. FSHr also has advantages in treating selected infertility problems such as ovaries with multiple small cysts (polycystic ovarian disease), but this situation rarely applies to the IVF setting.   Instead of influencing the hypothalamus and pituitary gland to produce more hormones to stimulate follicular development (as is the case with clomiphene and letrozole), gonadotropins act directly on the ovaries and do not inhibit the function of estrogen or the enzymes that act on the cells lining the follicles. If administered in sufficient amounts beginning early enough in the menstrual cycle, gonadotropins will prompt the growth of multiple follicles. Although the average number of eggs usually retrieved from a

woman younger than 40 after gonadotropin stimulation, provided she has two ovaries, is usually between four (4) and fifteen (15), retrievals of more than 20 eggs are not uncommon.

"TIGGERING WITH HCG VERSUS GnRH AGONIST

Commercial human chorionic gonadotropin (hCG) is either extracted from the urine of pregnant woman (hCGu) or is available as a genetically engineered product (hCGr), known as Ovidrel. It is administered following optimal follicle development to "trigger" egg maturation (meiosis) prior to fertilization.

Ideal egg development sets the scene for optimal egg maturation that occurs 36-42h prior to ovulation or egg retrieval. Without prior optimal egg development (ovogenesis), egg maturation will often be dysfunctional and many/most eggs will be rendered "incompetent" and unable upon fertilization to propagate viable embryos. In IVF, optimal ovogenesis requires the selection and implementation of an individualized approach to COS. Thereupon, at the ideal time, maturational "reproductive division" of the egg's chromosomes (i.e., meiosis) is "triggered" through the administration of hCG or by inducing an LH surge through the administration of a GnRHa (e.g., Lupron, Buserelin, Superfact, Decapeptyl. The dosage, type and timing of this "trigger shot" can profoundly affect the efficiency of meiosis as well as the potential to yield "competent (euploid) mature (M2) eggs, and as such it often represents a rate limiting step in the IVF process.

Until quite recently, the standard method used to "trigger" egg maturation was through the administration of 10,000 units of hCGu. Subsequently, a hCG-DNA recombinant (hCGr) available commercially as Ovitrelle or Ovitrel) was introduced and marketed in 250 mcg doses. But clinical

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experience soon strongly suggested that 250 mcg of Ovidrel was most likely not equivalent in biological potency, to the standard dosage of hCGu, 10,000 unit and as such might not be sufficient to fully promote meiosis, especially in cases where the woman had numerous follicles. For this reason, we hold that when hCGr is selected as the "trigger shot" the dosage should be doubled to 500 mcg at which dosage it will probably have an equivalent effect on promoting meiosis as would 10,000 units of hCGu. Failure to "trigger" with 10,000U hCGu or 500mcg hCGr, might increase the likelihood of disorderly meiosis, "incompetent (aneuploid) eggs" and introduce the risk of follicles not yielding eggs at egg retrieval ("empty follicles").

Some clinicians, when faced with a risk of OHSS developing will deliberately elect to reduce the "trigger" dosage of hCG in the hope that the risk of critical OHSS developing will thereby be lowered. However, this approach might not be optimal because a low dose of hCG (e.g., 5000 units, hCGu or 250mcg hCGr) is often inadequate to optimize the efficiency of meiosis particularly when it comes to cases such as this where there are many follicles. It has been suggested that the preferential use of a GnRHa "trigger" in women at risk of developing OHSS could potentially reduce the risk of the condition becoming critical and thereby placing the woman at risk of developing life-endangering complications. It is against this background that many RE's prefer to "trigger" meiosis by way of administering a GnRHa rather than through the use of hCG. The GnRHa promptly causes the woman's pituitary gland to expunge a large amount of LH over a short period of time and it is this induced "LH surge" that triggers meiosis. While this approach definitely reduces the risk of severe OHSS-related complications developing, it often comes at the expense of egg quality. *For this reason, we prefer to use a full dosage of 10,000U hCGu (or 500mcg hCGr) for the "trigger" after adequate duration of "prolonged coasting" and so reduce the risk of critical OHSS.*

Another "acceptable" approach is to reduce the dosage of hCG administered as a "trigger" and to combine this with a GnRHa and so reduce the risk of OHSS developing.

The timing of the "trigger shot "to initiate meiosis should coincide with the majority of ovarian follicles being >15 mm in mean diameter with several follicles having reached 18-22 mm. Follicles of larger than 22 mm will usually harbor overdeveloped eggs which in turn will usually fail to produce good quality eggs. Conversely, follicles less than 15 mm will usually harbor underdeveloped eggs that are more likely to be aneuploid and incompetent following the "trigger".

COMBINING CLOMIPHENE OR LETROZOLE WITH GONADOTROPIN

Some IVF programs administer a mixture of clomiphene/Letrozole and gonadotropins for controlled ovarian hyperstimulation. One of the reasons given for using this combination is because clomiphene/Letrozole increases the ovaries' sensitivity to gonadotropins, thereby reducing the dosage of gonadotropins that must be administered. In this way the overall cost of the fertility drugs can be diminished by reducing the required amount of expensive gonadotropins. However, addition of clomiphene or Letrozole can have significant drawbacks. First, because both clomiphene and Letrozole tend to increase pituitary LH output which in-turn increases ovarian testosterone, their use could compromise egg/embryo "competence", especially if used in older women and those with DOR. Second, clomiphene is an anti-estrogen that can negatively impact uterine lining development. Third, using a combination of clomiphene and gonadotropins may enhance the

risk for spontaneous ovulation even without the administration of hCG.

VARIATIONS IN RESPONSE TO GONADOTROPINS

Some women stimulate well after relatively small doses of gonadotropins. Others require two, three, or even four times that dosage to achieve the same effect. In the past, selecting the proper dosage was a trial-and-error process. Estimation of proper stimulation dosages is not an exact science but can be fairly accurate by taking into account a woman's current antral follicle count, anti-Mullerian hormone (AMH) level, and response to gonadotropins in prior stimulation cycles (should there be any).

RISKS AND SIDE EFFECTS OF GONADOTROPINS

Many women taking gonadotropins report breast tenderness, backaches, headaches, insomnia, bloating, and increased vaginal discharge, which are directly due to increased mucus production by the cervix.

Luteal Phase defect (LPD):

Endometrial biopsies have shown that the development of the uterine lining of patients stimulated with gonadotropins is usually a few days ahead of that which could be expected in unstimulated cycles. Thus, gonadotropins help to synchronize development of the endometrium with growth of the follicles and eggs. This synchronization is a critical prerequisite for successful implantation because IVF embryos are usually

transferred to the uterus slightly earlier than they would reach it under natural circumstances. Therefore, accelerated endometrial development enhances the chances that the young embryos will implant after their transfer to the uterus.

Severe Ovarian Hyperstimulation syndrome-OHSS (see above)

THE APPROACH TO COS WITH GONADOTROPINS

PREPARING FOR COH WITH GONADOTROPINS

It is preferable that at least one month be allowed to elapse ("resting cycle") between IVF treatment cycles, to allow the ovaries to fully recover. It is also important to ensure that the plasma E2 level is below 70 pg/ml (200pmol/L) following successful pituitary LH suppression (with GnRH agonist or antagonist), prior to initiating COH. The best time to measure the E2 level is soon after (i.e., within days) the onset of spontaneous or GnRHa-induced menstruation. The commonest cause of an elevated blood E2 level around this time is the existence of one or more ovarian follicular cysts. These should be allowed to absorb or be aspirated as soon as possible. Spontaneous absorption will usually occur with continued LH suppression using agonist/antagonist.

GnRH AGONISTS & ANTAGONISTS

Gonadotropin releasing hormone (GnRH) is produced by the hypothalamus, a specialized area in the brain. GnRH stimulates the pituitary to produce two natural gonadotropins: luteinizing hormone (LH) and follicle stimulating hormone (FSH). FSH, in turn, is responsible for the development of mature

follicles, which contain healthy eggs, and for the production by the follicles of estrogen necessary to build the uterine lining. LH is released at low concentration throughout the cycle, but at the time of ovulation, there is a rapid increase (a surge) in its blood concentration. The LH surge causes ovulation, and this is followed by the conversion of the follicle to the corpus luteum which produces progesterone and estrogen. Together progesterone and estradiol enhance the growth of the uterine lining to support implantation of the embryo and early maturation of the developing fetus.

GnRH-AGONIST

(e.g., Leuprolide Acetate; Superfact; Buserelin; Decapeptyl)

How GnRHa works: Leuprolide is very similar in structure to GnRH. As such, its initial effect, for 4-6 days or so, is to stimulate the pituitary gland to produce both LH and FSH. The omnipresence of the GnRHa however will ultimately profoundly reduce output of biologically active LH and FSH production. This is referred to as "pituitary down-regulation" and the effect continues for as long as leuprolide acetate therapy is maintained uninterrupted. The progressive decline in blood estrogen levels that accompanies the "down regulatory process" precipitates the onset of menstruation within 2-4 days (i.e., 6 to 10 days following the commencement of leuprolide therapy).

The reason leuprolide acetate is administered to women who receive gonadotropin therapy is to produce a sustained reduction in the release of biologically active luteinizing hormone by the pituitary and so prevent ovulation inducing rises in LH levels. LH surges would cause a premature ovulation and high-

normal LH levels have been shown to interfere with development of the egg(s) and follicle(s).

At SFS, we often start the woman on a birth control pill (BCP) for 7-21 days to suppress ovarian response to FSH/LH whereupon Lupron is administered in conjunction with the BCP for an additional 4-6 days whereupon the BCP is discontinued while daily Lupron injections are sustained. This approach reduces the incidence of cycle cancellation due to ovarian cyst formation. Menstruation will usually occur 6-10 days after stopping the BCP, whereupon daily gonadotropin injections are added to the Lupron regime. Both gonadotropins and Lupron are discontinued on the day of hCG. The egg retrieval (ER) is performed 34-37 hours following hCG administration.

Recipients of embryos derived from a third party (e.g., egg donation and gestational surrogacy) and thawed embryos (i.e., previously frozen) undergo a similar regime of BCP/GnRHa preparation, but instead of getting gonadotropins, they receive estrogen therapy. In such cases, the GnRHa is discontinued 5-7 days prior to embryo transfer (ET).

The administration of subcutaneous GnRHa is rarely associated with significant side effects. Some women experience temporary fluctuations in mood, hot flashes, nausea, and symptoms not vastly dissimilar from PMS. No serious long-lasting side effects have been reported.

The injection of GnRHa is given subcutaneously and is relatively painless. Unfortunately, the drug will incur a modest additional financial burden. Moreover, as mentioned above, the administration of GnRHa will almost always require that gonadotropins be administered for slightly longer than would otherwise be required had GnRHa not been administered. GnRHa administration spares many women the inconvenience and frustration of repeated cancelled treatment cycles with

gonadotropins. Indeed, when taking this into consideration, the administration of leuprolide acetate may reduce the overall cost of ovulation induction.

GnRH ANTAGONIST

(e.g., Ganirelix; Cetrotide; Orgalutron)

How GnRH antagonists' work: Unlike GnRHa, which lower blood levels of gonadotropins by depleting stored pituitary FSH and LH, GnRH antagonists elicit lower blood levels of endogenous gonadotropins by rapidly blocking the release of FSH and LH by the pituitary gland. Furthermore, while the agonist takes 4-10 days to achieve an adequate blood gonadotropin lowering effect, recipients of the agonist will have minimal blood levels within 1 day of initiating treatment.

The advantage of using GnRH antagonists: Since the administration of GnRH antagonist rapidly suppresses pituitary gonadotropin release, and menstruation occurs within two to five days, it can be administered starting in the middle of the cycle and still effectively prevent the dreaded pre-ovulatory rise in blood LH levels that could doom the treatment cycle to failure. The manufacturers recommend that treatment with the GnRH antagonist be initiated on cycle day 6 following the onset of spontaneous or induced menstruation. Thus, unlike with the use of GnRH agonists where the need for a week or more to initiate adequate pituitary "down-regulation", administration of antagonists allows for the initiation of ovarian stimulation to begin at short notice.

How GnRH antagonists are administered:

The use of GnRH antagonist as currently prescribed in ovarian stimulation cycles, i.e., the administration of 250 mcg daily from the 4th to7th day of stimulation with gonadotropins is problematic, especially for women with high steady state (tonic) release of LH and overgrowth (hyperplasia) of ovarian stromal (connective tissue). Examples of such cases include women over 40y, women with DOR, and some cases of PCOS. In such women, the initiation of pituitary suppression with GnRH antagonist so late in the cycle of stimulation fails to suppress high tonic pituitary LH in the most formative (early) stage of folliculogenesis. One of the roles of LH is to promote androgen (male hormone) production which in turn is essential (in small amounts) for optimal follicular growth to take place. In women with high steady (tonic) release of LH and/or ovarian stromal hyperplasia, the failure of conventional GnRH antagonist protocols to address this issue, results in the inevitable excessive exposure of follicles to androgens (mainly testosterone). This can adversely influence egg/embryo quality and endometrial development.

Presumably, the reason for the suggested mid-follicular initiation of high dose GnRH antagonist is to prevent the occurrence of the so-called   "premature LH surge", which is known to be associated with "follicular exhaustion" and poor egg/embryo quality. However, the term "premature LH surge" is a misnomer and the concept of this being a "terminal event" or an isolated insult is, erroneous. In fact, the event results from a culmination (end point) of the progressive escalation in LH ("a staircase effect") which results in increasing ovarian stromal activation with commensurate growing androgen production. Trying to improve ovarian response and protect follicular exhaustion by administering GnRH antagonist during the final few days of ovarian stimulation is like trying to prevent a shipwreck by collision, through removing the tip of an iceberg.

The use of such mid-follicular GnRH antagonist protocols in younger women or in normal responders will probably not produce such adverse effects because the tonic endogenous LH levels are low (normal) in such cases and such normally ovulating women rarely have ovarian stromal hyperplasia.

At SFS when prescribing a GnRH antagonist, we often start the antagonist much sooner than the 4th-7th day of stimulation with gonadotropins. The intent is to prevent a large amount of LH from reaching the circulation and inducing excessive ovarian testosterone production which can compromise follicular growth and egg maturation. Moreover, since testosterone also down-regulates estrogen receptors in the endometrium, an excess of testosterone can also have an adverse effect on endometrial growth.

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MALE FACTOR INFERTILITY: NON-SURGICAL TREATMENT

The treatment of male factor infertility is one of the true success stories in the field of reproductive medicine. Disorders of sperm quality range from a low count or motility to a complete absence of sperm production. Deformities of the sperm cell shape (morphology) are also important to its ability to fertilize the egg. Mild abnormalities of semen parameters can be effectively treated using techniques that "wash" out the seminal plasma and improve the concentration of normally shaped motile sperm, which are then transferred to the uterus via an intrauterine insemination. However, for more severe conditions this treatment is inadequate. With a total motile cell concentration of less than 10 million cells per ml or a normal

morphology of less than 4% by strict Kruger criteria, the chance of suboptimal fertilization is high, even with IVF.

Effective treatments for male infertility may involve:

- Hormonal therapy (clomiphene, Letrozole gonadotropins, corticosteroids, thyroid hormone)

- Non-hormonal drug therapy (Bromocriptine, antibiotics)

- Surgery (varicocelectomy, vasectomy reversal, surgical treatment of undescended testes, etc.)

- IVF-related procedures [intracytoplasmic sperm injection (ICSI), testicular sperm extraction/aspiration (TESE/TESA)]

As a general principle, if the male factor cannot be reversed in the man's body, by simple medical or surgical treatment, then IVF with ICSI represents the most rational approach. Intrauterine insemination is not an effective way of treating moderate to severe male infertility.

HORMONAL THERAPY

In a relatively small number of cases of male infertility, the failure to produce an adequate quality of sperm relates to reduced secretion of the gonadotropins FSH and LH by the pituitary gland. Luteinizing hormone's predominant function is to act on a particular variety of cells in the testicles that produces the male hormone testosterone. These cells are referred to as Leydig cells. A sustained reduction in FSH production, therefore, can result in male infertility. Usually, if there is a reduction in either one of the components, LH or FSH, the other one will also be low. In other words, if a man produces a normal amount of

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LH and has normal male hormone levels (testosterone, androstenedione, dehydroepiandrosterone), then it is very unlikely that he will have a reduced FSH production. Accordingly, if his sperm function is reduced, it is unlikely to be the result of reduced FSH production by the pituitary gland.

In men, the entire spermatogenic cycle, from initiation to the production of the most mature forms of spermatozoa, takes approximately 90-100 days.   Accordingly, any treatment administered to improve sperm production can only be properly assessed after waiting for a period this long. In the man, as with the woman, the pituitary gland releases FSH and LH in response to need.   In other words, if there is an abundance of male hormone being produced then the pituitary gland, through messages received from higher centers in the brain, reduces its production of LH. This push-pull mechanism, referred to as a feedback response, helps the body regulate exactly how much stimulation is needed to keep normal testicular function going.

To assess the potential of a male to respond to fertility drugs aimed at stimulating the testicles to produce more spermatozoa and/or male hormone, it is therefore necessary to first measure both FSH and LH as well as prolactin and the male hormones   testosterone,   androstenedione   and dehydroepiandrosterone.   Measurement of these hormones gives an indication as to the likelihood of the man responding to treatment aimed at: (1) inducing increased production of FSH or FSH/LH (e.g., clomiphene citrate), or (2) the direct administration of gonadotropins which comprises FSH, LH (e.g., Menopur, Gonal F) or hCG (e.g., Pregnyl, Novarel, Profasi, Ovidrel).

Clomiphene Citrate (The first approach: Clomiphene citrate is a hormone which, through its central action in the brain, stimulates the pituitary gland to produce natural FSH in large amounts. The FSH, in turn, as mentioned above, stimulates

spermatogenesis. The treatment is very simple and involves the administration of 25 mg of clomiphene citrate every other day for a period of 100 days. It is necessary to perform a baseline semen analysis, FSH, LH, and male hormone measurements immediately prior to initiating therapy, and then to serially repeat all of these tests throughout the treatment with clomiphene. The final assessment of response can only be made approximately 100 days after initiating therapy. This administration of clomiphene is essentially harmless to the man. He may experience some minor side effects such as spots in front of the eyes (scotomata), dryness of the mouth, headaches, slight changes in mood and, rarely, hot flashes. These side effects are all reversible upon discontinuation of therapy.

Gonadotropin Therapy. In cases where clomiphene therapy fails to be successful, or in certain situations where it is not possible for clomiphene to stimulate the pituitary gland into action, it is possible to administer FSH alone or in combination with LH in the hope of stimulating the testicles directly. This therapy, in certain cases of male infertility, might be combined with the administration of the hormone human chorionic gonadotropin (hCG), which is also a natural hormone and has a function like that of LH. The basis upon which hCG would be administered would be to further stimulate the production of male hormones in cases where failed masculinization is associated with reduced sperm production. Administration of these drugs is usually carried out 3 times per week, again for a period of about 100 days, and the same hormonal and sperm assessments as stipulated for clomiphene therapy would apply. The treatment is, again, relatively harmless and the minor side effects which might occur are all reversible upon discontinuation of therapy.

Other Hormonal Therapies. In some cases, there may be systemic conditions affecting other areas of the body which

indirectly impact upon the pituitary gland's ability to produce the hormones necessary to stimulate testicular function. Rare examples include administration of thyroid hormone in cases of involvement of the thyroid gland, severe diabetes mellitus, and collagen diseases amongst others. Sometimes the pituitary gland produces too much prolactin which, in turn, inhibits the ability of FSH and LH to act on the testicles. In such cases, it may be necessary to administer a drug called Parlodel or Dostinex (bromocriptine, cabergoline) to suppress prolactin production, and thereby remove the restraining effect that prolactin might have on the action of FSH upon the testicles. There are, of course, many other such examples of where treatment of unrelated conditions might improve overall male fertility. Testosterone treatment is contraindicated because prolonged use (more than 2-3 months) will have the reversed effect, compromising sperm count, motility and even morphology.

If the man is fortunate enough to respond to one of the above treatment modalities for enhancement of sperm production, then it is possible for several masturbation specimens of sperm to be collected and frozen in liquid nitrogen. These samples can be kept for several years, so there will always be relatively good quality sperm on hand, even if the fertility treatment is discontinued, and you subsequently revert to a relatively poor production of sperm. It is, of course, not practical to permanently treat an individual on potent medications such as clomiphene or gonadotropins.

INTRACYTOPLASMIC SPERM INJECTION (ICSI)

Although always a treatment of choice for male infertility, it was not until the introduction of ICSI in the mid 90's that IVF became more successful when applied in cases of male infertility than for female-related causes. ICSI is a procedure

where fertilization is achieved through the direct injection of a single sperm into the substance of each mature egg. Even high concentrations of anti-sperm antibodies attached to the sperm or severe sperm defects such as absence or abnormalities of the acrosome (the enzyme-rich attachment at the top of the sperm head) that enables the sperm to penetrate the zona pellucida, are offset by ICSI.

TESTICULAR SPERM INJECTION/ASPIRATION (TESE/TESA) (TESE/TESA)

TESE/TESA are procedures involving the extraction of sperm directly from the testicle(s) either by open biopsy (usually done under anesthesia) or through the introduction of a thin needle directly into the testicle(s), under local anesthesia, without making a skin incision. Sperm or hair-thin specimens of testicular tissue are extracted, and each egg is injected with a single sperm using the ICSI technique. It is most done in cases of spermatic duct (vas deferens) occlusion or absence but can also be performed in cases of ejaculatory dysfunction, such as might occur following spinal cord injuries, after prostatectomy, or in cases of intractable male impotency. (It can also be used as a last-resort approach in cases of testicular failure, but unfortunately, success rates are low.) TESE/TESA are simple, relatively low-cost, and safe procedures. Aside from the remarkable success rates with TESE/TESA/ICSI done for blockage of the sperm duct, the fact is that unlike surgical vasectomy reversal, these procedures allow the man to retain his vasectomy for future contraception.

SPERM CHROMATIN STRUCTURE ASSAY (SCSA)

The Sperm Chromatin Structure Assay (SCSA) and DFI tests are used for measuring clinically important properties of sperm nuclear chromatin integrity. The results correlate well with the potential of sperm from a given male to produce embryos that would be sufficiently "competent" to produce a live birth. The tests utilize the metachromatic features of acridine orange (AO), a DNA probe, and the principles of flow cytometry (FCM).

SCSA/DFI data are not well correlated with classical sperm quality parameters and have been solidly shown to predict sub/infertility and poor reproductive performance. These tests measure DNA damage. The degree of abnormalities in the genetic material of the sperm is expressed numerically as the DNA Fragmentation Index (DFI). DNA damage may be present in sperm from both fertile and infertile men. Therefore, this sperm DNA damage analysis may reveal a hidden abnormality of sperm DNA in infertile men classified as unexplained based on apparently normal standard sperm parameters. Infertile men with abnormal sperm characteristics exhibit increased levels of DNA damage in their sperm. Sperm from infertile men with normal-appearing sperm may have DNA damage to a degree comparable to that of infertile men with abnormal-appearing sperm. The data suggests that an abnormal DFI assay is more likely to occur in cases of abnormal semen parameters. Thus, the assay is ideally suited to assess male sperm DNA integrity as related to fertility potential and embryo development as well as effects of reproductive toxicants. Since SCSA/DFI parameters are somewhat independent of conventional semen parameters, results may allow physicians to identify male patients for whom IVF and ICSI will be far less likely to result in the initiation of a viable (>12 weeks) pregnancy.

Cancer treatments are well known to adversely affect male fertility. Reduction of sperm output arises from the cytotoxic effects of chemo- or radiotherapy upon the spermatogenic epithelium. However, even if the epithelium survives there is an association with infertility and poor reproductive performance. Optimal sperm chromatin packaging seems necessary for full expression of the male fertility potential.

The improvement seen in sperm motility after sperm separation and Percoll processing is not associated with a similar improvement in SCSA/DFI results. These data suggest that sperm processing techniques will not minimize sperm DNA damage and the potential transmission of genetic mutations in assisted reproductive cycles.

It is important to add that the most current data available on the significance of abnormal SCSA/DFI results in infertile couples seeking treatment has emanated from non-IVF pregnancies. A large database of information on the clinical role of the SCSA/DFI in patients suggests the following:

- The viable (>12 weeks) IVF pregnancy rate (and thus presumably also the birth rate) could be as much as 2 times lower in women under 33 yrs. of age, whose husbands have abnormal SCSA/DFI assays (with a DFI of >15%). Results become progressively worse with advancing maternal age such that at 35+ yrs., the viable pregnancy rate could be as much as 3-4 times lower.
- Although it is possible for abnormal DFI results to revert spontaneously to normal, this probably occurs quite infrequently.
- Although abnormal SCSA/DFI results are detected in men with apparently normal semen analyses, abnormal results are more commonly seen in cases of men who

have abnormal sperm parameters (abnormal sperm count, motility and/or morphology).

- There is some suggestion that the use of antioxidant therapy (Pycnogenol 200 mg daily, L-Carnitine 3 grams per day, acetyl carnitine 500 mg per day, Vitamin C 1,000 mg per day and Vitamin E (800 IU per day) taken for 8-10 weeks, can cause the SCSA/DFI assay to revert to normal. There is also some suggestion that men who have varicoceles (a collection of distended veins in the scrotum) associated with an abnormal DFI assay may experience a reversion of the DFI assay back to normal, 3-6 months following surgical or radiological ablation of the varicocele.

In summary, an abnormal SCSA/DFI assay augers poorly for the outcome of fertility treatment in general and IVF/ICSI in specific. In such cases, the fertilization rate and pregnancy rates are reduced, and the chance of early pregnancy loss appears to be increased significantly. The prognosis worsens progressively as the age of the egg provider advances beyond 33 yrs.

If despite "treatment" an abnormal SCSA/DFI result fails to revert to normal, the use of TESE/TESA sperm, which possesses less DNA breakage than ejaculated sperm, should be seriously considered, especially where the egg provider is over 35 and facing a "rapidly ticking" biological clock. Should that fail, then donor sperm seems like the next best step.

It is possible that the SCSA/DFI assays will, in time, become regarded as required baseline tests to be performed, regardless of their basic traditional semen analysis parameters (count, motility and sperm morphology). It will be performed in all cases of recurrent pregnancy loss and IVF where the sperm provider has not previously participated in a pregnancy that has

proceeded beyond the 12th week (the traditional point of likely viability).

SPERM ANTIBODIES

Anti-sperm antibodies (ASA) are immunoglobulins that attach to sperm. They are most encountered in semen, blood, cervical mucous and follicular fluid. Not all ASA bind to sperm. However, those that do so can inhibiting fertilization. Methods used to detect the presence of SAs in blood, in the seminal plasma of the ejaculate, or in the cervical mucus only measure those immunoglobulins that bind to sperm components. In about 1-4% of infertility cases the presence of antisperm antibodies (ASA) in the male or female appear to be the cause. Attempts to try and remove antibodies from sperm by allowing the sperm to swim through a column of beads are by and large unsuccessful. And, while there have been isolated reports that administration of corticosteroids will temporarily suppress antibody production, pregnancy rates are poor. In Vitro Fertilization (IVF) with intracytoplasmic Sperm injection (ICSI) is the best treatment option. Here each egg is injected with a single sperm and whether there are antibodies attached to the outer surface of the sperm becomes irrelevant. In fact, pregnancy and birth rates are the same as in cases where IVF is performed for reasons other than male factor infertility. IVF/ICSI success rates are also not unaffected by the concentration of anti-sperm antibodies.

VARICOCELE

The testicles are housed in the scrotum, a skin-covered sac that houses the two testicles as well as blood vessels that deliver blood to these glands, nerves, and lymphatics. An abnormality of the plexus of veins (the pampiniform plexus) that carry blood away from the testicles can result in their distention, proliferation, and enlargement within the scrotum.

About 15 percent of men have varicoceles. These generally form during puberty and are more commonly found on the left side of the scrotum, and ~ 40% of the time they are bilateral. Most males are diagnosed between the ages of 15 and 25 years of age. The increased scrotal blood flow often raises the temperature in the scrotum by 1-2 degrees. This in turn sometimes results in decreased sperm production and functionality as well as testicular shrinkage (atrophy), leading to male infertility.

Most varicoceles are not symptomatic. In fact, most are not ever detected. Those that are symptomatic present with scrotal enlargement, scrotal and/or lower abdominal pain, and infertility. The condition is often diagnosed by physical examination where, to the touch, a varicocele feels like a "bag of worms". However, ultrasound examination of the scrotum offers a more definitive diagnostic capability.

Contrary to popular belief, varicoceles seldom reduce sperm count. In fact, in many cases of low-sperm count, treatment of the varicocele rarely results in an improvement in sperm production. It is important to recognize that most men who have varicoceles do not have evidence of sperm dysfunction.   When a varicocele causes sperm dysfunction, this

most commonly manifests with a normal or even raised sperm count. However, there is usually markedly reduced motility and sperm progression, but a high percentage of sperm are found to be "immature". Collectively this is referred to as a "stress pattern". In many such cases the Sperm Chromatin Structure Assay (SCSA) will show an increase in the DNA Fragmentation Index (DFI). These are the cases where treatment of the varicocele will often improve sperm function, along with improving the likelihood of a viable pregnancy occurring naturally or following IVF with ICSI.

There are two approaches to treating varicoceles:

- Traditional treatment of a varicocele is through ligation (under general anesthesia) of one or both spermatic veins that carry blood from the pampiniform venous plexus ("varicocelectomy"). While surgery is in most cases curative, post-surgical recurrence is not uncommon.

- Another approach is interventional radiological obliteration of one or both the spermatic veins. This approach involves passing a balloon catheter via a groin blood vessel, under radiological view, into the spermatic vein and inflating the balloon in that position. This causes the spermatic vein to permanently occlude. Sometime later, the catheter is withdrawn, and the vein remains occluded causing the varicocele to collapse and the pampiniform plexus to shrink. This radiological approach is less costly, less traumatic, and seems equally as effective as the surgical approach. less likely to result in a recurrence than is spermatic vein surgical ligation.

Regardless of whether surgical or interventional radiological treatment is contemplated, it is in our opinion, advisable for the patient to take a male fertility blend that is rich

in antioxidants. This will sometimes result in an improvement in the DFI within 3-6 months. It should be taken for at least 12 weeks whereupon the SCSA should be repeated.

It is important to bear in mind that both surgical and radiological treatment will only improve sperm function in about 35% of men who have varicocele.

Under what circumstances will treatment be unlikely to succeed? The following, in our opinion, represent situations where treatment of a varicocele (whether surgical or radiologic) is unlikely to resolve the male infertility issue.

- FSH level (greater than 12MIU/ml)
- low sperm count (<20million/ml).
- DFI is below 15%

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PELVIC INFLAMMATORY DISEASE (PID) AND TUBAL DAMAGE

Pelvic inflammatory disease (PID) refers to pelvic structures including the uterus, fallopian tubes, ovaries, bowel, and the smooth membrane that lines the surface of the pelvic cavity (the peritoneum). PID follows infection which reaches pelvic structures as a result of: (1) sexual transmission via the vagina and cervix; (2) contamination from other inflamed structures in the abdominal cavity (appendix, gallbladder, kidneys, etc.); (3) a foreign body inside the uterus (i.e., intrauterine device – IUD); (4) contamination of retained products of conception following abortion or childbirth; and (5) rarely as a result of blood-born bacterial transmission (e.g., pelvic tuberculosis which is common in developing countries but rare in the United States).

It has been estimated that about 2,000,000 women develop PID annually in the United States. Less than one-third of these women present with acute pelvic inflammatory disease. The remaining cases usually go undetected until the woman presents with symptoms or infertility. In fact, more than 60% of patients who undergo surgery or IVF/ET for chronic PID-related complications/symptoms have no history of acute PID.

In most cases, PID results from the sexual transmission of infecting organisms such as Neisseria Gonorrhea and Chlamydia Trachomatis, which are readily eradicated through appropriate antibiotic therapy. Sexually transmitted bacteria first infect the cervix (the opening into the uterus) through which it ascends via the uterus to the fallopian tubes, ovaries, and other pelvic structures.

There are several important factors which predispose women towards developing PID. The first is exposure to an infected partner; the second is exposure to infection immediately prior to menstruation (menstrual blood provides an excellent growth and travel medium for bacteria); the third is relatively ill health and poor nutritional status. It is predominantly for this reason that PID runs a rampant course in lower socioeconomic groups; fourth is the fact that previously infected tissues are highly susceptible to re-infection; resulting in women with a history of PID being highly susceptible to recurrent attacks.

While sexually transmitted PID is certainly capable of causing endometritis (infection of the uterine lining) the uterus itself is not the focus of the inflammatory process. Cyclical shedding of most of the uterine lining with menstruation tends to remove infected tissue monthly, thereby preventing the inflammation from taking a hold and causing permanent damage to or scarring of the uterine lining (the endometrium). The main site of inflammation following sexually transmitted PID is the

fallopian tube via which other pelvic and abdominal structures may be infected.

In contrast, pelvic inflammatory disease that occurs following childbirth or abortion primarily targets the uterine lining, causing endometritis. Organisms such as Bacteroides, Peptostreptococcus, Beta hemolytic streptococcus, and E. coli readily proliferate in the products of conception that are sometimes retained in the uterus following childbirth and abortion. The delayed onset of menstruation after both childbirth and abortion provides an opportunity for the inflammatory process to take hold and progress, sometimes leading to the development of scar tissue in the uterine cavity. This may cause opposing surfaces of the endometrium to fuse together or produce scarring which obliterates the opening (in the uterus) into the fallopian tubes and might also damage a small, adjacent segment of the tubes. Less commonly, post-childbirth and post-abortal endometritis infects the entire fallopian tube(s) (salpingitis) as well as causing partial or complete blockage, and/or spreading into the pelvic cavity.

PID may also result from the use of the Intrauterine contraceptive device (IUD). This most commonly occurs in cases where the device is inserted in women concurrently infected with Gonorrhea or Chlamydia. The IUD causes local irritation which compromises normal defense mechanisms against infection. At the same time, the IUD string which protrudes through the cervix into the vagina may act as a "wick" via which infecting organisms gain entrance to the uterus. IUD-related PID, now a rare phenomenon due to better designed IUDs, is a potentially life endangering condition capable of causing pelvic abscess formation, the development of peritonitis (inflammation of the peritoneum), systemic infection (septicemia), and shock.

Acute PID

Pelvic inflammatory disease may present as an acute illness with fever, severe lower abdominal pain, accompanied by a yellow or blood stained nonirritant, vaginal discharge and vomiting which usually prompts the woman to seek urgent medical attention.

Subacute PID

More commonly, the onset of PID is gradual, less severe, and often goes unnoticed until superimposed acute PID occurs, or chronic incapacitating symptoms prompt the woman to seek medical attention (see below).

Chronic Pelvic Inflammatory Disease

Chronic PID is a consequence of untreated or unsuccessfully managed acute and/or subacute PID. The woman usually presents with symptoms of pelvic pain, heavy and painful menstrual periods, pain with intercourse (dyspareunia) and infertility.

HOW PID CAUSES INFERTILITY

Sexually transmitted PID caused by Gonorrhea or Chlamydia rapidly spreads via the cervix and uterus to the fallopian tube(s). These organs are highly specialized and are designed to promote the active passage of eggs, sperm, and embryos in a timely manner to and from the uterine cavity. They contain cells whose function is to protect and nurture eggs, sperm, and embryos in transit. At their ends, the fallopian tubes have small delicate finger-like projections (fimbriae) that approximate, envelope and "pick-up" the egg(s) at the time of ovulation. Infection-related inflammation damages and often destroys the specialized lining of the fallopian tube(s) and in

severe cases results in fusion of the fimbriae thereby blocking the ends of the tube(s) compromising their mobility and their potential to facilitate timely passage of eggs, sperm and embryos. Pus which accumulated inside the tube(s) often passes into the pelvic cavity producing peritonitis and results in the formation of scar tissue (adhesions) which further disrupts normal pelvic anatomy as well as the relationship between the tube(s) and the ovary(ies). This may prevent the fallopian tubes from collecting the egg(s) during ovulation.

In cases where the ends of the fallopian tubes are blocked, pus may collect and distend the tube(s). The pus is usually absorbed over time and replaced by clear straw-colored fluid. The resulting, occluded, fluid-filled, distended, and often functionless fallopian tube(s) is referred to as a hydrosalpinx.

Sexually transmitted PID almost invariably affects both fallopian tubes. Even in cases where a dye x-ray test (hysterosalpingogram) or laparoscopy (a procedure where a telescope-like instrument is passed through the belly button to visualize the pelvic structures) indicates that only one fallopian tube has been infected, the other tube is almost invariably involved.

SURGERY VERSUS IVF

IVF is the treatment of choice for almost all forms of tubal infertility. One possible exception   involves surgical reversal of tubal ligation. The chance of having a baby within years of such surgery is about 50%. However, in selective IVF centers of excellence, such as SFS, where the anticipated birth rate following a single attempt at IVF in women under 40 years is above the national average, and the birth rate following three IVF

attempts is in the order of 80%, this argument does not hold water.

Surgery to unblock fallopian tubes or clear adhesions resulting from an inflammatory process due to infections with Gonorrhea or Chlamydia is an exercise in futility. The chances of pregnancy occurring following such an undertaking are less than 2% per month and less than 25% in three years. We concur with a recent opinion that in the modern context, infertility surgery for fallopian tubes damaged by inflammation should be considered an "anachronism".

In our view, given the high incidence of ectopic pregnancy following tubal surgery (20-25%), the fact that surgery requires hospitalization, general anesthesia, pain, and a significant risk of post-operative complications, and in consideration of the statistical fact that more than 70% of patients undertaking such an escapade would ultimately need IVF anyway, there is no longer any medical justification to choose tubal surgery over IVF.

REMOVING HYDROSALPINGES IN PREPARATION FOR IVF

Damage to the fallopian tubes because of prior infection, endometriosis or previous pelvic surgery is one of the most common causes of fertility. Tubal blockage can occur in several locations. Often the ends of the tubes are obstructed, while the openings into the uterus are still patent. In many cases the tubes fill with fluid. We call this hydrosalpinges. While for patients with significant tubal disease surgery is not indicated to improve fertility outcome in the era of IVF, the exception is the presence of hydrosalpinges. Recent literature has shown that fluid in swollen tubes, which contains dead cells and other noxious products, is highly toxic to embryos. In addition, the fluid can

leak back into the uterus and cause a mechanical barrier to implantation.   Patients with hydrosalpinges should strongly consider having their tubes removed or ligated prior to undergoing treatment. It is often hard for patients to accept that their tubes will be gone, as it means that conception is impossible without assistance.   However, the presence of hydrosalpinges means that the tubes are non-functional and even if these tubes could be rendered patent (open), the likelihood of pregnancy occurring would be remote.

UTERINE FIBROIDS (LEIOMYOMATA)AND REPRODUCTION

The uterus is composed of a thick layer of smooth muscle (myometrium) surrounding a thin   lining (the endometrium) into which the embryo implants and which serves to protect and nourish a growing pregnancy. Approximately 20 to 40% of all reproductive age women will develop benign growths of the myometrium, referred to as fibroid tumors (leiomyomata). These tumors are rarely malignant (see below). They can be in the wall of the uterus (intramural), on the outside of the uterus (subserosal), within the uterine cavity (submucosal), on a thin stalk (pedunculated) or a combination of the above. Estrogen causes them to grow. African and African American women seem to have a much higher incidence of fibroid tumors. Fibroids usually grow very slowly (over a number of years). However, when growth occurs rapidly over a month or two, especially in older women who have large fibroids, it should raise the suspicion of a very rare but extremely serious malignancy called

sarcoma. Fibroid tumors rarely undergo a malignant change. The reported incidence is less than 1:2000.

PRESENTATION, SYMPTOMS AND SIGNS ASSOCIATED WITH UTERINE FIBROID TUMORS

Fibroid tumors, even large ones, can occur without producing any symptoms at all. However, they can also cause a variety of symptoms depending on their size, location and the absence or presence of complications such as torsion (twisting) or degeneration (such as might occur when a fibroid grows so fast that it starts running out of its blood supply). The most common symptoms are heavy cyclical menstrual bleeding (menorrhagia) accompanied by menstrual pain (dysmenorrhea). Sometimes, especially when a fibroid protrudes into the uterine cavity, it can cause erosion of the endometrial lining and produce irregular or continuous bleeding (menometrorrhagia). Sustained non-menstrual pelvic pain may point to torsion of a pedunculated fibroid that is attached to the inner or outer wall of the uterus, or to degeneration, other possible symptoms include pain with deep penetration during intercourse deep dyspareunia), bladder irritability, rectal pressure, constipation, and painful bowel movements (dyschezia).

EFFECT OF FIBROID TUMORS ON REPRODUCTION

For the most part, only those fibroids that impinge upon the endometrial cavity (submucosal) affect fertility. Exceptions include large intramural fibroids that block the openings of the fallopian tubes into the uterus, and where multiple fibroids cause abnormal uterine contraction patterns. Surgery to treat fibroids can also affect fertility in several ways. If the endometrial cavity is entered during the surgery, there is a possibility of postoperative adhesion formation within the uterine cavity. This should always be checked for through the performance of a hysteroscopy or fluid ultrasound prior to beginning fertility

treatment. Because of bleeding that may occur during a myomectomy, there is a high likelihood of abdominal adhesion formation, which could encase the ovaries and prevent the release of the eggs or block the ends of the fallopian tubes. For this reason, it is important that only accomplished surgeons, who are familiar with techniques to limit blood loss and prevent adhesion formation, perform myomectomies.

In some cases, multiple uterine fibroids may so deprive the endometrium of blood flow, that the delivery of estrogen to the uterine lining (endometrium) is curtailed to the point that it cannot thicken enough to support a pregnancy. This can result in early 1st trimester (prior to the 13th week of pregnancy) miscarriages. Large or multiple fibroids, by curtailing the ability of the uterus to stretch to accommodate the spatial needs of a rapidly growing pregnancy, may precipitate recurrent 2nd trimester (beyond the 13th week) miscarriages and/or trigger the onset of premature labor.

DIAGNOSIS

Sizable fibroid tumors are usually easily identified by a simple vaginal examination. However, even the smallest fibroid can be identified by transvaginal ultrasound. Sometimes it is difficult to tell if a fibroid is impinging on the endometrial cavity. In such cases, a hysteroscopy (where a telescope-like instrument is inserted via the vagina into the uterine cavity) or a hysterosonogram (where injected fluid distends the uterine cavity allowing for examination of its contour and inner configuration) can help distinguish between intramural and submucosal fibroids. Magnetic resonance imaging (MRI) can be used to distinguish between fibroid tumors and a related condition called adenomyosis, in which diffuse or localized foci of endometrium are found within the myometrium. Given the

often-diffuse nature of adenomyosis, it is difficult to remove surgically. This contrasts with fibroid tumors, which are well defined and are usually easily removed.

MANAGEMENT/TREATMENT

Surgical treatment of fibroid tumors: The mainstay for the treatment of fibroid tumors is surgical removal (myomectomy). Small, asymptomatic fibroids that do not impinge upon the endometrial cavity will usually not require treatment other than observation and vigilance. Large fibroids and submucosal fibroids should be removed prior to starting fertility treatments such as IVF to decrease the chance of implantation failure, miscarriage, pregnancy complications and premature labor. Intramural and subserosal fibroids are readily removable by laparoscopic resection or via an abdominal incision. The former allows for a more rapid convalescence and is ideal for the removal of small and accessible superficial fibroid tumors, while the latter approach is preferred for treating larger and less accessible fibroids.

Regardless of whether the laparoscopic or abdominal approach is employed, adequate closure of the uterine wall is essential to reduce the subsequent risk of uterine rupture during pregnancy or labor. This is one of the main arguments used against the use of laparoscopic removal of large, multiple, or remotely situated fibroids. While a laparoscopic myomectomy requires but a few days (at most) for post-operative convalescence, abdominal myomectomy usually requires 6-8 weeks of recovery time. When a myomectomy necessitates or results in the uterine cavity being entered (purposefully or inadvertently), a second-look hysteroscopy to rule out scar tissue formation may be considered in certain cases.

Uterine polyps (and in some cases, also submucosal fibroids), can often be removed hysteroscopically (through the

vagina). This eliminates the need for abdominal surgery and greatly reduces the recovery time. Hysteroscopic surgery is only useful if most of the fibroid protrudes into the endometrial cavity, ensuring that the tumor defect will not be too large. This surgery is often done under laparoscopic guidance, to reduce the risk of uterine perforation. After hysteroscopic surgery it is often advisable to prescribe cyclical hormonal therapy for a few months to encourage regeneration of the endometrial lining over the area of the tumor defect and healing of the uterine muscle. A 2nd look hysteroscopy should be performed a few months later in all cases, to rule out scar tissue formation even if it means delaying or deferring the initiation of definitive fertility treatment.

Medical treatment: The growth of fibroid tumors is estrogen dependent. Thus, when a woman enters the menopause and stops making female hormones, fibroids tend to shrink in size on their own. Conditions that mimic the menopause can also reduce the size of fibroid tumors. The most common of these treatments is with a medication such as leuprolide acetate (Lupron), which shuts off the communication of the brain with the ovaries, preventing hormone production. However, this type of medication can only be taken for a limited period (usually 6 months) and once the medication is stopped the fibroids will usually regain their original size within a few months. The medication is therefore only a "temporary fix" used mostly to decrease the size of large fibroids to make their ultimate surgical removal easier or to help a woman bridge the gap until spontaneous menopause sets in. For most women, there is no major benefit from Lupron therapy prior to surgery.

"**Embolization":** A myomectomy always carries the small risk of severe, uncontrollable intra-operative bleeding mandating the performance of a hysterectomy (complete removal of the uterus), as a life-saving measure, might be required. Moreover,

some women are poor surgical candidates for surgery. This is where a procedure known as "embolization" is beneficial. Embolization is a procedure in which small particles are injected into the arteries of the uterus under radiological guidance to shut off the blood supply to the fibroids, in the hope that they will "shrink" and perhaps even, disappear.

Embolization is relatively new to the field of gynecology, and little is known about its potential effects on future fertility. We are concerned that in the process of shutting off the blood supply to the uterus, it will permanently so reduce endometrial blood flow to compromise embryo implantation. There is also a known risk of collateral damage to the ovaries with subsequent DOR. For this reason, we currently do not recommend this therapy for women who still wish to conceive and carry a gestation in their uterus. At present, it seems best suited for symptomatic women who are finished with their childbearing or who are planning to use a gestational surrogate.

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POLYCYSTIC OVARIAN SYNDROME (PCOS) AND IVF

In the mid-1930's, Stein and Leventhal described a syndrome characterized by irregular or absent ovulation, amenorrhea (absent menstruation), obesity, short stature, an increase in body hair growth (hirsutism), acne, infertility, and evidence of slightly enlarged, "glistening white ovaries that contained numerous small cysts under the capsule". It subsequently became evident that infertility occurring in women with "polycystic ovaries" often occurs unaccompanied by many of the typical features of the so called "Stein-Leventhal Syndrome", leading to the condition being renamed polycystic

ovarian syndrome (PCOS). Women with PCOS often have insulin resistance, and/or a family history of diabetes mellitus. Women with PCOS also have a slightly increased risk of developing endometrial or ovarian cancer in later life, thus necessitating vigilance through regular annual examinations. Some enzymatic deficiencies (especially 21-hydroxylase deficiency) can create a syndrome that phenotypically mimics PCOS with symptoms of hyperandrogenism and anovulation. These syndromes create what is known as congenital adrenal hyperplasia (CAH). Here androgens such as testosterone and/or androstenedione are often also raised but here, the levels of dehydroepiandrosterone (DHEAS) and 17-hydroxyprogesterone are also raised, confirming the adrenal origin of this androgen.

Treating the anovulation/infertility associated with PCOS.

- PCOS patients, especially when obese, tend to have insulin resistance. Their ovulation/fertility issues have been shown to frequently respond to three months or longer of treatment with the insulin-sensitizing medication known as metformin.
- Anovulation related Infertility is most often treated with either oral or injectable ovarian stimulation medications (clomiphene, letrozole, gonadotropins.)
- Now for the most part considered an obsolete option, in isolated, selected cases, surgical "ovarian drilling" with cautery or laser of the ovarian cortex has been effectively utilized in PCOS with severely polycystic ovaries. However, the benefit is temporary and may diminish ovarian longevity.
- Congenital Adrenal Hyperplasia (CAH): Some enzymatic deficiencies (especially 21-hydroxylase deficiency) can create a syndrome of hyperandrogenism and

anovulation that mimic PCOS and may be considered by some to be an adrenal form of PCOS. Here, in addition to other androgens, the level of dehydroepiandrosterone (DHEAS) and 17-hydroxyprogesterone is also raised, confirming the adrenal origin of this androgen. CAH is treated with steroids such as prednisone or dexamethasone which will suppress adrenal androgen production, allowing regular ovulation to take place spontaneously.

Severe Ovarian Hyperstimulation Syndrome (OHSS): As previously discussed, there is an inevitable tendency for women with PCOS to hyper-respond to gonadotropin fertility drugs and in the process produce large numbers of ovarian follicles. If left unchecked this can lead to OHSS, a potentially life endangering condition. The key to OHSS is prevention and requires careful monitoring of each patient for signs of hyper-responsiveness.

Poor egg/embryo quality in PCOS patients: Women with PCOS are at risk to yield poorly developed ("dysmature") eggs at the time of egg retrieval. However, contrary to popular belief, this is not due to an intrinsic deficit in egg quality. Rather, the tendency on the part of PCOS women to produce eggs that have reduced fertilization potential, and poor-quality embryos relates to intra-ovarian hormonal changes brought about by hyperstimulation. This can often be significantly reduced through an individualized and selective approach to ovarian stimulation with gonadotropins that avoids overexposure of the developing follicles and eggs to an excessive amount of LH-induced androgens.

The detection of large numbers of developing ovarian follicles and rapidly rising plasma estradiol levels heralds the onset of OHSS. In such cases, the fear of impending OHSS often leads the treating physician to prematurely administer hCG to

abruptly arrest further follicle growth. However, the premature administration of hCG unfortunately also initiates meiosis (the final maturational process) in the developing egg prematurely. Since the ability of an egg to achieve optimal maturation upon hCG triggering is largely predicated upon it having achieved prior optimal developmental potential, the untimely administration of hCG often precipitates disorderly separation of the chromosomes during meiosis, resulting in structural and/or numerical chromosomal abnormalities (aneuploidy) of the egg. This leads to reduced fertilization potential, poor egg/embryo quality and low birth rates.

Another consideration is the fact that in PCOS the ovarian stroma (connective tissue that surrounds the follicles) is often characteristically overgrown (stromal hyperplasia), leading to an excessive production of androgen hormones. Since LH promotes stromal androgen production and many women with PCOS have increased pituitary production of LH, it should come as little surprise that such women often have high levels of androgens (predominantly, testosterone) in their ovarian and uterine blood systems. Excessive exposure to ovarian androgens compromises follicle and egg growth and development and compromises endometrial growth and development by down-regulating estrogen receptors in the uterine lining (endometrium).

The obvious remedy for these adverse effects on egg and endometrial development is to employ stimulation protocols that limit ovarian overexposure to LH while safely affording sufficient time for optimal follicle/egg development to occur prior to initiating the hCG trigger. This requires a reliable method of protecting the PCOS patient from the associated risks of OHSS while allowing the time necessary for the follicles/eggs to develop optimally, prior to administering hCG. Using a judiciously low amount of ovarian stimulatory medications, and

incorporating "prolonged coasting" as needed, can go a long way in this regard.

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OPTIMIZING EMBRYO QUALITY

There is an unfortunate tendency to place the blame for "poor quality embryos" on the embryology laboratory. The truth of the matter is that while the IVF embryology laboratory plays a pivotal role in achieving optimal fertilization and embryo quality, no embryologist, regardless of expertise, can produce "good quality embryos" from "poor quality eggs". Accordingly, to optimize egg quality, it is necessary for ART physicians to tailor the protocol for ovarian stimulation to meet the individual needs of each patient. Notwithstanding the above, it is important to recognize that "poor egg/embryo quality", although an important cause of IVF failure, is by no means the only one.

The potential of a woman's eggs to undergo orderly maturation, successful fertilization, and subsequent progression to "good quality embryos" that are capable of producing healthy offspring, is in large part, genetically predetermined. However, the expression of such potential is profoundly susceptible to numerous extrinsic influences, especially to intra-ovarian hormonal changes during the pre-ovulatory phase of the cycle.

FACTORS POINTING TO POOR EGG/EMBRYO QUALITY

- Failure after >2 consecutive IVF attempts.

- The egg provider is >39y of age

- History of "unexplained infertility"

- History of exposure to radiation or cancer chemotherapy.

- Failed IVF associated with:

  o A high percentage (more than 50%) of the eggs are classified as being immature (MI or germinal vesicles) in at least two consecutive IVF attempts.

  o Poor fertilization rates (less than 50% of mature eggs) occurring in at least two consecutive IVF attempts. A high percentage (more than 20%) of the non-cleaved embryos (i.e., zygotes) contain three or more pronuclei (i.e., polyspermia) in at least two consecutive IVF attempts.

  o A large percentage (more than 50%) of eggs are morphologically abnormal (i.e., misshapen, granular, dark, vacuolated, etc.) in at least two consecutive IVF attempts. More than 50% of the embryos (as assessed three days following fertilization) are morphologically deficient (i.e., comprise less than seven cells, have discordant cells, embryos contain more than 20% fragmentation, or the embryos are dark in appearance) in at least two consecutive IVF attempts.

  o Failure to produce blastocysts, or producing blastocysts that are all chromosomally abnormal by virtue of PGS/PGT-A. Of course, this will occur with ever greater frequency as a woman ages and is not technically an unexpected or abnormal finding in women in their mid-40s.

FACTORS THAT INFLUENCE EGG/EMBRYO QUALITY

The age of the egg provider: A woman is born with all the eggs she will ever have. After menarche (the age at which menstruation starts), a monthly process of using up numerous eggs continues until the menopause, when most of her eggs have been used up, and both ovulation and menstruation cease. When the number of eggs remaining in the ovaries falls below a certain threshold, ovarian function begins to wane rapidly over a 5-to-10-year period, referred to as the climacteric. With the onset of the climacteric, plasma follicle stimulating hormone (FSH) levels begin to rise and AMH concentrations fall and growing ovarian resistance to gonadotropins develops. Simultaneously, symptoms such as hot flashes, vaginal dryness and weight gain emerge and then progressively worsen the closer the woman gets to the menopause. While the age of onset of both the climacteric and the menopause is largely genetically predetermined (and may vary from woman to woman), other factors, such as exposure to environmental toxins, radiation, systemic and pelvic pathology, or surgery that compromises ovarian blood flow, can influence the timing of both events. Most American women will enter the climacteric in there early to mid -forties and enter the menopause around age 51.

Egg quality progressively declines after 35 years of age. A normal consequence of progressive aging is that an ever-increasing number of a woman's egg quota will, upon fertilization, have an abnormal chromosome number and/or structure (i.e., aneuploidy). As an example, approximately 30% of embryos derived from the eggs of a <35-year-old woman are likely to be aneuploid as compared with about 60-70% for a 40-year-old, 75% at 43 years and 95% at 45 years. While most aneuploid embryos often grade as "poor quality" on microscopic examination, it is quite common for many such embryos to have

a normal microscopic appearance. It is accordingly not possible to identify all aneuploid embryos by morphologic criteria alone. Therefore PGS/PGT-A testing has risen to prominence in that it can with great sensitivity detect chromosomal abnormalities in even "pretty" embryos.

When couples try to conceive naturally, there is a process of natural selection that prevents most aneuploid embryos from attaching to the uterine lining (i.e., "failed implantation"). Those that inadvertently attach, usually do so for such a brief period of time that the woman, in most cases, is unaware of the fact that she actually conceived. Infrequently, an aneuploid embryo will attach for a period of days or weeks only to be "expelled" or miscarried. It is only on rare occasions, that an implanted aneuploid embryo will develop into a viable fetus (e.g., some cases of Down syndrome). The increased prevalence of embryo aneuploidy with advancing age also serves to clarify why the incidence of "infertility", miscarriage, and chromosomal birth defects increases as women get older and why infertility treatment becomes less effective.

The misconception that an elevated FSH level is a cause of "poor quality" eggs/embryos arises from the fact that both commonly occur together during the climacteric. The young women who prematurely enter the climacteric commonly produce "good quality" eggs and embryos at IVF (albeit the number of both is likely to be low). Conversely, while older women (i.e., in their mid-forties) with normal FSH and AMH routinely yield surprisingly large number of eggs/embryos, these remain highly susceptible to quality-related deficiencies. Simply stated, it is age and not ovarian reserve that influences egg/embryo quality.

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EMBRYO IMPLANTATION DYSFUNCTION

Implantation dysfunction is unfortunately often overlooked as an important cause of IVF failure. This is especially relevant in cases of unexplained IVF failure, recurrent pregnancy loss (RPL), and in women with underlying endo-uterine surface lesions, thickness of the uterine lining (endometrium) and /or immunologic factors.

IVF success rates have been improving over the last decade. The average live birth rate per embryo transfer in the U.S.A for women under 40y using their own eggs is currently better than 1:3 women. However, there is still a wide variation from program to program for IVF live birth rates, ranging from 20% to near 50%. Based upon these statistics, most women undergoing IVF in the United States require two or more attempts to have a baby. IVF practitioners in the United States commonly attribute the wide dichotomy in IVF success rates to variability in expertise of the various embryology laboratories. This is far from accurate. In fact, other factors such as wide variations in patient selection and the failure to develop individualized protocols for ovarian stimulation or to address those infectious, anatomical, and immunologic factors that influence embryo implantation are at least equally important.

About 80% of IVF failures are due to "embryo incompetency" that is largely due to aneuploidy   usually related to advancing age of the woman and is further influenced by other factors such as the protocol selected for ovarian stimulation, diminished ovarian reserve (DOR), and severe male factor infertility. However, in about 20% of dysfunctional cases embryo implantation is the cause of failure.

This section will focus on implantation dysfunction and IVF failure due to:

ANATOMICAL ENDO-UTERINE SURFACE LESIONS

It has long been suspected that anatomical defects of the uterus might result in infertility. While the presence of uterine fibroids, in general, are unlikely to cause infertility, an association between their presence and infertility has been observed in cases where the myomas distort the uterine cavity or protrude through the endometrial lining. Even small fibroids that lie immediately under the endometrium (submucous fibroids) and protrude into the uterine cavity have the potential to lower embryo implantation. Multiple fibroids in the uterine wall (intramural fibroids) that encroach upon the uterine cavity can sometimes so compromise blood flow that estrogen delivery is impaired, and the endometrium is unable to thicken properly. This can usually be diagnosed by ultrasound examination during the proliferative phase of the menstrual cycle. It is likely that any surface lesion in the uterine cavity, whether submucous fibroids, intrauterine adhesions a small endometrial or a placental polyp, has the potential to interfere with implantation by producing a local inflammatory response, not too dissimilar in nature from that which is caused by an intrauterine contraceptive device (IUD).

Clearly, since even small uterine lesions have the potential to adversely affect implantation, the high cost (financial, physical, and emotional) associated with IVF and related procedures, justifies the routine performance of diagnostic procedures such as an HSG, hysterosonogram (fluid ultrasound examination), or hysteroscopy prior to initiating IVF. Identifiable uterine lesions that have the potential of impairing implantation usually require surgical intervention. In most cases, dilatation and curettage (D & C) or hysteroscopic resection will suffice. Some cases might require the performance of a laparotomy. Such intervention will often result in subsequent improvement of the endometrial response.

Sonohysterography [Fluid ultrasonography (FUS)]: Fluid ultrasonography is a procedure whereby a sterile solution of saline is injected via a catheter through the cervix and into the uterine cavity. The fluid-distended cavity is examined by vaginal ultrasound for any irregularities that might point to surface lesions such as polyps, fibroid tumors, scarring, or a uterine septum. If performed by an expert, a FUS is highly effective in recognizing even the smallest lesion and can replace hysteroscopy under such circumstances. FUS is less expensive, less traumatic, and equally as effective as hysteroscopy. The only disadvantage lies in the fact that if a lesion is detected, it may require the subsequent performance of hysteroscopy to treat the problem anyway.

Hysteroscopy: Diagnostic hysteroscopy is an office procedure that is performed under intravenous sedation, general anesthesia, or paracervical block with minimal discomfort to the patient. This procedure involves the insertion of a thin, lighted, telescope like instrument known as a hysteroscope through the vagina and cervix into the uterus to fully examine the uterine cavity. The uterus is first distended with normal saline, which is passed through a sleeve adjacent to the hysteroscope. As is the case with FUS, diagnostic hysteroscopy facilitates examination of the inside of the uterus under direct vision for defects that might interfere with implantation. We have observed that approximately one in eight candidates for IVF have lesions that require attention prior to undergoing IVF in order to optimize the chances of a successful outcome. We strongly recommend that all patients undergo therapeutic surgery (usually by hysteroscopy) to correct the pathology prior to IVF. Depending on the severity and nature of the pathology, therapeutic hysteroscopy may require general anesthesia and, in such cases, should be performed in an outpatient surgical facility or conventional operating room where facilities are available for laparotomy, a procedure in which an incision is made in the

abdomen to expose the abdominal contents for diagnosis, or for surgery should this be required.

THICKNESS OF THE UTERINE LINING (ENDOMETRIUM):

As far back as in 1989 we first reported on the finding that ultrasound assessment of the late proliferative phase endometrium can identify those candidates who are least likely to conceive. We noted that the ideal thickness of the endometrium at the time of ovulation or egg retrieval is >8 mm and that thinner linings are associated with decreased implantation rates.

More than 30 years ago we first showed that in normal and "stimulated" cycles, pre-ovulatory endometrial thickness and ultrasound appearance is predictive of embryo implantation (pregnancy) potential following ET. With conventional IVF and with FET, endometrial lining at the time of the "trigger shot" or with the initiation of progesterone needs to preferably be at least 8 mm in sagittal thickness with a triple line (trilaminar) appearance. Anything less than an 8mm endometrial thickness is associated with a reduction in live birth rate per ET. An 8-9mm thickness represents a transitional measurement…a "gray zone". Hitherto, attempts to augment endometrial growth in women with poor endometrial linings by bolstering circulating estrogen blood levels (through the administration of increased doses of fertility drugs, aspirin administration and by supplementary estrogen therapy) yielded disappointing results.

A "poor" uterine lining is usually the result of the innermost layer of endometrium (the basal or germinal endometrium from which endometrium grows) not being able to respond to estrogen by propagating an outer, "functional" layer thick enough to support optimal embryo implantation and development of a healthy placenta (placentation). The "functional" layer ultimately comprises 2/3 of the full

endometrial thickness and is the layer that sheds with menstruation if no pregnancy occurs.

The main causes of a "poor" uterine lining are:

- ❖ Damage to the basal endometrium because of:
  - *Inflammation of the endometrium (endometritis) most commonly resulting from infected products left over following abortion, miscarriage, or birth*
  - *Surgical trauma due to traumatic uterine scraping, (i.e. due to an over-aggressive D & C)*
  - *Insensitivity of the basal endometrium to estrogen due to:*
  - *Prolonged, over-use/misuse of clomiphene citrate*
- ❖ Prenatal exposure to diethylstilbestrol (DES). This is a drug that was given to pregnant women in the 1960's to help prevent miscarriage
- ❖ Over-exposure of the uterine lining to ovarian male hormones (mainly testosterone): Older women, women with diminished ovarian reserve (poor responders) and women with polycystic ovarian syndrome -PCOS tend to have raised LH biological activity. This causes the connective tissue in the ovary (stroma/theca) to overproduce testosterone. The effect may be further exaggerated when certain methods for ovarian stimulation such as "flare" protocols and high dosages of Menopur are used in such cases.
- ❖ Reduced blood flow to the basal endometrium: Examples include.
- ❖ Multiple uterine fibroids - especially when these are present under the endometrium (submucosal)
- ❖ Uterine adenomyosis (excessive, abnormal invasion of the uterine muscle by endometrial glands).

Vaginal Viagra: About 35 years ago, after reporting on the benefit of administering vaginal Sildenafil (Viagra) to women who had implantation dysfunction due to thin endometrial linings we announced the birth of the world's first "Viagra baby." Viagra administered vaginally, but not orally, in affected women improves uterine blood flow causing more estrogen to be delivered to the basal endometrium and increasing the endometrial thickening. Following vaginal administration, Viagra is rapidly absorbed and quickly reaches the uterine blood system in high concentrations. Thereupon it dilutes out as it is absorbed into the systemic circulation. This probably explains why treatment is virtually devoid of systemic side effects. It is important to recognize that Viagra will NOT be effective in improving endometrial thickness in all cases. In fact, about one third of women treated fail to show any improvement. This is because in certain cases of thin uterine linings, the basal endometrium will have been permanently damaged and left unresponsive to estrogen. This happens in cases of severe endometrial damage due mainly to post-pregnancy endometritis (inflammation), chronic granulomatous inflammation due to uterine tuberculosis (hardly ever seen in the United States) and following extensive surgical injury to the basal endometrium (as sometimes occurs following over-zealous D&C's).

- **Immunologic factors**: These also play a role in IVF failure (see "Immunologic factors and Implantation" …see below.

IMMUNOLOGIC IMPLANTATION DYSFUNCTION (IID)

Currently, with few exceptions, practitioners of assisted reproduction tend to attribute "unexplained and/or repeated" IVF failure(s), almost exclusively to poor embryo quality, advocating adjusted protocols for ovarian stimulation and/or gamete and embryo preparation as a potential remedy. The idea,

having failed IVF, that all it takes to ultimately succeed is to keep trying the same recipe is over-simplistic.

The implantation process begins six or seven days after fertilization of the egg. At this time, specialized embryonic cells (i.e., trophoblasts), that later become the placenta begin growing into the uterine lining. When the trophoblast and the uterine lining meet, they, along with immune cells in the lining, become involved in a "cross talk" through mutual exchange of hormone-like substances called cytokines. Because of this complex immunologic interplay, the uterus can foster the embryo's successful growth. Thus, from the earliest stage, the trophoblast establishes the very foundation for the nutritional, hormonal and respiratory interchange between mother and baby. In this manner, the interactive process of implantation is not only central to survival in early pregnancy but also to the quality of life after birth.

There is an ever growing realization, recognition, and acceptance of the fact that uterine immunologic dysfunction can lead to immunologic implantation dysfunction (IID) with "unexplained" infertility, IVF failure, and recurrent pregnancy loss (RPL).

DIAGNOSIS

Because immunologic problems may lead to implantation failure, it is important to properly evaluate women with risk factors such as:

- Unexplained or recurrent IVF failures

- Unexplained infertility or a family history of autoimmune diseases (e.g., rheumatoid arthritis, lupus erythematosus and hypothyroidism).

- Recurrent Pregnancy Loss (RPL)

- Endometriosis

- A personal or family history of autoimmune conditions, e.g., Rheumatoid Arthritis, Lupus erythematosus, autoimmune hypothyroidism (Hashimoto's disease) etc.

Considering its importance, it is not surprising that the failure of a properly functioning immunologic interaction during implantation has been implicated as a cause of recurrent miscarriage, late pregnancy fetal loss, IVF failure and infertility. A partial list of immunologic factors that may be involved in these situations includes:

- Antiphospholipid antibodies (APA)

- Antithyroid antibodies (ATA/AMA)

- Activated natural killer cells (NKa)

ACTIVATED NATURAL KILLER CELLS (NKa):

Following ovulation and during early pregnancy, NK cells and T-cells comprise more than 80% of the lymphocyte-immune cells that frequent the uterine lining. These lymphocytes (white blood cells) journey from the bone marrow to the uterus and under hormonal regulation, proliferate there. After exposure to progesterone (due to induced /spontaneous exogenous administration), they begin to produce TH-1 and TH-2 cytokines. TH-2 cytokines are humoral in nature and induce the trophoblast ("root system of the embryo") to permeate the uterine lining while TH-I cytokines induce a process referred to as apoptosis (cell suicide) thereby confining placental development to the inner part of the uterus. Optimal placental development (placentation) mandates that there be a balance between TH1 and TH-2 cytokines. Most of the cytokine production originates from NK cells (rather than from cytotoxic T-cells/Lymphocytes (CTL)). Excessive production/release of TH-1 cytokines, is toxic to

the trophoblast and to endometrial cells, leading to programmed death/suicide (apoptosis) and subsequently to IID.

Functional NK cells reach a maximal concentration in the endometrium by about t day 6-7 days after exposure to progesterone …. This timing corresponds with when the embryo implants into the uterine lining (endometrium).

It is important to bear in mind that measurement of the concentration of blood NK cells has little or no relevance when it comes to assessing NK cell activation (NKa). Rather, it is the NK cell activation that matters. In fact, there are certain conditions (such as with endometriosis) where the NK cell blood concentration is below normal, but NK cell activation is markedly increased.

There are several methods by which NK cell activation (cytotoxicity) can be assessed in the laboratory. Methods such as immunohistochemical assessment of uterine NK cells and/or through measurement of uterine or blood TH-1 cytokines. However, the *K-562 target cell blood test* still remains the gold standard. With this test, NK cells, isolated from the woman's blood using Flow Cytometry are incubated in the presence of specific "target cells". The percentage (%) of "target cells" killed is then quantified. More than 12% killing suggests a level of NK cell activation that usually requires treatment.

Currently, there are less than a half dozen Reproductive Immunology Reference Laboratories in the U.S.A that are capable of performing the K-562 target cell test reliably.

There exists a pervasive but blatant misconception on the part of many, that the addition of IL or IVIg to a concentration of NK cells could have an immediate down-regulatory effect on NK cell activity. Neither IVIg nor IL is capable of significantly suppressing already activated "functional NK cells". They are believed to work through "regulating" NK cell progenitors which only thereupon

will start to propagate down-regulated NK cells. Thus, testing for a therapeutic effect would require that the IL/IVIg infusion be done about 14 days prior to ovulation or progesterone administration… in order to allow for a sufficient number of normal (non-activated) "functional" NK cell" to be present at the implantation site when the embryos are transferred.

Failure to recognize this reality has, in our opinion, established an erroneous demand by practicing IVF doctors, that Reproductive Immunology Reference Laboratories report on NK cell activity before and again, immediately following laboratory exposure to IVIg and/or IL in different concentrations. Allegedly, this is to allow the treating physician to report back to their patient(s) on whether an IL or IVIG infusion will be effective in downregulating their Nka. But, since already activated NK cells (NKa) cannot be deactivated in the laboratory, effective NKa down-regulation can only be adequately accomplished through deactivation of NK cell "progenitors /parental" NK cells in order to allow them thereupon, to s propagate normal "functional" NK cells and his takes about 10-14 days, such practice would be of little clinical benefit. This is because even if blood were to be drawn 10 -14 days after IL/IVIg treatment it would require at least an additional 10 -14days to receive results from the laboratory, by which time it would be far too late to be of practical advantage.

ANTIPHOSPHOLIPID ANTIBODIES:

Many women who experience "unexplained" IVF failure, women with RPL, those with a personal or family history of autoimmune diseases such as lupus erythematosus, rheumatoid arthritis, scleroderma, and dermatomyositis (etc.) as well as women who have endometriosis ("silent" or overt) test positive for APAs. More than 30 years ago, we were the first to propose that women who test positive for APA's be treated with a mini-dose heparin to improve IVF implantation and thus birth rates. This

approach was based upon research that suggested that heparin repels APAs from the surface of the trophoblast (the embryo's "root system) thereby reducing its ant-implantation effects. We subsequently demonstrated that such therapy only improved IVF outcome in women whose APAs were directed against two specific IgG and/or IgM phospholipids [i.e., phosphatidylethanolamine (PE) and phosphatidylserine (PS)]. More recently low dosage heparin therapy has been supplanted using longer acting low molecular weight heparinoids such as Lovenox and Clexane. It is very possible that APAs alone do not cause IID but that their presence might help to identify a population at risk due to concomitant activation of uterine natural killer cells (Nka) which through excessive TH-1 cytokine production causes in IID: This is supported by the following observations:

- The presence of female APAs in cases of male factor cases appears to bear no relationship to IID.
- Only APA positive women who also test positive for abnormal NK activity appear to benefit from selective immunotherapy with intralipid/IVIg/ steroids.
- Most APA positive women who have increased NK cell activity also harbor IgG or IgM phosphatidylethenolamine (PE) and phosphatidylserine (PS) antibodies.

ANTITHYROID ANTIBODIES: (ATA).

A clear relationship has been established between ATA and reproductive failure (especially recurrent miscarriage and infertility).

Between 2% and 5% of women of the childbearing age have reduced thyroid hormone activity (hypothyroidism). Women with hypothyroidism often manifest with reproductive failure i.e., infertility, unexplained (often repeated) IVF failure, or

recurrent pregnancy loss (RPL). The condition is 5-10 times more common in women than in men. In most cases hypothyroidism is caused by damage to the thyroid gland resulting from of thyroid autoimmunity (Hashimoto's disease) caused by damage done to the thyroid gland by antithyroglobulin and antimicrosomal auto-antibodies.

The increased prevalence of hypothyroidism and thyroid autoimmunity (TAI) in women is likely the result of a combination of genetic factors, estrogen-related effects, and chromosome X abnormalities. This having been said, there is significantly increased incidence of thyroid antibodies in non-pregnant women with a history of infertility and recurrent pregnancy loss and thyroid antibodies [can] be present asymptomatically in women without them manifesting with overt clinical or endocrinologic evidence of thyroid disease. In addition, these antibodies may persist in women who have suffered from hyper- or hypothyroidism even after normalization of their thyroid function by appropriate pharmacological treatment. The manifestations of reproductive dysfunction thus seem to be linked more to the presence of thyroid autoimmunity (TAI) than to clinical existence of hypothyroidism and treatment of the latter does not routinely result in a subsequent improvement in reproductive performance.

It follows, that if antithyroid autoantibodies are associated with reproductive dysfunction they may serve as useful markers for predicting poor outcome in patients undergoing assisted reproductive technologies.

Some years back, I reported on the fact that 47% of women who harbor thyroid autoantibodies, regardless of the absence or presence of clinical hypothyroidism, have activated uterine natural killer cells (NKa) cells and cytotoxic lymphocytes (CTL) and that such women often present with reproductive dysfunction. We demonstrated that appropriate immunotherapy with IVIG or intralipid (IL) and steroids, subsequently often results in a

significant improvement in reproductive performance in such cases.

The fact that almost 50% of women who harbor antithyroid antibodies do not have activated CTL/NK cells suggests that it is NOT the antithyroid antibodies themselves that cause reproductive dysfunction. The activation of CTL and NK cells that occurs in half of the cases with TAI is probably an epiphenomenon with the associated reproductive dysfunction being due to CTL/NK cell activation that damages the early "root system" (trophoblast) of the implanting embryo. We have shown that treatment of those women who have thyroid antibodies + NKa/CTL using IL/steroids, improves subsequent reproductive performance while women with thyroid antibodies who do not harbor NKa/CTL do not require or benefit from such treatment

TREATMENT OF IID:

The mainstay of treatment involves the selective use of:

- Intralipid (IL) infusion
- IVIg therapy
- Corticosteroids (Prednisone/dexamethasone)
- Heparinoids (Lovenox/Clexane)

Intralipid (IL) Therapy: IL is a suspension of soybean lipid droplets in water and is primarily used as source of parenteral nutrition. When administered intravenously, IL provides essential fatty acids, linoleic acid (LA), an omega-6 fatty acid, and alpha-linolenic acid (ALA), an omega-3 fatty acid.

It is thought that fatty acids within the emulsion serve as ligands that activate peroxisome proliferator-activated receptors (PPARs) expressed by the NK cells. This is believed to decrease NK cell cytotoxic activity, and thereby enhance implantation A growing number of IVF programs, including ours, perform egg

retrieval under conscious sedation using Propofol, a short acting hypnotic agent.

Whatever the exact mechanism of action might be, Intralipid acts primarily to suppress NK cell over-production of TH-I cytokines. It exerts a modulating effect on certain immune cellular mechanisms largely by down-regulating cytotoxic /activated natural killer cells (NKa). This effect is enhanced through the concomitant administration of corticosteroids such as dexamethasone, prednisolone and prednisone which augment immune modulation of T cells. The combined effect of IL + steroid therapy suppresses pro-inflammatory cellular TH1 cytokines such as interferon gamma and TNF-alpha that are produced in excess by activated NK cells and cytotoxic lymphocytes/T-cells (CTL). IL will, in about 80% of cases, successfully down-regulate activated natural killer cells (NKa) over a period of 2-3 weeks. It is likely to be just as effective as IVIg in this respect but at a fraction of the cost and with a far lower incidence of side-effects. Its effect lasts for ~ 4-6 weeks when administered in early pregnancy.

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Can laboratory testing be used to assess for an immediate effect of IL on Nka suppression?   Since the downregulation of NKa through IL (or IVIg) therapy can take several weeks to become measurable, it follows that there is really no benefit in trying to assess the potential efficacy of such treatment by retesting NKa in the laboratory after adding IL (or IVIg) to the cells being tested.

IVIg Therapy: Until about a decade ago, the only effective and available way (in the US) to down-regulate activated NK cells was through the intravenous administration of a blood product known as immunoglobulin-G (IVIg). The fear (albeit unfounded) that the administration of this product might lead to the transmission of viral infections such as HIV and hepatitis C, plus the high cost of IVIG along with the fact that significant side effects occurred about 20% of the time, led to bad press and bad publicity for the entire field of reproductive immunology. It was easier for RE's to simply say "I don't believe IVIg works" and thereby avoid risk and bad publicity. But the thousands of women who had babies because of NK cell activity being downregulated through its use, attests to IVIg's efficacy. But those of us who felt morally obligated to many desperate patients who would not conceive without receiving IVIg were facing an uphill battle. The bad press caused by fear mongering took its toll and spawned a

malicious controversy. It was only through the introduction of IL less (about 15-20 years ago), that the tide began to turn in favor of those patients who required low cost, safe and effective immunotherapy to resolve their IID.

Corticosteroid Therapy (e.g., Prednisone, and Dexamethasone): Corticosteroid therapy has become a mainstay in the treatment of most women undergoing IVF. It is believed by most to enhance implantation due to an overall immunomodulatory effect. Corticosteroids reduce TH-1 cytokine production by CTL. When given in combination with IL or IVIG they augment the implantation process. The prednisone or dexamethasone therapy must commence (along with IL/IVIg) 10-14 days prior to egg retrieval and continue until pregnancy is discounted or until the 10th week of pregnancy.

Heparinoid Therapy: There is compelling evidence that the subcutaneous administration of low molecular heparin (Clexane, Lovenox) once daily, (starting with the onset of ovarian stimulation) can improve IVF birthrate in women who test positive for APAs and might prevent later pregnancy loss when used to treat certain thrombophilias (e.g., homozygous MTHFR mutation)

What About Baby Aspirin? In our opinion, aspirin has little (if any) value when it comes to IID, and besides, it could even reduce the chance of success. The reason for this is that aspirin thins the blood and increases the potential to bleed. This effect can last for up to a week and could complicate an egg retrieval procedure or result in "concealed" intrauterine bleeding at the time of embryo transfer, thereby potentially compromising IVF success.

TH-1 Cytokine Blockers (Enbrel, Humira): TH-1 cytokine blockers, (Enbrel and Humira) are in our opinion relatively ineffective in the IVF setting. There has to date been no convincing data to support their use. However, these blockers could have a role in the treatment of a threatened miscarriage thought to be due to CTL/NK activation, but not for IVF. The

reason is that the very initial phase of implantation requires a cellular response involving TH-1 cytokines. To block them completely (rather than simply restore a TH-1:TH-2 balance as occurs with IL therapy) so very early on could compromise rather than benefit implantation.

Leukocyte Immunization Therapy (LIT)*:* The subcutaneous injection of the male partner's lymphocytes to the mother is thought to enhance the ability for the mother's decidua (uterus) to recognize the DQ alpha matching embryo as "self" or "friend" and thereby avert its rejection. LIT has been shown to up-regulate Treg cells and thus down-regulate NK cell activation thereby improving decidual TH-1:TH-2 balance. Thus, there could be a therapeutic benefit from such therapy. However, the same benefit can be achieved through the use of IL plus corticosteroids. Besides, IL is much less expensive, and the use of LIT is prohibited by law in the U.S.A.

There are two categories of immunologic implantation dysfunction (IID) linked to NK cell activation (NKa).

Autoimmune Implantation Dysfunction: Here, the woman will often have a personal or family history of autoimmune conditions such as Rheumatoid arthritis, Lupus Erythematosus, and thyroid autoimmune activity (e.g., Hashimoto's disease) etc. Autoimmune as well as in about one third of cases of endometriosis, regardless of severity. Autoimmune sometimes also occurs in the absence of a personal or family history of autoimmune disease.

When it comes to treating NKa in IVF cases complicated by autoimmune implantation dysfunction, the combination of daily oral dexamethasone commencing with the onset of ovarian stimulation and continuing until the 10th week of pregnancy, combined with an initial infusion of IL (100ml, 20% Il dissolved in

500cc of saline solution, 10-14 days prior to PGT-normal embryo transfer and repeated once more (only), as soon as the blood pregnancy test is positive), the anticipated chance of a viable pregnancy occurring within 2 completed IVF attempts (including fresh + frozen ET's) in women under 39Y (who have normal ovarian reserve) is approximately 65%.

Alloimmune Implantation Dysfunction: Here, NK cell activation results from uterine exposure to an embryo derived through fertilization by a spermatozoon that shares certain genotypic (HLA/DQ alpha) similarities with that of the embryo recipient.

Partial DQ alpha/HLA match: Couples who upon genotyping are shown to share only one DQ alpha/HLA gene are labeled as having a "partial match". The detection of a "partial match" in association with NKa puts the couple at a considerable disadvantage with regard to IVF outcome. It should be emphasized however, that in the absence of associated Nka, DQ alpha/HLA matching whether "partial" or "total (see below) will NOT cause an IID. Since we presently have no way of determining which embryo carries a matching paternal DQ alpha gene, it follows that each embryo transferred will have about half the chance of propagating a viable pregnancy. Treatment of a partial DQ alpha/HLA match (+ Nka) involves the same IL, infusion as for autoimmune-Nka with one important caveat, namely that here we prescribe oral prednisone as adjunct therapy (rather than dexamethasone) and the IL infusion is repeated every 2-4 weeks following the diagnosis of pregnancy and continued until the 24th week of gestation. Additionally, (as alluded to elsewhere) in such cases we transfer a single (1) embryo at a time. This is because, the likelihood is that one out of two embryos will "match" and we are fearful that if we transfer >1 embryo, and one transferred embryos "matches" it could cause further activation of uterine NK cells and so prejudice the implantation of all transferred embryos. Here it should be emphasized that if

associated with Nka, a matching embryo will still be at risk of rejection even in the presence of Intralipid (or IVIg) therapy.

Total (complete) DQ alpha Match: Here the husband's DQ alpha genotype matches both of that of his partner's. While this occurs very infrequently, a total alloimmune (DQ alpha) match with accompanying Nka, means that the chance of a viable pregnancy resulting in a live birth at term, is unfortunately greatly diminished. Several instances in our experience have required the use of a gestational surrogate.

> *It is indeed unfortunate that so many patients are being denied the ability to go from "infertility to family" simply because (for whatever reason) so many reproductive specialists refuse to embrace the role of immunologic factors in the genesis of intractable reproductive dysfunction. Hopefully this will change, and the sooner the better.*

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RECURRENT PREGNANCY LOSS (RPL):

When it comes to reproduction, humans are the poorest performers of all mammals. In fact, we are so inefficient that up to 75% of fertilized eggs do not produce live births, and up to 30% of pregnancies end up being lost within 10 weeks of conception (in the first trimester). RPL is defined as two (2) or more failed pregnancies. Less than 5% of women will experience two (2) consecutive miscarriages, and only 1% experience three or more.

Pregnancy loss can be classified by the stage of pregnancy when the loss occurs:

- • Early pregnancy loss (first trimester)

- Late pregnancy loss (after the first trimester)

- Occult "hidden" and not clinically recognized, (chemical) pregnancy loss (occurs prior to ultrasound confirmation of pregnancy)

In more than 70% of cases, the loss is due to embryo aneuploidy (where there are more or less than the normal quota of 46 chromosomes). Conversely, repeated losses (RPL), are proportionally higher in their causality to non-chromosomal causes such as anatomical uterine abnormalities or Immunologic Implantation Dysfunction (IID).

Since most sporadic early pregnancy losses are induced by chromosomal factors and thus are non-repetitive, having had a single miscarriage the likelihood of a second one occurring is no greater than average. However, once having had two losses the chance of a third one occurring is double (35-40%) and after having had three losses the chance of a fourth miscarriage increases to about 60%. The reason for this is that the more miscarriages a woman has, the greater is the likelihood of this being due to a non-chromosomal (repetitive) cause such as IID. It follows that if numerical chromosomal analysis (karyotyping) of embryonic/fetal products derived from a miscarriage tests karyotypically normal, then by a process of elimination, there would be a strong likelihood of a miscarriage repeating in subsequent pregnancies and one would not have to wait for the disaster to recur before acting. This is precisely why we strongly advocate that all miscarriage specimens be karyotyped using state-of the art PCR technology that can eliminate confounding by maternal DNA contamination.

Late pregnancy losses (occurring after completion of the 1st trimester/12th week) occur far less frequently (1%) than early pregnancy losses. They are most commonly due to anatomical abnormalities of the uterus and/or cervix. Weakness of the neck

of the cervix rendering it able to act as an effective valve that retains the pregnancy (i.e., cervical incompetence) is in fact one of the commonest causes of late pregnancy loss, as are developmental (congenital) abnormalities of the uterus (e.g., a uterine septum) and uterine fibroid tumors. In some cases, intrauterine growth retardation, premature separation of the placenta (placental abruption), premature rupture of the membranes and premature labor can also cause late pregnancy loss.

Much progress has been made in understanding the mechanisms involved in RPL. There are two broad categories:

1.   Problems involving the uterine environment in which a normal embryo is prohibited from properly implanting and developing. Possible causes include:

- Inadequate thickening of the uterine lining

- Irregularity in the contour of the uterine cavity (polyps, fibroid tumors in the uterine wall, intra-uterine scarring and adenomyosis)

- Hormonal imbalances (progesterone deficiency or luteal phase defects). This most commonly results in occult RPL.

- Deficient blood flow to the uterine lining (thin uterine lining).

- Immunologic implantation dysfunction (IID). A major cause of RPL. Plays a role in 75% of cases where chromosomally normal preimplantation embryos fail to implant.

- Interference of blood supply to the developing conceptus can occur due to a hereditary clotting disorder known as Thrombophilia.

2.   Genetic   and/or   numerical   chromosomal abnormalities(aneuploidy) of the embryo are far away the commonest overall causes of miscarriages. But this only applies to sporadic pregnancy losses (which comprises most of all miscarriages. However, recurrent, (consecutive) pregnancy losses are much more likely due to implantation dysfunction than to embryo-related issues, where implantation dysfunction (usually anatomical or immunologic) factors usually underly the problem.

3.   Genetic or Structural chromosomal abnormalities (which only occur in about 1% of cases) can also cause RPL. This is referred to as an unbalanced translocation and they result from part of one chromosome detaching and then fusing with another chromosome. Additionally, genetic defects (unrelated to chromosomal abnormalities) can also affect embryo quality and pregnancy outcome. Damaged sperm DNA can sometimes be diagnosed using the SCSA (see before) which primarily measures the sperm DNA fragmentation index (DFI).

4.   Immunologic Implantation Dysfunction (see previous discussion of IID)

DIAGNOSING THE CAUSE OF RPL

Establishing the correct diagnosis is the first step toward determining effective treatment for couples with RPL.   RPL results from a problem within the pregnancy itself or within the uterine environment where the pregnancy implants and grows. Diagnostic tests useful in identifying individuals at greater risk for a problem within the pregnancy itself include:

- Karyotyping (chromosome analysis) both parents to assess for translocations/deletions
- Assessment of the karyotype of products of conception from miscarriage specimens

- Uterine cavity assessment with HSG, hysteroscopy, or saline sonogram
- thrombophilia workup: antiphospholipid antibody panel, Factor V Leiden assessment, Prothrombin gene mutation
- Full hormonal evaluation (estrogen, progesterone, adrenal steroid hormones, thyroid hormones, FSH/LH, etc.)
- Immunologic testing to include:
    - Antinuclear antibody (ANA) panel
    - Antithyroid antibody panel (i.e., antithyroglobulin and antimicrosomal antibodies)
    - Reproductive immunophenotype: NKa and DQalpa/HLA of both the male and female partners

TREATMENT OF RPL

Treatment for Anatomic Abnormalities of the Uterus:

This involves restoration through removal of local lesions such as fibroids, scar tissue, and endometrial polyps or timely insertion of a cervical cerclage (a stitch placed around the neck of the weakened cervix) or the excision of a uterine septum when indicated.

Treatment of Thin Uterine Lining:

A thin uterine lining has been shown to correlate with compromised pregnancy outcome. Often this will be associated with reduced blood flow to the endometrium. Such decreased blood flow to the uterus can be improved through treatment with sildenafil and possibly "baby" aspirin.

Modalities such as IL/IVIg, heparinoids (Lovenox/Clexane), and corticosteroids can be used in select cases depending on autoimmune or alloimmune dysfunction.

The Use of IVF in the Treatment of RPL

In the following circumstances, IVF is the preferred option:

• When in addition to a history of RPL, another standard indication for IVF (e.g., tubal factor, endometriosis, and male factor infertility) is superimposed.

• In cases where selective immunotherapy is needed to treat an immunologic implantation dysfunction.

The reason for IVF being a preferred approach in such cases is that to be effective, the immunotherapy needs to be initiated well before spontaneous or induced ovulation. Given the fact that in the absence of IVF the anticipated birthrate per cycle of COS with or without IUI is at best about 15%, it follows that short of IVF, to have even a reasonable chance of a live birth, most women with immunologic causes of RPL would need to undergo immunotherapy repeatedly, over consecutive cycles. Conversely, with IVF, the chance of a successful outcome in a single cycle of treatment is several times greater and, because of the attenuated and concentrated time required for treatment, IVF is far safer and thus represents a more practicable alternative. In addition, since embryo aneuploidy is a common cause of miscarriage, the use of PGS/PGT-A can provide a valuable diagnostic and therapeutic advantage in cases of RPL. PGT requires IVF to provide access to embryos for testing.

There are a few cases of intractable alloimmune dysfunction due to complete DQ alpha matching where gestational surrogacy or use of donor sperm could represent the only viable recourse, other than abandoning treatment altogether and/or resorting to adoption. Other non-immunologic factors such as an intractably thin uterine lining or severe uterine pathology might also warrant that last resort consideration of gestational surrogacy.

The good news is that if a couple with RPL is open to all the diagnostic and treatment options referred to above, a good outcome is ultimately achievable.

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EGG DONATION IVF

INTRODUCTION:

For many women, disease and/or diminished ovarian reserve precludes achieving a pregnancy with their own eggs. Since most women are otherwise healthy and physically capable of bearing a child, egg donation (ED) provides them with a realistic opportunity of going from infertility to parenthood. Egg donation is the process by which a woman donates eggs for purposes of assisted reproduction. Egg donation typically involves in vitro fertilization (IVF) technology, with the eggs being fertilized in the laboratory; unfertilized eggs may be frozen and stored for later use. Egg donation is a third-party reproduction as part of assisted reproductive technology (ART).

Egg donation is associated with definite benefits. Firstly, in most instances, more eggs are retrieved from a young donor

than would ordinarily be needed to complete a single IVF cycle. As a result, there are often supernumerary (leftover) embryos for cryopreservation and storage. Secondly, since eggs derived from a young woman are less likely than those from older women to produce aneuploid (chromosomally abnormal) embryos, the risk of miscarriage and birth defects such as Down's syndrome is greatly reduced.

Egg donation-related, fresh, and frozen embryo transfer cycles account for 10%-15% of IVF performed in the United States. Most egg donation procedures performed in the U.S involve older women with diminished ovarian reserve (DOR). While some are done for younger women with premature ovarian failure, the majority are undertaken in women over 40 years of age. Recurrent IVF failure due to "poor quality" eggs or embryos is also a relatively common indication for ED. A growing indication for ED is in cases of same-sex relationships.

Most egg donation in the U.S. is done through the solicitation of anonymous donors who are recruited through a state-licensed egg donor agency or frozen egg bank. It is less common for recipients to solicit known donors through the services of a donor agency, although this does happen on occasion. It is also not easy to find donors who are willing to enter such an open arrangement. Accordingly, in most cases where the service of a known donor is solicited, it is by virtue of a private arrangement.

Some recipients wish to know or at least to have met their egg donor. In the U.S. however, it is more common to seek the services of anonymous donors. Most, if not all, egg donor agencies and Egg Banks provide a detailed profile, photos, and a medical and family history of each prospective donor for the benefit and information of the recipient. Agencies generally have

a website through which recipients can access donor profiles in the privacy of their own homes to select the ideal donor.

Interaction between the recipient and the egg donor program/egg bank may be conducted in-person, by telephone or online in the initial stages. Once the choice of a donor or selection of banked eggs has been narrowed down to two or three, the recipient is asked to forward all relevant medical records to their chosen IVF physician. Upon receipt of her records, a detailed medical consultation and physical examination by a treating physician is scheduled. This entire process is usually overseen and orchestrated by one of the donor program's nurse coordinators who, in concert with the treating physician, will address all clinical, financial, and logistical issues, as well as answering any questions. At the same time, the final process of donor/donor banked egg selection and donor-recipient matching is completed.

Egg donor agencies as well as most egg banks usually limit the age of egg donors to women under 35 years with normal ovarian reserve to minimize the risk of ovarian resistance and negate adverse influence of the "biological clock" (donor age) on egg quality.

When using "fresh donors" recruited from agencies, no single factor instills more confidence regarding the reproductive potential of a prospective egg donor than a history of her having previously achieved a pregnancy on her own, or that one or more recipients of her eggs having achieved a live birth. Moreover, such a track record makes it far more likely that such an ED will have "good quality eggs". Furthermore, the fact that an ED readily conceived on her own lessens the likelihood that she herself has tubal or organic infertility. This having been said, the current shortage in the supply of egg donors makes it both impractical and unfeasible, to confine donor recruitment to

those women who could fulfill such stringent criteria for qualification.

SCREENING EGG DONORS

Genetic Screening:

The introduction of genetic screening panels has allowed most egg donor programs to test for a myriad of transmissible genetic disorders. Others follow the basic recommendations and guidelines of the American Society of Reproductive Medicine (ASRM) which advises routine genetic screening of prospective egg donors for conditions such as sickle cell trait or disease, thalassemia, cystic fibrosis and Tay Sachs disease. Consultation with a geneticist is available through about 90% of programs.

Psychological /emotional Screening:

Most recipient couples place a great deal of importance on emotional, physical, ethnic, cultural, and religious compatibility with their chosen egg donor. As such, egg donors are usually subjected to counseling and screening by a qualified psychologist. When it comes to choosing a known egg donor, it is equally important to make sure that she was not coerced into participating. We try to caution recipients who are considering having a close friend or family member serve as their designated egg donor, that in doing so, the potential always exists that the donor might become a permanent and an unwanted participant in the lives of their new family.

Drug Screening:

Because of the prevalence of substance abuse in our society, we selectively call for urine and/or serum drug testing of our egg donors.

Screening for Sexually Transmissible diseases (STDs):

FDA and ASRM guidelines recommend that all egg donors be tested for sexually transmittable diseases before starting a cycle of IVF. While it is highly improbable that DNA and RNA viruses could be transmitted to an egg or an embryo through sexual intercourse or IVF, women infected with viruses such as hepatitis B, C, HTLV, HIV etc., must be disqualified from participating in IVF with egg donation due to the (albeit remote) possibility of transmission, as well as the potential legal consequences of the egg donation process being blamed for their occurrence. Moreover, evidence of prior or existing infection with Chlamydia or Gonococcus introduces the possibility that the egg donor might have pelvic adhesions or even irreparably damaged fallopian tubes that might have rendered her infertile or place her at risk for infection post egg-retrieval.

SCREENING EMBRYO RECIPIENTS

Medical Evaluation:

While advancing age, beyond 40 years, is indeed associated with an escalating incidence of pregnancy complications, such risks are largely mitigated or rendered manageable through careful medical assessment and planning prior to pregnancy. The fundamental question namely: "Is the woman capable of safely engaging a pregnancy that would culminate in the safe birth of a healthy baby" must be answered in the affirmative, before any infertility treatment is initiated. For this reason, a thorough cardiovascular, hepatorenal, metabolic and anatomical reproductive evaluation must be done prior to initiating IVF in all cases.

Infectious Screening:

The need for careful infectious disease screening for embryo recipients cannot be overemphasized. The introduction

of an embryo transfer catheter via a so infected cervix might transmit the organism into an otherwise sterile uterine cavity leading to early implantation failure and/or first trimester miscarriage.

Immunologic Screening:

Certain autoimmune and alloimmune disorders can be associated with immunologic implantation dysfunction (IID). To prevent otherwise avoidable treatment failure, it is advisable to evaluate the recipient couple for autoimmune IID and also to test both the recipient and the sperm provider for alloimmune similarities that could compromise implantation.

DISCLOSURE AND CONSENT:

Preparation for egg donation requires full disclosure to all participants regarding what each step of the process involves from start to finish, as well as potential medical and psychological risks. This necessitates that significant time be devoted to this task and that there be a willingness to painstakingly address all questions and concerns posed by all parties involved in the process. An important component of full disclosure involves clear interpretation of the medical and psychological components assessed during the evaluation process. All parties should be advised to seek independent legal counsel to avoid conflicts of interest that might arise from legal advice given by the same attorney. Appropriate consent forms are then reviewed and signed independently by the donor and the recipient couple.

Most embryo recipients fully expect their chosen donor to yield a "large" number of mature, good quality eggs, sufficient to provide enough embryos to afford a good chance of pregnancy as well as several for cryopreservation (freezing) and storage. While such expectations ore often met, this is not always the case. Accordingly, to minimize the trauma of unexpected and

usually unavoidable disappointment, it is essential that in the process of counseling and of consummating agreements, the respective parties be fully informed that by making best efforts to provide the highest standards of care, the caregivers can only assure optimal intent and performance in keeping with accepted standards of care. No one can ever promise an optimal outcome. All parties should be made aware that no definitive representation can or will be made as to the number or quality of ova and embryos that will or are likely to become available, the number of supernumerary embryos that will be available for cryopreservation or the subsequent outcome of the IVF donor process.

TYPES OF EGG DONATION IVF

Conventional Egg Donation:

This is the basic format used for conducting the process of egg donor IVF. It involves synchronizing the menstrual cycles of both the recipient and the donor by placing the donor and the recipient on a birth control pill so that both parties start stimulation with fertility drugs simultaneously. This ultimately allows for precise timing of the fresh embryo transfer. Using this approach, the anticipated egg donation birth rate is >50% per cycle.

Eggs from a Donor Egg Bank:

In this scenario, viable eggs derived from young egg donors are stored and subsequently made commercially available for IVF and embryo transfer to women for whom egg donor-IVF provides the only means by which they can go from infertility to family. In the last few years, numerous frozen egg banks have sprung up, offering access to non-genetically tested cryobanked eggs. As a result, more and more egg donor agencies

are offering access to commercially available banked, donor eggs as an alternative to the use of fresh eggs derived through a designated and dedicated egg donor. Despite the convenience associated with the use of donor eggs available through commercial egg banks, aside from convenience, there is little financial benefit in using such banks. Frozen eggs in general are somewhat less likely than fresh eggs to generate viable embryos.

Being able to freeze and bank donor eggs solves many challenges. Through an electronic catalogue, recipients can select and purchase allotments of eggs (usually 6 to 10) from the comfort of their homes. Following ICSI fertilization of these eggs, the selective transfer of up to 2 embryos achieves about a 30-40% pregnancy rate without the risk of initiating high-order multiple pregnancies in the process. Through this process, the cost, inconvenience, and risks associated with "conventional" fresh egg donor cycles would also be reduced significantly. However, success rates using frozen eggs are often quoted as pregnancy or baby rate per embryo transfer. But this can be misleading because it reflects the baby rate per embryo transfer. In other words, it does not consider how many embryos are transferred at one time, nor the number of cases where no embryos are generated and therefore no embryo transfer procedure is performed. The fact is that the chance that any given frozen egg will thaw well, fertilize successfully, propagate a normal blastocyst and upon being transferred to the uterus result in a healthy baby is only about 8% (per frozen egg). Moreover, blastocysts derived through the fertilization of fresh (non-frozen) eggs, whether transferred directly to the recipient's uterus or first vitribanked and then and transferred to the uterus later yields an overall success rate that is about 25-30% higher than when embryos derived from cryobanked eggs are used. Thus presently, aside from the convenience factor and a marginal cost differential, using fresh eggs obtained through an agency-derived egg donor has considerable benefits. However, as technology

improves, the pendulum could well shift in the opposite direction. It is as well to recognize that the "cost" of IVF should not come down to the financial outlay associated with doing a procedure. Rather, it should be viewed as the "cost" of having a baby. And the cost is both financial and emotional, with the latter often being farm more depleting.

PGT-A IN EGG DONOR-IVF:

It is debatable whether the routine use of PGS/PGT-A to select embryos for transfer in IVF with ED is justifiable. Since most egg donors are under the age of 35y, ~60% of the embryos propagated through fertilization of their eggs will likely be euploid. Therefore, the transfer of one "untested" embryo derived from such donors should yield pregnancy rates comparable to that obtained using PGS/PGT-A embryo selection.

EGG DONATION WITH FET:

Improvement in embryo cryopreservation (vitribanking) technology to the point that the success rates are at least equivalent to that which can be achieved through the transfer of fresh embryos, has changed the way many of us conduct IVF with egg donation. In fact, many RE's as well as patients preferentially freeze all their blastocysts (whether PGS/PGT-A tested or not) and transfer them in a subsequent FET cycle. This is both more convenient and far less complicated to facilitate logistically and markedly improves delivery of the service.

FINANCIAL CONSIDERATIONS

The average fee paid to the egg donor agency per cycle usually ranges between $2,000 -and $12,000. This does not include the cost associated with psychological and clinical pre-testing, fertility drugs, and donor insurance, which commonly range between $3,000 and $6,000. The medical service costs of the IVF treatment cycle ranges between $8,000 and $14,000. The

donor stipend can range from $2,000 to as high $50,000 depending upon the "exotic requirements" of the recipient couple as well as supply and demand. Thus, the total out of pocket expenses for an egg donor cycle in the United States range between $15,000 and $78,000, putting egg donation outside the financial capability of most couples needing this service.

The growing gap between need and affordability has spawned several creative ways to try and make IVF with egg donation more affordable. Here are a few examples:

- Egg banking (see above)

- Egg Donor Sharing, where one comprehensive fee is shared between two recipients and the eggs are then divided between them. The downside is that fewer eggs are available embryos for transfer and/or cryopreservation.

- Egg Bartering, when during conventional IVF, a woman undergoing IVF remits some of her eggs to the clinic (who in turn provides it to a recipient patient) in exchange for a deferment of some or all the IVF fees. In my opinion, such an arrangement can be fraught with problems. For example, if the woman donating some of her eggs fails to conceive while the recipient of her eggs does, it is very possible that she might suffer emotional despair and even go so far as to seek out her genetic offspring. Such action could be very damaging to both her and the recipient, as well as the child.

- Financial Risk Sharing. Certain IVF programs offer financial risk sharing (FRS) which most recipient couples favor greatly. FRS offers qualifying candidates a refund of fees paid if egg donation is unsuccessful. FRS is designed

to spread the risk between the providers and the recipient couple.

GESTATIONAL SURROGACY

With gestational surrogacy, one or more embryos derived from the patient's eggs and her partner's sperm is transferred into the uterus of a surrogate. The surrogate in effect provides a host womb but does not contribute genetically. Despite original ethical, moral, and medicolegal reservations, gestational surrogacy has now gained widespread social acceptance. Candidates for IVF surrogacy can be divided into two groups: (1) women born without a uterus or who because of uterine surgery (hysterectomy) or disease are not capable of carrying a pregnancy to full term, and (2) women who cannot safely undertake a pregnancy because of systemic illnesses, such as diabetes, heart disease, and hypertension, or certain malignant conditions.

The process involves the genetic parents undergoing a thorough clinical, psychological, and laboratory assessment prior to selecting a surrogate. This is to exclude sexually transmitted diseases that might be carried to the surrogate at the time of embryo transfer. They are also counseled on the many issues confronting all IVF candidates such as the possibility of multiple births, ectopic pregnancy, and miscarriage. All legal issues pertaining to custody and the rights of the biological parents as well as the surrogate are discussed in detail and the appropriate consent forms are completed following full disclosure. It is advisable for the surrogate and the genetic parents to obtain separate legal counsel to avoid a conflict of interest that would arise were one attorney to counsel both parties.

SELECTING AND SCREENING THE SURROGATE

Many infertile couples who qualify for gestational surrogacy parenting solicit the assistance of empathic friends or family members to act as surrogates. Other couples seek surrogates by advertising in the media. Many couples with the necessary financial resources retain a surrogacy agency to find a suitable candidate. At SFS, we direct our patients to a reputable surrogacy agency with access to many surrogates. Because the surrogate gives birth, it is rarely possible or even realistic for her to remain anonymous. Whether recruited from an agency, family members, or through personal solicitation, the surrogate should be carefully evaluated psychologically as well as physically. This is especially important in cases where a relatively young surrogate or family member is recruited. In such cases, it is important to ensure that the surrogate has not been subjected to any pressure or coercion. She should also be counseled on issues faced by all IVF aspiring parents, such as multiple births. She will consult with one of our clinical coordinators, who will outline the exact process step by step. It is emphasized to the surrogate that she has full right of access to the clinic staff and that any questions or concerns will be addressed promptly. If pregnancy occurs, the surrogate will be referred to a competent, board certified obstetrician for pregnancy care and delivery. After the evaluations and counseling of both the couple and the surrogate have been completed, the three of them will meet and once all the evaluations have been completed, the couple will select a date to begin treatment.

If a viable pregnancy is confirmed by ultrasound recognition of a fetal heartbeat (at the sixth week), there is a better than 85% chance that the pregnancy will proceed normally to term. Once the pregnancy has progressed beyond the 12th week, the chance of a healthy baby being born is upward of 95%. Pregnancy rates are excellent when IVF is performed using a

gestational carrier. In fact, they are slightly higher than when conventional IVF is performed on a woman of a comparable age. The birthrate declines as the age of the egg provider advances beyond 40. It is important to note that there is no convincing evidence to suggest an increase in the incidence of spontaneous miscarriage or birth defects as a direct result of IVF surrogacy.

As soon as the egg provider starts taking gonadotropin injections, the surrogate will receive estrogen (estradiol valerate), by twice weekly injections, and then progesterone to help prepare her uterine lining for implantation. GnRHa is administered for a period of 7 to 12 days to prepare the ovaries prior to administration of estradiol valerate. The duration of GnRHa therapy is adjusted to synchronize the cycle of the woman undergoing follicular stimulation with that of the surrogate. Once the prospective mother commences follicular stimulation, the surrogate will be given estradiol and progesterone injections while continuing GnRHa therapy.

If the surrogate's blood pregnancy test is negative, treatment with estrogen and progesterone is discontinued, and she can expect to menstruate within 10 days. If the pregnancy test is positive, estrogen and progesterone therapy will continue for 4-6 weeks.

THE BIOETHICS OF IVF SURROGACY

The determination of ethical guidelines has not kept pace with the exploding growth and development in IVF. However, some leaders in the field are working together, sharing experiences and advice, to formulate a code of ethics. The genetic combination of the male and the female provide two of the essential elements that, along with gestation, are necessary to produce a human being. The "two-out-of-three rule" basically looks at these three elements: the egg, the sperm, and the gestational component. If possible, it is recommended that at

least two of these three components be contributed by the intended parents. If they can only contribute one, the other two should ideally not be contributed by the same person.

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MISCELLANEOUS TOPICS

ENDOMETRIOSIS AND INFERTILITY

Endometriosis is a condition that occurs when the uterine lining (endometrium) grows not only in the interior of the uterus but in other areas, such as the fallopian tubes, ovaries and the bowel. Endometriosis is a complex condition where, the lack or relative absence of an overt anatomical barrier to fertility often belies the true extent of reproductive problem(s).

All too often the view is expounded that the severity of endometriosis-related infertility is inevitably directly proportionate to the anatomical severity of the disease itself, thereby implying that endometriosis causes infertility primarily by virtue of creating anatomical barriers to fertilization. It is indisputable that even the mildest form of endometriosis can compromise fertility. It is equally true that, mild to moderate endometriosis is by no means a cause of absolute "sterility". Rather, when compared with normally ovulating women of a similar age who do not have endometriosis, women with mild to moderate endometriosis are about four to six times less likely to have a successful pregnancy.

Endometriosis often goes unnoticed for many years. Such patients are frequently erroneously labeled as having "unexplained infertility", until the diagnosis is finally clinched through direct visualization of the lesions at the time of laparoscopy or laparoscopy. Not surprisingly, many patients with so called "unexplained" infertility, if followed for several years, will ultimately reveal endometriosis.

Women who have endometriosis are much more likely to be infertile. There are several reasons for this:

• Ovulation Dysfunction: In about 25 - 30% of cases, endometriosis is associated with ovulation dysfunction. Treatment requires controlled ovarian stimulation (COS). The problem is that the toxic pelvic environment markedly reduces the likelihood that anything other than IVF will enhance pregnancy potential.

• Toxic pelvic environment that compromises fertilization: Endometriosis is associated with the presence of toxins in peritoneal secretions while it is tempting to assert that normally ovulating women with mild to moderate endometriosis would have no difficulty in conceiving if their anatomical disease is addressed surgically or that endometriosis-related infertility is confined to cases with more severe anatomical disease...nothing could be further from the truth. The natural conception rate for healthy ovulating women in their early 30's (who are free of endometriosis) is about 15% per month of trying and 70% per year of actively attempting to conceive. Conversely, the conception rate for women of a comparable age who have mild or moderate pelvic endometriosis (absent or limited anatomical disease) is about 5-6% per month and 40% after 3 years of trying. As sperm and egg(s) travel towards the fallopian tubes they are exposed to these toxins which compromise the fertilization process. In fact, it has been estimated that there is a 5-6-fold

reduction in fertilization potential because of these toxins which cannot be eradicated. Surgery will only prove helpful in this respect if every effort is made to detect and excise or ablate all endometriosis lesions. If much residual endometriosis is left, then only IVF, through removing eggs before they are exposed to the toxic pelvic environment, fertilizing them in-vitro, and then transferring the embryos to the uterus, will appreciably enhance pregnancy potential.

• Pelvic adhesions and Scarring: In its most severe form, endometriosis is associated with scarring and adhesions in the pelvis, resulting in damage to, obstruction or fixation of the fallopian tubes to surrounding structures, thereby preventing the union of sperm and eggs.

• Ovarian Endometriomas: Advanced endometriosis is often associated with ovarian cysts (endometriomas/chocolate cysts) that are filled with altered blood and can be large and multiple. When these are sizable (>1cm) they can activate surrounding ovarian connective tissue causing production of excessive male hormones (androgens) such as testosterone and androstenedione. Excessive ovarian androgens can compromise egg development in the affected ovary(ies) resulting in an increased likelihood of numerical chromosomal abnormalities (aneuploidy) and reduced egg/embryo competency. In my opinion, large ovarian endometriomas need to be removed surgically before embarking on IVF.

• Immunologic Implantation Dysfunction (IID): Endometriosis, regardless of its severity is associated with immunologic implantation dysfunction linked to activation of uterine natural killer cells (NKa) and cytotoxic uterine lymphocytes (CTL).

Advanced Endometriosis: In its most advanced stage, anatomical disfiguration is causally linked to the infertility. In such cases,

inspection at laparoscopy or laparoscopy will usually reveal severe pelvic adhesions, scarring and "chocolate cysts". However, the quality of life of patients with advanced endometriosis is usually so severely compromised by pain and discomfort, that having a baby is often low on the priority list. Accordingly, such patients are usually often more interested in relatively radical medical and surgical treatment options (might preclude a subsequent pregnancy), such as removal of ovaries, fallopian tubes, and even the uterus, as a means of alleviating suffering.

Moderately Severe Endometriosis: These patients have a modest amount of scarring/adhesions and endometriotic deposits which are usually detected on the ovaries, fallopian tubes, bladder surface and low in the pelvis, behind the uterus. In such cases, the fallopian tubes are usually opened and functional.

 Mild Endometriosis: These patients who at laparoscopy or laparotomy are found to have no significant distortion of pelvic anatomy are often erroneously labeled as having "unexplained" infertility. To hold that there can only be infertility attributable to endometriosis if significant anatomical disease is present is to ignore that biochemical, hormonal, and immunological factors can impact fertility.

TREATMENT

The following basic concepts apply to management of endometriosis-related infertility:

1. **Controlled Ovulation stimulation (COS) with/without intrauterine insemination (IUI**): Toxins in the peritoneal secretions of women with endometriosis exert a negative effect on fertilization potential regardless of how sperm reaches the fallopian tubes. This helps explain why COS with or without IUI

will usually not improve the chances of pregnancy (over no treatment at all) in women with endometriosis. IVF is the only way by which to bypass this problem.

2.	Laparoscopy or Laparotomy: Surgery aimed at restoring the anatomical integrity of the fallopian tubes does not counter the negative influence of toxic peritoneal factors that inherently reduce the chances of conception in women with endometriosis four to six-fold. Nor does it address the immunologic implantation dysfunction (IID) commonly associated with this condition. Approximately 30-40% of women under 35 years of age with endometriosis will conceive within two to three years following corrective pelvic surgery. Unless there are large quality of life issues, pelvic surgery may not be the best initial pathway for the treatment of infertility associated with endometriosis when the woman is more than 35 years of age. With the pre-menopause approaching, such women may wish to move to IVF as a first choice.

3.	**Sclerotherapy for ovarian endometriomas ("chocolate" cysts).** About 25 years ago we introduced "sclerotherapy", a relatively non-invasive, safe, and effective outpatient method to eliminate endometriomas without surgery being required. Sclerotherapy for ovarian endometriomas involves needle aspiration of the liquid content of the endometriotic cyst, followed by the injection of 5% tetracycline (or more recently, with ethanol via laparoscopy) into the cyst cavity. Treatment results in disappearance of the lesion within 6-8 weeks in ~ 75% of cases. Ovarian sclerotherapy can be performed under local anesthesia or under general anesthesia. It has the advantage of being an ambulatory office- based procedure, at low cost, with a low incidence of significant post-procedural pain or complications and the avoidance of the need for laparoscopy or laparotomy.

4.   **Selective immunotherapy for IID, as discussed earlier.**

5.   **IUI versus IVF**: As stated previously, the toxic pelvic environment caused by endometriosis, profoundly reduces natural fertilization potential. As a result, normally ovulating infertile women with endometriosis and patent fallopian tubes are much less likely to conceive naturally, or by using fertility agents alone (with or without intrauterine (IUI) insemination. The only effective way to bypass this adverse pelvic environment is through IVF. Not all women who have endometriosis require IVF but in cases further compromised by IID associated with NKa, and/or for older women (over 35y) who have DOR where time is of the essence, IVF is the treatment of choice.

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THE ROLE OF NUTRITIONAL SUPPLEMENTS

It is important to nurture and take care of yourself mentally and physically when preparing and going through your IVF journey. This starts with trying to have a positive attitude about what you are about to go through, creating a stress support system for yourself by using tools such as visualization, acupuncture and meditation, eating the right foods taking a few supplements (see below) and balancing exercise with sufficient rest. . Not only will it help your experience, but it may also help to increase your chances for IVF success

Nutrition is indeed a vital prerequisite for optimal reproductive function. However, a well-balanced diet that meets food preferences, coupled with modest vitamin, mineral, and antioxidant supplementation (as can be found in many prenatal vitamin preparations) should suffice. But the truth is that most

people do not have a balanced diet and are unwittingly often deficient in important nutrients. A balanced diet is one that is rich in good quality protein, low in sugar, salt, caffeine and industrially created trans-fats (trans-fatty acids or partially hydrogenated oils) and soy, uncontaminated by heavy metals, free of nicotine, alcohol, and recreational drugs. Therefore, routine supplementation with the following nutrients could enhance preconception readiness:

- Folic acid (400 micrograms daily)

- Vitamins D-3 2,000U daily; Vitamin A (2565 IU daily); B3/Niacin (250mg daily); B6 (6mg -10 mg daily); B12 (12-20 mcg per day); C- (2,000 mg a day for both men and women); E (both sexes should get 150-200U daily); Vitamin D3 (2000U daily)

- Co-enzyme Q10 (400-600mg daily)

- Amino acids such as L-Carnitine (3 grams daily) and L-arginine (1 gram per day)

- Omega 3 fatty acids (2,000mg per day)

- Minerals, mainly zinc (15mg per day); selenium (70-100mcg per day); iron (up to 20mg per day); magnesium (400mg daily)

There are likely to be significant reproductive health benefits (including enhanced fertility and intrauterine development) associated with the use of nutritional supplements. However, there are also certain potential pitfalls associated with their use. Some supplements are not as safe as they would seem. For example, excessive intake of fat-soluble vitamins (A, D, E and K) can even be dangerous to your health and may be associated with fetal malformations. Additionally, numerous supplements have been found to contain

contaminants such as toxic plant materials, heavy metals and even prescription medications that can compromise fetal development. Prior to the passage of the Dietary Supplement Health and Education Act of 1994, supplements (vitamins, minerals, amino acids, and botanicals) were required to demonstrate safety. However, since passage of "the Act", they are now presumed to be safe until shown otherwise, thus establishing a rather hazardous situation where a typical prenatal vitamin that will provide sufficient vitamins and minerals for a healthy early pregnancy and potentially dangerous supplements can and are being sold in the same store without product liability.

DHEA is a weak androgen hormone supplement that is metabolized to androstenedione and testosterone in the ovaries. While a small amount of ovarian testosterone is needed for optimal follicle and egg development, too much testosterone could be decidedly harmful. DHEA supplements probably won't do harm if taken by healthy young women who have normal ovarian reserve, but they probably would not derive any benefit either. However, while opinions differ regarding the use of DHEA, we are concerned that DHEA supplementation could be potentially harmful when taken by women with DOR, women who have polycystic ovarian syndrome (PCOS) and older women in their 40's as such women often already tend to have increased LH-activity, leading to increased ovarian testosterone. Additional ovarian testosterone in such women, could thus potentially compromise follicle development and egg quality/competency.

Maximizing reproductive performance and optimizing outcome following fertility treatment requires a combined strategy involving a balanced diet (rich in protein, low in sugars, soy and trans-fats), modest nutritional supplementation, limiting/avoiding foods and contaminants that can compromise reproductive potential, and adopting disciplined lifestyle modification such as not smoking, reducing stress, minimizing

alcohol intake, avoiding nicotine and recreational drug consumption, and getting down to a healthy weight through diet and exercise.

CHROMOSOMAL/GENETIC TESTING OF EMBRYOS

Preimplantation Genetic Screening/testing for aneuploidy (PGS/PGT-A):

Preimplantation Genetic Screening/testing for aneuploidy (PGS/PGT-A) is the process whereby the chromosomes in the cells of an embryo (or the polar body of an egg) are examined (karyotyped). Embryo cells that have all 46 chromosomes intact are termed euploid. Those with additional chromosomal material and those with deficient chromosomal material are aneuploid. In younger women, euploid embryos have better than a 60% chance of propagating a viable pregnancy. The chances in women >39y conceiving after receiving a euploid embryo decreases slightly over time but regardless of her age it is still around 50%. For those that conceive using such embryos, the miscarriage rate is below 10%.

Embryo aneuploidy and "mosaicism"

Human embryo development occurs through a process that encompasses reprogramming, sequential cleavage divisions and mitotic chromosome segregation and embryonic genome activation. Chromosomal abnormalities may arise during germ

cell and/or preimplantation embryo development and represents a major cause of early pregnancy loss. More than 15 years ago, we were the first to introduce full embryo karyotyping (identification of all 46 chromosomes) through preimplantation genetic sampling (PGS/PGT-A) as a method by which to selectively transfer only euploid embryos (i.e. those that have a full component of chromosomes) to the uterus. We subsequently reported on a 2-3-fold improvement in implantation and birth rates as well as a significant reduction in early pregnancy loss, following IVF. Since then, PGS has grown dramatically in popularity such that it is now widely used throughout the world.

Many IVF programs that offer PGS/PGT-A services, require that all participating patients consent to all their aneuploid embryos (i.e., those with an irregular quota of chromosomes) be disposed of. However, a growing body of evidence suggests that following embryo transfer, some aneuploid embryos will in the process of ongoing development, convert to the euploid state (i.e. "autocorrect") and then go on to develop into chromosomally normal offspring. In fact, I am personally aware of several such cases having occurred in my own practice. So clearly, summarily discarding all aneuploid embryos as a matter of routine we are sometimes destroying some embryos that might otherwise have "autocorrected" and gone on to develop into normal offspring. Thus, by discarding aneuploid embryos the possibility exists that we could be denying some women the opportunity of having a baby. This creates a major ethical and moral dilemma for those of us that provide the option of PGS/PGT-A to our patients. On the one hand, we strive "to avoid knowingly doing harm" (the Hippocratic Oath) and as such would prefer to avoid or minimize the risk of miscarriage and/or chromosomal birth defects and on the other hand we would not wish to deny patients with aneuploid embryos, the opportunity to have a baby.

The basis for such embryo "autocorrection" lies in the fact that some embryos found through PGS/PGT-A-karyotyping to harbor one or more aneuploid cells (blastomeres) will often also harbor chromosomally normal (euploid) cells (blastomeres). The coexistence of both aneuploid and euploid cells coexisting in the same embryo is referred to as "mosaicism."

It is against this background, that an ever-increasing number of IVF practitioners, rather than summarily discard PGS/PGT-A-identified aneuploid embryos are now choosing to cryobanking (freeze-store) certain of them, to leave open the possibility of ultimately transferring them to the uterus. In order to best understand the complexity of the factors involved in such decision making, it is essential to understand the causes of embryo aneuploidy of which there are two varieties:

1. *Meiotic aneuploidy"* results from aberrations in chromosomal numerical configuration that originate in either the egg (most commonly) and/or in sperm, during preconceptual maturational division (meiosis). Since meiosis occurs in the pre-fertilized egg or in and sperm, it follows that when aneuploidy occurs due to defective meiosis, all subsequent cells in the developing embryo/blastocyst/conceptus inevitably will be aneuploid, precluding subsequent "autocorrection". Meiotic aneuploidy will thus invariably be perpetuated in all the cells of the embryo as they replicate. It is a permanent phenomenon and is irreversible. All embryos so affected are thus fatally damaged. Most will fail to implant and those that do implant will either be lost in early pregnancy or develop into chromosomally defective offspring (e.g., Down syndrome, Edward syndrome, Turner syndrome).
2. M*itotic aneuploidy ("Mosaicism")* occurs when following fertilization and subsequent cell replication (cleavage), some cells (blastomeres) of a meiotically normal (euploid) early embryo mutate and become aneuploid.

This is referred to as "mosaicism". Thereupon, with continued subsequent cell replication (mitosis) the chromosomal make-up (karyotype) of the embryo might either comprise of predominantly aneuploid cells or euploid cells. The subsequent viability or competency of the conceptus will thereupon depend on whether euploid or aneuploid cells predominate. If in such mosaic embryos aneuploid cells predominate, the embryo will be "incompetent"). If (as is frequently the case) euploid cells prevail, the mosaic embryo will likely be "competent" and capable of propagating a normal conceptus.

Since *some* mitotically aneuploid ("mosaic") embryos can, and indeed do "autocorrect' while meiotically aneuploid embryos cannot, it follows that an ability to reliably differentiate between these two varieties of aneuploidy would potentially be of considerable clinical value. The recent introduction of a variety of preimplantation genetic screening (PGS) known as next generation gene sequencing (NGS) has vastly improved the ability to karyotype embryos reliably and accurately and thus to diagnose embryo "mosaicism".

Most complex aneuploidies are meiotic in origin and will thus almost invariably fail to propagate viable pregnancies. The ability of mosaic embryos to autocorrect is influenced by stage of embryo development in which the diagnosis is made, which chromosomes are affected, whether the aneuploidy involves a single chromosome (simple) or involves 3 or more chromosomes (complex), and the percentage of cells that are aneuploid. Many embryos diagnosed as being mosaic prior to their development into blastocysts (in the cleaved state), subsequently undergo autocorrection to the euploid state (normal numerical chromosomal configuration) as they develop to blastocysts in the Petri dish. This is one reason why "mosaicism" is more commonly detected in early embryos than in blastocysts. Embryos with segmental mosaic aneuploidies, i.e., the addition (duplication) or

subtraction (deletion), are also more likely to autocorrect. Finally, the lower the percentage of mitotically aneuploid (mosaic) cells in the blastocyst the greater the propensity for autocorrection and propagation of chromosomally normal (euploid) offspring. A blastocyst with <30% mosaicism could yield a 30% likelihood of a healthy baby rate with 10-15% miscarriage rate, while with >50% mosaicism the baby rate is roughly halved and the miscarriage rate double.

As stated, the transfer of embryos with autosomal meiotic trisomy, will invariably result in failed implantation, early miscarriage, or the birth of a defective child. Those with autosomal mitotic ("mosaic") trisomies, while having the ability to autocorrect in-utero and result in the birth of a healthy baby can, depending on the percentage of mosaic (mitotically aneuploid) cells present, the number of aneuploid chromosomes and the type of mosaicism (single or segmental) either autocorrect and propagate a normal baby, result in failed implantation, miscarry, or cause a birth defect (especially with trisomies 13, 18 or 21). This is why when it comes to considering transferring trisomic embryos, suspected of being "mosaic", I advise patients to undergo prenatal genetic testing once pregnant and to be willing to undergo termination of pregnancy in the event of the baby being affected. Conversely, when it comes to meiotic autosomal monosomy, there is almost no chance of a viable pregnancy. in most cases implantation will fail to occur and if it does, the pregnancy will with rare exceptions, miscarry. "Mosaic" (mitotically aneuploid) autosomally monosomic embryos where a chromosome is missing), can and often will "autocorrect" in-utero and propagate a viable pregnancy. It is for this reason that I readily recommend the transfer of such embryos, while still (for safety's sake) advising prenatal genetic testing in the event that a pregnancy results.

What should be done with "mosaic embryos? While the ability to identify "mosaicism" through karyotyping of embryos has vastly

improved, it is far from being absolutely reliable. In fact, I personally have witnessed a number of healthy/normal babies born after the transfer of aneuploid embryos, previously reported on as revealing no evidence of "mosaicism". However, the question arises as to which "mosaic" embryos are capable of autocorrecting in-utero and propagating viable pregnancies. Research suggests that that embryos with autosomal monosomy very rarely will propagate viable pregnances. Thus, it is in my opinion virtually risk-free to transfer embryos with monosomies involving up to two (2) autosomes. The same applies to the transfer of trisomic embryos where up to 2 autosomes are involved. Only here, there is a risk of birth defects (e.g., trisomy 21/18, etc.) and any resulting pregnancies need to be carefully assessed and if needed/desired, be ended. Regardless, it is essential to make full disclosure to the patient (s), and to ensure the completion of a detailed informed consent agreement which would include a commitment by the patient (s) to undergo prenatal genetic testing (amniocentesis/CVS) aimed at excluding a chromosomal defect in the developing baby and/or a willingness to terminate the pregnancy should a serious birth defect be diagnosed. Blastocysts with aneuploidies involving > 2 autosomes are complex abnormal and should in my opinion, be discarded.

Should PGS be done routinely in IVF?

When we first introduced PGS/PGT-A testing into the clinical IVF arena (2005) initial results were most-encouraging. Embryo implantation rates of >50% and birth rates of 50-60% when up to two euploid blastocysts were transferred. In addition, the reported incidence of miscarriages and chromosomal birth defects was likewise greatly reduced. In fact, we were so encouraged that most of us predicted that a time would come

where full embryo karyotyping through PGS/PGT-A would become a routine part of IVF. But alas, we were soon to be disappointed when following the widespread introduction of PGS testing, success rates started dropping. This was especially the case when PGS/PGT-A was performed on embryos derived from the eggs of older women and women with severely diminished ovarian reserve (DOR). With further investigation it began to dawn upon us that:

• Chromosomal numerical integrity, while being the most important determinant of embryo "competency" was likely not the only factor that impacted embryo "competency". Indeed, advancing age was revealed to increase the incidence of embryo aneuploidy, independent of embryo karyotype and this is probably linked to non-chromosomal, genetic and metabolomic factors that might also be age-related.

- Independent of embryo competency, there are many other variables, that can and do determine IVF outcome and these are often outside the control of the embryology/genetic laboratory. They include selection and implementation of individualized protocols for controlled ovarian stimulation (COS), endometrial factors that determine embryo implantation (e.g., anatomical an immunologic implantation dysfunction), the technical skill of the physician performing embryo transfer etc.

- Not all PGS-aneuploid embryos are "incompetent". Some are mosaic (see elsewhere) and these are often capable of "autocorrecting" upon being transferred to the uterus, and propagating healthy babies. In our experience, embryos that have additional or deficient chromosomal material affecting only one of the 23 chromosome pairs, are the ones most likely to be "mosaic", while those that

have absence or addition of >1 chromosomal material involving several chromosome pairs, are usually meiotically aneuploid.

Against this background, it is our considered opinion that PGS/PGT-A-embryo selection be primarily considered in the following circumstances:

- Women over the age of 39Y and those who, regardless of age have significant DOR, are running out of eggs and time, and need to "make hay while the sun shines"!

- Unexplained IVF failure.

- Certain cases of recurrent pregnancy loss (RPL).

- Family gender balancing cases

- Women who have alloimmune, IID with activation of uterine natural killer cells (NKa)…see elsewhere.

- Where karyotyping reveals that one of the partners has a chromosomal translocation

- Known or anticipated specific genetic abnormalities

PGS/PGT-A for Gender Selection and Family balancing:

Gender selection for family balancing has previously come under scrutiny for presumed ethical reasons as well as for the concern that such practice could distort the natural sex ratio, leading to a population gender imbalance. Given that in the United States most couples do not care about the gender of their offspring, and only a minority are interested in selecting the sex of their children, there is currently no risk that IVF sex-selection will impact the population gender balance. Thus, in our opinion by

and large, freedom of choice should prevail. We offer gender selection in the following circumstances:

- For cases associated with sex-linked genetic disorders or,

- Serious genetic disorders that are more likely to occur in one gender or the other.

- For family balancing

- For couples who have at least one child of the opposite gender to that which they choose for their IVF embryo transfer and,

- For those women who do not have any children at all but prefer to have a child of one or the other gender.

: Preimplantation Genetic T-M (PGT-M), previously known as Preimplantation Genetic Diagnosis, or PGD, is a genetic testing method, for monogenic/single gene defects that is performed prior to pregnancy to greatly reduce the risk of passing on a specific genetic condition. It should be considered in the following situations:

- If both partners are carriers of the same autosomal recessive condition (e.g., Sickle Cell Disease, Cystic fibrosis or Beta Thalassemia)

- If either partner has an autosomally dominant disorder (e.g., Myotonic Dystrophy, Marfan Syndrome; or Huntington/s disease)

- If one of the partners has a dominant X-linked condition (e.g., Duchenne Muscular Dystrophy)

- If a partner has a mutation associated with hereditary cancer (e.g., BRCA1 & 2 or Lynch Syndrome)

- If the patient has or has had a child or a pregnancy diagnosed with a single gene disorder

PGT-M t is designed for each family. It can be done for almost any single gene disorder, provided that the specific familial mutation is identified, and family members are available for test preparation.

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ASHERMAN SYNDROME

Asherman syndrome is a condition characterized by the presence of severe intrauterine adhesions (synechiae) that destroy most of the basal layer of the endometrium from which, under the influence of the hormones, estrogen and progesterone, the uterine lining (endometrium), develops. When most of the basal endometrium is incapacitated so that virtually no regeneration of the endometrium can take place, amenorrhea (cessation of menstruation) and infertility follows.

Asherman syndrome most commonly results from post-partum or post-abortal inflammation involving the uterine lining (endometritis), but it can also occur following uterine surgery such as removal of fibroid tumors (myomectomy) that encroach upon or penetrate the uterine cavity.

Treatment involves a procedure called hysteroscopic resection, whereby a telescope-like instrument is introduced via the vagina and cervix into the uterine cavity, to allow direct surgical resection of as much scar tissue as possible. The objective is to remove as much scar tissue as possible and to free adhesions that fuse the walls of the uterine cavity together to

uncover and enable viable basal endometrium to resume growth and progressively cover as much of the surface of the uterine cavity as possible. Post operatively, a small balloon is often placed in the uterine cavity for a day or two, to keep the opposing surfaces separated in the hope of preventing recurrence of adhesion formation. The woman usually receives supplemental estrogen, corticosteroid (prednisone), and antibiotic to encourage endometrial growth.

Endometritis of a severity sufficient to produce Asherman Syndrome usually scars and blocks the uterine entrance to the fallopian tubes. However, sometimes one or both tubes remain open (although there is usually always some degree of damage to the inner lining). While in such cases, the uterus is often incapable of allowing proper embryo implantation, a pregnancy could implant in a fallopian tube leading to an ectopic pregnancy, which unless it is diagnosed early (by ultrasound examination) and treated medically or surgically, will rupture with severe intra-abdominal bleeding.

Unfortunately, with Asherman syndrome, even after surgical removal of as much scar tissue as possible, the uterine lining often remains quite thin and insufficient to allow implantation. In these cases, there is such widespread destruction of the basal endometrium (from which fresh endometrial cells must be generated), that improving blood flow with Viagra is usually unsuccessful in improving estrogen-mediated endometrial development sufficient to achieve "adequate" improvement in endometrial growth. In such cases, women should consider adoption or resort to gestational surrogacy.

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ADENOMYOSIS

Adenomyosis is a condition where endometrial glands develop outside the uterine lining (endometrium), within the muscular wall of the uterus (myometrium). The condition should be suspected when a premenopausal woman presents with pelvic pain, heavy painful periods, pain with deep penetration during intercourse, "unexplained infertility", or repeated miscarriages and thereupon, when on pelvic examination she is found to have an often enlarged (bulky) soft tender uterus.

Definitive diagnosis of adenomyosis is difficult to make, but MRI and sonogram are very helpful imaging studies. Some suggestive features on sonogram include:

- Smooth generalized enlargement of the uterus.

- Asymmetrical thickening of one side of the (myometrium) as compared to another side.

- Thickening (>12mm) of the junctional zone between the endometrium and myometrium with increased blood flow.

- Absence of a clear line of demarcation between the endometrium and the myometrium

- Cysts in the myometrium

- One or more non discrete (not encapsulated) tumors (adenomyomas) in the myometrium.

Since there is no proven independent relationship between adenomyosis and egg/embryo quality, any associated reproductive dysfunction (infertility/miscarriages) might be

attributable to an implantation dysfunction. It is tempting to postulate that this is brought about by adenomyosis-related anatomical pathology at the endometrial-myometrial junction. However, many women with adenomyosis have children without difficulty. Given that 30%-70% of women who have adenomyosis also have endometriosis, a known cause of infertility, infertility caused by adenomyosis is likely linked to endometriosis where infertility is at least in part due to a toxic pelvic environment that compromises egg fertilization potential or fosters an immunologic implantation dysfunction (IID). Thus, women who are suspected of having adenomyosis-related reproductive dysfunction (infertility/miscarriages) should consider an investigation for endometriosis and for IID.

Conservative surgery to address adenomyosis-related infertility involves excision of portions of the uterus with focal or nodular adenomyosis and/or excision of uterine adenomyomas. It is very challenging and difficult to perform because adenomyosis does not have distinct borders that distinguish normal uterine tissue from the lesions. With the advancement of robotically assisted laparoscopy, such surgeries are growing in popularity as long as the disease doesn't appear to be diffuse and ubiquitous throughout the myometrium.

Medical treatment: There are three approaches:

- GnRH agonist (Buserelin/Lupron) therapy is the most common treatment modality and is frequently used as a three-month depot injection to precede embryo transfer. These medications lower estrogen levels and "starve" endometriotic implants regardless of location.

- Aromatase inhibitors such as Letrozole have also been tried with limited success

- Immunotherapy to counter IID: The use of therapies such as Intralipid (or IVIG)/steroids/heparin in combination with IVF might well hold promise in those women with adenomyosis who have NKa.

Fortunately, not all women with adenomyosis are infertile. For those who are, treatment presents a real problem. Even when IVF is used and the woman conceives, there is still a significant risk of miscarriage. Since the condition does not compromise egg/embryo quality, women with adenomyosis-related intractable reproductive dysfunction should consider gestational surrogacy.

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ECTOPIC ("TUBAL") PREGNANCY

An ectopic pregnancy is a gestation that occurs outside of the uterine cavity. The most common site is in the fallopian tube, but sometimes it can also occur in the ovary, the cervix, or even the abdominal cavity. Estimates put the incidence of ectopic pregnancy at about 1 in 200 pregnancies, but it occurs in about 3-4% of pregnancies following IVF. Ectopic pregnancy is one of the most dangerous complications of pregnancy. If undetected, the ectopic pregnancy will continue to grow and will ultimately burst through the wall of the fallopian tube, often resulting in potentially fatal intra-abdominal bleeding.

The introduction of sophisticated sonographic and hormonal monitoring technology now makes it possible to detect an ectopic pregnancy very early on, well in advance of it rupturing. A decade or two ago, the diagnosis of an ectopic pregnancy, ruptured or not, was an indication for immediate

laparotomy to avoid the risk of catastrophic hemorrhagic shock. This often resulted in the affected fallopian tube having to be completely removed, sometimes along with the adjacent ovary.

In the late 1980's, early conservative surgical intervention by laparoscopy began replacing laparotomy (a wide incision made in the abdominal wall) for the treatment of ectopic pregnancy, often allowing the affected fallopian tube to be preserved and shortening the period of post-surgical convalescence. In the 90's, early detection combined with the advent of medical management with methotrexate (MTX) has all but eliminated the need for surgical intervention in the majority of patients. If administered early enough, MTX will allow spontaneous absorption of the pregnancy and a dramatic reduction in the incidence of catastrophic bleeding. This was especially true in ectopic pregnancies arising from in vitro fertilization, where the early progress of pregnancy is usually carefully monitored with hormone levels and ultrasound

The fertilization of the human egg normally takes place in the fallopian tube. The embryo then travels into the uterus, where it implants into the endometrial lining 5 to 6 days after ovulation. Anything that delays the passage of the embryo down the fallopian tube can result in the embryo hatching and sending its "root system" into the wall of the fallopian tube and initiating growth within the tube. One of the most common predisposing factors is pelvic inflammatory disease (PID) in which microorganisms, such as Chlamydia, and Gonococcus damage the inner lining (endosalpinx) and eventually also the muscular walls of the tube(s) by the formation of scar tissue. The endosalpinx has a very complex and delicate internal architecture, with small hairs and secretions that help to propel the embryo toward the uterine cavity. Once damaged this lining can never regenerate. This is one of the reasons why women who manage to conceive following surgery to unblock fallopian

tubes damaged by PID, have about a 1 in 4 chance of a subsequent pregnancy developing within the fallopian tube.

Congenital malformations of the fallopian tube, associated with shortening of, or small pockets and side channels within, the tube can interrupt the smooth passage of the embryo down the fallopian tube, is another cause of an ectopic pregnancy.

Since the lining of the fallopian tube does not represent an optimal site for healthy implantation, a large percentage of pregnancies that gain early attachment to its inner lining will usually be absorbed before the woman even knows that she is pregnant. This is often referred to as a tubal abortion.

The classical picture of ectopic pregnancy is the following symptoms:

- Missed menstrual period. A missed period as in early pregnancy, although some patients will have spotting or other abnormal bleeding. The pregnancy test will be positive in such cases.

- Pain. This is typically cramp-like in nature, located on one or another side of the lower abdomen. It is caused by spasm of the muscular wall of the fallopian tube(s). In cases where the ectopic pregnancy has ruptured and bleeding has occurred into the abdominal cavity, the woman often experiences severe, abdominal pain that increases progressively. This is because blood irritates the peritoneal membrane, which envelops all abdominal-pelvic organs. Sometimes the woman will experience pain in the right shoulder tip. The reason is that that blood which tracts along the side of the abdominal cavity finds its way to the area immediately below the diaphragm, above the liver (on the patient's

right side), irritates the endings of the phrenic nerve, which supplies that part of the diaphragm. This results in the referral of the pain to the neck and shoulder.

- Vaginal bleeding. When a pregnancy inadvertently implants in the fallopian tube the lining of the uterus undergoes profound hormonal changes associated with pregnancy (primarily associated with the hormone progesterone). When the embryo dies, the lining of the uterus separates. Initially, vaginal bleeding is dark and usually is quite scanty, even less than with a normal menstrual period. In some cases of ectopic pregnancy, bleeding is more severe, similar to that experienced in association with a miscarriage. This sometimes leads to ectopic pregnancy initially being misdiagnosis as a miscarriage and is the reason that we often want to examine the material that is passed vaginally, for evidence of products of conception.

- Dizziness and/or fainting. This is usually a late symptom, indicative of tubal rupture and internal bleeding and impending shock. It is an indication to seek immediate medical attention.

The easiest and most common method of diagnosing an ectopic pregnancy is by tracking the rate of rise in the blood levels of the "hormone of pregnancy", human chorionic gonadotropin (hCG). With a normal intrauterine pregnancy, blood level of hCG will usually double every two days throughout the first nine to ten weeks. While an inappropriate rate of increase in hCG usually suggests an impending miscarriage, it might also point to an ectopic pregnancy. Thus, the hCG levels should be followed serially until a clear pattern emerges.

The diagnosis of an ectopic is often clinched by ultrasound examination, which if performed using a sensitive

ultrasound machine and with sufficient expertise, will often reveal an unruptured ectopic pregnancy within a fallopian tube. When an ectopic pregnancy occurs following infertility treatment, there is the added advantage that the physician will be on the lookout for the earliest possible signs of trouble. The performance of a vaginal ultrasound within two weeks of a positive blood pregnancy (hCG) test following IVF allows for early detection of the unruptured pregnancy and timely intervention with MTX and/or laparoscopy.

If the tube has already ruptured or internal bleeding has occurred, ultrasound examination will inevitably detect the presence of free fluid into the abdominal cavity. If there has been a significant amount of intra-abdominal bleeding, irritation of the peritoneal membrane will cause the abdominal wall to become tense and depending on the amount of blood in the abdomen, to distend. In such cases, any pressure on the abdominal wall will evoke significant pain and when a vaginal examination is done, movement of the cervix produces excruciating pain, especially on the side of the affected fallopian tube.

When a tubal ectopic pregnancy rupture it represents an abdominal catastrophe. The woman will often collapse with severe pain. She will be shocked, pale, have a rapid pulse with a low blood pressure and will often be breathing rapidly with demonstrable "air hunger". The clinical picture is often so typical that in most cases, the diagnosis will present no difficulty at all.

The most important conditions to differentiate from an ectopic pregnancy are:

- hemorrhagic cyst of the ovary

- appendicitis

- acute pelvic inflammatory disease (PID)

- an inevitable miscarriage.

In questionable situations, laparoscopy is usually performed for diagnostic purposes. If an ectopic pregnancy is detected, a small longitudinal incision over the tubal pregnancy will allow for its removal, without necessitating removal of the tube (linear salpingostomy). Bleeding points on the fallopian tube can usually be accessed directly and appropriately ligated through the laparoscope. Sometimes the damage to the fallopian tube has been so extensive that the entire tube will require removal. On occasions where very severe intra-abdominal bleeding heralds a potential catastrophe, a laparotomy is performed to stop the bleeding post haste. In such cases, a blood transfusion is usually required and may be lifesaving.

The introduction of methotrexate (MTX) therapy for the treatment of ectopic pregnancy has profoundly reduced the need for surgery in most patients. MTX is a chemotherapeutic that kills rapidly dividing cells, such as those present in the "root system" of the conceptus. Extremely low doses of MTX are used to treat ectopic pregnancy. Accordingly, the side effects that are often associated with such chemotherapy used for the treatment of other conditions are seldom seen. It is important to confirm that the ectopic pregnancy has not yet ruptured prior to administering MTX.

The administration of MTX is by intramuscular injection. Prior to its administration, blood is drawn to get a baseline blood hCG level. After the injection of MTX the patient is allowed to return home with strict instructions that she should always have someone with her and never be alone in the ensuing week. The concern is that were the patient to be on her own and an intra-abdominal bleed were to occur, she might not readily be able to access someone who could get her to the hospital immediately. Instructions are also given to look for early signs that might point

towards severe intra-abdominal bleeding such as the sudden onset of severe pain, light-headedness, or fainting.

The patient returns to the doctor's office four days later to check the blood hCG level. Three days later (7 days after MTX), the level is checked again. By this time the hCG level should have dropped at least 15% from the value on day 4. If not, a second MTX injection is given, and the blood levels are tested twice weekly until hCG level is undetectable. Once this occurs, vaginal bleeding will usually ensue within a week or two.

It is important to note, especially in cases where more than one embryo or blastocyst has been transferred to the uterine cavity or fallopian tube that implantation may occur in two sites simultaneously (i.e., in the fallopian tube as well as inside the uterine cavity). This is referred to as a heterotopic pregnancy. It is therefore important that before administering MTX, which will cause the death and absorption of any early pregnancy, that the physician makes certain that he/she is not dealing with a heterotopic pregnancy. In such cases, surgery is required to treat the tubal ectopic, while every precaution is taken to protect the pregnancy growing within the uterine cavity.

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STAGGERED IVF

Staggered IVF refers to the process whereby embryos are intentionally vitrified and cryobanked ("vitribanked") and then electively transferred in a different cycle to the one in which the eggs were harvested.

Many physicians treating infertile women over age 40 who have patent fallopian tubes still opt to start with the least invasive strategy rather than going straight to IVF. This usually begins with the prescribing of oral and then injectable fertility drugs with or without artificial insemination/IUI. The rationale for this approach is that IVF is significantly more expensive than such alternatives. While the cost of an IVF procedure is certainly more expensive, what is often ignored is that it is also far more likely to achieve the desired result of a live birth. When one considers that the real price (financial as well as emotional) resides in the cost of having a baby rather than the cost of a procedure, doing IVF often turns out to be less costly.

It is well established that regardless of a woman's age or the cause of her infertility, the chance of pregnancy following IVF is several-fold greater than with any other method of treatment including intrauterine insemination (with or without the use of fertility drugs) and that this difference becomes much more pronounced with advancing age. For example, the chance of an ovulating woman of under 35 who has patent Fallopian tubes and a fertile male partner, having a baby after a single attempt at IUI is <15%. For a woman in her mid-40s the chance is <3%. With IVF, the comparable chances of success would be about 40-45% and 10-15%, respectively.

This does not mean that all infertile women who have patent Fallopian tubes and fertile male partners should select IVF over less invasive and less expensive treatments. What it does suggest, however, is that women running out of time on the "biological clock" (i.e., women in their 40's, and women who have diminished ovarian reserve -DOR), should probably go directly to IVF rather than waste precious time on less effective treatment options. It must also be recognized that the older the woman, the greater the risk of miscarriage and of having a chromosomally abnormal baby. For example: At age 30 the risk of miscarriage is about 15% and the chance of a woman giving birth to a chromosomally abnormal baby (e.g., Down syndrome) is <1:1000. Conversely, in the mid-40's the comparable risk of miscarriage is >40%, and 1:60-80, respectively.

Making strong a case for Embryo banking with Staggered IVF and PGS/PGT-A embryo testing: Against this background, to try and put the "biological clock" on hold and provide older infertile women and those with DOR with an alternative to IVF/egg donation, we recently introduced Embryo Banking with Staggered IVF and Preimplantation Genetic Sampling (PGS) of embryos. This approach usually requires more than one IVF procedure, biopsy of all potentially viable embryos for PGS, holding all biopsied specimens until several (4-8) blastocysts have been cryobanked and then sending the biopsied DNA for PGS/PGT-A (using Next Generation Gene Sequencing-NGS).

Once the PGS/PGT-A results are known, the woman returns in a subsequent cycle for the selective transfer of up to two PGS/PGT-A-normal embryos to her uterus. The use of embryo banking with Staggered IVF and selectively transferring only PGS-normal embryos is an efficiency tool which significantly improves the baby rate per embryo transferred (to as high as 50%), reduces the miscarriage rate by a factor of 5-6-fold and

minimizes the risk of the birth of a baby who has chromosomal birth defects (e.g. Down syndrome).

The "competency" (numerical chromosomal integrity) of a woman's eggs declines rapidly with advancing age (about) 2:3 are normal in her early 30's as compared to 1:5 at 40Y and probably < 1:20 by 45Y) In addition, the older the woman gets, the more her total egg supply diminishes, ultimately resulting in DOR. For these reasons, advancing age and DOR reduce fertility, increase the risk of miscarriage and result in a rise of chromosomal birth defects (e.g., Down syndrome). IVF can markedly improve outcome by maximizing the number of eggs available for fertilization and making the resulting embryos available for genetic testing. The introduction by SFS physicians of Staggered IVF, Embryo Banking and PGS/PGT-A with the selective transfer of only "chromosomally competent" embryos represent an excellent "efficiency tool" which markedly improves pregnancy rate, reduces the chance of miscarriage an minimizes the risk of chromosomal birth defects. As such we strongly recommend this approach for the following women undergoing IVF:

- older women (>39y),

- women who (regardless of age) have DOR,

- women with known transmittable genetic defects

- chromosomal testing for gender selection,

- women with recurrent pregnancy loss and

- those with unexplained IVF failure with or without IUI.

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THROMBOPHILIA (HEREDITARY CLOTTING DEFECTS:

Thrombophilia (Hereditary Clotting Defect) is defined as the genetic predisposition to developing intravascular thrombosis. It is due to hypercoagulability of blood leading to impairment of initial vascularization that takes place during implantation.

Thrombophilia affects as many as one in five people in the United States and is responsible for pregnancy loss (most particularly after the 1st trimester) and "unexplained" infertility, as well as being a factor in some cases of "unexplained" IVF failure. Whether (and/or the extent to which) thrombophilia causes 1st trimester recurrent pregnancy loss (RPL) is the subject of debate and is controversial. In fact, first trimester RPL is far more likely to be due to immunologic implantation dysfunction (IID) and/or irregularities in the contour of the uterine cavity or insufficient thickness of its lining (a thin endometrium). Thrombophilia has also been associated with late pregnancy-induced complications such as preeclampsia, premature separation of the placenta (*abruptio placenta*), placental insufficiency with intrauterine growth retardation, and in "unexplained" intrauterine death.

This having been said, it is a fact that most women with a thrombophilia go on to experience healthy pregnancies.

Diagnosis of Throbophilia: Thrombophilia is diagnosed when one or more of the following is detected:

- Mutational defect involving methylenetetrahydrofolate reductase (MTHFR), which occurs in at least 20% of affected cases. Homozygosity for a common C677T mutation in the MTHFR gene that is associated with

hyperhomocysteinemia is the most common form of hereditary thrombophilia leading to a 3-fold increase in risk of complications.

- Mutation of factor V Leiden (FVL),
- A mutation of prothrombin G20210A,
- Deficiency of antithrombin III
- Deficiency of protein C
- Deficiency of protein S

Risk Factors

- Pregnant women with predisposing factors such as:
- A personal or family history of thromboembolism (deep vein thrombosis), pulmonary embolism (blood clot in the lung), cerebrovascular accidents (i.e. strokes)
- A personal history of pregnancy complications such as unexplained intrauterine death, preeclampsia, abruptio placenta, intrauterine growth retardation, placental insufficiency, should be tested for the condition.

Treatment: Treatment should be initiated as soon as possible after pregnancy is diagnosed biochemically (blood or urine hCG test) and be continued throughout gestation.

Severe thrombophilias (e.g., homozygous MTHFR mutations, protein C deficiency, prothrombin G20210A mutation) as well as cases of mild thrombophilias associated with one or more of the pregnancy complications mentioned above, are best treated with low-molecular weight heparin (LMWH) taken throughout pregnancy.

For other (milder) thrombophilias and no history of prior pregnancy complications: Low-dose aspirin with the B vitamins folic acid, B6 and B12.