EVALUATION OF INFERTILITY
EVALUATION OF THE INFERTILE COUPLE

INTRODUCTION: A definition of infertility is one year of unprotected intercourse without conception. Related terms with different definitions include symptomatic childlessness, sterility and recurrent abortion. In addition, there are relative issues relating to infertility that modify the way it is clinically approached: examples include high risk historical factors, particularly age of the couple and overt medical history.

WHEN TO BEGIN THE INFERTILITY WORKUP: This is commenced when a mutual understanding between the physician and the patient (or couple) is reached based on the various definitions described above.

OVERVIEW OF CAUSES OF INFERTILITY: As a couple, the male and female contributions to the infertility are 50% each. In some cases, the cause is absolute (blocked fallopian tubes or azoospermia) but the majority are relative (endometriosis, presence of a varicocele). In relative causes of infertility often a corresponding relative factor in the opposite partner accounts for the delay in conception.

CAUSES OF FEMALE INFERTILITY

<table>
<thead>
<tr>
<th>Category</th>
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<tbody>
<tr>
<td>Mechanical Factors</td>
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<tr>
<td>Ovulatory Dysfunction - Failure</td>
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<tr>
<td>Other Factors</td>
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<tr>
<td>Endometriosis</td>
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<td>Uterine (Intrauterine Synechiae)</td>
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<td>Cervical Factors (Poor Mucus)</td>
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<td>Immunologic Factors</td>
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<td>Miscellaneous Factors</td>
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CAUSES OF MALE INFERTILITY

<table>
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<tr>
<td>Semen Disorders</td>
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<tr>
<td>Varicocele</td>
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<td>Idiopathic</td>
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<tr>
<td>Obstruction</td>
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<tr>
<td>Miscellaneous</td>
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WORKUP

Initial history and physical examination of both partners

TESTS.
- Semen Analysis
- Endometrial Biopsy
- Post-Coital Test
- Hysterosalpingogram
- Laparoscopy
If the above table of tests including the history and physical examination are within normal limits, the couple will be diagnosed as having unexplained or idiopathic infertility. Up to 20% of couples will have this diagnosis at this stage. If age is excluded, more than half the patients in this category will have an identified factor relating to their infertility with the use of modern additional testing.

**OUTLINE OF TESTS**

**THE SEMEN ANALYSIS**

A semen sample is collected by masturbation after two days of abstention. The physical characteristics are assessed including volume, viscosity, time to liquefaction and microscopic assessments. The major parameters studied include:

- Concentration Per cc (60 million per cc)
- Initial Motility (60%)
- Morphology (60% WHO)

**ASSESSMENT OF OVULATORY FUNCTION**

Basal body temperature recording - Ovulatory levels of progesterone give a 0.4 (F) degree temperature elevation or more in the basal temperature. Ovulation occurs with the establishment of levels of progesterone of 2 nanograms per ml. The luteal phase should last twelve to sixteen days, with an average of 14 days of progesterone production and temperature elevation. Normal levels of progesterone are difficult to quantitate owing to the pulsatile fluctuation in progesterone and the frequency variation throughout the luteal phase. Typical mid-luteal levels are 5-15 ng/ml but normal levels have not been well documented.

The endometrial biopsy - This appears to be the better of the various tests for ovulatory function with its own limitations. A microscopic assessment of endometrial dating (range of accuracy equals two days) is made and the predicted onset of menses is calculated to an idealized 28 day cycle. Should the onset of menses occur three or more days earlier than the predicted date of menses, a luteal phase deficiency can be diagnosed. Of course, proliferative endometrium (absence of secretory endometrium) diagnoses anovulation in appropriately timed biopsies.

Luteal phase deficiency - In this situation, ovulation occurs but the progesterone effects on the endometrium lag behind the orderly and sequential changes characteristic of the glands and stroma. Causes include age, thyroid, prolactin and androgen disorders. Serum prolactin, thyroid functions, LH, FSH, testosterone and DHEAS should be considered.

**Anovulation:**

- WHO I
- WHO II
- PCO
- Ovarian Failure
- Miscellaneous Disorders

Evaluation of the Infertile Couple
History and physical examination, basal temperature record, endometrial biopsy, serum hormonal evaluations and bioassays will help diagnose the type of anovulation. Assessments are made as to whether the gonadotropins are high, normal or low in relation to estrogen status. A classic example would be low gonadotropins with low estrogen status and no withdrawal bleed to progestin challenge. This would be the classic WHO I example of hypothalamic amenorrhea. The other aspect of gonadotropins is the relationship of LH to FSH, the classic example being a high LH to FSH ratio in polycystic ovary disease (PCO).

**TREATMENT OF OVULATORY DISORDERS**

This involves treatment of the causative factor. One example would be a luteal phase deficiency treated with the progesterone that is lacking. The WHO I type amenorrhea can be treated by adding gonadotropins that are low (human menopausal gonadotropin, Pergonal treatments.) In several examples of ovulatory disturbance, Clomiphene Citrate (Serophene or Clomid) can be used to improve the output of gonadotropins (PCO, WHO type II). Surrogate LH surge can be created when required by giving hCG (human gonadotropin).

**THE HYSTEROSALPINGOGRAM**

This is an x-ray assessment of the cavity of the uterus, the patency of the tubes and the, presence of peritubular adhesions in some cases. The cavity of the uterus may be distorted by Mullerian anomalies such as bicornuate uterus, submucosal fibroids and intrauterine synechiae (Asherman's Syndrome), etc.

The tubes may be occluded proximally at the cornual area, isthmus, or the fimbria distally. Fimbrial occlusions appear to be the most common. Causes-of tubal occlusion include infection and endometriosis.

Management of the couple with abnormal uterine or tubal status will depend upon the nature of the finding and whether the fertility problem is absolute or relative. Clearly a relative problem for infertility can be managed by treating all aspects of the findings to date without proceeding to the most invasive (surgical) procedures. The age of the female determines how soon the more invasive treatment will be undertaken.

**THE POST-COITAL TEST**

For approximately three days prior to ovulation, the cervical mucus is most ripe. This means the mucus is copious, stretchy (spinnbarkeit) and produces more ferns with drying. These are all estrogen induced effects associated with mucus receptivity and the ability of the sperm to penetrate through the cervix. Intercourse takes place the night before the test (six to eighteen hours prior to the test) and the number of the sperm and their forward swimming motion counted per high power field. An essentially normal post-coital test can be regarded as ten sperm per high power field with at least 50% of the sperm having a forward swimming motion. Other aspects studied include the presence of white cells and the clarity of the mucus. Poor post-coital tests relate to cervical and sperm factors as well as combinations of these including immune factors (anti-sperm antibodies including auto-antibodies). Intrauterine insemination (IUI) can be used in most cases of poor post coital tests. To rule out infective disorders do chlamydia, GC, and mycoplasma cultures of cervix. About 50% of the time, the poor post-coital test is unexplained. Pregnancy appears to occur across a wide range of post-coital test findings.
THE LAPAROSCOPY

According to some definitions, a normal workup up until but excluding the laparoscopy can be regarded as the basic workup. In most centers, the basic workup includes the laparoscopy. This ambulatory surgical procedure involves examination of the pelvic anatomy transperitoneally. Essentially, the anatomy of the uterus and tubes with their relationship to the ovaries is studied. Minor or major laparoscopic treatments can be performed at the time of laparoscopy depending the expertise of the surgeon and the patient counseling prior to the procedure.

SURGICAL TREATMENTS

These can be done on the uterus (myomectomy, lysis synechiae), ovaries (e.g. removal of endometriomas), tubes (e.g. surgery to remove occlusion or salpingostomy), and peritoneum (lysis adhesions).

FURTHER MANAGEMENT OF THE INFERTILE COUPLE

At this stage, patients will either have a diagnosis, have an assessment of unexplained infertility and may have been on some treatments or no treatment to date. Stresses involving self perception of bodily failure, esteem, interpersonal relationships and work obligations all add up to the emotional components of infertility diagnosis and management. Sensitivity and counseling at all stages is required and the use of mental health providers should be liberally introduced.

UNEXPLAINED INFERTILITY

When the basic workup reveals no causes, the infertility is called unexplained or idiopathic. 50% of couples with unexplained infertility will conceive without treatment within one to two years. Studies showing the latter do not account for the age related decline in fertility. Such a period of waiting should not be suggested to patients age 35 or older and should not be the first choice in patients age 30 - 34. Today numerous additional tests can be done over and above the basic evaluation to help identify the cause of infertility.

ADDITIONAL TESTS

MALE FACTOR:

These include anti-sperm antibodies, sperm penetration tests of the hamster egg, sperm penetration through bovine mucus, acrosyn content of sperm, the hemi-zona assay, Tygerberg Strict Criteria of sperm morphology and others. The more strict assessment of sperm morphology suggests normal sperm function when 14% oval shaped sperm are present and a very poor prognosis if 4% or less such sperm are seen. Recent studies suggest that only these normal or oval shaped sperm by strict criteria are present in the normal post-coital test and are the sperm that are attached to the zona by the hemi-zona assay. In unexplained failed fertilization in IVF procedures, an assessment of Tygerberg Strict Criteria will help explain all but 11% of such failures.

ASSESSMENT OF OVARIAN RESERVE

Evaluation of the Infertile Couple
Early follicular phase (basal) FSH levels on day three of the cycles has become a useful test for assessing the oocyte factor in fertility. Day 3 FSH levels of 20 iU/ml or more (12 iU/ml in some labs) indicate poor prognosis (poor oocyte reserve).

**THE UTERINE FACTOR**

This can be further evaluated by hysteroscopy (e.g. subtle polyps; endometrial atrophy).

**THE TUBAL FACTOR**

This may be studied by a technique of fallopscopy (e.g. subtle intra-tubal adhesions; obliteration of portions of endosalpinx).

Implantation factors can be studied by assessing the antiphospholipid antibodies (lupus anticoagulant and the anticardiolipin antibodies).

In unexplained infertility or where explained infertility fails to respond to treatment of the causes, elective treatments are available. These elective treatments include Clomiphene plus intrauterine insemination, combination treatments and gonadotropin augmentation of ovulation (super ovulation) treatments. The former treatments may yield up to an 8s conception rate per month of treatment with the latter approximately 20% conception per treatment. The latter treatment begins to approximate the conception rate per month in the fertile population which has to be controlled for age. Once the above treatments have been completed, the assisted reproductive technologies such as gamete intrafallopian tube transfer (GIFT) and in vitro fertilization (IVF) will complete the elective options for treatment.
THE ASSISTED REPRODUCTIVE TECHNOLOGIES

The assisted reproductive technologies (ART) are defined as treatments for infertility involving surgical retrieval of gametes, egg or sperm. In the female the eggs can be retrieved from a natural cycle (single mature oocyte) or in the stimulated cycle (multiple mature oocytes).

The first in vitro fertilization (IVF) conception (Steptoe and Edwards) resulted in the birth of Louise Brown in 1978. This heralded the era of the assisted reproductive technologies. IVF showed the way for several parameters: retrieval of the oocyte and its survival and retention of function in vitro, the development of medium in which sperm can be suspended in the absence of seminal fluid with retention of fertilizing capability extra corporeally, identification of fertilization in vitro, return of the embryo to the uterine cavity with subsequent implantation, continuation of the pregnancy relatively normally and until term with normal delivery. Today, there are over 150,000 babies born world-wide through the ART's.

The following are additional ART's that have been developed from the IVF model:

- IMMATURE OOCYTE RETRIEVAL WITH MATURATION IN VITRO
- CRYOPRESERVATION OF EMBRYOS
- CRYOPRESERVATION OF OOCYTES
- CRYOPRESERVATION OF OVARIAN TISSUE
- GIFT (GAMETE INTRAFALLOPIAN TUBE TRANSFER)
- ZIFT (ZYGOTE INTRAFALLOPIAN TUBE TRANSFER)
- MESA (MICROSURGICAL EPIDIDYMAL SPERM ASPIRATION)
- TESE (TESTICULAR SPERM EXTRACTION/TESTICULAR BIOPSY RETRIEVAL OF SPERM)
- PESA (PERCUTANEOUS SPERM ASPIRATION)
- MICROMANIPULATION OF OOCYTES (ICSI/INTRACYTOPLASMIC SPERM INJECTION)
- ASSISTED HATCHING
- HETEROLOGOUS OOPASM TRANSFER (CYTOPLASMIC DONATION)
- OOCYTE NUCLEAR TRANSFER
- OOCYTE DONATION TREATMENT (CLASSICAL)
- GESTATIONAL SURROGACY
- PRE-IMPLANTATION DIAGNOSIS (EMBRYO BIOPSY)
IVF

IVF is indicated when the fallopian tubes are absent or irreparably damaged. Soon indications were extended to male factor, endometriosis and unexplained infertility. Today, IVF is utilized for all causes of infertility where more conventional treatments have failed.

IVF can be performed on the natural cycle or the stimulated cycle, (the most common approach). After a thorough evaluation and counseling, the couple go through a so-called cycle of treatment. We know that oocytes are released with ovulation approximately 36 to 48 hours after the initiation of the LH surge or the administration of hCG. The egg retrieval procedure is done by 36 hours and prior to ovulation. The oocyte matures with the onset of the LH surge (or its surrogate hCG). LH matures the germinal vesicle stage of the oocyte normally resting in the pro-phase immediately prior to the first meiosis. The first phase of meiosis is completed immediately prior to ovulation. The oocyte must be retrieved at the time of completion of the first meiosis and extrusion of the first polar body in order to maintain optimal fertilizing ability. The oocytes are pre-incubated for four to eight hours in vitro medium. The medium contains amino acids, is buffered and contains electrolytes mimicking those found in human tubular fluid.

Sperm is prepared by a washing and separation process (swim up) using similar media, removal of the seminal fluid with non-motile sperm and other debris. During this process, the sperm undergo capacitation. This is an influx of calcium and other membrane changes enabling the sperm to subsequently undergo an acrosome reaction. The acrosome reaction is initiated by the zona pellucida, particularly the zona pellucida protein #3 (ZP 3). The sperm enters the perivitelline space acrosome reacted and the process of fusion occurs with the penetration of the oolemma. Thirteen to twenty-four hours later, fertilization can be documented by the presence of two pronuclei. This morphologic appearance documents the presence of the diploid zygote. The zygote will divide into two then four cells by forty eight hours after retrieval.

An embryo transfer procedure is a simple placement of the embryo within a centimeter of the fundus of the uterine cavity. Implantation occurs six to seven days after egg retrieval and three to five days after embryo transfer depending on which day the embryos are returned to the uterus (day one, two or three post-retrieval). The luteal phase is preferably managed with progesterone or hCG support in the stimulated cycle. Currently the baby rate per embryo transfer is in the range of about 25% for women under forty. If fertilization is not documented, this adds to the information relating to the infertile couple. This pertains to oocyte and sperm parameters. Fertilization rate in normal couples is in the range of 90% per treatment cycle.

CRYOPRESERVATION OF OOCYTES

This is a difficult procedure as the ice crystals can damage the cytoskeleton. In particular, the spindle in the maturing oocyte is easily damaged by freezing. There is one report of mature oocytes being frozen then thawed with subsequent IVF and a term pregnancy. The freezing lasted some four hours only and the establishment of pregnancy from cryopreserved mature eggs has not been duplicated. Substantial work has found some promise in the cyropreservation of the immature oocyte. This is usually done at the germinal vesicle (GV) stage. Here the nucleus is at a resting stage with no spindle with the nuclear cytoplasmic ratio substantially larger where crystal damage to

Assisted Reproductive Technology
the cell is not as severe. No on-going pregnancies have been developed from these frozen immature oocytes but maturation of the oocyte upon thawing has now been described. Subsequent fertilization in these immature thawed embryos has also been developed with the use of ICSI (Intracytoplasmic sperm injection). Prior to ICSI, in vitro fertilization of eggs matured in vitro was very poor. There is substantial need for the improvement of immature oocyte maturation, immature oocyte cyropreservation in patients with cancer who need their ovaries removed or where salvage of the ovaries is needed prior to radiation or chemotherapy.

CRYOPRESERVATION OF OVARIAN TISSUE

Techniques of salvaging oocytes from cryopreserved ovarian tissue are currently being developed. There are still no good methods of freezing ovarian tissue with adequate oocyte salvage and subsequent in vitro fertilization capability of these eggs. Portions of the ovary are often too thick and newer techniques are developing thin wafers of ovarian tissue which may allow for better cyroprotectant penetration of the ovarian tissue and better subsequent freezing. This technique could be a major help for patients who wish to combat the aging process of infertility. A small portion of ovarian tissue can be frozen in women in their early thirties who wish to delay childbirth until a more opportune time when their ovarian reserve would otherwise be substantially diminished.

CRYOPRESERVATION OF EMBRYOS

This was first successfully performed with the establishment of alive baby in 1981 (Trounson). Embryos can be frozen in the pronuclei and subsequent developmental stages. A cryoprotectant is used (propanediol or DMSO). Various cooling techniques are utilized and after seeding, the embryos are plunged into liquid nitrogen for storage. There appears to be good salvage of embryos up to five years. Storage of embryos beyond this time awaits additional study. The embryos are thawed in a series of progressively normalized culture media and transferred to the uterine cavity as in a fresh cycle. The timing if the placement of embryos in the uterus is critical. The embryo should be the same age as the endometrium in relation to the time of ovulation or older than the endometrium (negative asynchrony). Fibronectin, laminin and other growth and adhesion proteins are laid down by the sixth day post-ovulation and implantation cannot occur beyond this date. Implantation of an embryo can occur only after hatching which occurs days five or six after ovulation. Embryos that are younger than the endometrium will therefore be hatching after the window of implantation has closed and therefore conception will not occur.

The age of the embryo is determined by the time at which they are frozen. The age of the endometrium is the time that has elapsed since the onset of the LH surge or the time of ovulation both of which have a fixed relationship to each other. The embryos are preferably thawed out twelve hours or so earlier in the patient's cycle. Example: embryos frozen at 48 hours after ovulation can be thawed when the patient is thirty-six hours after ovulation. This ensures that the embryos are older than the endometrium. Currently, pregnancy rates for frozen embryos are in the range of 20% per cycle. Numerous factors are involved including the 10% - 20% loss of embryos in the cryopreservation process. Generally good embryos by morphologic appearances have a higher rate of implantation in the fresh cycle and have a greater chance of survival and implantation post cryopreservation.

Assisted Reproductive Technology
GIFT

The placement of freshly retrieved oocytes in the stimulated cycle in the fallopian tube via laparoscopy along with washed sperm was first described in 1984. (Asch). It was performed initially for a wide range of indications including unexplained infertility, endometriosis, mild to moderate male factor, etc. Today it is indicated for all forms of infertility but for severe male factor provided at least one fallopian tube appears to function normally. The procedure is done laparoscopically although recent techniques describe a trans-cervical approach via tactile techniques or ultrasound guidance of a catheter to the cornu of the fallopian tube and 5 cm beyond. Baby rates per GIFT procedure are reported in the 30% range and higher for women under 40. Miscarriage rates are approximately 20%, ectopic pregnancy rates 6%, and multiple birth rates 20%. GIFT may be better than IVF for women over age 40.

ZIFT

This stands for zygote intra fallopian tube transfer and represents all of the technologies where a fertilized oocyte or later stage embryo is placed in the fallopian tube. JET or tubal embryo transfer). Initially, pregnancy rates appeared to be higher using these techniques than those found in a regular IVF. But, with on-going studies the pregnancy rates were no different from those seen in the GIFT procedure. ZIFT can be a useful choice in special situations where fertilization needs to be documented yet the advantage over tubal transfer is still desires. Most patients do not wish to undergo two surgical procedures during one cycle of treatment. (Egg retrieval followed a few days later by an embryo transfer through laparoscopy). Other advantages of ZIFT are where the return of embryos is difficult owing to severe cervical stenosis.

SPERM RETRIEVAL TECHNIQUES

MESA

In 1988 fertilization was documented using sperm retrieved from the epididymis in men with congenital absence of the vas deferens. This was a major new finding in that epididymal sperm was previously thought to be incapable of fertilization. It was thought that substantial maturation of the sperm was required through the 25 feet of the tubular structure that is normally packed in tightly coiled tubules to form the epididymis. The initial fertilization rates were reported to be 20%. The pregnancy rate was also reported in that range. With repeated use of this technique, it was clear that the fertilization rate was substantially lower than initially reported (8%). In order to improve the rate, specialized sperm enhancing treatments were used including the use of pentoxiphylline, a xanthine capable of improving sperm motility. Other techniques involved the concentration of sperm under microscopic droplets of fluid in order to increase the proximity of the high density of sperm close to the egg. Minor improvements in fertilization occurred. It was not until the development of ICSI in 1992 that epididymal sperm achieved fertilization in the 90% range. Subsequent pregnancy rates became a steady 20% per embryo transfer procedure.
TESE
The advent of ICSI spread to the use of sperm retrieved directly from the testicle. Relatively mature looking sperm can be so retrieved in the obstructive azoospermic patients. In addition, men with non obstructive azoospermia were soon found to have islets of developing sperm with haploid chromosome numbers. The biopsy of testicular tissue have allowed the discovery of pockets of developing sperm in a 50 - 80% of cases. Even immature sperm forms and occasionally non-motile such sperm are now capable of establishing fertilization in up to 50% of cases with a subsequent 10-20% pregnancy rate. New questions are now being asked relating to the genetics of oligospermia and azoospermia. The so-called DAZ gene complex on the Y chromosome (deletion in azoospermia) shows substantial sites of loss of genetic information. The chromosome and genetic analysis in the patient who is otherwise normal suggests these findings to be mutational . Other chromosomal abnormalities have also been identified including the well known Kleinfelter's Syndrome. Open questions remain on the ethics of having children that may have the same fertility problems as their parents.

PESA
This stands for percutaneous sperm aspiration, a technique developed to simplify the technology and costs of retrieving sperm mostly in obstructive azoospermic patients. This can be achieved without general anesthetic and simple placement of a fine needle in the testicular tissue and aspirating the sperm. Such sperm can then be used to fertilize oocytes using ICSI.

MICROMANIPULATION OF OOCYTES
This pertains to alterations in the oocyte in order to improve fertilization. Initially, the techniques included zona drilling using acid Tyrodes. In addition the PZD method (partial zona dissection) was a mechanical method of making a slit in the zona. Subsequently, SZI (subzonal insertion of sperm) was utilized to place sperm directly under the zona of the egg. All these techniques were beset with erratic fertilization rates, polyspermy and poor implantation rates. In 1992 the ICSI (intracytoplasmic sperm injection) procedure was developed to the point of a successful birth (Palermo, DeVroye, Van Steirteghem). Fertilization rates dramatically improved to the point of 90% of mature eggs showing fertilization. More recently, the pregnancy rates with typical ICSI (with the exception of epididymal or testicular sperm) showed pregnancy rates similar to regular IVF (25-30% per embryo transfer). ICSI remains currently the most important breakthrough in the ART's field in recent years. This has opened up options for treatment for the most severe male factor infertilities that heretofore have not been able to conceive. This also has enlarged major new areas for research on the role of sperm and oocyte interactions.

TREATMENTS FOR DEFECTIVE OOCYTES OR OOCYTE FACTOR.

• CLASSIC OOCYTE DONATION
In 1982 oocytes donated to a couple were fertilized by the husband's sperm and the embryos transferred to the uterus of the wife (Lutjen). The endometrium was prepared by a sequential treatment of estradiol followed by progesterone. An endometrial biopsy confirmed the normal development of post-ovulatory endometrium. Since then, repeated such treatments in various forms of ovarian failure and the absence of ovaries have confirmed that conception rates are very substantial (50%). It was discovered that...
pregnancies can also carry to term without functioning ovaries at all. Progesterone and estrogen support is generally continued until ten completed weeks. Normally, the corpus luteum supports the pregnancy with progesterone and other steroids for approximately six to ten weeks of gestation. The exact day of shift from corpus luteum to the placenta itself is variable from one patient to another but appears to be as early as 5 1/2 weeks of gestation. Once the placenta has taken over steroid support, pregnancy can maintain its own autonomous function without exogenous steroids.

**HETEROLOGOUS OOPLASM TRANSFER (CYTOPLASMIC DONATION)**

In 1997, the first baby born was reported from a technique of donating the ooplasm to a recipient egg (Jaques Cohen). Only six such patients have been treated with one term birth. The concept is that some defective oocytes still have normal nuclear DNA. It is postulated that the cytoplasm in these patients is insufficient for adequate fertilization cleavage and implantation. Studies have shown that with aging, oocytes have less ATP production and fewer mitochondria. In mice, transfer of cytoplasm from the eggs of younger rodents to those of older rodents yield improved fertilization and implantation. These findings are very preliminary and much more research will be needed.

**OOCYTE NUCLEAR TRANSFER**

This concept involves the extraction of the nucleus of the patient herself who is usually older. The nucleus is extracted from her own immature egg and injected into the donor's egg that is free from the donor's nucleus. This if successful, may allow a patient to conceive with her own nuclear genetic information. No pregnancies have been reported with this to date.

**GESTATIONAL SURROGACY (HOST UTERUS TREATMENT)**

This treatment was developed using donated egg technology. Here a patient with an absent uterus or severely damaged uterus who may still have healthy eggs can have her eggs treated by IVF and the embryos transferred to the uterus of a surrogate. The classic indication for this would be an absent uterus, such as status post hysterectomy for carcinoma for the cervix where the ovaries are preserved or the Rokitansky-Kuster-Hauser syndrome.

ART allows us to work with sperm, egg, embryo and uterus from different couples. The ethics and emotional aspects of parents, children and providers need to be evaluated, discussed, and further developed.

**PRE-IMPLANTATION DIAGNOSIS (EMBRYO BIOPSY)**

A blastomere can be removed from an early embryo and analyzed using YC:K (polymerase chain reaction) as well as the FISH technique (florescent in situ hybridization). The ideal timing of removal of the blastomere is at the eight cell stage. At this stage the embryo may survive with as few as half of the remaining blastomeres and in some circumstances as little as two blastomeres. Primers are being developed for specific diseases such as several forms of muscular dystrophy, cystic fibrosis, etc. In addition, primers against specific chromosomes have been developed (against the thirteen, eighteen, twenty-one and Y chromosome). In this way aneuploidy and other abnormalities can be identified prior to placement of embryos. In addition X-linked genetic disorders can be eliminated by replacing the female embryos only. New primers
are being developed with more diseases being identified in the pre-implantation stage. There are numerous problems that still need to be evaluated, including DNA contamination, mosaicism, etc. The ability to micro manipulate the embryo, or the blastomere including the work of knockin and knock-out genes, holds promise for the future and may substantially reduce the incidence of numerous diseases.