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## Male Aspects of Fertility

**Author: Jack H. Mydlo, MD, FACS**

One of the most sensitive and frustrating aspects of practicing urology is when couples are trying to conceive without success. Much has improved over the years with better techniques of semen analysis, collection, and in vitro fertilization of the egg. This brief review highlights some of the basic concepts of male infertility, and demonstrates the basic work-up of these patients.

**Statistics:**

- About 25% of couples will become pregnant after one month of trying to conceive.
- 85% of couples will become pregnant after one year of trying to conceive.
- 15% of couples will have difficulty achieving pregnancy during their first year.
- A male factor in infertility is found in about 30% to 50% of couples who are unable to conceive.

**Evaluation of the male in doing a fertility workup.**

1. Questions to ask in the present couple history
   - Duration of infertility.
   - Contraceptive methods and length of time used.
   - Length of time trying to conceive.
   - Number of pregnancies in present and previous relationships, including miscarriages, abortions, etc.

2. Previous marital history and relationships
   - Duration and numbers of pregnancies achieved.
   - This infertility may be a problem of both partners.

3. Sexual history
   - Frequency of intercourse. Overly frequent or infrequent can adversely affect the couple's ability to conceive.
   - Erectile dysfunction: this can be treated with PDE-5 inhibitors. However, the stress involved in trying to conceive has been associated with ED.
   - Problems with ejaculation, such as premature ejaculation, hypospadius or chordee, may affect the proper deposition of the sperm onto the cervix.
   - Dyspareunia, or painful intercourse, can cause problems with intercourse.
   - Lubricants can be spermicidal and can be a cause for infertility.
   - Timing of ovulation is important. Intercourse before ovulation rather than after ovulation might improve pregnancy rates.

4. GU history
   - Cryptorchidism, when bilateral, can be associated with impaired spermatogenesis. Even when corrected by orchidopexy so that potential tumors may be detected, spermatogenesis may already be decreased.
   - Infections that involve the genitalia may induce scarring and consequent fertility problems. In pelvic inflammatory disease, this includes the occlusion of fallopian tubes. In the male, this includes urethral strictures, vas occlusion, and mumps orchitis which may cause testicular atrophy, etc.
   - A history of testicular tumors, which required surgery, radiotherapy and/or chemotherapy.
   - Trauma or torsion may also result in scarring of the duct system and thus decrease the transporting of the spermatozoa.
   - Exposure to increased heat, in the form of continued exposure to hot tubs, hot baths, saunas, steam rooms, or via varicoceles, etc.,

***Continued ..... pg 2***

### WHAT'S INSIDE ➤➤



### UPCOMING EVENTS ➤➤

| | |
|---|---|
| 03/13-15 | **BCDVV:** 19th Biennial Conference on Diseases of the Vulva and Vagina |
| 03/16-17 | **ACOOG:** American College of Osteopathic Obstetricians & Gynecologists |
| 04/3-5 | **ACOG-OR:** American College of Obstetricians & Gynecologists Oregon Section Clinical Meeting |
| 04/4-5 | **TACOG:** Texas Section of the American College of Obstetricians & Gynecologists |
| 05/3-6 | **ACOG:** American College of Obstetricians & Gynecologists 2008 Annual Meeting |

## Female Infertility

**Author: Shlomo Stemmer, MD, FACS**

Infertility is defined as the failure of a couple to conceive after having unprotected intercourse without contraception for at least one full year. In primary infertility, pregnancy has never occurred. In secondary infertility, one or both members of the couple have previously conceived, but are unable to conceive again after a full year of trying. The probability for a normal couple to conceive per cycle is defined as fecundability and it is estimated at 25%.

**Incidence**

It is estimated that in the United States approximately 10% of women between 15-44 years of age have difficulty conceiving or carrying a pregnancy full term. Also, 25% of women will experience a period during which they are infertile during their reproductive life (1).

**Risk factors**

Age alone is a contributing factor to infertility. After age 30 to 32, female fertility rates decline with increasing age. At age 37 fertility declines more rapidly. Between the ages 40 to 45, fertility rates are 95% lower compared to women 20 to 24 years of age (2). Furthermore, with increasing age there is a significant increase in the incidence of spontaneous abortions and chromosomal abnormality in the fetus. Another risk factor is smoking which has been associated with decreased female fecundity and increased incidences of spontaneous abortion (3). Extremes in weight may reduce a women's fertility rate. Obesity is associated with polycystic ovarian syndrome (PCOS) which is known to cause ovulation dysfunctions. On the other hand being underweight may also contribute to infertility as it may disrupt the menstrual cycle.

**Causes**

Infertility may be due to both male and female partners. In approximately 40% of cases, a couple is unable to conceive because of a male factor; 40% of cases it may be due to the female partner; and 20% are caused by both partners. Female infertility may be due to an ovarian factor which usually involves problems with ovulation. Tubal factor refers to infertility resulting from damaged fallopian tubes commonly caused from pelvic inflammatory disease, endometriosis, or ectopic pregnancies. Uterine factors are a relatively uncommon source of female infertility and include congenital abnormalities, leiomyomas, chronic endometritis, and intrauterine adhesions. Cervical factor commonly relates to problems with the cervical mucus which may prevent the sperm from moving freely into the uterus.

**Diagnosis**

A detailed history should be obtained underscoring menstrual abnormalities, past medical and surgical history, medication usage, family history and lifestyle issues such as smoking, drug use and alcohol abuse. The patient should be weighed and her Body Mass Index (BMI) calculated. In addition to breast and pelvic exams, signs of thyroid disorder and excess androgen production, which may result in excessive facial and body hair growth, should be carefully evaluated.

Ovulatory dysfunction such as anovulation, the suspension or cessation of ovulation, may often be diagnosed by the patient's medical history. Urinary testing with an ovulation predictor kit measuring the presence of a luteinizing hormone (LH) surge confirms the diagnosis. High serum levels of LH and follicle stimulating hormone (FSH) in conjunction with low estrogen levels may indicate

***Continued ..... pg 4***

# Male Aspects of Fertility

*Continued from ..... pg 1*

may contribute to decreased sperm production.

- Exposure to radiation may also destroy sperm since these are particularly sensitive. This may be a new phenomenon seen in younger and younger men undergoing radiotherapy to the prostate to treat prostate cancer, yet still want to start families.
- Previous semen analysis is crucial to know the etiology of the infertile couple.
- It is important to know if the female has been pregnant before. The evaluation of the female, especially invasive procedures to evaluate the patency of the fallopian tubes, should be done after the complete workup of the male.

**Table 1.** Chemicals that may adversely affect fertility.

| |
|---|
| Alcohol |
| Arsenic |
| Aspirin in large quantities |
| Caffeine |
| Cimetidine |
| Colchicine |
| Diethylstilbesterol |
| Lead |
| Marijuana |
| Medroxyprogesterone |
| Nicotine |
| Niitrofurantoins |
| Phenytoin |
| Spironolactone |
| Sulfasalazine |
| Testosterone |

5. General medical history
   - Diabetes and hypertension can affect erectile function and consequently fertility.
   - Hernia repair is associated with injury to the vas deferens in 1% to 2% of cases.
   - Surgery on the bladder or prostate, or retroperitoneal surgery can result in problems with emission or ejaculation. In young men who are to undergo these types of operations, it is prudent to bank sperm for future use.

6. Environmental/Sociological factors
   - Severe stress can affect sperm count and the volume of ejaculate. Many men cannot change the stress in their jobs or careers. Therefore, sometimes meditation or exercise is a way to reduce the amounts of stress.
   - Alcohol, tea, coffee, cigarettes, and marijuana have been shown to be injurious to the gonads and to sperm production. Some of these same agents have also been shown to have a detrimental effect on erection function.

7. Physical Examination
   - Testis should be 4 cm in length and 2.5 cm in width, about 20 ml. These contain the seminiferous tubules which produce the spermatozoa into the lumen, where they are carried through the vas deferens to be ejaculated **(Figure 1)**.
   - Vas deferens should be palpable as a firm, distinct cord-like structure. In 1% to 2% of infertile men, one or both vas may not be palpable in the scrotum, which is a congenital condition.
   - A varicocele is an enlarged spermatic vein seen and felt along the cord, usually on the left side, because of the back pressure that the right angle insertion the spermatic vein makes upon the left renal vein. This increased spermatic vein can heat up the scrotum by 1-2 degrees and cause a decrease in sperm production. If seen on the right side, one should be concerned about a retroperitoneal mass compressing the spermatic vein.
   - Hernias or previous hernia surgery may have injured the blood flow to the cord or the vas and should be evaluated in the fertility work-up.

8. Semen Analysis
   - At least two semen analyses are needed to establish a baseline for a patient. If there is a large difference or discrepancy, then a third should be taken.
   - Each specimen should be taken after 3-5 days of abstinence. It is usually recommended to have the patient ejaculate in the rest room of the lab as opposed to at home and bringing it to the lab, so the most accurate assessment of sperm viability and motility is obtained.

Azoopsermia or low sperm count: if no sperm are seen on analysis, centrifugation should be done to rule out any sperm in the sample. If retrograde ejaculation is present, then one may find sperm in the urine. If this is the case, then oral alkalization and sympathomimetic agents can be used to promote antegrade ejaculation. Ductal obstruction must also be ruled out. A positive fructose test will rule out complete obstruction. LH, FSH, and testosterone levels can differentiate primary testicular failure from secondary testicular failure. A serum FSH which is >3 X normal, with atrophic testicles, indicates severe testicular failure. This rules out the need for a surgical biopsy to rule out complete obstruction. However, the small amounts of sperm found in these men can be used with IVF/ICSI by sperm extraction techniques.

If multiple abnormal parameters are seen in the sperm analysis, it is important to rule out stress, infections, heat (such as hot tubs, Jacuzzis, etc.) smoking, drugs, and varicoceles. If a varicocele is present, data has shown that a varicocelectomy, or ligation of the varicocele vein, may give as large as a 70% improvement rate in semen quality, which can be associated with a 40% pregnancy rate. These pregnancies can occur in about 7 or 8 months following varicocelectomy. In doing these operations, the best results are obtained when all the spermatic veins are ligated, including dilated and non-dilated. However, care must be taken not to injure the arterial blood supply to the testis, which could lead to atrophy.

**Table 2.** Semen analysis parameters.

| | |
|---|---|
| Ejaculate volume: | 1.5 - 5.0 cc |
| Sperm density: | > 20 million/cc |
| Motility: | > 50% motile |
| Forward progression: | > 2.0 (scale 0 - 4) |
| Morphology: | > 30% |

*- Jack H. Mydlo, MD, FACS, a scientific consultant for the Research & Development Department at Medical Diagnostic Laboratories, L.L.C., is also a Professor and Chairperson for the Department of Urology at Temple University School of Medicine.*

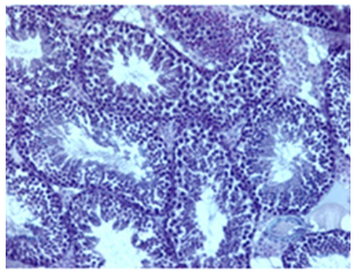


**Figure 1**. Cross section of seminiferous tubules of testis (photo courtesy of Dr. Stephen H. Pilder).

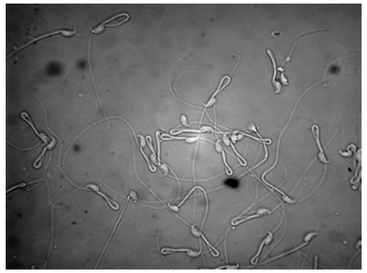


**Figure 2.** Motile sperm. (photo courtesy of Dr. Stephen H. Pilder)

**References:**


1. **Turek PJ.** Male Infertility. In Tanagho EA, McAninch JC, (eds): Smith's Urology, 15th ed Stamford, CT Appleton and Lange, 2000 Chapter 46, pp. 750-787.
2. **Sigman M, Howards SS.** Male infertility In Walsh PC, Retik A, Vaughan ED Jr, Wein A (eds): Campbell's Urology, 7th ed. Philadelphia, WB Saunders 1998, pp 1287-1330.
3. **Jarow JP, Sharlip ID, Belker AM et al.** Male Infertility Best Practice Policy Committee of the American Urological Association Inc, J Urol.167:2138-2144, 2002.
4. **Turek PJ.** Male infertility and sterility. In Hanno PM, Malkowicz SB, Wein AJ (eds): Penn Clinical Manual of Urology, Philadelphia, PA, Saunders Elsevier 2007, Chapter 20, 707-742.




## e-Quiz

For results to the electronic Epidemiology Quiz, please visit www.mdlab.com and click on the e-Quiz link.

1. What percentage of couples will have difficulty achieving pregnancy during their first year?

   a. 5%  c. 15%
   b. 10%  d. 20%

2. True or False. With increasing age there is a significant increase in the incidence of spontaneous abortions and chromosomal abnormality in the fetus.

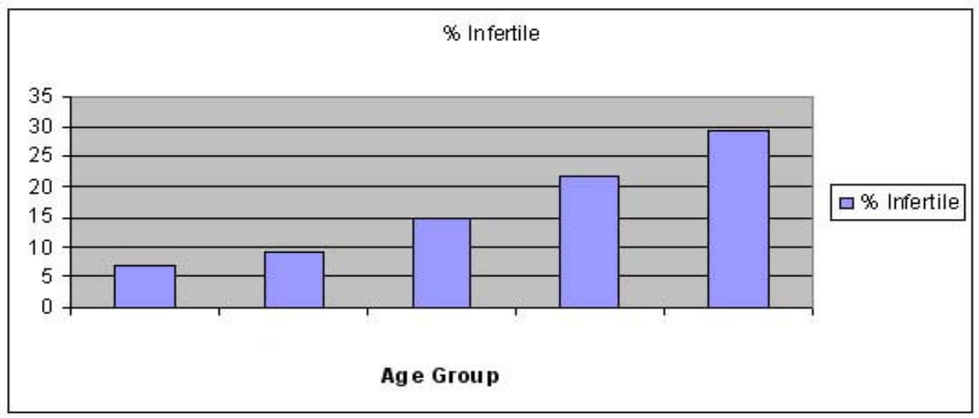
3. What is the only HLA gene expressed in the placenta and believed to protect the fetus from maternal natural killer cell attack?

   a. HLA-A  c. HLA-C
   b. HLA-DR  d. HLA-G

4. The probability of survival for human conceptions from fertilization to term is estimated to be less than _____ %

5. List the appropriate age group underneath the percentage of infertility:

Age Groups:
15 - 19
20 - 24
25 - 29
30 - 34
35 - 39
40 - 44
45 - 49




# Recent Publications

## Medical Diagnostic Laboratories, L.L.C.

**Abstracts**

1. **Prasad A, Basu P, Mordechai E, Adelson ME, Gygax SE.** PBP4 polymorphisms generate penicillin tolerance in Group B Streptococcus. 109th General Meeting of the American Society for Microbiology, Philadelphia, PA. May 17-21, 2009
1. **Vermitsky JP, Self MJ, Chadwick SG, Katiyar SK, Edlind TD, Gygax SE.** cAMP-PKA kinase Tpk3 negatively regulates *Candida glabrata* Pdr1. 109th General Meeting of the American Society for Microbiology, Philadelphia, PA. May 17-21, 2009
2. **Hilbert D, Paulish T.** Uropathogenic *Escherichia coli* inhibits the activity of nuclear-localized NF-kB to obscure a toll-like receptor-independent inflammatory stimulus. 109th General Meeting of the American Society for Microbiology, Philadelphia, PA. May 17-21, 2009
3. **McCourt P.** Molecular analysis of the *Candida albicans* Arv1 ortholog of *Saccharomyces cerevisiae*. 109th General Meeting of the American Society for Microbiology, Philadelphia, PA. May 17-21, 2009

**Peer-Reviewed Papers**

1. **Hoey JG, Valois-Cruz F, Goldenberg H, Voskoboynik Y, Pfiffner J, Mordechai E, Adelson ME.** 2009. Development of an IgM capture-based ELISA for detection of acute infection with *Bartonella henselae*. *Clin Vaccine Immunol*. 16(2): 282-284.

## HUMIGEN, L.L.C.

**Peer-Reviewed Papers**

1. **Ucisik-Akkaya E, Dorak MT. 2009.** A Study of Natural Killer Cell Lectin-like Receptor K1 Gene (KLRK1/NKG2D) Polymorphisms in a European Population Sample. *Tissue Antigens*. 73(2): 177-83.

# Journal Watch

**Ucisik-Akkaya E, Dorak MT.** 2009. A study of natural killer cell lectin-like receptor K1 gene (KLRK1/NKG2D) region polymorphisms in a European population sample. *Tissue Antigens*. 73(2): 177-83.

This study, which was performed at HUMIGEN, LLC., used a different approach to find a genetic marker predictive of pregnancy loss. Instead of examining genetic polymorphisms in women experiencing reproductive failure, newborns were examined for genotype frequency differences between males and females to obtain evidence for sex-specific prenatal loss. Natural killer (NK) cells regulate maternal immune system tolerance to the fetus. The gene encoding the major NK cell receptor (NKG2D) was analyzed in detail in 388 newborns. Healthy male newborns had a lower frequency of heterozygous combinations of alleles at more than one variant at the NKG2D gene. This finding suggests that male fetuses may be at higher risk than females carrying these genotypes. If confirmed, the NKG2D gene markers may be used in IVF clinics to predict the likelihood of success before implantation.

**Pellati D, Mylonakis I, Bertoloni G, Fiore C, Andrisani A, Ambrosini G, Armanini D.** 2008. Genital tract infections and infertility. *Eur J Obstet Gynecol Reprod Biol.* 140(1):3-11.

Infectious agents can impair various important human functions, including reproduction. Bacteria, fungi, viruses, and parasites are able to interfere with the reproductive function in both sexes. Infections of the genitourinary tract account for about 15% of male infertility cases. Infections can affect different sites of the male reproductive tract, such as the testis, epididymis, and male accessory sex glands. Spermatozoa themselves subsequently can be affected by urogenital infections at different levels of their development, maturation, and transport. Among the most common microorganisms involved in sexually transmitted infections that interfere with male fertility are *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. Less frequently, male infertility is due to non-sexually transmitted epididymo-orchitis, mostly caused by *Escherichia coli*. In the female, the first two microorganisms are certainly involved in cervical, tubal, and peritoneal damage, while Herpes simplex cervicitis is less dangerous. The overall importance of cervical involvement is still under discussion. Tubo-peritoneal damage seems to be the foremost manner in which microorganisms interfere with human fertility. *C. trachomatis* is considered the most important cause of tubal lacerations and obstruction, pelvic inflammatory disease, and adhesions. *N. gonorrhoeae*, even though its overall incidence seems to decline, is still to be considered in the same sense, while bacterial vaginosis should not be ignored, as causative agents can produce ascending infections of the female genital tract. The role of infections, particularly co-infections, as causes of the impairment of sperm quality, motility, and function, needs further investigation.

**Cetin I, Cozzi V, Antonazzo P.** 2008. Infertility as a cancer risk factor - a review. *Placenta*. Oct; 29 Suppl B:169-77.

Ovarian, endometrial, and breast cancers are associated with several risk factors, such as low parity, infertility, early age at menarche, and late age at menopause. Frequently, most of these risk factors coexist in infertile patients and some studies suggest that the different infertility causes can be involved in cancer risk development. In particular, case-control and cohort studies investigated the possible role of ovulatory disorders, endometriosis, and unexplained infertility in increasing the risk of this disease. Most studies have shown no overall increased risk in invasive ovarian cancer in subfertile patients, although nulliparity has been consistently associated with increased rates of ovarian tumor, in particular with borderline and endometrioid cancers in patients with a history of endometriosis. Different studies reported that infertile women are not at risk for breast cancer. However, women affected by infertility may be more at risk for endometrial cancer, particularly if affected by ovulatory disorders. Moreover, infertility is now often treated with medical devices that could by themselves modify the hormonal environment and be cofactors in the cellular changes towards cancer development. However, although early studies suggested that infertility medications were associated with an increased risk of ovarian cancer, subsequent studies although mainly reassuring, suggest that type and duration of medical treatment can increase the malignancy risk. An increased risk of endometrial cancer in patients undergoing infertility treatment has been reported, as expected by the similar structure shared by clomiphene and tamoxiphene. Since breast cancer is widely recognized as having a hormonal etiology, a possible role of fertility medications to promote cancer has been hypothesized. However, many large studies were not able to find an associated risk of breast cancer. In conclusion, firm answers about the precise effects of infertility and its treatment on cancer risk are not available but findings are generally reassuring. Further studies about fertility drug treatments on larger populations may offer longer follow-up and more precise data with better adjustments for confounding factors.

**Practice Committee of American Society for Reproductive Medicine.** 2008. Obesity and reproduction: an educational bulletin. *Fertil Steril.* 90(5 Suppl):S21-9.

Obesity is the most common chronic disease in the United States. In 1998, the National Institutes of Health defined obesity in relation to body mass index (BMI); overweight was defined as a BMI between 25 and 29.9 kg/m2, obesity as a BMIR30 kg/m2, and morbid obesity as a BMIR40 kg/m2 (Fig. 1). Using those definitions, data collected between 1999 and 2002 indicated that 31% of non-Hispanic white women, 38% of Hispanic women, and 49% of non-Hispanic black women in the United States were overweight or obese. Obesity often begins **in** childhood and becomes increasingly more common during the reproductive years. The purpose of the present study was to describe the effects of obesity on reproductive function and to outline contemporary treatments for obesity. Obesity is associated with menstrual dysfunction, decreased fertility, and increased risk of miscarriages, decreases fecundity, even in ovulatory women, increases the risks of obstetric and neonatal complications and is associated with abnormal semen parameters and may adversely affect male fertility. Preconceptual counseling for obese women should address the medical, obstetric, and neonatal consequences of obesity and its longer-term implications for offspring. Lifestyle changes involving a diet and exercise program are the first-line treatment for obesity. Adjunctive medical therapy for obesity is indicated when lifestyle changes prove to be inadequate or fail. When combined with a low-calorie diet, metformin may result in weight loss, restore ovulation, and improve fecundity in women with PCOS. Bariatric surgery is more effective than other treatments for weight loss and improves comorbidities related to obesity in patients with a BMI R40 kg/m2.

**Matzuk MM, Lamb DJ.** 2008. The biology of infertility: research advances and clinical challenges. *Nat Med.* 14(11):1197-213.

Reproduction is required for the survival of all mammalian species, and thousands of essential "sex" genes are conserved through evolution. Basic research helps to define these genes and the mechanisms responsible for the development, function and regulation of the male and female reproductive systems. However, many infertile couples continue to be labeled with the diagnosis of idiopathic infertility or given descriptive diagnoses that do not provide a cause for their defect. For other individuals with a known etiology, effective cures are lacking, although their infertility is often bypassed with assisted reproductive technologies (ART), some accompanied by safety or ethical concerns. Certainly, progress in the field of reproduction has been realized in the twenty-first century with advances in the understanding of the regulation of fertility, with the production of over 400 mutant mouse models with a reproductive phenotype and with the promise of regenerative gonadal stem cells. Indeed, the past six years have witnessed a virtual explosion in the identification of gene mutations or polymorphisms that cause or are linked to human infertility. Translation of these findings to the clinic remains slow, however, as do new methods to diagnose and treat infertile couples. Additionally, new approaches to contraception remain elusive. Nevertheless, the basic and clinical advances in the understanding of the molecular controls of reproduction are impressive and will ultimately improve patient care.





# New Test Announcements

**Now available on *OneSwab*®**


**Genetic Carrier Screening**

1217 Factor XI Deficiency by Bio-Plex
1218 Kennedy's Disease by Fragment Analysis
1216 Sickle Cell Anemia by SNP Genotyping with Pyrosequencing
1215 Torsion Dystonia by Real-Time PCR

# Quality Assurance Q&A

***Q: I have a male patient who needs to be tested for sexually transmitted diseases associated with infertility; however, he refuses to allow our office to collect a urethral swab. Is there an easier way?***

**A:** Yes. MDL's ***UroSwab***® specimen collection platform provides a simple, painless, noninvasive alternative to traditional urethral swabs. The simple 1-2 specimen collection instructions will allow the patient to confidently and conveniently collect the specimen.

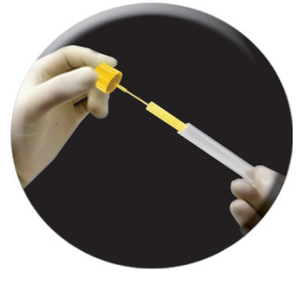
**Step 1**: Simply have your patient void urine directly onto the sponge swab. They may also void into a collection cup and then dip the sponge-swab into the cup to collect urine.

**Step 2**: Insert the sponge-swab into the transport vial and recap the vial tightly.

The specimen is now ready to be packaged and transported in accordance with MDL's specimen collection and transport requirements.

**Step 1:**

**Step 2:**




If you have a question you would like addressed in future issues, please email your question(s) to QAQ&A@mdlab.com

# Female Infertility

*Continued from ..... pg 1*

premature ovarian failure while a high LH/FSH ratio along with elevated androgen levels may be indicative of Polycystic Ovary Syndrome (PCOS). Measurements of serum levels of thyroid stimulating hormone (TSH) and prolactin should be obtained to rule out disorders of the thyroid gland and hyperprolactinemia, which may cause unovulation. Corpus luteum deficiency is diagnosed by measuring levels of progesterone one week prior to menses or on cycle day 21.

Tubal factor may be diagnosed by performing hysterosalpingography (HSG). However, false negative results may occur due to occasional tubal (cornual) spasm. Laparoscopy combined with chromotubation is more informative and is considered more accurate in diagnosing tubal pathology. Furthermore, laparoscopy allows for evaluation and treatment of other pelvic disorders contributing to tubal factors such as pelvic adhesions and endometriosis.

Uterine factor may be diagnosed in some patients by performing HSG which may reveal developmental abnormalities such as bicornuate uterus or uterine septum. Filling defects due to submucosal leiomyomas or endometrial polyps may also be seen on HSG. Pelvic ultrasound, specifically trans-vaginal is the standard imaging technique for evaluating the uterus and ovaries. Sonohysterography which uses ultrasound along with infusing saline into the uterine cavity allows for imaging of smaller lesions like polyps. Hysteroscopy can better differentiate between submucous leiomyomas and endometrial polyps and can be used with a resectoscope to dissect uterine adhesions, uterine septum, endometrial polyps, and submucosal leiomyomas.

Cervical factor may be diagnosed by examining the cervical mucus under the microscope, specifically the interactions between the sperm and the cervical mucus called post-coital test. This test is performed around the expected time of ovulation and a few hours after coitus, observing motile sperm in the cervical sample is considered a normal test.

**Treatment**

Treatment for infertility is directed towards the underlying medical problem which may be causing infertility. Life style changes, medications, and/or surgery are often used to correct factors contributing to infertility. Life style changes include maintaining a healthy body weight, smoking cessation, avoidance or reduction of alcohol use, and the elimination of unnecessary medications.

Ovulatory dysfunction may be treated with medications that stimulate the ovaries such as clomiphene, letrazole, human menopausal gonadotropin, FSH, and LH. Human chorionic gonadotropin (hCG) is used to induce oocyte maturation. Gonadotropin-releasing hormone (GnRH) agonists and antagonists are used to prevent premature ovulation. Women with polycystic ovary syndrome (PCOS) may start ovulating with weight loss alone. Insulin sensitizing agents such as metformin may restore ovulation and may be combined with clomiphene for better results.

Infertility caused by tubal factor may be treated surgically with reconstruction of the fallopian tubes or using in vitro fertilization (IVF). Younger patients with mild to moderate tubal damage may benefit from reconstructive surgery while older women with significant tubal pathology should be offered IVF.

Congenital uterine abnormalities such as septate uterus may be treated with hysteroscopic septoplasty. Submucosal leiomyomas may be resected hysteroscopically as well as intrauterine adhesions (Asherman's syndrome). Abdominal myomectomy for subserosal or intramural leiomyomas is controversial and should only be performed for large leiomyomas that significantly distort the uterine cavity.

Cervical factor is commonly treated by bypassing the cervix using intrauterine insemination (IUI). Other situations where IUI is used are if the partner's sperm count is low or if unexplained infertility is present. IUI combined with clomiphene has been shown to improve pregnancy rates in couples with unexplained infertility. Intracytoplasmic sperm injection (ICSI) is mainly used when there is a low sperm count. Some doctors may use ICSI in women who have failed prior IVF trials (4).

In many couples infertility is due to multiple factors and may involve both partners. Therefore, treatment choices involve several modalities utilized simultaneously to correct all underling infertility factors.

**Reference:**


1. **Greenhill E, Vessey M.** 1990. The prevalence of subfertility: a review of the current confusion and a report of two new studies. *Fertil Steril*. **54(6)**:978-83.
2. **Schwartz D, Mayaux MJ, Federation CECOS.** 1982. Female fecundity as a function of age: results of artificial insemination in 2,193 nulliparous women with azoospermic husbands. *N Engl J Med*. **306**:404-6.
3. **Weisberg, E**. 1985. Smoking and reproductive health. Clin. Reprod. *Fertil.* **3**:175-186.
4. **Jain T, Gupta RS.** 2007. Trends in the use of intracytoplasmic sperm injection in the United States. *N Engl J Med*. **357(3)**:251-7.


# Genetics of Recurrent Pregnancy Loss

**Author: M. Tevfik Dorak, MD, PhD.**

Recurrent pregnancy loss (RPL) is described as three or more consecutive documented pregnancy failures in couples trying to conceive. Around 5% of couples of reproductive age suffer from the mental and physical burden of RPL (1). Its etiology includes anatomic, endocrinologic, immunologic, hematologic and genetic causes but a proportion of RPL remains unexplained despite exhaustive diagnostic workup (2). Animal experiments have made substantial progress in understanding the genetic basis of male and female infertility (3), yet this information awaits application to clinical practice. The same does not apply to RPL where the problem is not failure to conceive but failure to maintain pregnancy. Still, the most promising developments have concerned the discovery of genetic markers of unexplained RPL (2).

It is recognized that inconsistencies in the results of RPL research have been discouraging. These inconsistencies are attributed to flaws in research methodology (2). Most research findings come from small studies that have not been replicated, but there are also established causes that need to be investigated in women with RPL. The evidence-based etiologic clues that require consideration are family history, body mass index, parental karyotypes, hysterosalpingography or hysteroscopy findings, and blood tests (lupus anticoagulant, anticardiolipin, antinuclear antibody and mannose-binding lectin) (2). Investigations that need more research are the following: thrombophilia screening, uterine or peripheral blood natural killer cell analysis, homocystine level and maternal HLA-DR / HLA-G typing (2). The only genetic test included in the definite list is parental karyotyping to rule out chromosomal abnormalities as the main genetic cause of RPL. There is however recent progress in genetic polymorphisms involved in susceptibility to RPL as presented below.

RPL research may benefit from a different approach. Etiologic studies on RPL logically focus on couples or women experiencing this condition since it is not possible to directly study aborted embryos. The probability of survival for human conceptions from fertilization to term is estimated to be less than 25%. Since the primary male-to-female ratio at the time of fertilization is believed to be 120-165:100 and decreases to 106:100 at the time of birth, more males than females must be lost during pregnancy. This inference is in agreement with most observations made directly on failed embryos although few reports based on late pregnancy losses suggested otherwise. This potential sex effect aside, studying couples experiencing RPL in order to find genetic markers is unlikely to be useful since miscarriages represent a minority of total prenatal loss. An alternative approach is to examine genotype frequency differences between male and female newborns, i.e., the end-products of successful pregnancies. This reasoning has already produced some results that are outlined below (4,5).

*"The probability of survival for human conceptions from fertilization to term is estimated to be less than 25%"*

The immunogenetic etiology of RPL is widely acknowledged. The role of the HLA complex genes and genes regulating natural killer cell activity has been explored extensively (1,2). Investigations of healthy newborns to detect signatures of fetal selection using gene frequencies generated some results that may be applicable to both infertility and RPL research. In the first study, HLA class II genes were examined in newborns (4). This region of the HLA complex has been shown to be most relevant in reproduction. Since parental HLA sharing and by inference fetal HLA homozygosity is also a risk marker for RPL, homozygosity for HLA class II lineages was examined. As also observed in animal experiments, there was a deficit for homozygosity for HLA class II haplotypes. This proof of principle study was followed up by a study of the major natural killer cell receptor gene NKG2D polymorphism in the same group of newborns (5). Again there was a difference in genotype frequencies between male and female newborns. These preliminary results need to be confirmed in larger cohorts and in prospective studies in IVF clinics. In any case, extracting clues from healthy newborns seems to be a viable option to expand the research on genetic basis of RPL.

The whole HLA complex is believed to be rich in genes involved in embryonic development. Specifically, the non-classic HLA gene, HLA-G, is thought to be the functional homolog of the mouse pre-implantation embryonic development gene Ped. There are also many transcription factors and other embryo-expressed genes encoded within the HLA complex. Several studies have shown the relevance of HLA-G genotypes in fetoplacental growth. Since HLA-G is the only HLA gene expressed in placenta and believed to protect the fetus from maternal natural killer cell attack, these results are encouraging and may lead to clinical use.

Besides immunogenetic markers, several other genetic polymorphisms of reasonable candidate genes belonging to the DNA repair and anti-oxidant pathways are associated with embryonic survival or miscarriages. The p53 molecule is the major guardian of the genome and orchestrates DNA repair. A functional polymorphism of the p53 gene (R72P) has shown associations with implantation failure and RPL. Likewise, another DNA repair pathway gene BRCA2, which is also

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involved in breast and ovarian cancer susceptibility, seems to influence prenatal viability in a sex-specific manner. A common polymorphism (N372H) of BRCA2 shows a frequency difference between males and females attributed to its sex-specific effects on prenatal viability. Embryonic survival is closely linked to its ability to counter oxidative stress. One of the important players in the anti-oxidative pathway is the heme oxygenase enzyme

encoded by HMOX1 whose polymorphism is associated with unexplained RPL. These findings concerning variants of genes in biologically relevant pathways are encouraging and promise to contribute to understanding the causes of unexplained RPL. We may soon have a panel of genetic markers to assess the risk of RPL in women or survival chances of an embryo in IVF clinics. This outcome is only possible by appreciating the multifactorial etiology of RPL and by using a combination of alternative approaches to unravel them.

**REFERENCES**


1. **Sierra S, Stephenson M**. 2006. Genetics of recurrent pregnancy loss. *Semin Reprod Med.* **24(1)**:17-24.
2. **Christiansen OB**. 2006. Evidence-based investigations and treatments of recurrent pregnancy loss. Curr Opin *Obstet Gynecol*. **18(3)**:304-12.
3. **Matzuk MM, Lamb DJ**. 2008. The biology of infertility: research advances and clinical challenges. *Nat Med.* **14(11)**:1197-213.
4. **Dorak MT, Lawson T, Machulla HK, Mills KI, Burnett AK**. 2002. Increased heterozygosity for MHC class II lineages in newborn males. *Genes Immun*. **3(5)**:263-9.
5. **Ucisik-Akkaya E, Dorak MT**. 2009. A study of natural killer cell lectin-like receptor K1 gene (KLRK1/NKG2D) region polymorphisms in a European population sample. *Tissue Antigens*. **73(2)**:177-83.


*"The Centers for Disease Control (CDC) estimate more than one million cases of PID occur annually with more than 100,000 cases leading to infertility"*

Table 1. Pathogens Associated with Infertility

| Pathogen | Male Infertility | Female Infertility |
|---|---|---|
| | **Bacteria** | |
| *Chlamydia trachomatis* | No known direct effect; Transmission to female | Chronic salpingitis leading to tubal infertility or ectopic pregnancy |
| *Mycoplasma genitalium* | Transmission to female | Salpingitis; Pelvic Inflammatory Disease |
| *Mycoplasma hominis* | Sperm motility, morphology and fertilization potential | |
| *Neisseria gonorrhoeae* | Urethritis; Epididymo-orchitis | Acute Pelvic Inflammatory Disease; Salpingitis; Endometritis; tubo-ovarian abscess |
| *Ureaplasma urealyticum* | May impair sperm motility | Non-gonococcal urethritis; Embryo implantation |
| | **Parasites** | |
| *Trichomonas vaginalis* | Non-gonococcal urethritis; prostatitis; impaired sperm motility | Impaired sperm motility |
| *Toxoplasma gondii* | Hypogonadotropic hypogonadism | Antibodies generated against sperm |
| | **Viruses** | |
| HIV | Infection and antiretroviral therapy affect semen quality | Negative affect through unknown mechanism |
| Human Papillomavirus | Low sperm count; reduced motility | Cervical intraepithelial neoplasia; tubal factor infertility |
| Herpes Simplex Virus | Low sperm count; reduced motility | Possible association with PID |



# Infection-induced Infertility

**Author: Kathryn Iacono, PhD.**

Mammalian reproduction is a complex process that relies upon multiple pathways and biological systems working in timely conjunction with one another. The reported success rate of a fertile couple in their twenties conceiving each month is only 25% and, with age, the success rate drops precipitously. It has been known for some time now that, as we age, hormonal levels fluctuate, egg integrity decreases, and the interplay between systems are adversely affected, making conception even more difficult. Within the United States, approximately 6 million couples are affected by infertility, which equates to roughly 10% of the reproductive aged population. Generally, infertility is defined as the inability to become pregnant within a certain period of time during which unprotected sex is regularly performed; for women under the age of 35 this time frame is typically 12 months and for those aged 35 and over it decreases to 6 months. While this particular definition emphasizes the role of the female in this process, medicine has known for many years now that fertility is an issue that affects both sexes equally. Infertility can result from a multitude of factors, ranging from physical to physiological, but this article will focus solely upon the role of infectious disease.

Two questions that will be addressed are: 1) who are the main infectious culprits associated with infertility and 2) how do they mechanistically affect the process reproduction? First, a number of bacterial, viral, and parasitic pathogens have been categorized as adversely affecting fertility (Table 1). Despite the diversity of these etiologic agents, they can be more generally grouped with one another under the general heading of sexually transmitted diseases (STDs). The two most common culprits within the United States are the bacterial species *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. What makes *C. trachomatis* particularly insidious is its ability to asymptomatically infect both men and women. *C. trachomatis* is not known to have a direct effect on any one aspect of male reproduction but is problematic when asymptomatic infections are transmitted to sexual partners who in turn could develop Pelvic Inflammatory Disease (PID). PID, a general term for any infection involving the reproductive organs, is a serious and widespread disease. The Centers for Disease Control (CDC) estimate more than one million cases of PID occur annually with more than 100,000 cases leading to infertility. Despite the generality of PID, there is a lot more known about the mechanism of infection-induced infertility in women. Most studies have highlighted the role of increased scar tissue, which builds with repeated infection, and possibly the immune response to the infections themselves as integral to the process of induction of infertility. Depending on the location, scar tissue serves as a physical barrier that can impede the migration of sperm to the Fallopian tubes, the site of fertilization. In other reports some women were found to actually generate antibodies against sperm and have also implicated the generation of reactive oxygen species, a by-product of certain immune cells, as another potential mechanism within females. Infertility issues stemming from infection in men seem to be a little more direct, usually affecting the number and motility of sperm, although there have been reports of males generating antibodies against their own sperm as well.

Infertility cases have been on the rise in recent years. Many factors have been attributed to adding to this growth as varied as increased daily stress to individuals putting-off starting families until their 30's. As a result, a lot of emphasis and research has been focused upon the issue and from this a better understanding of the various processes affecting fertility and how it can be treated has been discerned. Fertility treatment and techniques are constantly advancing and improving. Evaluating couples with fertility issues for suspected infections has become more mainstream. There are also initiatives to make testing for certain pathogens, like *C. trachomatis,* more routine to prevent these long-term adverse effects for highly treatable infections. Until such time, self education is paramount and could save a lot of heartache in the long run.