

Effect of Aerobic Bacterial Infection to The Semen Parameters♦

* Asma'a J.Al-Tae **Abbas Al- Dajaily

*The State Company for Drug Industries and Medical Appliances

**Tikrit University/ College of Medicine

الملخص

اجريت فحوصات السائل المنوي وزرع السائل المنوي في هذه الدراسة على 105 مريض من خلال مراجعتهم مركز العقم الكائن في مستشفى الخنساء في الموصل اشتملت الفحوصات فحص السائل المنوي وفحص زرع السائل المنوي والتي تضمنت ما يلي فحص السائل المنوي ومدى الاماعة وعدد النطف وحركتها وشكلها على التوالي . وقد تضمنت الدراسة أيضا زرع السائل على الأوساط الزرع عيه الخاصة للحالات التي شخص فيها وجود خلايا قحيه (خلايا بيضاء ميتة) مما يزيد عن خمسة خلايا في الحقل المجهرى الواحد ز تم اجراء زرع السائل المنوي واجراء فحص الحساسيه وقد تبين من هذه الدراسة ان 21 مريض لديهم خلايا قحيه وان اكثر مسببات البكتيرية شيوعا هي بكتريا العنقودية الموجبة لصبغة بنسبه 16% ، ومن ثم وبالدرجه الثانية البكتريا المعويه بنسبة 4% واخيرا المكورات السبحيه النوع المحللة للدم نوع بيتا بنسبه 1% وتم ايضا من خلال هذه الدراسة قياس عدد الجيامن للملتر الواحد بالإضافة لقياس حيويه الجيامن الحيه والخامله والميته وكذلك فحص وتقدير شكلها النطف طبيعى وغير طبيعى ، وقد لوحظ انه في حاله وجود أي خلل في إحدى المقاييس الطبيعى للسائل المنوي يكون مصاحبا بوجود أصابه بكتريا.

Abstract

Semen analysis and culture assay were done (100) patients suspected to be subfertile men attended the male infertility clinic at Al-Khansa'a hospital in Mosul. The following parameters of semen are studied such as: semen volume liquefaction time sperm count, sperm motility and sperm morphology.

Culture have been made in patients with abnormal semen and have pus cells more than 5 cells in High Field Power (HFP).The most common abnormalities in the semen are decreased sperm count and /or decreased sperm motility. Culture and differential stain of patients' semen of

♦Presented at the first conference on Biology , University of Mosul ,college of Education , 4-5 September 2007

infertile men shows a significant negative correlation to sperm count and sperm motility (When sperm count decreases sperm motility increases), Azoospermia and Oligospermic men show a significant increase infection happens as compared to their non infected

Introduction

Infertility is defined as the inability to conceive and it is due to various socio-biological factors consultations for inability have been increased in the last few years . the man alone is cause of inability in about one fifty of cases (1).

Since inability in man can result from a wide range of various causes , man should be evaluated history , complete physical examination, examination of the ejaculate . for the purpose of physical diagnosis , semen analysis can give much information about the state of inability. Sperm count is helpful in azoospermia and severe oligospermia when the physician knows the latter is a barometer of testicular damage

A couple is considered to be infertile after unsuccessfully attempting pregnancy for 1 year. Infertility is termed primary when it occurs without any prior pregnancy and secondary when it follows a previous conception. Some conditions such as azoospermia. Endometriosis and tubal occlusion are more common in woman with primary inability but virtually all conditions occur in both settings making the distinction of little benefit (2) . male fertility depends on the production of adequate numbers of healthy spermatozoa by the seminiferous tubules their subsequent delivery into the upper vagina from where a small proportion of them will penetrate the cervical mucus travel through the uterus and then to the ampullary portion of the fallopian tubes where fertilization normally takes place.

Semen it is a fluid composed of spermatozoa and seminal plasma. The former originating in the testicles contribute to about 50% or less of the seminal fluid volume , as it had been shown by measurement of the packet sperm volume (PSV).The seminal plasma is mainly made of secretions from the epididymis , seminal vesicles , prostate and bulbourethral glands .

The composition of human semen fructose which is concentrated by the epididymis from blood serves as energy source for sperm to develop motility . Secretions from the seminal vesicles (about 60%) contain fructose and prostaglandins which are important for sperm motility .

These secretions also contain fibrinogen-like substance responsible for coagulation of semen .The prostatic secretion (about 20%) contain fibrinolysin enzyme responsible for liquefaction of semen within 10-30 minutes . These secretions also contain acid phosphatase, calcium, zinc,

citric acid and spermine with its bacteriostatic action. Many attempts have been made to define normal sperm count in the post, sperm count less than 60 million/ml were considered as oligospermia, subsequently the number was lowered to less than 40 million/ml and nowadays counts of 20 million/ml are considered to be the lower limit of normal count.

Review of literature

Causes of inability can be classified into pretesticular, testicular and post testicular (3)

1. pretesticular causes

1.2. hypogonadotropic

It is characterized by cryptorchidism, gonadotropin deficiency and with or without olfactory defect the defect is at the hypothalamic level with absence or inadequate release of LHRH and there is lack of responsiveness to exogenous gonadotropin administration

1.3. Estrogen excess

In patients with liver cirrhosis have increased estrogen level in blood which results in inhibition of gonadotropin secretion by the anterior pituitary gland and secondary testicular failure.

1.4. Androgen excess

Excess testosterone causes inhibition of spermatogenesis by its negative feedback inhibition of FSH secretion by the anterior pituitary. High testosterone causes a reversible reduced fertilizing capacity of the sperms in serves as a reversible male contraceptive agent.

1.5. hyperprolactinemia

This can be due to pituitary adenoma, hypothalamic dysfunction, drug induced or idiopathic. Hyperprolactinemia may cause secondary testicular dysfunction. High prolactin may result in low semen volume, low or normal sperm motility, impotence, galactorrhea and infertility. In cases of hypothalamic the seminiferous tubules may be associated with low serum testosterone concentration found the mean prolactin level in the serum of infertile men was higher than that of fertile men. Impaired spermatogenesis is found in hypothalamic men however found that there was no difference in mean prolactin levels in the serum of men with normal sperm count and with oligospermia or azoospermia.

1.6. Glucocorticoid excess

Hypercorticism in Cushing's syndrome decreases LH secretion decreases testosterone secretion and leads to secondary testicular failure. Patients who are under steroid therapy for other diseases may develop oligospermia and infertility.

1.7. hypothyroidism

Patients with hypothalalamia may have decreased spermatogenesis
hypothyroid patients often restore fertility after thyroxine supplement .

1.8. Hyperthyroidism

it has been noted that thyrotoxicosis is associated with low sperm count and maturation arrest . It is associated with decreased sperm count , serum volume and increased serum testosterone . These changes are secondary to elevated estrogen levels .

1.8 Diabetes mellitus

Uncontrolled diabetes mellitus may result in impotence and Retrograde ejaculation. Semen is contaminated by urine , this result in great decrease of sperm motility and in fertility .

1.9 Stress

stress of any kind may result in infertility due to poor semen quality such as abnormal forms or an increase in immature forms (3).

1.10 General illness

Uremia patients develop infertility either by their action on adrenergic nervous system causing disturbance of ejaculation and erection or by causing hyperprolactinemia ureamic patients have decreased testosterone production with elevated gonadotropins indicating a defect at testicular level. These patients are also zinc deficient .

2. Testicular

2.1. Maturation arrest

because of ischemia which may result from trauma or torsion of the testis , there is damage of the spermatogenic epithelium including the sertoli cells, while leydig cells are resistant and remain functionally normal . patients present with infertility have reduced size of the testis azoospermia with a high serum FSH level

2.2. Hypospermatogenesis

proportional hypoplasia of all germ cells .

2.3 germinal aplasia : (sertoli cells only syndrome)

here only sertoli : cells are present in the tubule , this may be associated with small but compensated leydig cell failure, the raised serum FSH level. But serum testosterone and LH are usually normal .

2.4 Klinefelter syndrome

This is a genetic disorder characterized by the destruction of the germinal epithelium as well leydig cells . the patient usually presents with small testis , raised serum FSH level but normal LH level (4) .

2.5 XYY syndrome

2.6 Other sex chromosomal or autosomal anomalies with gonadal involvement

2.7 Cryptorchidism

By the effect of abdominal temperature on the undescended testis, there is atrophy of germ cells and small immature tubules. if the changes are bilateral, the patient will have azoospermia, raised serum FSH but normal LH level (5).

2.8 Radiation damage

Exposure to radiation as in cases of tumor of in the germ region or in cases of lymphoma results in the destruction of germ cells especially the spermatogonia while leydig cells are resistant. The degree of damage is dose dependent . below 600 rads the changes can be reversible and above this level the change can be permanent resulting in azoospermis and infertility . fractionated radiation may have more destructive effect than does a single radiation . men treated with radioactive iodine may also have impairment of spermatogenesis and elevation of serum FSH level.

2.9 Chemotherapy toxic agents

Many types of drugs cause damage to the seminiferous epithelium . the most harmful ones are anti-cancer agents especially alkylating agents this may be associated with decreased serum testosterone level and increased serum testosterone level and increased serum LH due to impairment of leydig cells function . monoamine oxidase inhibitors cause reduction in sperm count even to the degree of azoospermia . furadantin causes temporary reduction in spermatogenesis resulting in a decrease sperm count and affect sperm motility . Cimetidin causes temporary reduction in spermatogenesis elevated serum testosterone level with no effect FSH level. Sulphasalazine is related to infertility because removal of the drug results in improvement of quality and may also affect sperm motility .

2.10 mumps orchitis

Bilateral pubertal and postubertal mumps orchitis results in permanent damage to the seminiferous tubules leydig cells are usually preserved .The patients present with severe oligospermia or azoospermia unilateral involvement of the testis results in reduced sperm count but is compatible with fertility .

2.11 Alcohol

Alcohol induces testicular atrophy by inhibition of gonadotropin secretion and destruction of the seminiferous tubules. In addition it causes leydig cell failure which is manifested by loss of libido , impotence and infertility.

2.12 Smoking

Decreased sperm motility and count, low testosterone, high FSH levels were found in smokers. Nicotine depresses androgenic steroid. Production at testicular level by affecting leydig cells function. Serum estradiol levels were significantly higher in heavy smokers as compared to non-

smokers. High percentage of morphologically abnormal forms of spermatozoa are seen in the ejaculate of smokers.

2.13 Age

The percentage of seminiferous epithelium in the parenchyma of the testis and the volume of somniferous epithelium per man is lower in old men (6).

3. Posttesticular causes

3.1 block of the duct leading away from the test

Either congenital aphasia of the vas deferens or acquired obstruction after infection as gonorrheal epididymitis lead to azoospermia and infertility. These causes have normal FSH level and testicular biopsy necessary for accurate diagnosis.

3.2 Impaired sperm motility

Various factors play a role in impairment of sperm motility and these include .

3.3 prolonged abstinence

long periods of abstinence affect semen quality including sperm motility.

3.4 Pyospermia

Pus from any source has deleterious effect on sperm motility especially in patients with proven E.coli infection can reduce fertility are poorly understood.

3.5 partial obstruction

Characterized by severe oligospermia and severe asthenospermia with normal serum FSH level .

3.6 Poly zoospermia

Due to a large number of spermatozoa in the ejaculate fructose is insufficient to maintain their motility.

3.7 sperm agglutination and antibody

The clumping spermatozoa and the presence of sperm antibodies impair motility of sperms and their penetration of cervical mucus.

3.8 Biochemical abnormalities of the seminal plasma

Fructose is the main sugar in the seminal plasma and substrate for the metabolism of spermatozoa. The determination of fructose in seminal plasma provides a quantitative measure of seminal contribution to the make-up of human semen. It also serves as a diagnostic detection of occlusion of the ejaculatory ducts. Fructose free azoospermic ejaculates were encountered in man with aplasia of vasa deferentia. Also determination of seminal and citric acid serve as indicators of the androgenic status of the male because secretory activity of the seminal vesicles depends upon the stimulatory effect of testosterone. Low levels of fructose in semen is sometimes associated with hypogonadism. Men whose prostatic fluid contains increased number of leucocytes, were

found to have low concentration of zinc, magnesium, acid phosphate. However, found no difference in the mean concentration of zinc, magnesium copper, alkaline, phosphate in seminal plasma of infertile men as compared to fertile men.

3.9 Varicocele

Varicocele occurs when there is dilation of the pampiniform plexus resulting from incompetence of the valves of the internal spermatic vein. Varicocele is one of the most common causes of infertility in the male.

In most cases Varicocele does not deteriorate the spermiogenic function only but also lower the sperm level of testicular androgens.

Patients with Varicocele have low sperm count, abnormal sperm morphologic features, increased baseline serum gonadotropins as compared with fertile men without Varicocele.

However, some workers demonstrated that there was no significant difference in serum FSH, LH and testosterone between infertile men with Varicocele and normal fertile men.

classification of causes of male infertility pretesticular causes .

1. Hypogonadotropism
 2. prepubertal onset
 - 2.1. organic lesions in or near pituitary (tumors, cysts, trauma, etc)
 - 2.2. genetic defects in gonadotropin secretion (hypogonadotropic eunuchoidism)
 - 2.3 Postpubertal onset :
 - organic lesions of pituitary tumors or trauma to pituitary fossa)

Estrogen excess

1. Endogenous
 - a. Estrogen producing tumor (of the adrenal cortex for example)
 - b. Cirrhosis of the liver)
2. Exogenous
 - Hyperproliferation
 - Glucocorticoid excess
1. Endogenous
 - Cushing's syndrome
2. Exogenous
 - a. treatment for ulcerative colitis
 - b. treatment for chronic asthma
- Hypothyroidism
- Hyperthyroidism
- Diabetes mellitus

3. Testicular causes

Maturation arrest

Hypospermatodosis(proportional hypoplasia of all germ cells).

Absence of germ cells(sentoli –cell-only-syndrome)

Klinefelter 's syndrome (XXY karyotype and it's variant's)

XYY syndrome

Other sexchromosomal or autosomal anomalies with gonadal involvement.

Cryptorchism

Radiation damage

Chemotherapy

Mumpsorchitis

4. Posttesticular causes

Block of ducts leading away from the test

4.1 Congenital

a. Aplasia of vasa deferentia

b. plasia of epididymis

4.2. Acquired:

a. Infection:

(1) Gonorrheal epididmitis

(2) Tuberculous epididmitis

(3) others

b. Vas ligation

(1) Voluntary

(2) Iatrogenic

Impaired sperm motility (sperm count adequate ; testicularbiopsies normal)

1.Faulty maturation or improper storage of spermatozoa in the epididymis

2.Biochemical abnormalities of the seminal plasma

3.Genetic defects of the sperm tail (the immotile cilia syndrome)

Materials and Methods

1. Microscopic Examination

1.1 Sperm count

a. A diluting fluid is prepared by mixing 16 gm of sodium bicarbonate and 4 gm of phenol in 400 ml distilled water.

b. White blood cell pipette.

c. Improved Neubauer hemocytometer chamber.

d. Clicker – counter.

The semen is mixed thoroughly and drawn up to the mark 0.5 of the white blood cell pipette. The diluting fluid is drawn up to the mark at the top of the bubble chamber. By this method 1:20 dilution is obtained the

pipette is shaken well for 2 minutes few drops of the fluid are discarded from the pipette. Neubauer chamber is cover slipped, then both sides of it are filled with pipette mixture.

When a drop of semen is examined directly on cover slipped slide and the number of sperms are few, then 1:10 dilution is made by drawing up semen to the mark 1 then the pipette is filled with diluting fluid using clicker counter and X400 magnification the sperms are counted within 5 blocks, each block contain 16 small squares sperms overlying the upper and left lines of the square are eliminated and those overlying the right and lower lines of the square are counted. The total number within the 5 blocks 180 squares is obtained the sperm count/ml is calculated as follows:

- a. For the 1:20 dilution, six zeros are added to the total number in the 5 blocks.
- b. For the 1:10 dilution by 2, then six zeros are added to the figure obtained.
- c. For the 1:10 dilution when the number of sperms is very few, all sperms present in the 25 block, are counted and five zeros are added to the figure.

1.2 Sperm motility

It is evaluated within 1/2 to 1 hour after collection. The specimen is mixed well, then a drop of semen is placed on an ordinary microscope slide cover slipped and then examined using a magnification of X400 the percentage of spermatozoa moving with normal progressive motility is noted. In addition, the percentage of immotile sperms is recorded.

1.3 Agglutination

A drop of semen is placed on a microscope slide covered by a cover slip and observed for sperm agglutination can be observed if present.

1.4 Sperm morphology

Eosin stain is prepared by addition of 100 ml distilled water to 5 gm of eosin ymayer's hematoxylin stain is prepared by dissolving 50 gm of potassium alum in 1000 ml distilled water, without heat, 1 gm of hexatoxylin crystals are dissolved in this solution then 0.2 gm of sodium iodine, 1 gm citric acid and 50 gm of chloralhydrate are added, the solution is shaken well. The final color of the stain is reddish-violet.

Tincture of iodine is prepared by dissolving 50 gm iodine and 100 gm potassium iodine in 100 ml distilled water and the solution is completed 1000 ml by distilled water.

Preparation smear

A drop of semen is placed near the end of an ordinary microscope slide and with another slide the semen is spread from one side of the slide to the other side.

1. Fixation

- a. The following steps are used for fixation of the smear while wet, the smear is immersed in a solution composed of 2 parts 7% corrosive mercuric chloride and 7 part absolute, alcohol for 1 min.
- b. Then the slide is immersed in 50% alcohol for ½ min.
- c. Then immersed in 100 ml distilled water and 2 drops tincture of iodine for ½ min .
- d. Finally it is washed in distilled water.

2. Staining

- a. The slide is immersed in 5% aqueous eosin for ½ min.
- b. Then it is immersed in 100 ml 50% alcohol and 2 drops concentrated HCl for 1 min.
- c. It is washed in distilled water.
- d. The slide is then immersed in hematoxylin for 2-5 min.
- e. After wards it is washed in distilled water, dried and examined.

The slide is examined using oil immersion lens, and the percentage of morphologically normal sperms is recorded in addition, the percentage of abnormal forms like tapered head, rounded head double head, immature cells, coiled tail are recorded.

Semen culture

All media culture prepared depend to the information from company produced to the media which is used and then sterilized in the autoclave in 121°C temperature under 15 pound/in² pressure about 1 min. these media are:

Blood Agar

MacConkey Agar

Chocolate Agar

Nutrient Agar

Diagnosis gram positive bacteria

The isolated bacteria diagnosed depended up on material provided from media and chemical material these are following:

1. Gram Stain

Tested the samples which are response to gram stain by its solution described from.

Complete B-haemolysis on solid blood media: Isolated colonies surrounded by complete zone of blood haemolysis (about regein haemolysis in diameter 2-4 ml) diagnosed its characteristics.

2. Diagnosis gram negative bacteria

Diagnosis depended upon

- a. gram stain
- b. tested the colony cultured above the MacConkey media from lactose formentation.

Results and Discussion

Hundred patients (male) were participated in this study. The age of the subjects rang from 20 to 35 years. All of them were married and suffered from infertility. (Table 1) shows the sperm count for all patients, 10 patients have a zoospermia, while 48 patients have a sperm count less than 20 million /ml, 20 patients have 20-40 million /ml, 10 patients have 40-60 million/ml, 10 patients have 60-80 million/ml, 2 patients have 80-100 million/ml out of total 100 cases. (Table 2) shows the morphology of semen, the abnormal ratio was 80.1-90% (210 which the less was at 40.1-50% which is considered normal morphology. The result of microorganism that isolated from culture of semen is shown in table, staphylococcus aureus was found the most common bacteria that present in 16% of patient's semen. Isolated microorganism 1% then staphylococcus. B-hemolytic Streptococcus 4%, while E. coli was the less predominant. (Table 4) shows the microscopical finding in semen: pus cells were found in 20 cases under 5 cell in fitting while R.B.C in 13 cases, epithelial tissue (debris) 17, candida sp. In 2, spermatocytes 5, urate arystale 8, from total cases.

(Table 5) and (Table 6) shows the susceptibility pattern of E. coli and Staph aureus to different anti biotics respectively.

The cases without receiving antibiotic were highly susceptible to antimicrobial agents. shows the results of semen activity in this study, 60% was non motile, 25% active and 15% sluggish. The susceptibility test of isolated micro-organism appeared in the (Table 5) for 4 patients with E. coli infection appeared susceptibility to Tetracycline more than other antibiotics, the susceptibility of Staph aureus micro-organism appear to the ampicillin more than other antibiotics, and B. hemolytic streptococci

appear susceptibility to rifampicin only.

Table(1) : Abnormal Sperm Count

Sperm count range (million/ml)	No. of cases
Azoo spermia	10
0-20	48
20.1-40	20
40.1-60	10
60.1-80	10
80.1-100	2
Total	100

Table (2) Abnormal Morphology of semen

Percentage%	No. of patients
10-20	4
20.1-30	2
30.1-40	14
40.1-50	3
50.1-60	25
60.1-70	5
70.1-80	10
80.1-90	27
90.1-100	10
total	100

Table(3): types of isolated microorganism

Types of microorganism	No. (total)
E. coli	4
Staphylococcus aureus	16
β -haemolytic Streptococci	1
Total	21

Table (4): Microscopical findings

Findings	No.
Red blood cells	13
Epithelial tissue(debris)	17
Pus cells	20
Candida Spp .	2
Spermatocytes	5
Urate crystals	8

Reference

- 1.Wn F.C. ,and Bancroft H.j. Br.Med.J 290: 1417-1420. (1985)
- 2.Hacher, Moore. Essentials of obstrices and Gynecology, 2nd Ed. W.B. Saunders company, U.S.A. (1992).
- 3.Giblin P. T.W. Poland M.L., Olson J.M., and Moghissi I.S. Fertil.49: 127-132.(1988)
- 4.Booth J., Merriam G.R. Clark R. V., Loriaux L.D. and Sherins R. J. J. Clin. Endocrinol. Metab.64 : 1194-1198 (1987).
- 5.Kandeel F. R., and swerdloff R.S. Role of temperature in regulation of speratogenesis and the use of heating as method for contraception . Fertile. Sterile.49 : 1-23.(1988)
- 6.Johnson L.,Abdo J.G.,petty C.S and Neaves W.B. Fertil . Steril 49:1045-1051(1988)
- 7.Auroux M D D ,Semen examination Chapter 22.In : Gradwohls' clinical laboratory methods and diagnosis , volume one. Ed Sonnewirth & Jarret, pp.501,Mosby-London.(1980)
- 8.Bar-Chama N., Fisch L., Infection and pyospermia in male infertility. Department of Urology, Montefiore Medical, Center , Albert Einstein College of Medicine, Bronx. N.Y. World L. Urol.11 (3) :76-81 (1993).
- 9.Preveden, T; Jovasvic , J R and Stic, D. Abstract (1996).