www.jmscr.igmpublication.org
Index Copernicus Value: 79.54
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v7i6.111

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Original Research Article

Pathological analysis of semen in cases of male Infertility of patients attending in tertiary care hospital at NMCH, Patna, Bihar

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Abstract
Objective: *In the assessment of unexplained infertility the evaluation of seminal characteristics is primary and Paramount, as far as the male is concerned .Present study was undertaken to determine the role of seminal examination in cases of male infertility.*
Material and Methods: *A total of 124 male infertile patients were included in the studies. The collection of semen samples and examination were according to standardized methodology as per the current recommendation of CAP and 5th edition of WHO guidelines. Sample collection through masturbation after 3 to 5 days of abstinence periods. Physical parameters examined were volume, appearance, viscosity, contaminants, PH, coagulation and Liquefaction. Quantitative and Qualitative analysis were done.*
Result: *Majority of infertile men were 25 to 35 years of age and had been married for 1- 3 years, when they were submitted themselves for fertility assessment. Physical parameters were important cause, depletion in semen volume, altered color and PH changes were found informative. We also found the microscopic arm of semen analysis very useful in pin pointing existing deficits in the male. Some significant correlation was also observed between abnormal microscopic parameters and clinical conditions.*
Conclusion: *Semen analysis is the preliminary screening procedure which if carried out with standard methodology and criteria, is very valuable in separating a significant percentage of infertile male who would benefit from routine medical and surgical intervention for restoration of their infertility.*
Keywords: *Semen, Infertility, sample collection, Methods.*

Introduction

Inability of male's to cause pregnancy in a Fertile Female refers to as a Male infertility and it affects approximately 7% of all men and accounts for 40-50% of Infertility.

Reproduction (making a baby) is simple and natural experience for most couples. However, for some couples it is very difficult to conceive. A men's fertility generally relies on the quantity and quality of his sperm, If the sperm count is low and sperm are of a poor quality it will be difficult and sometimes impossible for him to cause a pregnancy. Male infertility is diagnosed when after testing both partner, reproductive problems have been found in the male.

Infertility is a wide spread problem. For about one in five infertile couples the problems lies solely in the male partner. It is estimated that one in 20 men has some kind of fertility problems with low numbers of sperm in his ejaculate**.** However, only about one in every 100 men has no sperm in his ejaculate.

In most cases there are no obvious sign of infertility. Intercourse, erection and ejaculation will usually happen without difficulty. The quantity and appearance of the ejaculated semen generally appears normal to the necked eye.

Male infertility is usually caused by either sperm production or sperm transport. About two-thirds of infertile man has a problem with making sperm in the testes. Either low numbers of sperm are made and/or the sperm that are made do not work property.

Sperm transport Problems are found in about one in every five patients including after vasectomy and Blockage in the tube. Less common are sexual problem (one in 100 in infertile couples), Low level of Hormone (one in 100 infertile men) and sperm antibodies (one in 16 infertile men).

Known cause of male infertility is: 1. **Sperm production Problems**- chromosomal or genetic causes, Undescended testes. Torsion of testes, varicocele, medicine and chemicals, Radiation damage and Unknown cause**. 2. Blockage of Sperm transport-** Infection, Prostate related problems, Absence of vas deference, vasectomy. **3. Sexual Problem (erection and ejaculation Problems)-** Retrograde and Premature ejaculation, Failure of a ejaculation, erectile dysfunction, Infrequent inter course, spinal cord injury Prostate surgery, Damage to nerves, some Drugs. **4. Hormonal-** Pituitary tumors, congenital Lack of LH or FSH, Anabolic (androgenic) steroid abuse. **5. Sperm antibodies-** Vasectomy, injury or infection in the epididymis, Unknown cause.

The male genital system includes testes, ducts or tubes and other glands that open into the ducts**.** The brain plays an important part in the control of male reproductive system. The pituitary gland and the hypothalamus, located at the base of brain, control the production of male hormone and sperm. LH and FSH are the two messenger hormones made by the pituitary gland that act on the testes. Testes make sperm and the male sex hormones testosterone. In about 70 days sperm becomes mature and able to fertilize an egg.

Sperm when released from the testes, the sperm spend 2 to 10 days passing through the epididimis where they gain the vital ability to swim strongly and to attach to and penetrate the egg. At orgasm, waves of muscles contractions transport the sperm, with a small amount of fluid, from the tested through to the vas deferens. The seminal vesicles and prostate contribute extra fluid to protect the sperm. This mixture of sperm and fluid (semen) travels along the urethra to the tip of penis where it is ejaculated.

The urge and the existential need to procreate are as old as life itself. Infertility though a bane of many a desirous couple is still largely a nebulous concept. Yet in any fertility assessment protocol, the primacy of a semen analysis is unchallenged. The criteria prescribed by the WHO syllabus have given investigators some solid ground, such that men whose semen quality fulfills certain criteria prescribed in the above syllabus are deemed fertile in their own right; in such men the fertility of the female is under question and this cannot admittedly be evaluated accurately in all instances. Yet, the sub fertile male, who has semen parameters lacking in some aspects, should be subjected to a closer scrutiny, so as to arrive at a best choice modality for a pregnancy to occur. It goes without saying that for many abnormalities detected in a stringently carried out semen analysis no ready physical association can be forwarded, yet in a sizable and important subgroup of infertile males the putative causal association is with an eminently treatable condition. It is for this subgroup of individuals that semen evaluation assumes more importance, in that directed therapeutic intervention would result, in a large majority, regaining the fertile status.

Materials and Methods

The present study was carried out in the Department of pathology, Nalanda Medical College, Patna, by the help of obstetrics and Gynecology Department, during the period of September 2018 to May 2019, where the couples presented with in fecund marriages of varying durations. A total of 124 infertile males were evaluated. After detailed clinical history and clinical examination samples were collected.

Sample collection was through masturbation after a 3 to 5 day abstinence period. A pre and post ejaculation urine sample was also collected for routine and microscopic examination. Adequate safety guidelines were followed, because of the potential risk of infection with sexually transmitted disease. Physical parameters evaluated were volume, color, odor, pH, coagulation, and liquefaction. Quantitative and qualitative analysis of semen included sperm density, using a conventional Neubaur's chamber. The sperms were counted in the erythrocyte counting area. The diluents used contained sodium bicarbonate 5 g and neutral formalin 1 ml in 100 ml of distilled water'.

Wet mount was utilized in studying motility, no grade allocation was made. A minimum of 200 sperms were observed and percentage with forward motility recorded.

Thin layer smears, after fixation with methyl alcohol and Giemsa staining were used for studying morphology. Supravital staining with eosin, for assessing sperm viability was used. Fructose test were done. The entire test was done as per the current recommendation of CAP and 5th edition of WHO guidelines.

Results

The sub fertile males undergoing semen evaluation in this study mostly belonged to the age group of 25-35 years and had mostly presented within 1-3 years of marriage.

Table shows SEMENONGRAM of infertile patients (n=124)

Physical parameter and microscopic Examination of Semen	Abnormalities	No. of Patients (n=124)	Clinical Correlation
volume	Increase	n=14	Pyospermia(n=4) Probable non compliance over abstinence(n=10)
	Decrease(<4ml)	n=7	obstruction(n=1),Prostatitis (n=2)
Color	Decreae intensity	n=8	Increase in volume
	yellowish	n=5	Acute infection (pyospermia)
	Brownish	n=3	Blood+, Bleeding undetected (vescicles)
Odor	Putrid	n=1	Vesiculitis +/-
PH	>7.8	n=4	Pyogenic Prostatitis
	< 7.2	n=3	Chronic Prostatitis (n=2)
Coagulation	Absent	n=3	Chronic Prostatitis (n=2)
Liquefaction	absent at 1 hour	n=5	Chronic Prostatitis.(n=2) Undetected cause, Decrease enzyme
Sperm density	Azoospermia	n=7	Germ cell aplasia (n=1), biopsy documented
	<20x10^6/ml(oligospermia)	n=27	Partial pathway obstruction, detected in 7 patients, by clinical, Radiological and biochemical tests.
Motility	<50% sperm with forward motility	n=28	Epididimitis and epididymo-orchitis detected (n=9)
Morphology	>75% abnormal mostly with multiple defects	n=36	Unknown cause, concomitant infection(n=8)
Viable sperm	>25 % sperms Dead	n=14	High correlation with infection(n=9)
Agglutination	Fusion of Sperm in various orientations	n=8	Chronic prostatitis(n=4),sub-acute epididymo-orchitis(n=2),unknlwn cause(n=2)
Inflammatory cells	> 1x10^6/ ml	n=14	Infection in genito- urinary tract (n=14)

Discussion

The trend that most of the males were in their third decade and had presented within 3 years of marriage may have its origins in the anxiety and families social pressures, which a married couple has to countenance in the immediate years after marriage, for procreation, in our country.

Increased volume of semen perhaps not an ominous finding, was probably mostly due to default over abstinence, in 4 patients volume increase could be attributed to the presence of pus. In the 7 patients who had decreased volume 1 had a urethral polyp causing obstruction and another with chronic prostatitis. The first association has been cited as an important cause of lower genitourinary obstruction and non renal haematuria in males. Color alteration from the normal grey white to yellowish was seen to be associated with pyospermia in 5 patients. A dirty soil brown hue in 2 patients was associated with semen positive for blood. 1 sample, among the 5 with pyospermia had a putrid odor, replacing the characteristic musty smell of semen. Altered pH was detected in only 7 patients, 4 (pH > 7.8) had associated pyogenic foci and 3 (pH < 7.2) harbored chronic prostatitis. The normal pH range for semen is between 7.2-7.8, an increased pH usually indicates acute infection and a decreased pH the reverse, or obstructed ejaculatory ducts . Abnormalities of coagulation and liquefaction (8 cases) were concomitant with chronic prostatitis in 2 instances. Further assessment of these unliquefied samples could only be accomplished after use of chymotrypsin digestion. A failure to liquefy may indicate a condition adversely affecting physiologic prostatic secretion, the preceding often being the case in chronic prostatitis.

Azoospermia (7 patients) could be correlated with germ cell aplasia in 1 case, documented through testicular biopsy, in a patient with history of peri pubertal mumps orchitis, this condition has been considered an important cause of germ cell loss . An increased endogenous estrogen level may also cause the preceding. Oligospermia (<20x10^6/mL) was evident in 27 patients, of whom partial pathway obstruction was detected in 7 patients. Pathway obstruction constituents important etiology in oligospermic patients, more so because usually the underlying condition is amenable to surgical correction. Reduced motility (<50% sperm with forward mobility) was seen in 28 patients, 9 of these showed evidence of infection. Reduced sperm motility may also result from improper collection and technique, extremes of temperature and functional disturbances in the sperm especially submicroscopic or defects in sperm maturation and facilitation. The critical percentage of morphologically normal sperm has been set out as more than 4%, below which pregnancy rates drop precipitously. We found the previous threshold being overstepped in 36 patients, only 8 of these could be attributed to a tangible cause, namely infection. Sperm viability, by eosin dye penetration in percentage of immotile sperms, was seen to be reduced (<75% viable sperms) in 14 cases, a high correlation was observed with concomitant (9 cases) of acute infection. Agglutination of spermatozoa, in various configurations was set in 8 cases; follow up revealed chronic prostatitis in 4 patients and subacute epididymorchitis in 2 patients. Apart from agglutination occurring in presence of infection, it may be suggestive of an immunologic cause of infertility. Unequivocal identification of inflammatory cells (>1x10^6/mL) was possible in 14 cases, of which 14 had corroborative findings of probable infection in the genitourinary tract through examination of either urine or, prostate massage fluid. More than 40 leucocytes per high power field in the latter have been suggested as a very likely coeval of prostatic inflammation.

Conclusion

Semen analysis as evidenced by our study forms a low cost, least labor and resource intensive, preliminary screening procedure, which if carried out within confines of precaution and standard criteria is very valuable in separating a significant percentage of infertile males who would benefit

from routine clinical or surgical intervention for restoration of their fertility. Those cases in which the etiology is more obscure can then be referred for specialized, comprehensive anthological analysis, and strategy then crafted for the best possible option for them.

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