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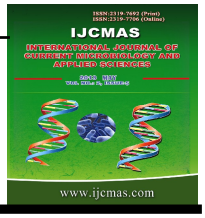

Original Research Article

An in-vitro study of effectiveness of Uropathogenic yeast on Male infertility

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Keywords

Urogenital tract; Yeast infections; Sperm motility; spermatozoa; Physical characteristics.

A B S T R A C T

Male urogenital tract infection plays an important role in men infertility. Yeast infections in sperm have been paid attention as a major cause of male infertility. There was a significant relation between the yeast infection in sperm and the rate of no motile and morphologically abnormal sperm. The quality of sperm motility was significantly decreased in contaminated semen; the percentage of morphologically normal sperm was lower abnormal sperm was lower. Highly motile preparation of spermatozoa was co-incubated for 2 hours with various uropathogenic organisms. The interaction between various uropathogenic organisms and disturbances on sperm motility has impact on male infertility. Pre diagnosis and antibiotics treatment which could helps to take advance treatment for microbial infected male partner. In our study reveals yeast infection in semen which plays major role in male infertility while in infection, and pre-diagnosis with antibiotic treatment is ideal to treat male partner.

Introduction

Fertilization is a sequence of events that's begins with contact between spermatozoa and an oocytes, leading to their fusion for embryo, which leads to the formation of a zygote and ends with the initiation of its cleavage. Infertility is a condition in which conception does not occur even after one year of regular unprotected sexual intercourse, which are induced by genital, trauma, occupational, microbial infection and these are infertility gametes of is due to the absence dysfunction of morphology, character etc., obtained regularly. Infection is one of the causes of infertility. The reproductive organs of male comprise of testis, epididymis, vas deference, efferent duct and accessory sex glands (prostate, seminal vesicles, bulbo-urethral glands and penis). The germ cell that originates in testis undergoes a series

of transformation within testis as well as the rest of the reproductive tract to form spermatozoa, capable of fertilizing the oocytes. This process takes about 70-80 days (Pandiyan, 1999).

A sperm cell or spermatozoa [pl. spermatozoa in greek; sperm: semen and zoon-alive] is the haploid cell that is the male gamete. It is carried in fluid called semen and is capable of fertilizing an egg cell to form a zygote. A zygote can grow into a new organism, such as a human. Sperm cells contain half of the genetic information needed to create life. Generally, the sex of the offspring is determined by the sperm, through the chromosomal pair, 'XX' for female or 'XY' for male sperm cells were first observed by Antoni Van Leeuwenhoek in 1969. Sperm are produced in the seminiferous tubules of the testis; the differentiation of spermatids into sperm cells is called spermiogenesis. It corresponds to the final part of spermatogenesis and comprises the following individual processes the partially preoceed at the same time.

Nuclear condensation: Thickening and reduction of the nuclear size, condensation of the nuclear contents into the smallest space.

Acrosome formation: Forming a cap (acrosome) containing enzymes that play an important role in penetration through the pellucid zone of the oocytes.

Flagellum formation: Generation of the sperm cell tail.

Cytoplasmic reduction: Elimination of all unnecessary eytoplasm.

During sperm cell production considerable individual variations exist that are also partially influenced by psychological factors. Per day roughly 100 million sperm cells are produced. It is said that in each ejaculate an average number of 50-200 million sperm cells. Cells are present (WHO standard value; over 40 million). The bulk of ejaculate immediately turns into a gelatinous coagulum because of the action of clotting enzymes and structural proteins, seminogelin derived from seminal vesicles. The degradation of these proteins by prostate specific antigen results in liquefaction of semen within 15-20 minutes. Production of spermatozoa is continuous throughout a man's life however each spermatozoa has a limited life span within the male testicle they remain for a month. Once in the uterine cavity, they have a life span of 2 to 7 days. The mature sperm cell is approximately 60μm long and completely enveloped by the plasma membrane.

A normal sperm should have following parameters (Table-1)

The head or core contains the genetic information. The neck is the engine that gives the power. The tail is the element that allows movement.

Head: Smooth and oval configuration, Length 3 to 6 microns, Diameter 2.5 to 3.5 microns. Acromosome must comprise 40-70% of the sperm head.

Mid-piece: Slender, axially attached. Less than 1 micron in width and approximately 1.5 micron length. No eytoplasmic droplet larger than 50% of the size of the sperm head.

Tail: Single, unbroken, straight without or coil. Appromimately 45μm is length.

Table.1 Shows Parameters and Normal range of semen

Parameters	Normal range
Volume	≥ 2ml
pH	7.2 – 8.5
Concentration	≥ 20 x 10^6
Motility	50%
WBC	≤ 1 x 10^6
Morphology	≥ 30%

Semen analysis is still today a fundamental stage of male infertility diagnosis. But a semen analysis with normal parameters does not assure male infertility. Except the cases of azoospermia, if does not distinguish fertile men, several parameters and the normal values of semen like viscosity, morphology and appearance are noted. A specimen of semen is examined microscopically in the laboratory to determine the number of sperms as well as their size, shape and motility. Examination of the reproductive tract and genital organs may necessary. WHO 4[15] edition (1999) normal references used.

Male infertility

The inability to conceive after a year of unprotected intercourse is called infertility. More than 30% of infertility cases are attributed to the male factor alone i.e., fertility problems in men. Male infertility could be caused by one or more of the following factors:

Sperm disorders

Azoospermia : Semen contains no sperm

Oligospermia: Semen has low or insufficient sperm count, abnormally shapped sperm, sperm with poor motility.

Hormonal imbalance: Lower production fo sex hormones due to physiological or genetic factors.

Functional problems: Impotence, testicular problems, due to injury or disease, difficulties in ejaculation, antibodies that hinder normal sperm activity.

Idiopathic infertility: Infertility due to imbalance causes.

Varicocele	12%
Primary idiopathic testicular failure	10%
Male accessory gland infection	7%
Abnormal sperm morphology	6%
Other seminal fluid observations	4%
Decreased sperm motility	4%
Immunological causes	3%
Sexual problems	2%
Azoospermia owing to obstruction	1%
Endocrine and other causes	4%
No demonstrable cause	47%

The previous study reveals the male accessory sex gland infections are considered to hazard for male infertility. Various path physiologic concept evolving from experimental and clinical studies have began to explain the effects of yeast and immunologic events on spermatozoa. Besides the known indirect influence due to spermatogenesis inhibition resulting from testicular damage, autoimmune process induced by inflammation and leucocytospermia with its secondary influence on ejaculate parameters and with direct effect on the fertile properties of seminal fluid due to decrease in the number of spermatozoa, suppression of motility, changes in morphology and fertilizing capacity are also considered. Several inflammatory and reactive alterations on sperm quality seem to be proven, many microorganisms are isolated

from seminal fluid sample of infertile patients, but the significance of yeast is uncertain because many males lack symptoms associated with yeast infection of the reproductive tract. Unfortunately it is not clearly established as to whether any of the organisams isolated actually cause infertility. The negative impact of some microorganisms relevant to genital infections on sperm function has been claimed but could not actually be confirmed (Mustsfa berktas *et al*., 2006).

The microorganism plays major role in the no demonstrable cause of infertility by, inhibiting sperm motility, Secreting extracellular productions that immobilize the sperm, Agglutinating spermatozoa, The association of organism in sperm may also result in chromosomal. Abnormalities apart from various spermia which has been known for long time. The most commonly found organisms in infertile males was *Candida albicans, Candida tropical is, Cryptococcus neoformans, Saccaromyces* Cerevicieae and other bacterial like *Stphylococcus aureus, Enterrococci, Gonococci, Klebsiella, Pseudomonas aeruginosa, Ureaplasma urealyticum, Mycoplasma hominis, Streptococci, Eschrichia coli, Staphylococcus epidermis* (Golshnai *et al.,* 2006)

Semen is a mixture of spermatozoa and fluids derived from the epididymis and the bulbo urethral, and prostate glands (Mawhinneg, 1983). Each of the areas, that contribute to a semen sample are considered sterile areas, yet the culturing of semen for yeast is usually positive (Hilher *et al.,* 1990) although sterility of the internal urethra is maintained primarily by the normal flow of Urine, the distal urethra is not considered a sterile area, infections process may lead to deterioration of spermatogenesis, impairment of sperm function and/or obstruction of the seminal tract, detection of yeast in semen does not necessarily signifies infection since yeast cells in the sperms may represent contamination, colonization or infection (Bar-Chama fiseh 1993; Purves and Christiansen 1996).

Acute and chronic infections of the genital urinary tract may play a role contributing in male factor infertility. In the male genital tract reactive oxygen species are generated by spermatozon and leucocytes including neutrophils and macrophages. ROS involved in the regulation of sperm function such as capacitation and the acrosome reaction. Infection lead to an excessive ROS production, resulting in an oxidative burst from neutrophils/macrophages as a first lone defence mechanisms. ROS produced during infections of the testis and epididymis is especially harmful to Spermatozoa due to the longer contact and the lack of antioxidant protections. Cytokines play an important role in intercellular communication. They are involved in numerous physiological and pathological processes and have important functions in the reproductive physiology of men (Rees, 1992 Rutanen 1993, Fiocchi *et al*., 1994) there in evidence that some of these polypeptide are directly involved in the regulation of testicular .

Teratozoospermia: The ejaculate contains sufficient spermatozon with sufficient motility but less than 30% normal morphology.

Oligoasthenozoospermia: Significance disturbance of all the three variables, the enjaculate with less than 40 million spermatozon less than 30% normal morphology and less than 50% progressive motility.

Azoospermia: No spermatozon in the ejaculate, which is the direct indication for donor semen.

Aspermia: No ejaculate.

Nechrospermia: All sperms present in semen are dead.

Globospermia: The ejaculate that contains the sperms with reacted acrosome, which shows the round headed sperms due to acrosome failure.

Leucospermia: The ejaculate contains more than 1 million WBC.

The presence study deals with, the effects of certain uropathogenic organisms (*Candida aibicans, Candida tropicalis, Cryptococcus neoformans, Saccaromyces cereviceae* and bacteria like *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Mycoplasma hominis*) on human sperm motility characteristics were studies in vitro, semen sample were aseptically obtained from Normozoospermia patients, the required uropathogenic yeast were obtained from out patients with genitourinary tract infection, sperm suspension was incubated with various unrpathogenic yeast at various concentration and incubation. The effectiveness of yeast on sperm motility was examined.

Materials and Methods

Study material

The semen specimen was obtained from normozoospermia donors by means of masturbation in sterile manner, and following selected uropathogenic yeast obtained from out patients with genitourinary tract infection, *Candida albicans, Candida tropicalis, Cryptococcus neoformans, Saccharomyees cerevisiae.*

Semen collection

Semen sample of 40 patients attending to Billroth infertility centre were collected in the clinic, by masturbation after 3 days abstinence period, patient should not take any antibiotic from one week before collecting the sample, and patients must wash their hands and genital area with soap and saline water. Samples were collected in sterile plastic containers. The samples were delivered to the laboratory within less than 10 minutes and kept in the incubator adjusted to body temperature.

Spermatozoa analysis

Collected samples were subjected to the analysis of microscopic, physical and morphological characters of sperm (Table 2).

Analysis of physical characters of semen

After the collection of samples, it is allowed to liquefy for 45 minutes. Color of the sample is observed. Volume of total semen ejaculation is measured – pH of the semen was checked by use of the specific pH paper. These parameters were compared with the normal semen parameters to find the abnormalities in sperm (Table 3).

Microscopic observation of semen sperm motility

Sperm motility was evaluated soon after liquefaction, 10μl of thoroughly mixed semen is placed on a Makler counting

chamber and the cover slip placed on it and the motility was observed as the average percentage of sperm moving in atleast four random high power microscopic fields. The quality of motile sperm movement was classified based on the pattern displayed by the majority of motile spermatozoa and ranged from 4 (excellent forward progression) to 0 (no movement). A forward progression of 4 is denoted to spermatozoa moving rapidly moving in a straight line. A forward progression of 3 is denoted to spermatozoa similarly moving linearly but a slower velocity. Sperm movement with a forward progression of exhibited angular displacement or to varying degrees while a progression of 1 is denoted only tail motion without progression. Zero progression represented no movement at all (Table 2).

Calculation of motile and immotile sperm percentage

The concentration is expressed in values per cubic mm counted motile. Immotile sperms in vertical 10 squares were subjected to the formula and the percentage of motility and total sperm count is format out.

Number of motile sperms = Motility percentage/Number of sperms X 100

Total Sperm count = Number of motile sperms x volume

When the volume of the semen sample was taken out for count, other than the spermatozoa the presence of round cells, red blood cells and yeast contamination were noted when spermatozoa count was made.

Microbiological investigation of infertile patient semen

Culture Plate Method

A routine microbiological investigation of semen was carried out for patient who comes to Billroth fertility research centre, Chennai, Tamilnadu, India, to take treatment infertility. The sample obtained from the infertile patient was streaked on the following appropriate media. The semen sample obtained from the infertile patient was streaked on the above medium in sterile manner. The plates were incubated at 37°C for 24 - 48 hours. After incubation, the microbial colony morphology on the plates were observed recorded.

Staining for uropathogenic yeast and bacteria

The uropathogenic organisms obtained from genitourinary tract infected patient were confirmed through Gram's staining method and Lacto phenol cotton blue (LPCB) staining method.

An in vitro experiment on sperm motility

For this in vitro studies, reanalyzed normozoospermia. Semen sample were used to evaluate sperm motility changes after co-incubation with various uropathogenic organisms. Within the first 30 minutes the sperm suspension was divided into portions each about 0.5 ml. These sub samples were artificially infected with microorganisms in concentration varying from $2x10^3$-2 x 10^6 microorganisms/ml. Uninfected suspensions of spermatozoa served as controls. In all samples sperm motility was examined after 30 minutes, 1 hour, 1½

hours and 2 hours. Thus in vitro deletions effect of different microorganisms with different concentration on sperm motility were examined. To climinate the rapid growth of the microorganisms in the incubator the infected semen specimens were kept at room temperature after being infected as were controls. *Candida albicans, Candida tropicalis, Cryptococcus neoformans, Saccharomyees crevisiae, Staphylococcus aureus, Escherichiacoli* and *Klebsiella pneumonia* were used as microbial agents. These are the most frequently encountered strains in semen culture and are known as agents that cause urinary tract infections.

Motility changes in each sample were recorded and clarify the real effect of the same agent in various concentrations on motility. The motility index (Ml), defined as the sum of degree 3 and 4 motilities was determined.

Results and Discussion

Mean patient age was 33.2 years, with a range of 28 – 38 years sperm concentration ranged from 67 million to 190 million, with a mean of 122 million, the mean of initial motility index was calculated to be 79.6%, in the absence of infectious agents, the motility index was calculated after being infected with selected uropathogenic organisms in concentrations varying from $2x10^3$=$2x10^6$ microorganisms/ml and different incubation time ranged from ½ an hour to 1 hour.

Candida albicans

Table 4 shows during the incubation concentration of $2x10^3$-$2x10^6$ microorganisms/ml there was significant decline in the percentage of spermatozoa with forward motion, from ½ an hour to 2 hours, the deleterious effect of the yeast was found to be significant at all concentration, after ½ an hour incubation.

Candida tropicalis

Table 5 experimentally shows that *Candida tropicalis* strain indicated significant influence on sperm motility parameter from the 1½ an hour incubation. However 2 hours incubation of fresh specimen with concentration of $2x10^3$, $2x10^4$, $2x10^5$, and $2x10^6$ microorganisms/ml significantly decreased.

Cryptococcus neoformans

Table 6 indicates that, sperm motility was moderately affected even from the 1 hour of incubation of fresh specimen with concentration of $2x10^4$, $2x10^5$, and $2x10^6$ microorganisms/ml, interestingly 1 hour and 1 ½ hour incubation indicated very less significant decrease of motility.

Saccharomyces cerevisiae

Table 7 shows the significant inhibitory effect of *Saccharomyces cerevisiae* was detected in the samples with even from minimal concentration from $2x10^3$ to $2x10^6$ microorganisms/ml.

Klebsiella pneumonia

Table 8 shows the moderate inhibitory effect to *Klebsiella pneumonia* was detected in the sample with concentration of $2x10^3$ to $2x10^6$ microorganisms/ml, sperm motility was moderately affected comparatively other incubated specimen.

Microbial infection are thought to be found more frequently in semen samples of asymptomatic infertile patients then in those from fertile men.

Table.2 Semen Parameter Analysis – Motility Counting

S.No.	Sample	Age	Sperm Count Million / Cubic	Total Motility	Active	Moderate	Sluggish	Immotile
1	Patient 1	30	18	28	12	16	0	72
2	Patient 2	40	14	41	7	7	30	67
3	Patient 3	35	4	78	25	40	50	15
4	Patient 4	28	197	59	17	30	13	41
5	Patient 5	30	108	48	9	33	6	52
6	Patient 6	29	143	70	6	53	11	30
7	Patient 7	31	60	77	3	62	12	23
8	Patient 8	37	157	54	4	44	6	46
9	Patient 9	38	Nil	Nil	Nil	Nil	Nil	Nil
10	Patient 10	36	2	50	0	50	0	50
11	Patient 11	30	123	73	5	54	14	27
12	Patient 12	42	40	60	8	15	37	40
13	Patient 13	33	4	50	0	25	25	50
14	Patient 14	41	75	60	16	22	22	40
15	Patient 15	33	82	77	26	39	12	23
16	Patient 16	27	130	77	28	37	14	25
17	Patient 17	37	59	8	0	8	0	92
18	Patient 18	35	13	31	0	23	8	69
19	Patient 19	39	31	Nil	Nil	Nil	Nil	Nil
20	Patient 20	36	131	86	24	53	9	14
21	Patient 21	26	77	55	13	50	12	45
22	Patient 22	35	22	1	0	0	1	0
23	Patient 23	41	71	45	5	15	25	55
24	Patient 24	36	131	86	24	53	9	14
25	Patient 25	35	75	60	16	22	22	40
26	Patient 26	27	64	61	9	44	8	39
27	Patient 27	44	97	54	5	27	22	46
28	Patient 28	38	67	69	30	35	4	31
29	Patient 29	36	15	20	0	13	7	80
30	Patient 30	29	140	80	30	37	13	20
31	Patient 31	33	62	58	34	36	21	22
32	Patient 32	40	59	50	32	30	20	18
33	Patient 33	31	69	54	37	31	23	20
34	Patient 34	28	124	82	29	51	10	9

35	Patient 35	30	102	78	30	45	15	13
36	Patient 36	37	76	52	29	37	18	32
37	Patient 37	30	87	53	27	32	20	35
38	Patient 38	42	55	48	30	28	20	18
39	Patient 39	35	80	51	32	31	18	19
40	Patient 40	32	82	49	34	32	21	23

Table.3 Semen Parameter Analysis – Physical Character

S. No	Sample	Age	Colour	Volume (ml)	pH	Reaction	Viscosity	Liquefaction	Count Million /Cubic	Motility %	WBC Million / ml	Impression
1	Patient 1	30	Grey Yellow	3.7	7.4	Alkaline	Thin	Normal	18	28	0.7	Oligoasthenozoospermia
2	Patient 2	40	Grey Opaque	4.5	7.6	Alkaline	Thin	Normal	14	43	0.5	Oligoasthenozoospermia
3	Patient 3	35	Grey Opaque	2	7.6	Alkaline	Viscous	Normal	4	65	0.4	Oligoasthenozoospermia
4	Patient 4	28	Grey Opaque	0.6	7.6	Alkaline	Thin	Normal	197	59	0.4	Normo Zoospermia
5	Patient 5	30	Grey Opaque	1.2	7.6	Alkaline	Thin	2½ Hours	108	48	0.6	Astheno zoospermia
6	Patient 6	29	Grey Opaque	2.8	7.6	Alkaline	Viscous	Normal	143	70	0.7	Mild astheno zoospermia
7	Patient 7	31	Grey Opaque	2	7.6	Alkaline	Thin	Normal	60	77	0.6	Mild astheno zoospermia
8	Patient 8	37	Grey Opaque	1.6	7.4	Alkaline	Thin	Normal	157	54	0.6	Mild astheno zoospermia
9	Patient 9	38	Grey Opaque	3	8	Alkaline	Thin	Normal	Nil	Nil	Plenty	Azoospermia
10	Patient 10	36	Grey Opaque	1.7	7.8	Alkaline	Thin	Normal	3	50	0.6	Severe oligo Zoospermia
11	Patient 11	30	Grey Opaque	1.5	7.6	Alkaline	High Viscous	Normal	144	71	0.3	Astheno zoospermia
12	Patient 12	42	Grey Opaque	1.3	7.6	Alkaline	Thin	Normal	140	60	0.5	Mild astheno zoospermia
13	Patient 13	33	Grey Opaque	2.5	7.8	Alkaline	Viscous	Normal	4	50	0.7	Oligoastheno ospermia
14	Patient 14	41	Grey Opaque	2.6	7.6	Alkaline	Viscous	Normal	6	25	0.6	Oligoastheno ospermia
15	Patient 15	33	Grey Opaque	2.5	7.6	Alkaline	High Viscous	Abnormal	82	77	0.6	Normo Zoospermia
16	Patient 16	27	Grey Opaque	3.4	7.6	Alkaline	Thin	Normal	82	77	0.6	Astheno zoospermia
17	Patient 17	37	Grey Opaque	1.2	7.6	Alkaline	Thin	Normal	59	8	0.6	Asthenozoospermia

S. No	Sample	Age	Colour	Volume (ml)	pH	Reaction	Viscosity	Liquefaction	Count Million /Cubic	Motility %	WBC Million / ml	Impression
18	Patient 18	35	Grey Opaque	1.8	7.6	Alkaline	Thin	Normal	13	31	0.3	Oligoastheno ospermia
19	Patient 19	39	Grey Opaque	0.9	7.6	Alkaline	Thin	Normal	31	Nil	0.3	Azoospermia
20	Patient 20	36	Grey Opaque	1	7.6	Alkaline	Thin	Normal	31	86	0.5	Normo Zoospermia
21	Patient 21	26	Grey Opaque	0.8	7.6	Alkaline	Thin	Normal	77	55	0.6	Mild astheno zoospermia
22	Patient 22	35	Grey Opaque	0.4	7.8	Alkaline	Viscous	Normal	2	1	0.6	Oligoasthenozo ospermia
24	Patient 24	36	Grey Opaque	1.0	7.6	Alkaline	Thin	Normal	131	86	0.5	Normo Zoospermia
25	Patient 25	35	Grey Opaque	2.8	7.8	Alkaline	Thin	Normal	75	60	0.6	Mild astheno zoospermia
26	Patient 26	27	Grey Opaque	2.4	7.6	Alkaline	Thin	Normal	64	61	0.6	Mild astheno zoospermia
27	Patient 27	44	Grey Opaque	1.5	7.8	Alkaline	Thin	Normal	97	54	0.6	Normo Zoospermia
28	Patient 28	38	Grey Opaque	0.4	7.6	Alkaline	Thin	Normal	67	69	0.6	Normo Zoospermia
29	Patient 29	36	Grey Opaque	1.8	7.2	Alkaline	Thin	Normal	15	20	0.4	Oligoasthenozo ospermia
30	Patient 30	29	Grey Opaque	2.6	7.2	Alkaline	Thin	Normal	140	80	0.5	Normo Zoospermia
31	Patient 31	33	Grey Opaque	1.6	7.2	Alkaline	Thin	Normal	75	69	0.6	Normo Zoospermia
32	Patient 32	40	Grey Opaque	2.4	7.6	Alkaline	Thin	Normal	96	53	0.5	Oligoasthenozo ospermia
33	Patient 33	31	Grey Opaque	2.5	7.8	Alkaline	Thin	Normal	125	75	0.6	Normo Zoospermia
34	Patient 34	28	Grey Opsulent	0.6	7.2	Alkaline	Thin	Normal	65	67	0.6	Normo Zoospermia
35	Patient 35	30	Grey Opsulent	1.5	7.8	Alkaline	Thin	Normal	93	50	0.5	Mild astheno zoospermia
36	Patient 36	37	Grey Opaque	0.4	7.6	Alkaline	Thin	Normal	69	67	0.6	Mild astheno zoospermia
37	Patient 37	30	Grey Opaque	2.3	7.6	Alkaline	Thin	Normal	123	78	0.5	Normo Zoospermia
38	Patient 38	42	Grey Opaque	1.8	7.4	Alkaline	Thin	Normal	17	23	0.5	Normo Zoospermia
39	Patient 39	35	Grey Opaque	2.4	7.8	Alkaline	Thin	Normal	67	69	0.3	Oligoastheno ospermia
40	Patient 40	32	Grey Opaque	1.5	7.2	Alkaline	Thin	Normal	85	52	0.5	Oligoasthenozo ospermia

Table.4 Motility index at the beginning and ½ - 2 Hours incubation with *Candida albicans*

Test organisms	Inoculation doses (microorganisms/ml)	Initial motility index	Incubation (hour)				Control
			½	1	1 ½	2	
Candida albicans	2 x 10^3	69	49	42	20	00	un inoculated
	2 x 10^4	69	34	20	00	00	
	2 x 10^5	69	30	10	00	00	
	2 x 10^6	69	30	05	00	00	

Table.5 Motility index at the beginning and ½ - 2 Hour incubation with *Candida tropicalis*

Test organisms	Inoculation doses (microorganisms/ml)	Initial motility index	Incubation (hour)				Control
			½	1	1 ½	2	
Candida tropicalis	2 x 10^3	77	67	40	15	00	un inoculated
	2 x 10^4	77	52	34	14	00	
	2 x 10^5	77	42	15	01	00	
	2 x 10^6	77	42	10	00	00	

Table.6 Motility index at the beginning and ½ - 2 Hours incubation with *Cryptococcus neoformans*

Test organisms	Inoculation does (microorganisms/ml)	Initial motility index	Incubation (hour)				Control
			½	1	1 ½	2	
Cryptococcus neoformans	2 x 10^3	86	60	32	10	00	un inoculated
	2 x 10^4	86	56	28	04	00	
	2 x 10^5	86	5750	20	00	00	
	2 x 10^6	86	48	15	00	00	

Table.6 Motility index at the beginning and ½ - 2 Hours incubation with *Saccharomyces cerevisiae*

Test organisms	Inoculation doses (microorganisms/ml)	Initial motility index	Incubation (hour)				Control
			½	1	1 ½	2	
Saccharomyces cerevisiae	2×10^3	86	60	35	20	04	un inoculated
	2×10^4	86	45	30	14	00	
	2×10^5	86	43	21	04	00	
	2×10^6	86	37	14	00	00	

Table.7 Motility index at the beginning and ½ - 2 Hours incubation with *Klebeiella pneumoniae*

Test organisms	Inoculation doses (microorganisms/ml)	Initial motility index	Incubation (hour)				control
			½	1	1 ½	2	
Klebsiella pneumoniae	2×10^3	80	65	40	25	06	un inoculated
	2×10^4	80	60	35	15	00	
	2×10^5	80	56	26	05	00	
	2×10^6	80	42	20	00	00	

Yeast infection in sperm cells is also a common problem of male partners from couples undergoing *in-vitro* fertilitition. However, the reason for infertility are thought to be inhibiting sperm motility either by directly or by agglutinating sperm motility or by secreting extracellular product that immobilize the sperm and also due to the secretion of exogenous and endogenous microbial enzymes which lead to the motility and morphological defects and changing biochemical characteristics of semen results in increase in the number of dead sperm apart from the testicular part. If the microbial infection occurs in testicular part complete spermatogenesis cycle will get affect.

In this study highly motile-normozoospermia patient semen sample were inarbated for 2 hours with various uropathogenic organism to eliminate rapid growth of microorganism in the incubator adjusted to body temperature. The inoculated specimens were kept at body temperature with controls. This resulted rapid motility loss and survival time was much shorter than compare to controls. Many contradictory reports have been found in the literature, most of these studies are difficult to interpret, because there is no control group as well as of incubation in short and microorganisms are also belongs to uropathogenic group.

In this study reports have been interpret with control group duration of incubation hours long, as well as studies are belongs to urapathogenic groups. Incubation of spermatozoa with Candida albicans, at $2x10^3$ to $2x10^6$ microorganisms/ml concentration resulted in 48.25% of motility in ½ an hour incubation, 5.25% of motility in 1 hour incubation, 1.25% in 1½ an hour incubation, 0% of motility with 2 hour incubation and control motility has no significant change up to 2 hour incubation have been found. Experiments with Candida tropicalis indicated 60.75% of motility with ½ hour incubation, 24.75% of motility in 1 hour incubation, 7.5% of motility with 1½ hour incubation, 0% of motility with 2 hours incubations, and control motility has no significantly change up to 2 hours incubation have been found.

Incubation of spermatozoa with *Cryptococcus neoformans* 55.75% of motility with ½ hour, 25.75% at 1 hour, 10.25% in 1½ an hour, 0% motility with 2 hours of incubation, there is no significant changes on control motility up to 2 hours incubation. Experiments on co-incubation with *saccharomyces cereviside* indicated 68.75% of motility with ½ hour, 37.75% with 1 hour, 25.75% with 1 ½ an hour and 5.5% with 2 hours of incubation and control motility has no significant decline up to 2 hours incubation.

Experiment with *Klebsiella pneumonia*, motility rate decline 55.75% with ½ hour, 30.25% with 1 hour, 11.25% with 1 ½ an hour, 1.5% with 2 hours of incubation, and control motility has no significant decline up to 2 hours incubation have been found. Other authors of attributed decreased spermatozoa motility in ejaculates incubated with microorganisms because of increased fructose consumption similar to other, in this invitro studies comparison between *Candida albicans, Candida tropicalis, Cryptococcus neoformans, Saccharomyees crevisiae, Klebsiella pneumonia*, of concentration with ½ an hour to 2 hour incubation shows gradual deficiency in the sperm motility, particularly *Candida albicans* has the significant deficiency in sperm motility even from ½ an hour to 2 hours incubation in all concentration. No one these phenomena that are evident in *in vitro* have clearly been documented in semen specimens of patient with male accessory gland infection. Thus the observation derived from experimental *in vitro* studies which have reveals the uropathogenic microbial infection in semen which plays a major role in infertility factors.

Male Urogenital tract infection plays an important role in men infertility. Yeast infections in sperm have been paid attention as a major cause of male infertility. Microbiological investigation of semen sample of infertile men attending to infertility clinic and evaluation of the effects of yeast based on semen quality. 40 infertile men were evaluated by standard culture plate method; standard semen analysis was performed according to WHO guidelines.

There was a significant relation between the yeast infection in sperm and the rate of no motile and morphologically abnormal sperm. The quality of sperm motility was significantly decreased in contaminated semen; the percentage of morphologically normal sperm was lower.

In this study, highly motile preparation of spermatozoa was co-incubated for 2 hours with various uropathogenic organisms. Thus providing new definitions of the

interaction between various uropathogenic organisms and disturbances on sperm motility and subsequently we have idea, how they impact on male infertility. Pre-diagnosis and antibiotics treatment which could helps to take advance treatment for microbial infected male partner, which will prevent the decreasing semen count and motility.

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