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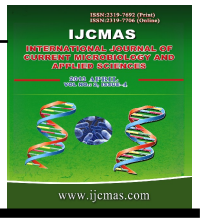

Original Research Article

An Investigation of DNA Fragmentation and Morphological Changes caused by Bacteria and Fungi in Human Spermatozoa

S.Sasikumar[1]*, D.Dakshayani[1] and D.Sarasa[2]

[1]Life care DKJ Hospital, Villivakkam, Chennai, Tamilnadu, India.
[2]Department of Zoology, Quaid-e-Millath Government College for Women, Chennai- 600 002, Tamil Nadu, India.
*Corresponding author: *sasikumarsundararajan1@gmail.com*

Keywords

DNA fragmentation; human spermatozoa; bacteria and fungi; Fertilization; *Chlamydia trachomatis.*

A B S T R A C T

The main aim of this study verifies the prevalence of semen Bacterial contamination and whether the contamination could cause the DNA fragmentation towards decreased sperm quality. Spermiogram, Toluidine blue staining, semen culture for bacteria, papanicolaus staining for sperm morphology assessment and sperm DNA fragmentation has been performed. Sperm morphology, seminal DNA fragmentation data were elaborated using a semen culture report and semen morphology and DNA fragmentation analysis – able to define that semen sample as whether normal or abnormal and percentage of sperm morphology and DNA fragmentation. Patients data were divided according to the sperm DNA fragmentation by toluidine blue staining, assessment of sperm morphology by papanicolaus staining and semen for Bacteria and Fungi isolation. According to results comparison significantly sperm quality was decreased, in terms of motility, DNA defect, acrosome defect, multiple morphology defects and sperm necrosis were seen in sperm DNA fragmentation, > than 30% morphological defect, Bacteria and Fungi isolated specimen. The DNA fragmentation and morphological changes of the spermatozoa might adversely affect the human in vivo fertilization of Embryo or fertilization through the assisted reproductive techniques.

Introduction

Fertilization is the process of penetration of male gamete into female gamete. The completion of this process and subsequent embryo development depend in the inherent integrity of the sperm DNA (Ahmadi *et al*., 1999). Fertility issue affects the ability to become pregnant. Infertility is defined as inability to Conceive after a year of regular unprotected intercourse. Infertility is a temporary failure of reproduction. It is a major problem today. As recently as 1980 only an estimated 10-15 couples were affected. Now, most survey puts the percentage of couples remaining child less involuntarily at above 20% in developing countries. The primary infertility is due to

male factor and secondary infertility due to female factor.

The male infertility factors are sperm disorder, noonan syndrome, hemachromatosis, sickle cell disease, heavy alcohol consumption, lack of sperm, sperm DNA damage, abnormal sperm morphology, congenital defect, blocked epidermis, impaired ejaculation, cystic fibrosis and testicular damage.

The female infertility factors are absence of ovulation, absence of egg formation, viability of fallopian tubes and uterus, endometriosis and frequent miscarriage. Both partners can also be affected by hormonal imbalance, anti-immune disorders and microbial infection. Fortunately medical advance come up with answer to most infertility problems such as IUI, IVF (ICSI).

The sperm DNA fragmentation causing bacteria and fungi includes *Staphylococus aureus, Eschericia coli, Pseudomonas aeruginosa, Chlamydia trachomatis, Ureplasma urealyticum, Mycoplasma* spp. *and Candida albicans*. Sperm DNA damage due to apoptosis. Bacteria induce the expression of apoptosis has been observed during inflammatory processes in the male genital tract. In this *Eschericia coli, Staphylococus aureus* will induce apoptosis in the absence of reactive oxygen species is proved (Villegas *et al*., 2005). The porin from *Pseudomonas aeruginosa* induces apoptosis in an epithelial cell line derived from rat seminal vesicles.

Chlamydia trachomatis is responsible for a widespread of sexually transmitted infection. In men is associated with a wide clinical spectrum casing infertility. The serovar E infection of the bacteria decreases the motility and increase in non viable sperm. It also causes sperm DNA fragmentation (Satta *et al*., 2005). The incidence of *U.urealyticum* infection in the semen of infertile men is variable (7% - 42%). The aim of this study was to specifically investigate the effects of *U. urealyticum* infection on sperm chromatin stability and DNA integrity, which are known to be correlated to pregnancy outcome (Reichart *et al*., 2000).

Sperm DNA fragmentation in infertile men with genitourinary infection by *Chlamydia trachomatis* and *Mycoplasma spp* (Gallegos *et al*., 2008). Porins and lipopolysaccharide increase the spontaneous apoptosis in the spermatozoa. In that in vitro study, porins and LPS were extracted from Salmonella enteric serovay *typhimurium* and *Pasturella multocida* mixed with spermatozoa and apoptosis is detected (Gorga *et al*., 2001).

The increased sperm DNA fragmentation in an infertile patient with the male accessory gland infection is due to *Candida albicans* (Burrello *et al*., 2004). It will didn't affect the sperm parameter, but, increased the sperm chromatin packaging damage.

Influence of reactive oxygen species produced by activated leukocytes at the level of apoptosis in mature human spermatozoa. In that, *Eschericia coli* showed significant increase in apoptosis on spermatozoa. *Eschericia coli* induce the alteration of human spermatozoa. Except microbial infection other parameter also causes DNA fragmentation and morphological changes in spermatozoa. It includes the effect of repeated sequential ejaculation on sperm DNA integrity in subfertility males with asthenozoospermia (Hussein *et al*., 2008). Sperm DNA

damage, it will affect the early post implantation embryo development in assisted reproductive technology. Sperm DNA fragmentation decreases the pregnancy rate in an assisted reproductive technique. The oxidative stress will damage to DNA in human spermatozoa does not preclude pronucleus formation at Intra cytoplasmic sperm injection (ICSI).

The sperm morphological change caused by the bacteria includes *Staphylococus aureus, Eschericia coli, Proteus mirabilis, Proteus vulgaris, Klebsiella pneumonia and Pseudomonas aeruginosa* (Ibadin and Ibeh, 2008).

The main objective of this study helps doctor to select the choice of treatment to infertile couples who coming for clinic and to treat the couples either by Intra uterine insemination (IUI) or Invitro fertilization (IVF) / Intra cytoplasmic sperm injection (ICSI) and the DNA fragmentation assessment helps to select the morphologically & genetically good sperm for fertilization and to give more intrauterine implantable embryos also. This assessment will help as alternative treatment for recurrent abortion and early miscarriage patients as selective sperm injection and to produce good embryos in terms of morphologically and genetically.

Materials and Methods

Specimen

The human semen was collected from the patients for infertility studies and processed in the fertility research centre of Billroth hospitals, Chennai, Tamil Nadu, India.

Requirements

Glass wares, Toludine blue staining, Papanicolaus staining

Microbial stains

Gram's staining kit, Lactophenol cotton blue (LPCB) or KOH wet mount

Culture media

Nutrient agar, Blood agar, MacConkey agar, Sabouraud dextrose agar (SDA).

Semen collection

Thirty semen specimens were collected from the patient attending infertility analysis in fertility research centre of Billroth Hospitals, Tamilnadu, India. Before specimen collection the patient's were instructed to abstain from sex for atleast 3-5 days. The specimen was obtained by masturbation and ejaculated into clean wide mouthed sterile plastic container or vials. After collection of the specimen was labeled as per WHO instruction.

Semen analysis

The collected semen specimens it was allowed to liquefy for 30-45 minutes and analyzed microscopically.

Microscopic studies

The microscopic examination of the semen as follows:

Sperm concentration

5-10ml of thoroughly mixed semen is placed on Makler counting chamber then covered with the cover glass and observed under microscope. The total number of sperms in vertical 10 squares was counted in order to find the count ad slide viewed under 40 x objectives to assess the motility (Rapid, progressive, moderate and

sluggish). Immotile, WBC, Epithelial cells and agglutination of sperms 3-4 columns were counted and average of it is taken and this gives concentration in millions per ml. The microscopic parameter were compared with the normal semen parameters. It is used to decide the infection of microorganisms in sperms.

Calculation of motile and immotile sperm (%)

Spermatozoa motile or immotile were determined by the microscopic examination using makler counting chamber. This concentration gives the values per cubic mm counted motile, immotile sperms in vertical 10 squares were subjected the formula and the % of motility and total sperm count is calculated.

$$\text{Motility percentage} = \frac{\text{Number of motile sperm}}{\text{Total number of sperm}} \times 100$$

Where,

Number of sperm defect in DNA = Normal sperm – DNA defected sperm

Total number of defect sperm = Number of DNA defect + number of acrosome defect

Papanicolaus staining

The smeared slides are immersed in alcohol for 30 minutes, then rinsed in water for 50 minutes. The smear was stained in Harris haematoxylin for 5 minutes and then rinsed distilled water for 5 minutes. The smear was immersed in alcohol for 5 minutes and then rinsed in distilled water for 5 minutes. The smear was stained in Pap orange (2a) stain for 2 minutes and then the slide was rinsed in alcohol for 3 minutes. Then, the smear was stained in Pap Ea 50 for 3 minutes and then rinsed in alcohol for 3 minutes. Finally the smear is kept in xylene for 10 minutes.

Observation

After staining, the stained slides were air dried for 10-15 minutes. Then one drop of DPX moundant was placed and the slide was covered with cover glass and observed under the oil immersion objective (100X) of light microscopic.

Calculation of morphology in %

The morphology of human spermatozoa calculated at least 100.

$$\text{Sperm Morphology defects} = \frac{\text{H/N/CD/T}}{\text{Abnormal sperm count}} \times 100$$

Where,
H – Head, N – Neck, CD – Cytoplasmic droplet, T – Tail

Bacteriological and Mycological Investigation of the same semen specimen

Identification of the isolates

Macroscopy

After, incubation the grown colony morphology of the bacteria and fungi isolates (consistency, size, pigmentation) was observed and results were recorded.

Microscopy

Gram's staining

Gram's staining is used to differentiate gram positive or gram negative bacteria. It is used to identify the budding yeast cells.

KOH wet mount

Take clean microscopic slide, then place one drop of KOH and isolate mingled with in that, and covered with cover glass and observed under 40X objective.

Catalase test

The bacterial colony is introduced into hydrogen peroxide give rapid evolution. The presence of catalase shows bubble formation, catalase negative shows to bubble formation.

Biochemical characterization

The isolated bacterial colonies were obtained from anyone of the medium and used biochemical test for identification. As follows:
Indole test, Methyl red test, Voges proskauer test, Citrate test, Triple sugar iron agar, Urease test.

Attachment of *Candida albicans* to spermatozoa

The invitro effects of *Candida albicans* in ultrastructure of human spermatozoa from healthy donors studied. The semen specimen was collected from healthy donors through masturbation infertility research centre of Billroth hospitals. *Candida albicans* isolated from semen is mixed with normal semen and incubated at 22-37oC for 30 minutes. After incubation, a thin smear was made on microscopic slide and air dried. Then the slide was stained with papanicolaus staining procedure. After staining the slide was observed under oil immersion and identified whether *Candida albicans* attaches to spermatozoa or not.

Results and Discussion

A study of DNA fragmentation and morphological changes caused by bacteria and fungi in human spermatozoa was studied.

Normal criteria of semen specimens

30 semen specimens were assessed for the semen parameters like count and motility and the results were tabulated in (Table 1). Out of 30 semen samples, 27 had changes in their normal criteria and the rest of three considered as normozoospermia.

Assessment of sperm DNA fragmentation by toluidine blue staining

Toluidine blue staining was performed for all the 30 semen specimens to assess the sperm DNA fragmentation. Out of 30 samples, 20 samples were found to have defective DNA and rest of the samples have aneuplid level of defective DNA. The percentage of DNA defect is tabulated in (Table 2).

Assessment of sperm morphology by papanicolaus staining

The morphological identification of sperm is done by Papanicolaus staining. Out of 30 semen samples, 17 samples were found to have defective sperm morphology (Head, mid piece, cytoplasmic droplet ad tail) and rest of the 13 samples have aneuplid level of defective morphology of sperm. The percentage of abnormal morphological defects is tabulated in (Table 3).

Identification of isolated bacteria and fungi

All the 30 semen specimens were plated on culture media like blood agar. Nutrient agar, macconkey agar and sabourauds dextrose agar. It showed bacterial growth on 20 out of 30 semen samples, and then the isolated bacteria were identified by using standard microbiological methods such as colony morphology preliminary tests and biochemical tests. The results are tabulated (Table 4). The percentage of bacteria is *Staphylococcus aureus* (43.33%). *Klebsiella* spp (10%). *Proteus mirabilis* (6.66%), *Escherichia coli* (3.33%) and *Pseudonomas aeruginosa* (3.35%). The samples 2 and 21 showed creamy white colour colonies on sabourauds destrose agar. The colonies obtained were subjected to Grams staining and Germ tube test showed Gram positive budding yeast cells and Gram tube positive. Based on results, the percentage of identified yeast like fungi is *Candida albicans* (6.66).

Attachment of *Candida albicans* to spermatozoa

In addition an *in-vitro* study of isolated *Candida albicans* from the semen to sperm cells was studied. The results showed the attachment of *Candida albicans* to spermatozoa through head. The frequently isolated *Staphylococcus aureus* and *Candida albicans* were checked for their antibiotic sensitively. The results showed that *Staphylococcus aureus* is highly sensitive to ceftrixone, gatifloxacin, azithromycin, piperacillin / tazobactam / tamobactam, cefazolin, linezolid, and cotrimaxazole and resistant to amaxyclave, sparofloxacin, amoxicillin, ceftazidime and oxacillin. *Candida albicans* is highly sensitive to nystatin, amphotericin, moderatively sensitive to intraconazole and resistant to fluconazole. In this study, Bacteria and Fungi were isolated and their effects on sperm count, motility, morphological defects and sperm DNA fragmentation were studied. From the a comparative results, it was clear that dominantly Bacteria and Fungi includes *Staphylococcus aureus, Klebsiella* species, *Proteus mirabilis* and *Candida albicans* were responsible for sperm DNA fragmentation and morphological defects. Since *Escherichia coli* and *Pseudomonas aeruginosa* were present in lowest population (3.33%), they were not taken in to account. But, they also caused sperm DNA fragmentation and morphological changes in spermatozoa. The dominant DND fragmentation and morphological defects caused Bacteria were *Staphylococcus aureus* and Fungi is *Candida albicuns.*

In previous studies (Ibadin and Ibeh, 2008),out of 87 processed specimen, 36 (41.4%) yielded Bacterial growth with *Staphylococcus aureus, Staphylococcus saprophyticus* and *E.coli* having the highest incidence rate of 16.9%,9.1% and 6.9% respectively. Trailing behind these were *Proteus mirablis* 3.4%, *Klebsiella pneumonia, Proteus vulgaris* recorded 2.3% and *Pseudomonas aeruginosa* had abnormal 1.1% each respectively. The rate of non-motile sperm cells and abnormal morphology was also established through WHO 1999 evaluation techniques and was found to be affected by these Bacterial isolates (Ibadin and Ibeh, 2008).

Bacteria directly increase the PS externalization in ejaculated human sperm. This way of inducing apoptosis does not require external reactive oxygen species (ROS) and may result from anyone of the molecular mechanism that account for

changes in motility, vitality and DNA integrity, that are characteristics of spermatozoa in male genital tract infection (Villegas *et al*., 2005).

Table.1 Normal Criteria of Semen

Samples	Count (Million/ ml)	Motility in %	Active	Moderate	Sluggish	Im-motile	Opinion
Sample 1	20	45	00	25	20	55	Asthenozoospermia
Sample 2	07	71	00	14	57	29	Oligoasthenozoospermia
Sample 3	09	74	05	10	59	32	Oligoasthenozoospermia
Sample 4	75	47	04	14	29	53	Asthenozoospermia
Sample 5	31	61	25	20	16	39	Mild Asthenozoospermia
Sample 6	34	47	03	24	20	53	Asthenozoospermia
Sample 7	11	55	09	19	27	45	Oligoasthenozoospermia
Sample 8	45	51	11	33	07	49	Leukozoospermia
Sample 9	101	72	21	32	19	28	Normozoospermia
Sample 10	54	63	31	19	13	37	Normozoospermia
Sample 11	18	33	00	22	11	67	Oligoasthenozoospermia
Sample 12	16	38	00	13	25	62	Oligoasthenozoospermia
Sample 13	65	68	09	30	29	32	Mild Asthenozoospermia
Sample 14	75	47	04	30	13	53	Asthenozoospermia
Sample 15	21	61	00	23	38	39	Asthenozoospermia
Sample 16	105	57	08	28	21	43	Asthenozoospermia
Sample 17	43	77	00	27	50	23	Asthenozoospermia
Sample 18	89	51	11	22	18	49	Asthenozoospermia
Sample 19	62	58	11	23	24	42	Mild Asthenozoospermia
Sample 20	05	40	00	00	40	60	Oligoasthenozoospermia
Sample 21	62	64	12	14	38	55	Mild Asthenozoospermia
Sample 22	60	80	08	47	25	20	Mild Asthenozoospermia
Sample 23	64	81	00	44	37	19	Asthenozoospermia
Sample 24	33	57	06	36	15	43	Asthenozoospermia
Sample 25	91	45	02	27	16	55	Asthenozoospermia
Sample 26	73	77	22	36	18	23	Normozoospermia
Sample 27	48	79	02	56	21	21	Mild Asthenozoospermia
Sample 28	92	51	16	14	21	49	Asthenozoospermia
Sample 29	70	57	04	32	21	43	Asthenozoospermia
Sample 30	73	73	04	36	33	27	Mild Asthenozoospermia

Table.2 Assessment of Sperm DNA Fragmentation by Toluidine Blue Staining

Samples	Normal sperm	DNA Defect Sperm	No Acrosome	% of DNA Defect
Sample 1	11	80	9	77.5
Sample 2	22	38	40	20.51
Sample 3	20	48	32	35.0
Sample 4	21	08	71	16.45
Sample 5	18	66	16	58.53
Sample 6	50	38	12	24.0
Sample 7	26	52	22	35.13
Sample 8	04	74	22	72.9
Sample 9	15	79	07	75.58
Sample 10	45	52	03	12.72
Sample 11	34	30	36	6.06
Sample 12	20	36	44	20.0
Sample 13	30	62	08	45.7
Sample 14	12	40	48	31.8
Sample 15	18	42	40	29.26
Sample 16	30	40	30	14.28
Sample 17	26	58	16	43.24
Sample 18	12	52	36	45.45
Sample 19	26	50	24	32.43
Sample 20	18	38	44	24.39
Sample 21	26	54	20	37.83
Sample 22	18	66	16	58.5
Sample 23	19	41	40	27.16
Sample 24	14	42	44	32.55
Sample 25	30	62	08	45.71
Sample 26	12	52	36	46.51
Sample 27	20	72	08	65.0
Sample 28	28	62	10	47.22
Sample 29	18	52	30	41.46
Sample 30	10	73	17	70.0

Table.3 Assessment of Sperm Morphology by Papanicolaus Staining

Samples	Normal Sperm	Abnormal Sperm	Head in %	Mid Piece in %	Cytoplasmic Droplet in %	Tail in %
Sample 1	28	72	41.66	2.77	1.388	54.16
Sample 2	20	80	50.0	12.5	6.25	31.25
Sample 3	22	78	61.5	5.12	3.84	29.48
Sample 4	32	68	63.23	2.94	4.411	29.4
Sample 5	12	88	38.6	6.81	2.27	52.27
Sample 6	37	63	31.74	3.17	1.58	63.49
Sample 7	27	73	54.79	4.109	2.73	38.35
Sample 8	36	64	40.62	3.125	4.68	51.56
Sample 9	31	69	33.33	2.89	4.34	59.42
Sample 10	48	52	57.69	1.92	7.69	19.23
Sample 11	34	66	21.2	4.54	1.51	72.72
Sample 12	53	47	53.19	4.25	2.12	40.42
Sample 13	32	68	17.64	2.94	5.88	70.58
Sample 14	18	82	41.16	7.31	2.43	51.21
Sample 15	29	71	28.16	2.81	1.408	67.60
Sample 16	18	82	57.31	13.41	3.65	25.60
Sample 17	26	74	29.72	5.405	2.70	62.16
Sample 18	38	62	19.35	25.80	6.45	48.38
Sample 19	27	63	44.44	4.761	3.174	47.61
Sample 20	08	92	52.17	10.86	2.17	34.78
Sample 21	28	72	48.61	5.55	2.77	43.05
Sample 22	26	74	24.32	2.70	8.10	64.86
Sample 23	18	82	29.26	6.09	7.31	57.31
Sample 24	38	62	16.12	8.06	4.83	70.96
Sample 25	30	70	25.71	14.28	2.85	57.14
Sample 26	38	62	19.35	25.80	6.45	48.38
Sample 27	20	80	27.5	10.0	3.75	58.75
Sample 28	22	78	17.94	12.82	5.12	64.10
Sample 29	14	86	45.34	9.30	4.651	40.69
Sample 30	37	63	84.12	1.58	3.17	11.11

Table.4 Cultural and Biochemical Identification of Isolated Bacteria

Samples	Preliminary examination							Biochemical test					Coagulase tests	Identification of Bacteria
	Nutrient Agar	Blood Agar	Mac Conkey Agar	Gram's Staining	Motility	Oxidase	Catalase	Indole	Methyl red	Voges Proskauer	Citrate	TSI		
Sample 1	Small, Circulate, White Colour Colonies	Alpha haemolytic colonies	LF Colonies	Gram positive cocci	Non-Motile	-	+	-	+	+	-	A/A, G-, H_2S	+	*Staphylococcus aureus*
Sample 2	Small, Circulate, White Colour Colonies	Alpha haemolytic colonies	LF Colonies	Gram positive cocci	Non-Motile	-	+	-	+	+	-	A/A, G-, H_2S	+	*Staphylococcus aureus*
Sample 3	Small, Circulate, White Colour Colonies	Alpha haemolytic colonies	LF Colonies	Gram positive cocci	Non-Motile	-	+	-	+	+	-	A/A, G-, H_2S	+	*Staphylococcus aureus*
Sample 4	Small, Circulate, White Colour Colonies	Alpha haemolytic colonies	LF Colonies	Gram positive cocci	Non-Motile	-	+	-	+	+	-	A/A, G-, H_2S	+	*Staphylococcus aureus*
Sample 5	Small, Circulate, White Colour Colonies	Alpha haemolytic colonies	LF Colonies	Gram positive cocci	Non-Motile	-	+	-	+	+	-	A/A, G-, H_2S	+	*Staphylococcus aureus*
Sample 6	Small, Circulate, White Colour Colonies	Alpha haemolytic colonies	LF Colonies	Gram positive cocci	Non-Motile	-	+	-	+	+	-	A/A, G-, H_2S	+	*Staphylococcus aureus*
Sample 7	Large, Circulate, mucoid Colonies	Beta haemolytic colonies	LF Colonies	Gram negative cocci	Non-Motile	-	+	-	+	+	-	A/A, G-, H_2S	-	*Klebsiella spp*
Sample 8	Small, Circulate, White Colour Colonies	Alpha haemolytic colonies	LF Colonies	Gram positive cocci	Non-Motile	-	+	-	+	+	-	A/A, G-, H_2S	+	*Staphylococcus aureus*

Sample 9	-	-	-	-	Nil	-	-	-	-	-	-	-	-	No growth
Sample 10	Small, Circulate, White Colour Colonies	Alpha haemolytic colonies	LF Colonies	Gram positive cocci										
Sample 11	-	-	-	-	Nil	-	-	-	-	-	-	-	-	No growth
Sample 12	-	-	-	-	Nil	-	-	-	-	-	-	-	-	No growth
Sample 13	Small, Circulate, White Colour Colonies	Alpha haemolytic colonies	LF Colonies	Gram positive cocci	Non-Motile	-	+	-	+	+	-	A/A, G-, H_2S	+	*Staphylococcus aureus*
Sample 14	-	-	-	-	Nil	-	-	-	-	-	-	-	-	No growth
Sample 15	-	-	-	-	Nil	-	-	-	-	-	-	-	-	No growth
Sample 16	-	-	-	-	Nil	-	-	-	-	-	-	-	-	No growth
Sample 17	Large, Circulate, mucoid Colonies	Beta haemolytic colonies	LF Colonies	Gram negative cocci	Non-Motile	-	+	-	+	+	-	A/A, G-, H_2S	-	*Klebsiella spp*
Sample 18	Mucoid colonies	Swarming growth and beta haemolytic colonies	NLF colonies	Gram negative bacilli	Motile	-	+	+	+	-	-	A/A, G-, H_2S	-	*Proteus mirabilis*
Sample 19	Small, large mucoid, grey colour colonies	Alpha haemolytic colonies	LF Colonies	Gram negative bacilli	Motile	-	+	+	+	-	-	A/A, G-, H_2S	-	*Escherichia coli*
Sample 20	-	-	-	-	Nil	-	-	-	-	-	-	-	-	No growth
Sample 21	Small, Circulate, White Colour Colonies	Alpha haemolytic colonies	LF Colonies	Gram positive cocci	Non-Motile	-	+	-	+	+	-	A/A, G-, H_2S	+	*Staphylococcus aureus*
Sample 22	Mucoid colonies	Swarming growth and beta haemolytic colonies	NLF negative bacilli	Gram negative bacilli	Motile	+	-	-	+	-	-	K/A, G-, H_2S	-	*Proteus mirabilis*

Sample 23	-	-	-	-	Nil	-	-	-	-	-	-	-	-	No growth
Sample 24	Large, Circulate, mucoid Colonies	Beta haemolytic colonies	LF Colonies	Gram negative cocci	Non-Motile	-	+	-	+	+	-	A/A, G-, H_2S	-	*Klebsiella spp*
Sample 25	Small, Circulate, White Colour Colonies	Alpha haemolytic colonies	LF Colonies	Gram positive cocci	Non-Motile	-	+	-	+	+	-	A/A, G-, H_2S	+	*Staphylococcus aureus*
Sample 26	-	-	-	-	Nil	-	-	-	-	-	-	-	-	No growth
Sample 27	Small, Circulate, White Colour Colonies	Alpha haemolytic colonies	LF Colonies	Gram positive cocci	Non-Motile	-	+	-	+	+	-	A/A, G-, H_2S	+	*Staphylococcus aureus*
Sample 28	-	-	-	-	Nil	-	-	-	-	-	-	-	-	No growth
Sample 29	Small, Circulate, White Colour Colonies	Alpha haemolytic colonies	LF Colonies	Gram positive cocci	Non-Motile	-	+	-	+	+	-	A/A, G+, H_2S	-	*Klebsiella spp*
Sample 30	Small, Circulate, White Colour Colonies	Alpha haemolytic colonies	LF Colonies	Gram positive cocci	Non-Motile	-	+	-	+	+	-	A/A, G-, H_2S	+	*Staphylococcus aureus*

In - vitro study of isolated *Candida albicans* from semen showed its attachment to spermatozoa which indicates that the spermatozoa act as a vector for transferring the infection.

The effects of *Candida albicans* on sperm parameters and the outcome of infertility treatment are unclear. This report describes a lack of fertilization after assisted reproductive techniques and increased sperm DNA fragmentation in an infertile patient with male accessory gland infection due to *Candida albicans*. In conclusion, *Candida albicans* did not affect sperm parameters, but increased sperm chromatin packaging damage and apoptosis that might have caused fertilization failure after assisted reproduction treatment in this coulples (Burrello *et al*., 2004)

The DNA fragmentation and morphological changes of the spermatozoa might adversely affect the reproductive outcomes. The study analyzed in patients, who have DNA damage (>than 30%) due to bacteria and fungi have less chance for fertility potential and so they are advised to undergo IVF or ICSI treatment.

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