Effect of Female Genital Candidiasis on Semen Parameters Female Genital Candidiasis and Semen

Original Article

Yousry Khalaf[1], Ayman Farrag[2], Eman K.Z. Shaeer[3]

[1,2]Faculty of Science, Al-Azhar University, [3]Department of Gynecology and Obstetrics, Kasr El Aini Faculty of Medicine, Cairo University. Egypt.

ABSTRACT

Purpose: Female genital candidiasis is a common gynecological complaint. Studies demonstrated its negative impact on sperm motility. In the current study we aimed at determining the effect of vaginally extracted *Candida albicans* on different semen parameters.

Study Design: Vaginal swabs were obtained from 25 females suffering from vaginitis and semen samples from 18 normozoospermia healthy males. Semen samples were inoculated with different concentrations of vaginal isolates of *Candida albicans* (0 (control), 20000, 60000 and 100000 cfus/0.5ml) for two incubation periods (5 and 15 hours). Semen was tested for sperm motility, vitality, morphology, and fertilizing capacity as well as seminal fructose, seminal level of reactive oxygen species (ROS) and pH.

Results: Significant concentration and duration dependent decrease in sperm motility, vitality, normality and sperm fertilizing capacity was observed following incubation with *candida*. The drop in the percent of normal forms was within the acceptable range for normal semen. Level of ROS decreased significantly only at high *candida* concentration (100000 cfus/0.5 ml) and duration of incubation (15 hours). Seminal fructose was mainly affected by *candida* concentration while pH decreased only at low incubation time. However, the observed change in pH was still within the normal semen pH range.

Relation between patient's results and types of graft used showed no statistically significant differences between them.

Conclusion: Vaginally extracted *Candida albicans* may have negative impact on different semen parameters affecting mainly sperm motility, vitality as well as sperm fertilizing capacity. To what extent this effect may influence couple fertility needs further studies.

Key Words: *Candida albicans*, capacity, sperm fertilizing, sperm motility; sperm normality; sperm vitality.

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Corresponding Author: Eman Shaeer, MD, Department of Gynecology and Obstetrics, Kasr El Aini Faculty of Medicine, Cairo University, Egypt. **Tel.:** (202)01006661139, **E-mail**: emanshaeer@yahoo.com

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INTRODUCTION

Infertility is an ongoing health problem that affects as many as 9% -15% of the population worldwide [1,2]. Etiology is variably shared between the male and female partners, besides a vague category which is unexplained infertility [3]. Genital infections have a major impact on fertility and contribute to more than 10% of gynecologic outpatient consultations [4,5]. It has been observed that sperm damaging microorganisms may indwell in the genital tract of asymptomatic females and contribute to infertility [6].

Genital Candidiasis is a common cause of female genital infections. It has been estimated that 75% of females may have experienced at least one episode of fungal genital infection, and that 40-45% have had recurrent attacks during their reproductive life [7,8]. *Candida* is a normal commensal of the genitourinary tract. However, under certain conditions of disturbed local biological host defense mechanisms, it becomes virulent and pathological [9]. Male genital candidiasis may contribute to male infertility through its detrimental effect on sperm quality and function [10-12].

During its journey in the female genital tract, sperms are exposed to various chemicals in the vagina and seminal fluid that negatively influence semen parameters and sperm functions [13]. In-vivo studies on the effect of female genital *Candida* isolates on semen parameters and sperm function are lacking [6]. The current study is one of few studies using *Candida* isolates extracted from female vagina to examine the impact of genital candidiasis on different semen parameters, sperm fertilizing capacity as well as the microenvironment surrounding the sperm through evaluation of reactive oxygen species (ROS), fructose and pH levels.

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PATIENTS AND METHODS:

This study was conducted at the outpatient gynecology clinic of Cairo University Hospital and Kamal Shaeer infertility Centre, Egypt. Ethical approval was obtained before conducting the study and all patients gave informed consents.

Isolation and identification of bacteria and Candida Albicans:

High vaginal swabs were obtained from 25 women presenting with vaginitis, but otherwise healthy. Swabs were placed in sterile tubes containing normal saline to maintain the swabs moist until being transported to the laboratory for culture. Each specimen was then inoculated on blood agar, nutrient agar, chocolate agar, Hicrom agar and MacConkey′s plates. All plates were incubated aerobically at 37°C for 24 - 48 hours.

The isolation and identification of bacterial opportunistic pathogens in patients' vaginal swabs were performed in three steps:

1-Colony morphology of bacterial isolates.

2-Microscopic examination with Gram stain.

3-Biochemical tests.

Candida albicans was isolated and grown on Sabouraud's dextrose agar at 37°C (Oxoid, Milan, Italy). Specimens were left overnight under aerobic conditions and *Candida* was identified by API20 test system (Biomerieux/France).

Candida isolates were then divided into different concentrations 0(control sample), 20000, 60000 and 100000 cfus/0.5ml. *Candida* concentrations were confirmed by spectrophotometry.

Semen Preparation:

Semen samples were collected from eighteen healthy and recently fertile men, after 3-5 days of abstinence. Only normal semen samples were used as per WHO criteria 2010 [14]. Samples showing evidence of leukocytospermia (leukocyte concentration more than 1×106 /ml) were excluded.

Semen Candida Interaction:

Each sample was divided into four equal aliquots each containing 15×10^6 spermatozoa and incubated with different concentrations of *Candida* 0 (control sample), 20000, 60000 and 100000 cfus/0.5ml) for 15 hours. Samples were then evaluated after 5 hours and at the end of the incubation period (15 hours).

At the end of incubation time, semen samples incubated with *Candida* isolates were tested for sperm motility, morphology, vitality, hypo-osmotic swelling test (HOS), acrosin assay (A.A), reactive oxygen species (ROS), fructose concentration and pH.

STATISTICAL ANALYSIS:

Statistical analysis was performed using SPSS software version 22. Normal Distribution was tested by Shapiro-Wilk test. Data were analyzed by multivariate regression analysis and ANOVA followed by post hoc comparisons by Tucker's test. Null hypothesis was rejected when *p-value* was less than 0.05. Data were expressed as mean ± standard deviation (SD).

RESULTS:

Twenty-five vaginal swabs were collected from which 18 showed positive cultures. Micro-organisms isolated included Staphylococcus sciuri (27.7%), Staphylococcus hemolyticus (22.2%), *Candida albicans* (22.2%), Esherichia coli (16.6%) and Acromobacter xylosoxidans (11.1%). Only *Candida* was further evaluated for its effect on semen.

At five hours incubation time, semen aliquots co-incubated with *Candida* showed a concentration dependent, statistically significant, progressive decrease in sperm motility, vitality, normal forms, HOS, AA, seminal fructose and pH compared to control. ROS concentration showed minimal change with little rise at 100000 cfu/ml *candida* concentration, however this was non – significant (Table 1).

Increasing incubation time to 15 hours resulted in a concentration-dependent, progressive, statistically significant drop in all semen parameters, except for pH which was non-significant (Table 2).

A highly significant inverse correlation was observed between rising *Candida* concentration and sperm motility, vitality, normality, HOS, AA and fructose concentration (r = -0.676, - 0.507, - 0.540, -0.486, -0.379 and -0.696 respectively, $P < 0.001$). *Candida* concentration showed non-significant inverse correlation with ROS (r = - 0.002, *P 0.989*) and non-significant direct correlation with pH (r= 0.027*, P 0.808*).

On increasing the duration of incubation from 5 to 15 hours significant reduction in sperm motility, vitality, HOS and AA was observed regardless the concentration of *Candida*. The drop detected in fructose and ROS levels was only significant at high *Candida* concentration (100000 cfu/ml). Other semen parameters did not show significant change (Table 3).

Table 1: Changes in semen parameters at 5 hours incubation time with different concentrations of Candida

Variable	0	20000 cfu/ml	60000 cfu/ml	100000 cfu/ml	*P value*
Motility %	48 ± 7.2	44.67 ± 6.1	43.33 ± 8.8	27 ± 11.1	0.007
Vitality %	66.67 ± 5.7	64.33 ± 3.7	63 ± 6.2	52.73 ± 4.8	0.001
Normal forms %	27.67 ± ٦,٤	27 ± 6.9	25.33 ± 7.5	16.27 ± 5.5	*<0.001*
HOS %	58 ± 1.7	55 ± 2	52.33 ± 2.5	45.36 ± 4.4	0.014
AA µIU/ml	27.33 ± 1.1	26 ± 1	25 ± 1	22.6 ± 2.2	0.005
Fructose mg/ml	437 ± 82.0	384.67 ± 47.1	351.6 ± 52.9	164.6 ± 97.6	*<0.001*
ROS	0.783 ± 0.2	0.770 ± 0.1	0.730 ± 0.2	0.878 ± 0.1	NS
pH	8 ± 0.0	7.90 ± 0.1	7.76 ± 0.0	7.70 ± 0.1	0.004

Values are means ± SD. NS = not significant
HOS, Hypo-osmotic swelling test, AA, Acrosomal Assay, ROS, Reactive oxygen species.

Table 2: Changes in semen parameters at 15 hours incubation time with different concentrations of Candida

Variable	0	20000 cfu/ml	60000 cfu/ml	100000 cfu/ml	*P value*
Motility %	30.33 ± 4.619	26 ± 6.5	18 ± 2.6	3.33 ± 6.2	*< 0.001*
Vitality %	47.33 ± 4.619	38.6 ± 8.1	40.33 ± 4.7	21.92 ± 10.7	0.001
Normal forms %	27.67 ± 3.055	25 ± 4.3	19.67 ± 2.8	13.83 ± 5.5	0.001
HOS %	39.33 ± 4.933	34 ± 5.2	32.33 ± 2.5	18.25 ± 10.8	0.005
AA µIU/ml	20 ± 2.645	18 ± 2.6	17.33 ± 1.1	8.875 ± 6.1	0.005
Fructose mg/ml	398.33 ± 44.814	294.33 ± 79.5	250 ± 90.1	65.83 ± 66.5	*< 0.001*
ROS	0.913 ± 0.110	0.846 ± 0.2	0.760 ± 0.1	0.562 ± 0.0	*< 0.001*
pH	8 ± 0.00	5.496 ± 4.0	7.733 ± 0.8	7.63 ± 0.1	NS

Values are means ± SD. NS = not significant
HOS, Hypo-osmotic swelling test, AA, Acrosomal Assay, ROS, Reactive oxygen species.

Table 3: Changes in semen parameters at fixed Candida concentrations and different durations of incubation

	20000 cfu/ml			60000 cfu/ml			100000 cfu/ml		
Variable	5	15	*P value*	5	15	*P value*	5	15	*P value*
Motility%	44.67±6.11	26±6.5	0.04	43.33±8.8	18±2.6	0.012	27.09±11.1	3.33±6.2	*< 0.001*
Vitality%	64.33±3.78	38.6±8.1	0.01	63±6.2	40.33±4.7	0.013	52.73±4.8	21.92±10.7	*< 0.001*
Normal forms%	27±6.9	25±4.3	NS	25.33±7.5	19.67±2.8	NS	16.27±5.5	13.83±5.5	NS
HOS%	55±2	34±5.2	0.003	52.33±2.5	32.33±2.5	<0.001	45.36±4.4	18.25±10.8	*< 0.001*
AA µIU/ml	26±1	18±2.6	0.004	25±1	17.33±1.1	0.004	22.6±2.2	8.875±6.1	*< 0.001*
Fructose mg/ml	384.67±47.1	294.3±79.5	NS	351.6±52.9	250±90.1	NS	164.6±97.6	65.83±66.5	0.018
ROS	0.770±0.19	0.846±0.2	NS	0.730±0.2	0.760±0.1	NS	0.878±0.1	0.562±0.0	0.001
pH	7.90±0.1	5.496±4.0	NS	7.766±0.0	7.733±0.8	NS	7.7±0.1	7.63±0.1	NS

Values are means ± SD. NS = not significant
HOS, Hypo-osmotic swelling test, AA, Acrosomal Assay, ROS, Reactive oxygen species.

DISCUSSION

One of the most common causes of infertility globally is genital infections. Many micro-organisms are involved through various mechanisms [15]. Candida albicans is one of those organisms [10].

The current study showed strong inverse correlation between Candida concentration and sperm motility, vitality and sperm fertilizing capacity (HOS and AA). Concentration and duration-dependent decrease in these parameters was observed. Several studies reported significant decrease in sperm motility under the effect of experimentally induced Candida albicans infection, which was attributed to an increase in sperm agglutination as well as structural abnormalities, mainly tail abnormalities [10,11]. Vander *et al,* reported that intravaginal inoculation of female mice by sperm immobilizing Candida albicans strains rendered them infertile without any observed clinical or pathological changes in their reproductive organs apart from the deleterious effect on semen [6].

In addition, Candida can cause significant reduction in mitochondrial membrane potential (MMP) and membrane phosphatidyl serine (PS) externalization that correlated significantly with sperm motility and viability [11-16]. MMP and PS externalization are considered early signs of apoptosis in somatic cells [17]. Candida may cause loss of acrosomal function when in contact with sperms through the ultrastructural changes induced in the acrosomal membrane that ends in acrosomal rupture [10]. Moreover, Candida may reduce sperm fertilizing capacity without affecting other semen parameters through inducing high DNA fragmentation and increasing apoptosis [18]. One of the virulence factors of the organism is Farnesol (nonsterol isoprenoid). This is a Quorum sensing molecule produced by Candida which has detrimental effects on sperms through inducing premature acrosomal loss and DNA fragmentation even at low concentrations. At high concentrations, it reduces sperm motility and increases apoptosis [19].

In the current study, Candida did not affect sperm morphology to the extent of impairment of male fertility. Despite the significant decrease in the percent of normal spermatozoa, it was still within the acceptable range for normal semen: >4% (WHO 2010) [14]. Studies have reported that sperm ultrastructural lesions induced by Candida mostly impaired motility rather than causing gross sperm abnormalities [10-20].

Fructose is a major nutrient in the seminal fluid on which sperms depend as a source of energy for good motility [21]. We observed concentration-dependent significant drop in fructose levels in tested semen. Candida undergoes metabolic adaptation in different host environments extracting carbon from different sources including fructose. Carbon extracted from fructose is an essential element for Candida virulence and stress resistance [9].

ROS showed non-significant concentration-dependent rise with significant drop observed as the duration of incubation with Candida increased. This could be explained by the fact that virulent Candida can produce oxidative stress scavenging factors in response to environmental stress, as glutathione and trehalose, as a mechanism of stress protection. This is much related to glucose availability in the host microenvironment on which Candida is largely dependent [9]. Thus, at low duration of incubation especially with low Candida concentration, oxidative stress factors might have been produced by concomitant organisms causing rise in ROS level. With increasing duration of incubation and Candida concentration, Candida starts producing stress scavenging factors that results in the observed drop in the levels of ROS.

In the current study, Candida caused minimal changes in seminal pH. This might be attributed to the presence of other microorganisms as Staphylococcus sciuri and Staphylococcus hemolyticus that render vaginal pH alkaline. Although the pH change was within the acceptable range for normal semen, still it is the pH range favored by Candida. It is known that the female genitourinary tract hosts many microorganisms that may synergistically act to create a hostile environment for spermatozoa. Bacterial vaginosis is a common female genital infection characterized by disturbance in the non-pathogenic micro floral environment, resulting in a rise in vaginal pH and predominance of pathogenic anaerobic and facultative organisms which are deleterious to sperms [19-22, 23].

It has been observed that postcoital semen exhibits strong fungicidal activity, attributed to the presence of a potent candidacidal protein that is only active under acidic pH which is the normal vaginal pH. Seminal plasma having neutral or slightly alkaline pH lacks this fungicidal activity. Thus, under conditions where there is mixed vaginal infection with other organism that may change vaginal pH towards the alkaline side, this candidacidal protein becomes inactive and Candida becomes virulent, increasing its transmission rate and causing infertility through its deleterious effect on postcoital semen [24].

CONCLUSION

In conclusion, Candida albicans strains extracted from females suffering from genital candidiasis have negative

impact on normal semen, affecting mainly sperm motility, vitality and sperm fertilizing capacity. Whether these findings may impact couple fertility needs further wider studies.

CONFLICT OF INTEREST

There are no conflicts of interest.

REFERENCES

1. Boivin J, Bunting L, Collins JA, Nygren KG. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. Hum Reprod. 2007;22:1506-12.

2. Mascarenhas MN, Flaxman SR, Boerma T, Vanderpoel S, Stevens GA. National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys. PLoS Med. 2012;9:e1001356.

3. Tabong PT, Adongo PB. Understanding the social meaning of infertility and childbearing: a qualitative study of the perception of childbearing and childlessness in Northern Ghana. PLoS One. 2013;8:e54429.

4. Parsapour R, Majlessi F, Rahimiforoushani A, Sadeghi R. Determination of factors affecting relapse of vaginitis among reproductive-aged women: An experimental study. Electron Physician. 2017;9:3499-507.

5. Karaer A, Boylu M, Avsar AF. Vaginitis in Turkish women: symptoms, epidemiologic - microbiologic association. Eur J Obstet Gynecol Reprod Biol. 2005;121:211-5.

6. Vander H, Prabha V. Evaluation of fertility outcome as a consequence of intravaginal inoculation with sperm-impairing micro-organisms in a mouse model. J Med Microbiol. 2015;64:344-7.

7. Karasz A, Anderson M. The vaginitis monologues: women's experiences of vaginal complaints in a primary care setting. Soc Sci Med. 2003; 56:1013-21.

8. Sutton M, Sternberg M, Koumans EH, McQuillan G, Berman S, Markowitz L. The prevalence of Trichomonas vaginalis infection among reproductive-age women in the United States, 2001-2004. Clin Infect Dis. 2007;45:1319-26.

9. Brown AJ, Brown GD, Netea MG, Gow NA. Metabolism impacts upon Candida immunogenicity and pathogenicity at multiple levels. Trends Microbiol. 2014;22:614-22.

10. Tian YH, Xiong JW, Hu L, Huang DH, Xiong CL. Candida albicans and filtrates interfere with human spermatozoal motility and alter the ultrastructure of spermatozoa: an in vitro study. Int J Androl. 2007;30:421-9.

11. Burrello N, Salmeri M, Perdichizzi A, Bellanca S, Pettinato G, D'Agata R, *et al*. Candida albicans experimental infection: effects on human sperm motility, mitochondrial membrane potential and apoptosis. Reprod Biomed Online. 2009; 18:496-501.

12. Sobel JD. Vulvovaginal candidosis. Lancet. 2007; 369:1961-71.

13. Cincik M, Ergur AR, Tutuncu L, Muhcu M, Kilic M, Balaban B, *et al*. Combination of hypoosmotic swelling/eosin Y test for sperm membrane integrity evaluation: correlations with other sperm parameters to predict ICSI cycles. Arch Androl. 2007;53:25-8.

14. Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HW, Behre HM *et al*. World Health Organization reference values for human semen characteristics. Hum Reprod Update. 2010;16:231-45.

15. Pellati D, Mylonakis I, Bertoloni G, Fiore C, Andrisani A, Ambrosini G, *et al*. Genital tract infections and infertility. Eur J Obstet Gynecol Reprod Biol. 2008;140:3-11.

16. Auger J, Leonce S, Jouannet P, Ronot X. Flow cytometric sorting of living, highly motile human spermatozoa based on evaluation of their mitochondrial activity. J Histochem Cytochem. 1993;41:1247-51.

17. Kroemer G, Zamzami N, Susin SA. Mitochondrial control of apoptosis. Immunol Today. 1997; 18:44-51.

18. Burrello N, Calogero AE, Perdichizzi A, Salmeri M, D'Agata R, Vicari E. Inhibition of oocyte fertilization by assisted reproductive techniques and increased sperm DNA fragmentation in the presence of Candida albicans: a case report. Reprod Biomed Online. 2004;8:569-73.

19. Rennemeier C, Frambach T, Hennicke F, Dietl J, Staib P. Microbial quorum-sensing molecules induce acrosome loss and cell death in human spermatozoa. Infect Immun. 2009;77:4990-7.

20. Tian YH, Liu J, Xu HM, Hu L, Xiong CL. [Influence of Candida albicans on the motility and ultrastructure of human spermatozoa in vitro]. Zhonghua Nan Ke Xue. 2005;11:179-84.

21. Al-Daghistani HI, Hamad AW, Abdel-Dayem M, Al-Swaifi M, Abu Zaid M. Evaluation of Serum Testosterone, Progesterone, Seminal Antisperm Antibody, and Fructose Levels among Jordanian Males with a History of Infertility. Biochem Res Int. 2010;2010:409640.

22. Donders GG, Caeyers T, Tydhof P, Riphagen I, van den Bosch T, Bellen G. Comparison of two types of dipsticks to measure vaginal pH in clinical practice. Eur J Obstet Gynecol Reprod Biol. 2007;134:220-4.

23. Krauss-Silva L, Almada-Horta A, Alves MB, Camacho KG, Moreira ME, Braga A. Basic vaginal pH, bacterial vaginosis and aerobic vaginitis: prevalence in early pregnancy and risk of spontaneous preterm delivery, a prospective study in a low socioeconomic and multiethnic South American population. BMC Pregnancy Childbirth. 2014;14:107.

24. Edstrom Hagerwall AM, Rydengard V, Fernlund P, Morgelin M, Baumgarten M, Cole AM, *et al*. beta-Microseminoprotein endows post coital seminal plasma with potent candidacidal activity by a calcium- and pH-dependent mechanism. PLoS Pathog. 2012;8:e1002625.