Sperm Agglutinating Activity of *Saccharomyces cerevisiae* and *Candida albicans* as a Potential Causative Factor of Infertility in Mice (*Mus musculus*)

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RESEARCH ARTICLE

Abstract

Background and Objective: Vaginal yeast infections in women are usually caused by *Candida albicans* and, to a lesser extent, by *Saccharomyces cerevisiae*. Studies on *C. albicans* have shown that it can cause sperm agglutination which can lead to lowered fertility. This study was conducted to compare the effect of *S. cerevisiae* and *C. albicans* on the fertility of ICR mouse (*Mus musculus*) through sperm agglutination.
Methodology: Sperm agglutinating activity was examined by mixing different concentrations of *S. cerevisiae* (10^4, 10^6, and 10^8 CFU/mL) and *C. albicans* (10^4, 10^6, and 10^8 CFU/mL) separately with semen from male mice of ICR strain. Determination of the effect of *S. cerevisiae* and *C. albicans* on the fertility outcome of female mice was done by intravaginal inoculation of 20 µL of 10^4, 10^6, and 10^8 CFU/ml of the two yeast organisms and later allowed to mate.
Results and Conclusion: The study showed a statistically significantly higher percent sperm agglutination by *S. cerevisiae* than *C. albicans* at 10^4 CFU/ml but no difference was observed at 10^6 and 10^8 CFU/ml. No significant difference was observed in the number of sperm per agglutinate between the two yeast species at α=0.05. The concentration that exhibited the highest percentage of agglutinated sperm is 10^6 CFU/mL for both yeast. The most frequent type of agglutination observed in *S. cerevisiae* is the mixed type, while head-to-head type is most frequent in *C. albicans*. Both yeasts were able to cause a decline in the number of births in mice starting at 10^4 CFU/ml. While sperm agglutination could be one of the reasons for the infertility observed in mice, there may be other processes, mechanisms, and/or activities that could contribute to such an outcome.

Keywords: *sperm agglutination, sperm analysis, Candida albicans, Saccharomyces cerevisiae, mouse infertility*

Introduction

One of the most common female genital conditions is vaginitis or vaginal yeast infection where nearly 75% of adult women experience it at least once in their lifetime. *Candida albicans* is the most frequent cause of vaginitis which accounts for 80% to 90% of yeast infection incidences [1,2]. Also, *C. albicans* has also been reported to lower fertility in mice by causing sperm agglutination [3,4,5].

Sperm agglutination is the aggregation or clumping of sperm cells [6]. It is caused by the reaction and subsequent attachment of the antibodies produced by the immune system of the organism to the sperm cells forcing them to aggregate. The coat of antibodies hinders the movement of sperm and covers the receptors on its surface involved in fertilization of the egg cell [7]. In addition to contributing to decreased viability and motility of the sperm, antibodies can also interfere with zona binding and acrosome reaction which are prerequisites for fertilization [8].

Sperm agglutination is determined through semen analysis wherein the sperm cells and their quality are evaluated. The parameters which are used to describe the extent of sperm agglutination are the number of agglutinates, type of agglutination and grade of agglutination. Sperm motility and the presence of morphological abnormalities, which are possible consequences of sperm-agglutination, can also be used to further illustrate sperm agglutination activity of an organism [8].

Some microorganisms which can cause sperm agglutination are *Staphylococcus aureus* and *Escherichia coli* [9,10], *Streptococcus viridans*, *Haemolyticus*, *Micrococcus*, *Staphylococcus albus*, *enterococci* and *diptheroids* [11], *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Mycoplasma*, *Candida albicans*, *Myxoviruses* and *Gonococcus* [12]

Another organism which can cause vaginal yeast infection is *Saccharomyces cerevisiae* but is considered a rare occurrence. Epidemiological studies presented that the prevalence of vaginal yeast infection due to *S. cerevisiae* is only up to 1% [13,14]. However, in the study of vaginal yeast infection in Italian women caused by *S. cerevisiae,* the prevalence was reported at 5.5% [15]. In another study, it was reported that 5.8% of women referred to the Microbiological Service at the University in Rome were positive for *S. cerevisiae* vaginal infection [16]. Also, vaginitis due to *S. cerevisiae* in Greek women had an incidence of 6.1% [17]. At present, no studies have been done to determine the effect of *S. cerevisiae* to fertility. This study compared the sperm agglutination property of *S. cerevisiae* and *C. albicans* and their consequential effect on fertility of mice. With the increasing prevalence of vaginitis caused by *S. cerevisiae,* the results of this study may have potential implications on the fertility of a human female with this ongoing infection.

Methodology

Sources of Experimental Organisms

A total of 28 male mice and 21 female mice of the ICR strain of the same genetic stock which were obtained from the Food and Drug Administration (FDA) were used in the study. The culture of *S. cerevisiae* and *C. albicans* ATCC-10453 were obtained from the Department of Medical Microbiology of the College of Public Health, University of the Philippines, Manila.

Preparation of Yeast Inoculum

A viable cell density versus optical density chart was prepared for both yeast cultures grown in Sabouraud Dextrose Broth (SDB) incubated overnight at room temperature. Subsequent yeast culture population densities were estimated using this chart and by using a spectrophotometer (Genesys 20) at 595 nm to obtain a concentration of 10^8 CFU/ml. The yeast cultures were then centrifuged at 5,000 g for 10 minutes and the pelleted yeasts were resuspended in phosphate buffer saline (PBS) to the following concentrations 10^4, 10^6, and 10^8 CFU/ml. These suspensions were used to determine the ability to induce sperm agglutination and infertility on mouse.

Extraction of Mice Sperm

Prior to dissection, the mouse was injected with 5 mg/kg Zoletil® intraperitoneally. In order to obtain the caudal epididymis, a small incision was made on the scrotum. The caudal epididymis was incised together with the fat pad and kept moist in a PBS solution [18]. The contents of several caudal epididymis were aspirated with an insulin syringe and were pooled together. Several set-ups with a final mixture of 0.1 ml aspirate to 0.1 ml PBS were prepared and slowly mixed together.

Determination of Sperm Agglutination by Yeast

A 0.1 ml volume of different yeast concentrations (10^4, 10^6, and 10^8 CFU/mL) were mixed with the sperm aspirate in PBS. The mixture was placed onto a hemocytometer and observed under a light microscope after 30 minutes have lapsed for reactions to occur. The movement and the type of agglutination were observed and graded according to the sperm agglutination standards from the World Health Organization Laboratory Manual for the Examination of Human Sperm [8].

Effect of Yeast on Mice Fertility

Three groups, each consisting of three female mice were inoculated intravaginally with 20 µL containing 10^4, 10^6, and 10^8 CFU/ml of *S. cerevisiae* in PBS using a sterile micropipette, respectively. The same set-up was also prepared for *C. albicans*. A single group with three female mice was inoculated with 20 µL sterile PBS as negative control [19]. After 24 hours, vaginal swabbing was performed daily to determine the presence of the inoculated yeast as proof of vaginitis.

The inoculated female mice were allowed to mate with the male mice during their oestrus at a ratio of one male mouse to one female mouse. The presence of a vaginal plug signified successful mating of the pair. After coitus, female mice were placed in a separate cage to allow for gestation. The number of offsprings by the inoculated female mice was recorded.

Data Presentation and Statistical Analysis

The data were summarized as tables with frequencies and percentage equivalents, and were presented as tables and bar graphs. Two-way Analysis of Variance (ANOVA) and Tukey's test as post hoc test at alpha = 5% was conducted to determine significant differences due to yeast species, concentration, and interaction effect. The type of agglutination (head-to-head, tail-to-tail, and mixed) and grade of agglutination were analysed using Mann-Whitney and Kruskal-Wallis tests. For the effect of yeast on the fertility of mice, the results were summarized as frequencies and percentage equivalents.

Results

Sperm agglutination was observed with the addition of yeast cells of *S. cerevisiae* and *C. albicans* to the sperm sample (Figure1). However, this was observed to be significantly higher in *S. cerevisiae* than in *C. albicans* at a concentration of 10^4 CFU/ml but not at 10^6 and 10^8 CFU/ml. There was no significant difference in the percent agglutination due to the different concentrations of *C. albicans*. In contrast, a significant difference was observed in terms of different concentrations of *S. cerevisiae* with 10^6 CFU/ml causing the highest percent agglutination. No interaction effect observed.

Table 1. *Number and percentage of agglutinated sperm in different concentrations (n=6)*

Concentration	*Saccharomyces cerevisiae*		*Candida albicans*		PBS	
	Number of Agglutinated Sperm	Percentage (%)	Number of Agglutinated Sperm	Percentage (%)	Number of Agglutinated Sperm	Percentage (%)
10^4	324	40.89^A	51	23.54	0	0
10^6	225	60.61^B	558	42.79		
10^8	386	27.76^A	1085	30.88		

note: A and B refer to statistical significant grouping of concentration

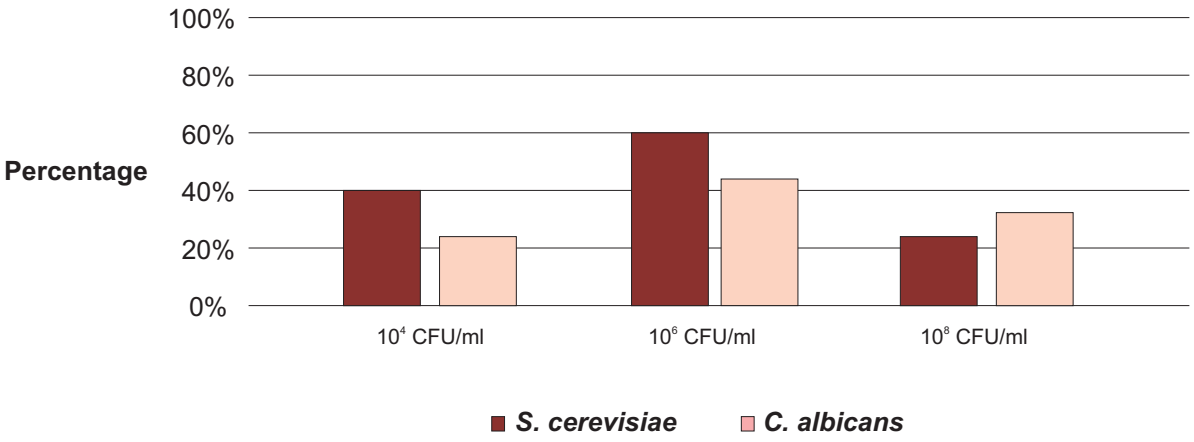

Figure 1. *Comparison of percent sperm agglutination of S. cerevisiae and C. albicans at different concentrations*

No significant difference was seen in the average sperm per agglutinate between *S. cerevisiae* and *C. albicans*. A significantly higher average sperm per agglutinate and grade was observed for both yeasts at a concentration of 10^8 cfu/ml. No variable interaction effect was observed between yeast species and concentration.

For *S. cerevisiae*, it was observed that there were more sperm agglutination of the mixed type compared to head-to-head or tail-to-tail. While the general type of sperm agglutination observed for *C. albicans* was that of the head-to-head. The different concentration of *S. cerevisiae* was able to show a significant difference only in the tail-to-tail category. The different concentrations of *C. albicans* showed a significant difference in all three categories of types of agglutination.

Table 5 shows a lower number of successful births in all concentrations of *S. cerevisiae* and *C. albicans* compared to the negative control (PBS). While no trend can be observed to describe the effect of concentration on mice fertility, it can be said that there is an observable reduction in fertility starting at a concentration of 10^4 CFU/ml for both yeasts.

Table 2. *Average sperm per agglutinate and its corresponding grade in different concentrations (n=6)*

Concentration	*Saccharomyces cerevisiae*		*Candida albicans*	
	Average Sperm per Agglutinate	Grade	Average Sperm per Agglutinate	Grade
10^4	7.13^A	1	2.48^A	1
10^6	6.78^A	1	6.64^A	1
10^8	14.86^B	2	17.80^B	2

Note: A and B refer to statistical significant grouping of concentration

Table 3. *Frequency and percentage types of agglutination in the different concentrations of Saccharomyces cerevisiae*

Concentration	Head to head		Tail to tail		Mixed		Total Number of Agglutinates
	Number of Agglutinates	Percentage (%)	Number of Agglutinates	Percentage (%)	Number of Agglutinates	Percentage (%)	
10^4	6	15.62	5	$12.07^{A,B}$	32	72.32	43
10^6	7	13.01	12	21.43^A	38	65.56	57
10^8	4	12.4	1	4.85^B	23	82.75	28

note: A and B refer to statistical significant grouping of concentration

Table 4. *Frequency and percentage types of agglutination in the different concentrations of Candida albicans*

Concentration	Head-to-head		Tail-to-tail		Mixed		Total Number of Agglutinates
	Number of Agglutinates	Percentage (%)	Number of Agglutinates	Percentage (%)	Number of Agglutinates	Percentage (%)	
10^4	14	73.44^A	2	$8.37^{P,Q}$	4	18.19^X	20
10^6	33	37.24^B	17	18.24^P	38	44.53^Y	88
10^8	40	54.14^A	8	8.53^Q	22	37.34^Y	70

Note: A, B, P, Q, X, and Y refer to statistical significant grouping of concentration

Table 5. *Effect on intravaginal inoculation on Saccharomyces cerevisiae, Candida albicans and PBS on fertility outcome of mice*

Concentration of Microorganism (CFU/20 mL)	Number of female mice with successful births					
	S. cerevisiae	%	*C. albicans*	%	PBS	%
10^4	1/3	33	1/3	33	3/3	100
10^6	0/3	0	1/3	33		
10^8	1/3	33	0/3	0		

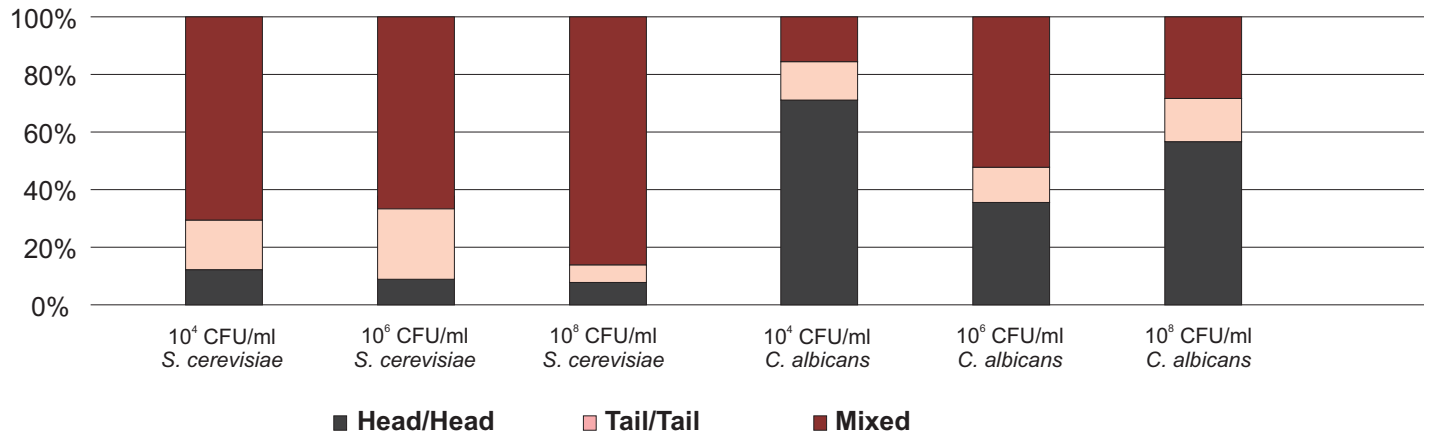

Figure 2. *Comparison of breakdown of type of sperm agglutination at different concentrations by S. cerevisiae and C. albicans*

Discussion

The large range difference in the number of agglutinated sperm in the results of Table 1 is an indication of variation in sperm sample collection and/or poor homogenization of the pooled semen from the mice. Hence, interpretation is best done by analysing the percentage of sperm agglutination instead. The observed percentage of sperm agglutination of *C. albicans* is similar to the results of past studies with an increasing trend up to a concentration of 10^6 CFU/ml as inoculum [3,4,5]. However, at 10^8 CFU/ml there was an observed decrease in this value for both yeasts. While having a higher yeast density involves more sperms per agglutinate as seen in Table 2, there are also more yeast cells involved per aggregate that somehow limits the overall number of sperm to bind and cause agglutination. It appears that a concentration of 10^6 CFU/ml is the ideal concentration to observe optimal sperm agglutination activity.

While both *S. cerevisiae* and *C. albicans* have comparable values of percentage in sperm agglutination activity, they differ in the type of sperm agglutination. The explanation for this is dependent on the surface receptors, adhesion, and/or proteins expressed by the microorganisms. It is known that head-to-head agglutinating antibodies react more strongly with higher molecular weight antigens, whereas tail-to-tail agglutination is more evident in lower molecular weight antigens [21]. In the case of *E. coli* surface adhesion, the presence of P fimbriae is associated with tail-to-tail sperm agglutination. On the other hand, the type I fimbriae is associated with head-to-head sperm agglutination and the presence of both fimbriae presented a mixed reaction. [22]. Also, the sperm heads carry a higher positive charge than the sperm tails [23]. A study to identify the different protein receptors and or surface adhesions associated with sperm agglutination present on the two yeast species is highly recommended. This study validated the previous studies on *C. albicans* as linked with head agglutination of sperm cells [5]. A research into identifying the reactive surface protein of *S. cerevisiae* that is associated with sperm agglutination may explain why majority of sperm binding is of the mixed type.

While there was an observed reduction in mice fertility, these values were not positively correlated and did not peak at 100% infertility as previously reported [24]. In the case of *C. albicans*, infertility in humans was suggested to be caused by decreased sperm motility as affected by the presence of sperm agglutination. Other possible reasons for infertility included multiple ultra-structural lesions on the sperms head [5], reduced mitochondrial membrane potential, and promotion of apoptosis [25]. For this experiment, it can be suggested that *S. cerevisiae* was able to cause infertility possibly due to sperm agglutination since it was observed to be present. However, there may be other factors, activity, and/or mechanisms possibly similar to that of *C. albicans* that caused the mice infertility.

As for the absence of a trend in the mice infertility due to yeast concentration, there is the likely presence of potential confounding factors in this experiment, such as individual genetic differences in the mice, difference in the level of immunological responses to yeast infection, varying degrees of grooming behaviour, and difference in the breeding condition. It is recommended that an experiment involving a larger number of female mice can be conducted to determine the relationship between concentration and percent infertility.

Conclusion

Both *S. cerevisiae* and *C. albicans* were able to induce sperm agglutination in mice. Also, *S. cerevisiae* showed a higher percentage of agglutination compared to *C. albicans* at 10^4 CFU/ml. No significant difference was observed between the two yeasts in terms of average sperm cells per agglutinate and reduction in the fertility of mice. The cells of *S. cerevisiae* produced more mixed type sperm agglutination while *C. albicans* produced more of the head to head type. Both yeasts were able to induce mice infertility starting at 10^4 CFU/ml. It is concluded that *S. cerevisiae* can induce mice infertility possibly by sperm agglutination but it may be just one of the many possible mechanisms that contribute to the causation of reduced fertility.

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