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ORIGINAL ARTICLE

The Effect of Intrauterine Candida Colonization on Pregnancy Outcome Following Intra-cytoplasmic Sperm Injection (ICSI)

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ABSTRACT

Background: *Infertility is caused by numerous anatomical and hormonal factors; however, the role of ascending colonization from vaginal microbiota in infertility is not properly addressed. C.albicans, the most common yeast retrieved from vagina is associated with 61.53% cases of infertility while C.species is associated with 38.46% [1]. Intrauterine Candida colonization and its effect on ICSI are still under study and need to be explored further.* ***Objective:*** *This work aims is to detect the effect of Candida colonization of the uterus in patients undergoing ICSI cycle and clinical pregnancy rate.* ***Methodology****: Menstrual blood flow sample at day two induction cycles is subjected to culture and identification of growing organisms. Growth and identification was correlated to Success of ICSI. Predetermined criteria were set up for selection of patients.* ***Results****: 69.2% of females with positive growth had failed the ICSI trial with p value 0.04, while 94.4% of patients with no culture growth had successful ICSI with p value 0.0001. Samples with growth of mixed Candida species with bacterial isolates either gram positive and /or gram negative showed 100% failed trials. Samples showed pure growth of Candida albicans or Candida spp. had positive ICSI with p value <0.001.* ***Conclusion****: Pure Candida colonization does not seem to affect ICSI outcome; in contrary, mixed Candida with gram positive and/or negative colonization affect pregnancy rate negatively. This could be due to microbial interactions and increasing the ability of Candida to turn into the pathogenic state when mixed with other microorganisms. Screening of females and administration of antifungals and/or antibiotics before ICSI is recommended.*

INTRODUCTION

Infertility is described as a couple's failure to conceive after one or two years of frequent unprotected heterosexual intercourse [2]. It affects between 60 and 80 million couples at reproductive age worldwide [3]. For nulliparous women, infertility is classified as "primary," whereas for parous women, it is classified as "secondary"[4]. 56% of infertile couples seek medical assistance to conceive[5]. The success rates of assisted reproductive technologies remain rather stable, with around 25% of live births each cycle happening until the age of 35, before rapidly declining[6]. ICSI, a kind of assisted reproductive technology (ART), has become the most often utilized technique of fertilization[7]. Changes in the microbiota of the female vaginal system may result in infertility. The impact of pathogenic bacteria such as *MRSA* and *E.coli* on fertility may potentially be connected to variations in *lactobacilli* levels[8]. *Lactobacilli* predominate in women of reproductive age's natural vaginal microbiome. Unbalanced microbiota in the vaginal tract, such as reduced *lactobacilli* and/or increased fungal or bacterial populations, might impair fertility and result in ascending colonization and /or infections [9]. *Candida* is a well-known colonizer and opportunistic vaginal pathogen [10]. This yeast is found in around 10%–20% of asymptomatic women of reproductive age [11]. While *Candida* may coexist with the host, it can develop a pathogenic condition when combined with other bacteria [12]. Alterations to the vaginal ecology may result in a shift in the balance of the many populations that comprise the flora, as well as the dominance of the infective *Candida* population, which results in vulvovaginal candidiasis (VVC). *Candida* infection is the second most prevalent cause of vaginal infections (between 20% and 25%)[13]. Around 90% of *Candida* vaginal strains are *Candida albicans*, however other *Candida* species such as *Candida tropicalis*, *Candida krusei*, and *Candida parapsilosis* may also cause illness[14]. The overgrowth of *Candida* spp. and the depletion of *Lactobacillus* species may lead to a

microbial imbalance that can precipitate in an ascending genital colonization and/or infection [15].This study was done to detect the effect of *candida* colonization on success or failure of ICSI and the pregnancy outcome.

METHODOLOGY

Study design:

A prospective study was conducted on all women underwent intra-cytoplasmic sperm injection (ICSI) in the In vitro fertilization (IVF) Unit in kasr Al-Ainy Hospital between November 2018 and October 2019.All Infertile females less than 35 years old with no previous trials and no chronic diseases were included; provided that, their partner have normal semen analysis. Females were subjected to general examination including vital signs, abdominal and vaginal examination, blood sampling for FSH, LH, E2, prolactin, and TSH measurements and Ultrasonography as a basal scan.

Sample size:

Sample size was estimated as a convenient period sample. All patients who were seeking Kasr Alainy Teaching Hospital with infertility were investigated. 60 females were recruited who met the predetermined criteria and underwent ICSI with long protocol induction. The study was approved by the ethics committee on research involving human subjects of Kasr Al-Ainy Hospital. A consent was obtained from each case. Menstrual blood flow sample at day 2 of cycle of induction was withdrawn and cultured. Based on culture they were divided into 3 groups: *Candida* positive cultures group (n=13), no growth group (n=18) and other pathogenic microorganisms group (excluded) (n=29). *Candida* positive culture group was further divided to 2 groups; pure *Candida* culture (n=4), *Candida* culture mixed with other organisms (n=9) and compared to the no growth group.

Sampling technique:

Menstrual blood sampling was withdrawn from all infertile female patients meeting the inclusion and exclusion criteria and had long protocol induction. Menstrual flow samples were collected on day 2 of the induction cycle using a 12-sized plastic catheter attached to a 10-ml plastic syringe. The plastic syringe was introduced transcervical into the uterine cavity, and intrauterine aspiration was conducted under negative pressure created by a syringe pump's manual suction [16].

Collection of samples, culture and isolation of microorganisms:

Menstrual blood samples were collected from all women undergoing ICSI using intrauterine sterile catheter (IUI). Sample collection occurred following aseptic precautions and inserted in BD BACTEC [17] blood culture bottles immediately. Menstrual blood samples were sub-cultured after BACTEC alarm on a panel of culture media including Blood agar, Chocolate agar, MacConkey agar, and Sabouraud dextrose agar for microbial isolation and identification. *Candida* colonies isolated on Sabouraud dextrose agar were further identified to *Candida albicans* or *candida spp.* by germ tube test where *Candida* colonies incubated in human serum (> 0.5 ml) and mixed well by rubbing, incubated in 37 for 3 hours and then examined under the microscope for filamentous outgrowth extending from yeast cells. It is positive for *Candida albicans* and *Candida dubliniensis* and negative for other species[18]. CHROMagar [19] medium was used for further differentiation between *Candida spp.* according to the manufacture (Oxoid England, Ref CM100)[20] with distinct color due to the change in chromogenic substrates existing in the medium.

Identification and antibiotic susceptibility of bacterial isolates mixed with candida:

Bacterial isolates mixed with candida were isolated and identified by colonies' color, morphology, microscopic examination with Gram staining, Biochemical tests such as; Catalase, Oxidase, Coagulase, Triple sugar iron agar (TSI), Motility Indol ornithine Medium test (MIO), Lysine–iron agar, Urease and Citrate test. Antibiotic Sensitivity test was done for identified colonies following CLSI 2021 guidelines [21].

Statistical analysis:

All obtained data were revised to ensure their accuracy and logical coherence. Pre-coded data was entered on the computer using the Microsoft Office Excel Software Program 2019. After transferring and entering pre-coded data into the Statistical Package of Social Science Software, version 26 (SPSS) for statistical analysis for quantitative variables, mean and standard deviation were calculated and compared using the independent t test, with a p value of less than 0.05 considered significant

RESULTS

Descriptive statistics of female patients: 100% were less than 35 years with no intra cytoplasmic sperm injection (ICSI) trials before. All females had normal hormonal profile, normal general and vaginal examination. All their male partners had normal semen analysis.

On classifying the microbial growth in menstrual blood samples obtained from patients prepared for ICSI, the following was detected as shown in figure 1; 22% (n=13) showed growth of *candida* either pure or mixed with other microorganisms, 30% (n=18) of samples were no growth and 48 % (n=29) showed growth of other pathogenic microorganisms. After excluding the other pathogenic microorganisms group, 31 females were included in the study and were divided into 3 groups: pure *candida* growth (n=4), *candida* growth mixed with other organisms (n=9) and no growth group (n=18).

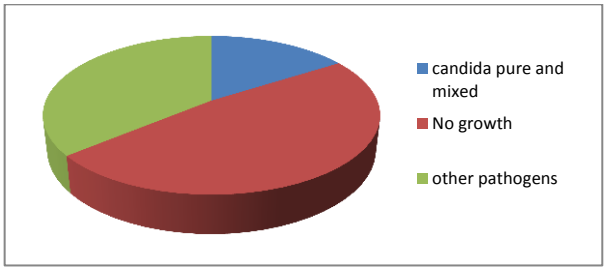

Fig. 1: Classification of intrauterine microbial growth

Identification of candida isolates by germ tube and chromagar is shown in (table 1). Germ tube detected that 77% of *Candida* isolates were *Candida albicans* and 23% were other *Candida* spp. Further identification of *candida spp.* by Chromagar showed that all species were *Candida tropicalis*.

Table 1: Identification of *Candida* by Germ tube and Chromagar

Candida type	Cases number	Percentage
Candida albicans	10	77%
Candida tropicalis	3	23%

Further identification of bacterial isolates in the group of *candida* mixed with other organisms (n=9) and their resistant pattern is shown in (table 2). 45% of isolated organisms mixed with *candida* was *E.coli* and all harbour ESBL pattern of resistance , 23% was *Klebsiella* species with all showed ESBL resistance pattern, 23% *Pseudomonus aerogenousa* and all showed Ampc resistance and 11% *MRSA*.

Table 2: Identification of isolates mixed with *Candida* and their resistant pattern

Isolated organism mixed with Candida	Percent (n=9)	Pattern of resistance
E.coli	45%	ESBL
Pseudomonus aerogenousa	23%	Ampc
Klebsiella pneumoniae	23%	ESBL
Methicillin resistant staph aureus	11%	MRSA

The relationship between growth of microorganisms and pregnancy rate is shown in the (table 3). There is a significant relationship between *Candida* colonization and failure of trials. 69.2% of females with positive growth had failed trial with P value= 0.04. In contrast 94.4% of patients with no growth in their culture had successful trials with P value= 0.0001. This indicates the strong inverse relation between growth of microorganisms and pregnancy rate.

Table 3: Relationship between growth of microorganisms and pregnancy rate

	Clinical pregnancy rate				P value	Sig.
	Positive		negative			
No growth	17	94.4%	1	5.6%	0.0001	HS
Candida spp.	4	30.7%	9	69.2%	0.04	S

Chi_2 test, P value is considered (S) significant <0.05, (HS) highly significant P value <0.01.

Further analysis of relationship between purity of *Candida* growth and pregnancy rate is shown in (table 4); i.e. pure *Candida* growth or *Candida spp.* mixed with other microorganisms. This analysis identified a highly significant relationship where *Candida* mixed with bacterial infection either gram positive and /or gram negative show 100% negative outcome and failed trials. On the other hand, trials in patients with pure growth of *Candida albicans* or other *Candida* species have positive pregnancy trial with P value <0.001. This gives a clue about increasing the pathogenicity of *candida* when mixed with other microorganisms and the synergistic bacterial fungal interactions.

Table 4: Relationship between purity of Candida isolates and pregnancy rate

	Negative		Positive		P value	Sig.
	No.	%	No.	%		
Mixed Candida and bacteria (Gram-positive or – negative)	9	100.0%	0	0.0%	0.0001	HS
Candida albicans or Candida tropicalis	0	0.0%	4	100.0%		

Chi_2 test, P value is considered (S) significant <0.05, (HS) highly significant P value <0.01.

DISCUSSION

Infertility is a condition affecting male or female reproductive system that is characterized by the inability to conceive after 12 months or more of frequent unprotected sexual contact [22].Causes of infertility include female and male factors which could be divided into; tubal factors (7.7%), endometriosis (4.2%), ovulatory dysfunction (6.8%), reduced ovarian reserve (11.5%), uterine factors (1.4%), male factor (18.8%), unexplained infertility (13.5%) and other causes (7.7%)[23]. *Candida albicans* is a yeast that colonizes the

oral cavity, gastro-intestinal tract and vagina in most humans [21]. It is however also an opportunistic pathogen, able to cause infections, especially if co-exists with bacteria, including *Staphylococcus* species and enterobacteriacea [22].

To the best of our knowledge, this is the first study in Egypt to investigate the effect of candida colonization on ICSI success; in addition, a limited number of international literatures were found addressing this topic. In this research, other causes of infertility were excluded. Infertile females less than 35 years old with no previous trials and no chronic diseases were included; provided that, having normal general examination, no symptoms or signs of genital infection, normal hormonal profile and their partner have normal semen analysis.

From the 60 patients recruited, culture of 22% (n=13) showed *Candida* growth, 30% (n=18) with no growth and 48% (n=29) showed growth of other pathogenic microorganisms and this group was excluded. The group of *Candida* growth was further divided into 31% (n=4) with pure growth of *Candida*, and 69% (n=9) *Candida* mixed with gram negative or positive bacteria. Seventy seven (77%) of isolated *Candida* was *Candida albicans* while only 23% were *Candida* species which were all *Candida tropicalis* and this is in agreement with previous studies which detected that *Candida albicans* was the predominant organism 61.53% recovered from vaginal swabs of infertile females while other *Candida* species were 18%[1].

94.4% of females with no growth cultures had positive pregnancy outcome while 69.2% of females with microbial growth failed the trial. This is in agreement with Lim et al.[24] who verified that candidiasis, whether clinical or subclinical, has a detrimental effect on implantation and pregnancy rates. Additionally, according to the research, the implantation rate was considerably greater in individuals treated with clotrimazole one week before to embryo transfer (67.0%) than in those who were not treated with clotrimazole (53.1%), with a P value of 0.05.

Another study confirmed the same findings and detected that 63% of cases with vaginal *Candida* species developed chorioamniotnitis following IVF and this was explained by the potential introduction of the yeast into the uterus at the time of embryo transfer causing infection not colonization. It has subsequently been suggested that a high vaginal swab should be performed and antifungal treatment should be initiated in women with positive culture results prior to ICSI [25].

Further analysis detected that 100% of cases of pure candida growth got pregnant while all cases of mixed *Candida* with bacterial colonizers either gram positive or gram negative organisms failed to get pregnant which enforces the hypothesis of fungal bacterial synergism. This is in agreement with Pammi et al. [26] who discovered that attachment of bacteria to *Candida albicans* results in the formation of a more widespread biofilm with 30-fold greater adhesion rates and pathogenicity than the yeast form alone. This might explain why mixed fungal bacterial colonizers have a greater likelihood of trial failure. Our results are also consistent with previous reports which revealed that bacterial colonization is highly associated with recurrent implantation failure [27].

The pregnancy rates in our study do not seem to be affected by pure *Candida* growth; however, inoculating *Candida* during ICSI could cause infection and detrimental consequence results in negative outcome as shown by previous research [25]. Pregnancy rates are much affected by fungal-bacterial co-colonization. This could be explained by two mechanisms: first, inoculation of endocervical microorganisms into the uterine cavity during sperm injection, thus altering the ultrastructural characteristics of the endometrium. Secondly, the high concentration of microorganisms in the uterus causes poor uterine receptivity.

CONCLUSION

Screening is highly recommended before ICSI to detect colonization and prevent infection. Prophylactic antifungal and or antimicrobials based on antimicrobial susceptibility testing should be included in the ICSI protocol to elevate the success rate.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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