Relationship between the Recovery of Aerobic Bacteria from Semen and Male Infertility in Jordan

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Abstract

Background: The role of bacteria detected in semen, on male's fertility is still a matter of debate.

Objective: This paper provides a comparative study on the significance of bacterial presence in semen derived from fertile and non-fertile Jordanian males.

Method: Semen specimens included in this study were collected from 80 males with fertility problems and from 40 volunteers with known fertility. All specimens were investigated for total microbial count, recovery of aerobic bacteria, sperm count, sperm motility and morphology using standard techniques. Out of 80 semen specimens collected from males with infertility problems, 45 (56.25%) of specimens harbored bacteria in counts $>10^2$ CFU / ml, and in 75.55% of these specimens bacteria were recovered in counts exceeding 10^4 CFU / ml. Common bacterial isolates were coagulase negative *Staphylococci* (31.11%), *Enterococcus faecalis* (24.44%), *Micrococcus* species (15.55%) and coagulase positive *Staphylococci* (13.33%). The least isolated aerobic bacteria belonged to the family Enterobacteriaceae.

Results: By comparing results of semen specimens derived from fertile and infertile individuals, it has been found that the total bacterial count was significantly higher in the semen collected from males with infertility problems. The infertile group also harbored *Ent. faecalis* and coagulase positive *Staphylococci* in a higher proportion of specimens. Certain types of bacteria when isolated from semen can provide an indication regarding the fertility potentials of males. Other isolates are most probably contaminants with no clinical impact on fertility.

Conclusion: Total bacterial count should become a routine practice whenever semen culture is performed.

Keywords: Semen, Sperm Quality, Aerobic Bacteria, Diagnostic Significance, Fertility.

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Introduction

Infertility may occur in 10 to 20% of couples of childbearing age. [1] Male contribution to infertility may vary in different communities and in Jordan, 31% of infertility cases are attributed to male factors. [2] The cornerstone for the evaluation of male fertility potential is semen analysis. This test can provide valuable information regarding several parameters

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including sperm count, sperm motility and sperm morphology. [3, 4]

Semen is composed of secretions derived from prostate, Bulbourethral gland (Cowpers), seminal vesicles and spermatozoa from the vas deference and distal epididymis. [5] Thus, the isolation of any microorganism from semen may indicate the presence of infection in any of the anatomical entities that secrete the various fractions of the ejaculate. [6] Genitourinary infection and varicocele induced an inflammatory effect which could play a detrimental role in spermatogenesis, revealed by a decrease in sperm motility and the fertility index, concomitant with an increase in immaturity mainly in varicocele and necrosis in infection [7] Weidner et al. (1999) [8] indicated that the impact of these sperm alterations on male fertility remains in many cases unclear.

The relationship between various semen characters and the recovery of bacteria from semen is still a matter of debate. Some investigations have suggested that bacterial isolation from semen may suggest defective sperm quality, with negative consequences on fertility. [9, 10] Others claimed that, the recovery of bacteria from semen did not affect sperm count, motility or morphology. [11, 12] Men with genital tract infections have a high incidence of antibodies, reactive with spermatozoa, which is associated with reduced fertility. [13] Ligospermia and azoospermia are the common causes of male factor infertility which has been attributed to bacterial infections. [14] Although, these contradictory conclusions have led to uncertainty regarding the role of bacteria in semen on the fertility potentials of males, it is important to keep in mind that bacteria isolated from semen were not always similar. For instance, in one investigation, *Staphylococcus* spp constituted 68% of the isolates, [15] while in another study *Ent. faecalis* was the dominant isolate and *Staphylococcus* spp. were not recovered. [16]

The presence of *Ent. faecalis* in semen resulted in poor sperm quality, whereas the presence of *Micrococcus* spp or alpha haemolytic *Streptococci* did not have any detrimental effect on the quality of sperms. [17] Onemu and Ibeh (2001), concluded that the presence of pathogenic microorganisms in semen might provide an early warning signal of impairment of male fertility. Infertile patients infected with *Helicobacter pylori* showed a low sperm quality respective to uninfected patients. [17] Particularly, in CagA-positive patients, we have observed a significant reduction in sperm motility and in the fertility index, while apoptosis and necrosis were increased. In these patients, the means of systemic TNF-alpha levels were higher than those of uninfected patients. The negative influence of CagA-positive *Helicobacter pylori* infection on sperm quality may help to understand the role of chronic infections in reproductive disorders. [17]

The question remains, what microorganisms should be considered as pathogenic with negative impact on fertility and what microorganisms should be considered otherwise.

It is clear that bacteria isolated from the semen of infertile males differ considerably and each isolate may exert different effects on sperm quality. Therefore, each community should be treated as a separate entity; bacterial agents should be isolated, identified and their relationship to sperm quality should be determined. The objectives of this study were to compare types as well as frequency of occurrence of aerobic bacteria recovered from semen collected from Jordanian males with and without fertility problems. Results were used to differentiate bacteria with possible clinical significance from those that may occur as mere contaminants.

Methods

A total of 80 males with known defective sperm quality as established in a previous work [2] were included in this investigation. All males were married, but sired no children and received no medication for at least 3 months prior to specimen collection. These precautions were taken to ensure that sperm quality in the collected semen specimens was not altered and bacterial isolation was not hampered. Each male observed 4 days of abstinence before collecting the specimen in the laboratory by masturbation.

Semen specimens were studied for sperm count, sperm activity and morphology. The techniques used to perform these tests were typically the same as described by the Word Health Organization (WHO, 1992). [3] After 60 minutes of collection, each specimen was mixed to ensure homogeneity and 1.0 ml aliquots were taken for serial dilution in sterile phosphate buffer pH 7.0. From the appropriate dilution 0.1ml quantities were separately plated on Blood Agar and MacConkey agar plates using the traditional spread plate technique. All plates were incubated at 37 ˚C for 24 hours before grown colonies were counted and expressed as Colony Forming Units per milliliter (CFU/ml) semen. Specimens that harbored bacteria < 10^2 CFU/ml were excluded from this work as they were believed to be contaminants rather than genuinely related to pathological condition. The most dominant bacterium was picked off for purification and identification. Thus, one type of bacteria was recovered from each specimen. The primary identification of the isolates was based on colonial morphology and gram stain. Further identification tests were performed according to the diagnostic tables given by Baron et al. (1994). [18]

Semen specimens were also collected from 40 candidates of proven fertility and were tested as for those collected from the infertile males. The lowest value obtained for various semen parameters from the fertile semen was taken as a cut off value for the confirmation of the infertility of individuals included in the other group. Thus, male infertility as used in this paper is directly related to sperm quality.

The student t-test statistics were performed using a Sigma Plot package to establish the statistical significance of the results obtained when required.

Results

The semen parameters were established by testing the count, motility and shape of semen samples of 40 fertile males. The lowest, highest and mean values of semen parameters for the fertile males are shown in Table (1).

The lowest readings given in this table were taken as cut off values to differentiate between normal and abnormal semen quality. It is clear from this table that all males included in the infertile group had sperm count below 23 million /ml, progressive motile sperms <42% and/or normal sperm morphology <55%. Infertility is attributed mainly to the sperm defects, sperm samples were tested for major defects giving abnormal sperms including Azospermia, Oligospermia, Poor motility and abnormal morphology. The types of sperm defects shown in Table (2) encountered in the infertile individuals emphasize that all males in this group gave semen specimens with at least 1 defective sperm character. It is apparent that the majority of specimens were with poor motility and low sperm count.

Table (1): Lowest, highest and mean values of semen characters derived from 40 males of known fertility.

Sperm Parameter	***Lowest***	***Highest***	***Mean***
Sperm count (Million/ml)	*23*	*183*	*69*
Progressive motility %	*42*	*93*	*67*
Normal sperm shape %	*55*	*94*	*82*

In order to test the association between the recovery of aerobic bacteria in both fertile and infertile individuals and infertility, the semen specimens were investigated for the isolation of these bacterial species. The recovery rate of aerobic bacteria from semen specimens was found to be almost the same regardless the fertility potential of the donor, the results of isolation of aerobic bacteria are shown in Table (3). A total of 45 out of 80 (56.25%) specimen taken from the infertile individuals harbored bacteria in counts exceeding 10^3 CFU/ ml, while 19 out of 40 (47.50%) specimens from the fertile group revealed the presence of bacteria in significant count. Only 12.5% of specimens in the fertile group harbored bacteria in count >10^4 CFU/ml., whereas 42.5% of specimens in the infertile group, contained bacteria in count >10^4 CFU/ml (Table 3). There was a significant difference between the fertile and infertile total bacterial count >10^4 CFU/ml ($P < 0.05$).

Table (2): Sperm defects encountered in semen specimens collected from 80 infertile males (refer to table 1 for cut off values).

Sperm defect	Number of cases
Azospermia	*3*
Oligospermia	*52*
Poor motility	*57*
Abnormal morphology	*8*
Total defects	*120*

Table (3): Total aerobic bacterial counts in semen specimens derived from infertile and fertile individuals.

Total bacterial counts (CFU/ ml) semen	Number of specimen	
	Infertile	Fertile
$<10^2$	35 (43.75)†	21 (52.50)†
$> 10^2$ but $<10^3$	5	9
$> 10^3$ but $< 10^4$	6	6
$> 10^4$ but $< 10^5$	16	3
$> 10^5$	18	2
Total	80	40

† Numbers in parenthesis indicate percentage

In this study, seven different bacterial species were isolated from the semen specimens, these are Coagulase−ve *Staphylococci*, *E.faecalis*, *Micrococcus spp*., Coagulase + ve *Staphylococci*, *Beta hemolytic Streptococci*, *Escherichia coli* and *Klebsiella spp*.. The results of the occurrence and recovery rate of aerobic bacteria from semen specimens derived from males with and without fertility problems are given in Table (4). It was clear that the most frequent isolated aerobic bacteria was coagulase negative *Staphylococci*. This organism was isolated from 14 (31.11%) and 9 (47.37%) of specimens from the infertile and the fertile individuals, respectively. Frequency of isolation of *Ent. Faecalis* from infertile and fertile individuals was (11(24.44%) and 2 (10.53%), respectively, while the frequency of isolation of coagulase positive *Staphylococci* from infertile and fertile individuals was (6 (13.33%) and 2 (10.53%), respectively. There was a significant difference between the frequence of isolation of both *Ent. Faecalis* and coagulase positive *Staphylococci* from the semen of infertile individuals as compared to specimens collected from the fertile volunteers ($P < 0.05$).

Table (4): Frequency of occurrence of aerobic bacteria in semen specimens derived from infertile and fertile individuals.

Type of bacterial	Frequency of Isolation	
	Infertile	Fertile
Coagulase −ve Staphylococci	***14 (31.11)*** †	***9 (47.37)*** †
E.faecalis	***11(24.44)***	***2(10.53)***
Micrococcus spp	***7(15.55)***	***5(26.31)***
Coagulase + ve Staphylococci	***6(13.33)***	***2(10.53)***
Beta hemolytic Streptococci	***2(4.45)***	***0***
Escherichia coli	***3(6.67)***	***1(5.26)***
Klebsiella species	***2(4.45)***	***0***
Total number of isolates	***45***	***19***

† Numbers in parenthesis indicate percentage

Discussion

The WHO definition of seminal tract infection does not clearly differentiate between infection, contamination and colonization of the genital tract. Semen that passes through the genital tract is routinely contaminated with Gram positive cocci such as *Staphylococcus*, *Streptococcus* and *Diphteroids.* [19] Standards of WHO (1992), regarding semen parameters are available, but due to the variation in semen quality obtained from individuals in different geographical locations, it is recommended to establish standards for each community independently.[20, 21] Therefore, the lowest value established for the sperm quality of the fertile volunteers was used to assess results obtained for the infertile individuals. According to figures given in table (2), all males considered as infertile gave semen specimens defective in at least 1 character. Correlation between infertility and various sperm defects is beyond the scope of this paper as this subject was covered in a previous publication. [2]

Merino et al. (1995) found that 66% of semen specimens collected from males with infertility problems harbored bacteria. Bacterial recovery rates as high as 73% and 95% were also reported by Reheway et al. (1979) and Hillier et al. (1990), [22, 23] respectively. In this work, semen specimens from the infertile and the fertile groups revealed the presence of bacteria in counts

exceeding 10^2 / ml, in 56.25% and 47.50% of specimens, respectively. This difference was not found to be of any statistical significance, thus it is prudent to suggest that the mere isolation of bacteria from semen does not provide significant indication regarding the fertility potential of the male.

Krause and Weidner (1982) stated that in order to diagnose infection in the genital tract, particularly semen pathways, it is essential to provide evidence of bacterial presence in count exceeding 10^5 organisms / ml ejaculate. As table (3) shows, bacterial count was significantly higher ($P < 0.05$) in the semen derived from the infertile group. Therefore, it is concluded that the higher the bacterial count in semen, the more likely to find defective sperm quality.

In medical laboratories, semen culture is performed to identify the causative agent of infection and to determine its antibiotic sensitivity, however, total bacterial count is not usually considered. Since bacterial count was found in this work to be related to the fertility potentials of males, it is highly recommended to carry out bacterial count whenever semen culture is requested.

Many authors have reported the isolation of mixed bacterial cultures from semen specimens. Merino et al. (1995) isolated 157 aerobic bacteria from 123 specimen (average of 1.27 bacteria /specimen), while Hillier et al. (1990) recovered 113 isolates from 36 specimens (average 5.2 isolates/semen specimen). Munuce et al. (1999) concluded that the number of aerobic bacterial species detected in a single semen specimen could not be considered as diagnostic in differentiating between semen specimens derived from fertile or infertile males. During the course of this study, mixed bacterial culture was observed in the majority of cases. However, concern was directed towards the dominant bacteria, which could be related to inflammatory process or may have a direct impact on fertility potentials.

Table (4) illustrates types and frequency of aerobic bacteria recovered from semen derived from fertile and infertile individuals. It is apparent that the most commonly isolated aerobic bacterium from semen was Coagulase negative *Staphylococci*. This organism was isolated from 31.11% and 47.37% of specimens derived from infertile and fertile individuals, respectively. The second most frequent isolate was *Ent. faecalis* that occurred in 24.44% and 10.53% of specimens collected from infertile and fertile males, respectively. The type and frequency of occurrence of these isolates is very difficult to compare with published literature as results varied from one work to another. [9, 15, 16, 23] This variation was probably one of the main reasons, which stimulated the authors to carry out this work.

Coagulase negative *Staphylococci* occurred in a higher percentage of specimens derived from fertile individuals as compared to those taken from the infertile group (table 4). Therefore, these organisms were believed to have no detrimental effect on sperm quality. Cottel et al. (2002) [25] indicated that gram-positive microbes in semen were most commonly contaminants. This is probably the case with the coagulase negative *Staphylococci* recovered in this investigation. It is believed that these organisms were derived from the skin micro flora and gained access into specimens during collection. This conclusion is substantiated by the findings of Kim and Goldstein (1999)[26] who demonstrated that using antibacterial skin preparations prior to specimen collection decreased the incidence of false positive semen culture by at least 50%.

Nevertheless, statistical significance ($P < 0.05$) was found to exist between the isolation of *Ent. Faecalis* as well as coagulase positive *Staphylococci* and the fertility potential of semen donors. These results are in agreement with those made by Onemu and Ibeh (2001) and Sanocka-Maciejewska et al. (2005). It is generally accepted that *Staphylococcus aureus* which are coagulase positive is regarded as pathogenic and should be treated and the presence of this microorganism can no longer be ignored. [14] The latter authors also reported that *E. coli*, when

isolated from semen, could have a negative impact on sperm quality. This observation could not be ascertained in the present work as the rate of *E. coli* recovery was low (table 4).

Other bacteria of concern in relation to sperm quality include *Gardnerella vaginalis*, *Mycoplasma genitalium*, *Ureaplasma urealyticum* and *Chlamydia trachomatic.* [27] All of these bacteria were isolated by many authors in a much lower rate than the aerobic bacteria and many believe that they are not related to sperm quality or fertility. [12, 15] Hence, they were not considered in this work.

In conclusion, negative impact on male fertility was found when bacterial count in semen was high and when *Ent. faecalis* or coagulase positive *Staphylococcus* was among the isolates. On the other hand, the presence of *Micrococcus* spp and coagulase negative *Staphylococci* in semen was not related to male fertility. These observations may explain some of the still existing uncertainty regarding the role of aerobic bacteria on the fertility potentials of males.

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العلاقة بين علاج البكتيريا الهوائية في السائل المنوي والعقم عند الرجال في الأردن

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الملخص

ان دور البكتيريا المكتشفة في السائل المنوي على الخصوبة عند الرجال ما زال محل جدل ونقاش. هذه الدراسة توضح اهمية الوجود البكتيري في السائل المنوي الذي تم جمعه من مجموعتين من الذكور احداهما تعاني من مشاكل في الخصوبة واخرى طبيعية. عينات السائل المنوي التي تم جمعها كانت من 80 رجل يعانون من مشاكل في الخصوبة و40 متطوع لا يوجد عندهم أي مشكلة في الخصوبة. لقد تم دراسة العد البكتيري الاجمالي، عزل البكتيريا الهوائية، عدد وحركة وشكل الحيوانات المنوية باستخدام طرق مخبرية معتمدة. لقد اظهرت الدراسة ان(56.25%) 45 من العينات الثمانين التي تم جمعها من رجال يعانون من مشاكل في الخصوبة لديهم عد بكتيري $< 10^2$ CFU/ml ،وفي 75.55% من هذه العينات كان العد البكتيري يتجاوز 10^4CFU/ml. لقد كانت البكتيريا المعزولة كالتالي coagulase negative *Staphylococci* (31.11%), *Enterococcus faecalis* (24.44%) *Micrococcus* species (15.55%) و(13.33%) coagulase positive *Staphylococci*. اقل الانواع البكتيرية التي تم عزلها هي من عائلة Enterobacteriaceae. بمقارنة النتائج التي تم الحصول عليها من هذه الدراسة بين الاشخاص الذين يعانون من مشاكل في الخصوبة والذين لايعانون من تلك المشاكل فقد تبين ان الاشخاص الذين يعانون من مشاكل الخصوبة لديهم عد بكتيري اكبر ممن لايعانون من مشاكل الخصوبة. ان المجموعة التي تعاني من مشاكل في الخصوبة ايضاً لديها وجود واضح ومتميز عن المجموعة الثانية لانواع اخرى من البكتيريا مثل *Enerococcus faecalis* and coagulase positive *Staphylococci*. وجود بعض العزلات البكتيرية الاخرى في الغالب يمكن اعتبارها ناتجة عن تلوث وليس لها أي قيمة مرضية. في الخلاصة يمكن القول ان العد البكتيري الاجمالي يمكن ان يكون ذا فائدة تشخيصية عند اجراء أي تحليل للسائل المنوي لذا يوصى بادراجه ضمن الفحص الروتيني للسائل المنوي في المختبر التشخيصي.

الكلمات الدالة: السائل المنوي، نوعية الحيوانات المنوية، البكتيريا الهوائية، أهمية التشخيص، الخصوبة.