The effect of cell-free supernatant (CFS) of *Escherichia coli* on human sperm motility

تأثير الطاف الخالي من الخلايا البكتيريه للاشريكيه القولونيه على حركة الحيوانات المنويه البشريه

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Abstract

***Escherichia coli* were isolated from vaginal and cervical samples during vaginal examination. Fifteen semen samples have been gathered through masturbation after three days of sexual abstinence. The Cell-Free Supernatant (CFS) of Escherichia has been collected by removing the cells from overnight broth cultures and filtered. The effect of (CFS) of *Escherichia coli* on spermatozoa motility was determined by using Earl's balanced solution to prepare1:2 to 1:16 dilutions of CSF. Results conducted there was a significant difference in the count number of motile spermatozoa in 15 seminal fluid samples after being treated with CSF at the dilutions1:2 ($p \leq 0.05$) ,1:4 ($p \leq 0.05$) and 1:8($p \leq 0.05$). The experiment showed a successive significant increase in the count number of motile sperms compatible with the lowering concentration of CSF until the count number of motile sperms reached the highest in the fourth dilution 1:16 ($P \geq 0.05$) . This demonstrates the real effect of CSF on the vitality and activity of sperm. On the other hand, the study showed that the *Escherichia Coli*, causing genital tract infection, could have a bad effect on the motility and transportation of men's spermatozoa and might be responsible for infertility.**

Key words: spermatozoal motility, Cell-Free Supernatant, *Escherichia coli*

الملخص

عزلت الإشريكية القولونية من عينات المهبل وعنق الرحم خلال الفحص المهبلي. فضلا عن جمع خمسة عشر عينة من السائل المنوي من خلال الاستمناء بعد ثلاثة أيام من الامتناع الجنسي. تم جمع السائل الخالى من الخلايا وذلك بفصل الخلايا البكتيريه بعد حضن الوسط الزرعى لمده 24ساعه وثم فلترته . تم ايجاد تاثير السائل الخالي من الاشريشيا كولاى على حركة الحيوانات المنوية باستخدام محلول إيرل المتوازن بتحضير تخافيف من 2:1الى 1:16 أظهرت النتائج وجود اختلاف معنوي في عدد الحيوانات المنوية المتحركة في 15 عينة من السائل المنوي بعد معالجتها بالسائل الخالى من البكتريا حيث وجد عند التخفيف 1 : 2 ($p \leq 0.05$) ، 1: 4 ($p \leq 0.05$) 1:8 ($p \leq 0.05$) أظهرت التجربة زيادة متواصلة في عدد الحيوانات المنوية المتحركه والتى تزامنت مع انخفاض تركيز السائل الخالى من الخلايا إلى أن وصل عدد الحيوانات المنوية المتحركة إلى أعلاه في التخفيف الرابع 1:16 .($P \geq 0.05$) وهذا يدل على التأثير الحقيقي للسائل الخالى من الخلايا على حيوية ونشاط الحيوانات المنوية. ومن ناحية أخرى ، أظهرت الدراسة أن الاشريشيا القولونيه التى تصيب الجهاز التناسلي ، يمكن أن يكون لها تأثير سيء على حركه وانتقال الحيوانات المنوية للرجال وربما تكون مسؤولة للعقم.

الكلمات المفتاحية : حركية الحيوانات المنوية ،التاثير السمي ، طاف خالٍ من الخلايا ،الإشريكية القولونية

Introduction

The causing of uropathogen isolated from women case study with urinary tract infections is *Escherichia coli*, which has been known to form biofilms of the urogenital tract [1,2,3].

Mahdavi (2007) reported that the bacterial vaginosis (BV) is more common in female with UTI [4].As well, the release of proinflammatory cytokines associated with vaginal bacterial infection [5].numerous studies have shown that infections of the reproductive tract both in male and female may obstruct reproductive function [3,6]. *In vivo* experimental studies confirmed that the infertility might be accordance to presence of sperm agglutinant strains *E. coli* and *S. aureus* in the vagina [7,8].

The comparison of semen characteristics between infected and non-infected men showed that spermatozoa motility and viability will be lower when the pathogenic microorganisms were present in the semen. It would appear that the bacteria can have a direct effect on semen quality with negative consequences in fertility [9]. *E. coli* probably represents the common repeatedly isolated bacteria in genitourinary disease [10].

It rapidly adheres to human spermatozoa in vitro, resulting in agglutination of spermatozoa. A increasingly decline in motility of spermatozoa is obvious over time induced by high changes in sperm morphology [11].

However, *E. coli has* been shown to extend greater noxious effects on human spermatozoa [12,13].The effective of *E. coli* on sperm was demonstrated through *in vitro* studies performed by directly incubating both cells. It has

been demonstrated by several authors that *E. coli* coming into contact with spermatozoa causes decreased sperm motility [14,15].

Materials and Methods

Samples

Fifteen semen samples were collected by masturbation after 3 days of sexual abstinence. *Escherichia coli* were isolated from Vaginal and cervical samples during vaginal examination.

Cell-Free Supernatant (CFS) preparing

Nutrient broth was inoculated by *Escherichia coli* vaginal sources discharge of a woman with a history of genital and urinary tract infections .The culture was incubated for 24 hrs at 37± °C. After incubation, cells were removed from overnight broth cultures by centrifugation at 4000 rpm for 20 min, The CFS were collected and filtered through a filter membrane (pore size 0.22 µm) [16].

Determination the effect of (CFS) *Escherichia coli* on Spermatozoal Motility

Earl's balanced solution was used to prepare the dilutions by mixing 50 microliter of filtered CSF with 50 microliter of Earle's solution in the first well of micro serological plate to prepare 1:2 dilutions. After those 50 micro liters was transferring to the second well to prepare 1:4 dilution, the process was repeated till the 1:16 dilution and finally discarding 50 microliters. 50 microliters from each of the four wells of CSF dilution was mixed with 50 microliters of liquefied mixed seminal fluid, and just 50 microliters of filtered nutrient broth was mixed with 50 microliters of the seminal fluid to use it as control for each test. The serological plate should be incubated for 30 minute at 37± °C. After incubation, 20 microliters of the mixture was transferred to a slide and covered by a coverslip, and by reading about 20 fields the mean number of motile sperm in each dilution was obtained.

Statistical Analysis

Data were analyzed by using the student's t-test, in order to determine whether or not the significance difference existed between the number of motile sperms in the control and study group at different dilutions of CFS, and a *P* value ≤0.05 was considered as statistically significant.

Results and Discussion

The different dilutions (1:2 to1:16) of CFS was mixed with human spermatozoa , showed that the high sperm immobilization could be noticed at the dilution of 1:2 after 30 minutes of incubation, while the minimum sperm immobilization was revealed at the dilution1:16, which was chosen as the minimum concentration of CSF Table (1). Results revealed there was a significant difference ($p \leq 0.05$) in the number of motile spermatozoa in 15 seminal fluid samples after being treated with CSF at dilutions 1:2, 1:4 and 1:8. Besides the experiment showed a continuous significant increase in the number of motile sperms coincided with the low concentration of CSF until the motile sperms reached the highest level in the fourth dilution 1:16 ($P \geq 0.05$) to be an insignificant difference compared to the control group Table (2). Cell-free supernatant CFS seemed to contain sperm immobilization factor released into the extracellular medium causing sperm immobilization. Several studies have reported the negative influence of various *E. coli* strains on human spermatozoa in *in vitro* experiment [17, 18]. The results obtained from this study indicated that the Escherichia *Coli*, causing genital tract infection, could have a negative role in the motility of men's spermatozoa and might be responsible for human infertility.

Table (1): Sperm motility characteristics from 15 patients during treatment with different concentrations of \111111. (CFS) of *E. Coli*

No. of samples	The number of motile sperms at different dilutions of CFS				control
	1:2	1:4	1:8	1:16	
1	1	4	7	10	17
2	1	3	9	9	15
3	4	9	11	18	24
4	1	2	7	7	11
5	4	3	11	25	31
6	6	20	20	23	29
7	9	12	15	15	32
8	16	20	35	44	48
9	8	25	23	33	40
10	6	13	33	21	45
11	16	33	40	45	55
12	1	4	7	10	15
13	4	9	11	18	24
14	1	2	7	7	13
15	8	3	15	25	31

Table (2): Mean ± SE of motile sperms of control and study groups at different dilutions of CFS in fifteen seminal fluids

CSF dilutions	Control groups Mean ± SE	Study groups Mean ± SE	P value
1:2	28.67 ±3.52	5.73 ±1.30	< 0.0001
1:4	28.67 ±3.52	10.80 ±2.49	<0.0003
1:8	28.67 ±3.52	16.73±2.87	<0.013
1:16	28.67 ±3.52	20.67±3.17	>0.102

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