An extract of protein pili and filtrat of human spermatozoa: A chemical review

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Abstract. This study aims to determine the effect of E. coli pili protein, filtrate media culture of E. coli and mixture of E. coli pili protein and E. coli filtrate on the motility and vitality of human sperm in an in vitro. The method for obtaining E. coli pili protein is E. coli prepared by do culture on TCG media to grow E. coli pili, then cutting the Pili E. coli using Omni mixer. Pili that has been obtained then dialysis, electrophoresis and electroelution. E. coli filtrate was obtained by the method of culturing E. coli on Mc Conkey media for 48 hours and then doing culture on BHI media for 48 hours. E. coli culture on BHI media was then centrifuged. E. coli cultures in BHI media were centrifuged and the supernatant produced from centrifugation was filtered using a 0.22µm milipore filter. 10 samples of normal donor sperm according to 1999 WHO criteria, in preparation using Percoll method. Spermatozoa were incubated with E. coli pili protein, E. coli filtrate and a mixture of E. col pili proteins and E. coli filtrate on U-base microplate. Incubation was performed for 1 hour. Observations by calculating the motility and vitality of spermatozoa each at 100 spermatozoa under a light microscope. Data analysis was done with SPSS. The results of this study found that Protein Pili E. coli, E. coli filtrate and a mixture of E. coli pili proteins and E. coli filtrate had significant effect on sperm motility (p = 0.000) and on sperm vitality (p = 0.000). Thus it can be concluded that E. coli pili protein and E. coli filtrate are potentially used for spermicidal materials.

1. Introduction

Sperm is a highly specialized cell that must express diverse arrays of properties including motility, acrosome reaction, zona recognition, and fusion with oocyte [1]. Among all, sperm motility and viability are most essential for successful fertilization. Motility is an expression of the viability and structural integrity of the cell as the fertile life of a spermatozoon can be measured by the duration of its motility [2]. Hence, spermatozoa dysfunction is the single most important cause of infertility. The negative impact of some microorganisms relevant to genital infections on sperm function has been claimed [3]. Some possible pathomechanisms of the development of infertility linked with infection are considered: direct effect on sperm function (motility, morphology, etc.), deterioration of spermatogenesis, autoimmune processes induced by inflammation, and dysfunction of accessory sex glands [4]. Recent studies have shown that the simple presence of bacteria in semen samples may compromise the semen quality [5]. The above-said facts were further justified by an observation made by a group of scientists wherein improvement of semen quality following eradication of infection was seen [6]. Escherichia coli probably represent the most frequently isolated microorganism in genitourinary infections with mainly negative influence on sperm motility and morphology [7]. E. coli rapidly adheres to human spermatozoa.
in vitro, resulting in agglutination of spermatozoa [8]. Rapidity and extent of sperm-\textit{E. coli} agglutination indicates receptor-ligand interaction. Although a number of authors have suggested that direct interaction between bacteria and spermatozoa represents the bacterial mechanism that facilitates immobilization of spermatozoa but other investigators have reported evidence for soluble spermatotoxic factors produced and secreted by pathogenic bacteria. Paulson & Polakoski investigated the mechanism of how bacteria immobilize spermatozoa and they reported a soluble, dialyzable, heat stable, spermatotoxic factor, apparently excreted by \textit{E. coli} which immobilizes spermatozoa without agglutinating it [9]. The increase in population growth rate warrants the development of additional contraceptive methods that are widely acceptable, free from side effects and less expensive. Besides the availability of the present methods of birth control, the population explosion and unintended pregnancies continue to pose major public health issues worldwide. At the present time, many of spermicidal products have been marketed. Most of them contain a detergent as an active ingredient, such as Isononyl-phenyl- polyoxyethylene ether or Nonoxynol 9, p- methanyl- phenyl- polyoxyethylene ether or Menfegol, and Isooctyl-phenyl-polyoxyethylene ether or Octoxynol 9 [10, 11]. The major drawback of using N-9 or other currently used spermicidal surfactants is their detergent-like action on epithelial cells and normal vaginal flora [12]. The repeated use of this surfactant as a vaginal contraceptive has been associated with an increased risk of vaginal or cervical infection, irritation and ulceration [13]. Detergent type spermicides alter vaginal bacterial flora, and such disturbance of the vaginal microbial milieu can lead to opportunistic infections which in turn increases the chance of UTI and also HIV/STI transmission. Several large studies have demonstrated first-year pregnancy rates of 11–31%, making N-9 ~75% effective in preventing pregnancy [14]. Therefore, development of new vaginal spermicide is required; lacking membrane toxicity and that may offer 100% inhibition of pregnancy over the currently marketed detergent type spermicide. Few peptides have been explored for their contraceptive potential. Subtilosin, a bacteriocin produced by \textit{Bacillus subtilis}, was shown to eliminate the motility and forward progression of human spermatozoa in a dose-dependent manner [15]. Peptides found in the skin secretions of frogs, such as dermaseptins and magainins, possess a potent spermicidal activity against human sperm along with their large spectrum of action against sexually transmitted pathogens [16]. Besides, these toxic and synthetic agents some other substances are available which block or retard the motility of spermatozoa and can act as vaginal contraceptive agents in future, e.g. Lysenin, Immotilin and Nisin can act as successful vaginal contraceptive agents. Reddy \textit{et al.}, were the first to report the contraceptive efficacy of nisin both \textit{in vitro} and \textit{in vivo} [17]. Nisin showed a time and dose dependent effect on sperm motility with limited activity against STI causing pathogens [18]. Like these substances, some bacteria are also able to inhibit the sperm motility. They can act by directly attaching to sperm or by secreting some extracellular substances that immobilize the sperms. Diemer \textit{et al.}, reported that immobilization of spermatozoa is also associated with tight adhesions between bacteria and spermatozoa resulting in agglutination of spermatozoa [19]. \textit{E. coli} also agglutinates human spermatozoa \textit{in vitro}. It agglutinates human spermatozoa by tight adhesions on both head and tail region. The purpose of this study was to examine the impact of isolated proteins from \textit{E. coli} and \textit{E. coli} filtrate on the motility and viability of human spermatozoa.

2. Materials and method

2.1. Propagation of \textit{Escherichia coli} and enriching pili
\textit{E. coli} from stock is rejuvenated first by making culture in medium for Mc Conkey temperature 370 C for 24 hours. Then the culture of the medium was inoculated to Erlenmeyer containing 500 ml of BHI medium, and incubated 24 hours, then bacteria poured into 50 bottles which already contained 25 ml TGC medium (medium to enrich bacterial fimbrae), each 10 ml. 370 C incubated for 48 hours. Furthermore, bacterial cultures are collected together in a 1000 ml Erlenmeyer tube and prepared for pili cutting.

2.2. Pili cutting
The liquid culture was transferred into a 100 ml centrifuge tube, plus TCA 3%, then centrifuge of 6000 rpm for 15 minutes. The precipitate was suspended with PBS pH 7.4; 3-5 times the volume, done pili cutting using Omni mixer tool at 4°C. The sample was rotated in a 4°C centrifuge of 12,000 rpm for 15 minutes. The supernatant was separated and the precipitate was suspended with PBS pH 7.4 to taste, then another pivot cut. This process is repeated up to 5 times.

2.3. Fractionation pili
Pili in the dialysis process can be done using PBS solution pH 7.4 at 4°C for 2x24 hours to remove the remaining TCA. Furthermore, the dialysate was precipitated with 35% ammonium sulphate, rotated 6000 rpm 4°C, the supernatant was removed, the precipitate was suspended with PBS sufficiently and dialysis was performed again.

2.4. Electrophoresis with SDS PAGE method
Pili fraction was performed electrophoresis by SDS PAGE method. Electrophoresis. Gel result of SDS-PAGE 12, 5% as many as 10 sheets containing pili protein ribbon cut horizontally on the top and bottom of the ribbon. Then electroelution was done using horizontal apparatus electrophoresis, with buffer running electrophoresis buffer, 125 volts for 2 hours. Electroelution results were dialyzed with PBS solution for 2x24 hours, the solution was changed every 24 hours. The eluate is applied to an absolute cold ethanol solution overnight, allowing precipitated pure protein ready for use in the test. Prior to use for the test, calculation of protein concentration using nanodrop spectrophotometer.

2.5. Isolation of filtrate from E. coli culture medium
E. coli isolate infertile male cement was grown on liquid BHI under water bath shaker condition at 37°C for 72 hours. The culture was centrifuged at 10,000 g for 15 min at 4°C and the cell free supernatant was prepared by supernatant in the filter using 0.22μm milipore. The supernatant is then precipitated with ammonium sulphate. The resulting precipitation results are dissolved in a number of PBS.

2.6. Human spermatozoa preparation
Spermatozoa used were normal spermatozoa according to WHO criteria obtained from the donor. Subsequently prepared by Percoll stratified columns method.

2.7. Treatment
The preparation sperm is divided into 5 groups. In group I sperm incubated with 100 μg / ml SIF, II: sperm was incubated with 100 μg / ml SAF, III: sperm incubated with 100 μg / ml mixture of SIF and SAF. Incubation was performed on a base microplate U with an incubation time of 1 hour. The parameters observed were motility, vitality, morphology. Performed 10 repetitions.

2.8. Observation of motility
Taken one drop on the glass object then closed the cover glass and observed under a microscope with 400 times magnification. Motility is determined by category according to WHO (1999). Tests were performed on 100 spermatozoa.

2.9. Observation of vitality
Taken one drop on object glass, drops one drop of Eosin Y and mixed. Observations were made after 30 seconds under a microscope with a magnification of 400 x. Spermatozoa live unpainted and dead red sperm. Calculated on 100 spermatozoa.

2.10. Morphological observation
One drop of spermatozoa was smeared on the object glass in dry fixation in air, then dipped in methanol (3 minutes), safranin (3 minutes), buffer (3 dip) and violet crystal (5 minutes) then observed under a microscope. Normal and abnormal morphology was observed in 100 sperm.

3. Results and discussion

3.1. Isolation and purification of protein of pili E. coli

![Figure 1. SDS PAGE of protein pili E. coli.](image)

3.2. Motility of human spermatozoa category a+b

![Figure 2. Motility of human spermatozoa category a+b.](image)

3.3. Vitality of human spermatozoa
3.4. Data analysis

| Table 1. ANOVA. |
|-----------------|
| motility        | Sum of Squares | df  | Mean Square | F     | Sig.  |
| Between Groups  | 96.167         | 34  | 2.828       | 11.068| .000  |
| Within Groups   | 3.833          | 15  | .256        |       |       |
| Total           | 100.000        | 49  |             |       |       |
3.5. Discussion
The negative impact of some microorganisms relevant to genital infections on sperm function has been claimed. Changes in sperm parameters that could account for infertility include reduced cell counts, reduced motility, or morphological alteration [19]. It is already known that these parameters play a vital role in the fertility potential of a man. Moreover, if spermatozoa prematurely lose motility, they also lose their natural fertilization potential since they cannot travel to meet oocyte. Among various microorganisms associated, *E. coli* appears to be the most important pathogen isolated from the ejaculate [20]. The direct inhibitory effect of *E. coli* on progressive motility of spermatozoa is being reported. Inhibition of motility is either directly by agglutination or indirectly by the secreted products leading to immobilization. This study presents agglutination of spermatozoa by *E. coli* obtained from the ejaculate of males attending infertility clinic. Serotyping of selected *E. coli* strain revealed it to be of rough type. The strain was capable of causing 100% sperm agglutination within 2 h of incubation. The agglutination was of mixed type, that is, head to head, head to tail, and tail to tail.

The result of this research is that there is decrease of motility and vitality of spermatozoa both in human sperm incubation treatment with *E. coli* pili protein, spermatozoa incubation treatment with *E. coli* filtrate and sperm incubation treatment with pili protein mixture and *E. coli*.

4. Conclusion
The conclusion of this study is *E. coli* pili protein and *E. coli* filtrate are potentially used for spermicidal materials.

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