

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

## 4,800

Open access books available

## 122,000

International authors and editors

## 135M

Downloads

Our authors are among the

## 154

Countries delivered to

## TOP 1%

most cited scientists

## 12.2%

Contributors from top 500 universities

**WEB OF SCIENCE™**Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.

For more information visit [www.intechopen.com](http://www.intechopen.com)

---

# Effect of Uropathogenic *Escherichia coli* on Human Sperm Function and Male Fertility

---

Juana V. Villegas, Rodrigo Boguen and  
Pamela Uribe

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.68312>

---

## Abstract

Infections of the reproductive tract represent nearly 15% of male infertility cases. The most frequently isolated bacterium in the ejaculate of infertile men is *Escherichia coli* (*E. coli*), which causes between 60 and 85% of cases of chronic bacterial prostatitis leading to sperm damage. The aim of this chapter is to discuss the negative effects of *E. coli* on sperm quality and male fertility. The *E. coli* isolated from semen is uropathogenic (UPEC) and can damage sperm in different ways. UPEC induces activation of polymorphonuclear leukocytes with the release of cytokines and reactive oxygen species, the latter being harmful due to their ability to induce lipid peroxidation and early sperm capacitation. Also, UPEC decreases sperm motility, vitality and mitochondrial membrane potential through direct contact or mediated by its soluble metabolites. The negative effects are higher with strains with specific characteristics such as hemolytic capacity. *In vivo* studies with mice models have shown that UPEC inoculated into the epididymis induces inflammatory damage with testicular mass decrease and low sperm concentration. Future studies are needed to clarify the molecular mechanisms by which *E. coli* damages sperm. This knowledge will make it possible to take measures to avoid deleterious consequences on the fertilizing potential of men.

**Keywords:** sperm motility, mitochondrial function, reactive oxygen species, infertility

---

## 1. Introduction

Infertility is currently a highly prevalent disease, defined by the failure to achieve a successful pregnancy after 12 months or more of appropriate, unprotected intercourse or therapeutic

---

donor insemination [1]. Approximately 50% of infertility cases are attributed to the male [2, 3] due to conditions such as varicocele, cryptorchidism, obstructive problems, hormonal disorders, ejaculatory dysfunction as well as infectious causes classified under male genital tract infection (MGTI).

MGTI accounts for 15% of male infertility cases [4]. MGTIs are an important problem in male reproductive health because they cause negative changes in semen parameters [5]. A consequence of MGTI is the inflammatory response evidenced by leukocytospermia, where a treatment with antibiotics and anti-inflammatory drugs may be helpful to try to recover the patient's fertilizing potential [6]. Principal causes of MGTI are bacteria such as *Staphylococcus epidermidis*, *Streptococcus viridans*, *Staphylococcus aureus* and *Escherichia coli* (*E. coli*), which have a negative effect on the fertilizing potential of a man and are strongly associated with MGTI [7, 8]. However, *E. coli* has been shown to exert greater damaging effects on human spermatozoa [7, 9–11].

*E. coli* is the causal agent in 65–80% of cases of chronic bacterial prostatitis [12], and these bacteria have been isolated from semen in 69% of the patients with this pathology [13]. Moreover, *E. coli* causes diverse MGTIs such as urethritis, epididymitis and orchitis, and it is the most frequently isolated bacterium [4].

With this background, this chapter discusses the negative effect of *E. coli* on sperm quality and male fertility. The chapter will be developed by first addressing the uropathogenic *E. coli* strains associated with male infertility. Then, the evidence of the *in vitro* effects of *E. coli* on the male gamete will be analyzed. Evidences will be described, obtained after *in vitro* co-incubation of normal human spermatozoa with *E. coli* and with polymorphonuclear leukocytes, emulating infection. Next, the effects of *E. coli* on human sperm functions observed by direct incubation of spermatozoa with bacteria and without leukocytes will be discussed. Also, evidence of the effects of the metabolic and soluble products of *E. coli* on human sperm function will be analyzed. To complete the picture of the evidence of the effects of this bacterium on male fertility, some studies on animal models will be reviewed.

## 2. Uropathogenic strains of *E. coli* are associated with male infertility

Among the different pathogenic strains of *E. coli*, the uropathogenic strains are mainly associated with urinary tract infections (UTI). The pathogenic *E. coli* is classified according to the O antigens. Some of these have been associated with uropathogenic *E. coli* (UPEC) being the serotypes O1, O2, O4, O6, O7, O8, O16, O18, O25, and O75 preferentially associated with these strains [14]. Similarly, the most frequently O antigens associated among the serotypes isolated from patients with prostatitis are O1, O2, O4, O16, O18, O22, O25 and O75 serotypes [15], which coincide with *E. coli* strains isolated in cases of infections to the urinary tract. Regarding to the strains isolated from semen of infertile men, the described prevalent antigens are O1, O2, O4 and O6 [16]. These data indicate that the *E. coli* that infects the male reproductive tract is uropathogenic strain, which is not surprising considering the proximity of the urinary and reproductive tracts.

### 3. Effect of *E. coli* on human spermatozoa is induced through seminal leukocytes

One way *E. coli* induces damage in spermatozoa is by mediating seminal leukocyte activation. The increased presence of leukocytes in semen, known as leukocytospermia, is defined by a concentration equal to or greater than  $1 \times 10^6$  leukocytes per ml of semen [17]. Proinflammatory cytokines, which are usually released by leukocytes during the inflammatory response, are able to decrease sperm motility by themselves [5, 18, 19]. This damaging effect seems to be mediated by polymorphonuclear (PMN) leukocytes instead of by other leukocytes such as lymphocytes or monocytes [20]. The decrease in sperm quality is also mediated by reactive oxygen species (ROS) released by PMN after bacteria-induced activation, with *E. coli* being able to induce a higher response of increased ROS production than other bacteria [9]. Other consequences of ROS released by leukocytes are lipid peroxidation, which affects sperm plasma membrane [21, 22], and early induction of sperm capacitation, which should normally occur later in the female reproductive tract [23].

### 4. *E. coli* directly affects human sperm function

The direct effect 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 [7, 24–26]. The decrease in sperm motility due to *E. coli* has been attributed for many years to an agglutinating effect on sperm [27]. Sperm agglutination can be caused by bacterial type 1 and P fimbriae; specifically, the type 1 fimbriae of *E. coli* cause a pattern of head-head type agglutination because they bind mannose residues in the head region of sperm. Instead, type P fimbriae of *E. coli* cause a head-tail agglutination pattern because they bind gal-gal receptors present along the sperm [28].

Electron microscopy has revealed that this bacterium causes damage primarily in the head of spermatozoa, such as rupture of the plasma membrane, vesicle formation and rupture of the inner and outer membranes of the acrosome [29].

Consistent with the plasma membrane damages observed, another report showed that *E. coli* per se causes phosphatidylserine translocation, a cell death indicator [9]. From the point of view of sperm cell death, it was observed that *E. coli* endotoxins such as lipopolysaccharide (LPS), peptidoglycan and porins produce loss of sperm viability [30]. Moreover, it has been shown that LPS and porins cause sperm DNA fragmentation [31]. The effects of these endotoxins are mediated by the toll-like receptor (TLR)-2 and TLR-4, both present in sperm. After TLR-2 and TLR-4 stimulation, sperm damage highlighting DNA fragmentation can be observed [32].

Another direct effect of *E. coli* is at the level of mitochondrial membrane potential ( $\Delta\Psi_m$ ). The *in vitro* observation that contact with *E. coli* decreases the  $\Delta\Psi_m$  together with the motility in

spermatozoa [33] was followed by another report showing, also *in vitro*, that *E. coli* directly reduces sperm  $\Delta\Psi_m$  and alters plasma membrane stability [34]. From the point of view of sperm function, it has been observed that  $\Delta\Psi_m$  is positively correlated with sperm motility *in vivo* [35, 36]. However, after contact with some strains of *E. coli*, which decrease sperm motility *in vitro*, they had no effect on  $\Delta\Psi_m$  [37]. This study also found that some *E. coli* isolated from different patients was unable to decrease sperm motility, remarkably even an O6, which is thought to be a highly uropathogenic strain in urinary tract infections [38]. These facts contrast with the notion that *E. coli* in general alters sperm function. These differences could be attributed to the fact that different strains bear specific but different characteristics from other *E. coli* strains. Evidence confirming this was observed in our work, when sperm were incubated with a hemolytic strain of *E. coli*. This strain caused a decrease in motility,  $\Delta\Psi_m$ , vitality and an increase in intracellular ROS in normal spermatozoa. These effects were not observed with other strains non-hemolysis producers [39]. These differences among strains highlight the importance of knowing what kind of toxins are effectively produced by the *E. coli* strain infecting a patient, because it could indicate the level of sperm damage to be expected. As example, hemolytic *E. coli* strains produce the alpha-hemolysin (HlyA) toxin [40], a calcium-dependent pore-forming toxin which has intracellular effects, inactivating pathways related to cell survival [41]. This toxin can be highly relevant, particularly if we consider that between 40 and 50% of *E. coli* strains isolated from patients with epididymitis release this toxin [42].

Evidence of *E. coli* effects on human spermatozoa shows that this bacterium impairs sperm quality, principally causing decreased motility; nevertheless, there are other consequences for sperm quality, specifically the incubation of sperm with *E. coli* decreases the ability of the male gamete to penetrate the oocyte, the most important step in the function of the spermatozoon [43].

## 5. Soluble products of *E. coli* also affect human sperm function

The effects of *E. coli* soluble products on sperm have been studied using supernatants of *E. coli* culture as a source of bacterial metabolic product. It has been reported that although the direct contact with *E. coli* was able to alter sperm motility, the metabolic products of *E. coli* had no effect on decreasing motility in human spermatozoa [24]. However, after this report, it was shown that incubation with the soluble factors of *E. coli* reduced sperm motility and  $\Delta\Psi_m$  [33]. Added to this, the ability of *E. coli* soluble factors to decrease motility, viability,  $\Delta\Psi_m$  and increase ROS in spermatozoa can be prevented by lactobacilli. In an *in vitro* experiment, after adding lactobacilli to simulate the normal condition in the female genital tract, the harmful effects of *E. coli* soluble factors were inhibited [44].

Among the soluble factors of *E. coli*, a component called spermatozoa immobilization factor (SIF) was first described in 1977. The effect of SIF, as the name implies, is to immobilize spermatozoa, and this effect could be reversed by washing the spermatozoa [45]. Years later, an apparently similar SIF of 56 kDa was isolated and purified from supernatants of a strain of *E. coli*. SIF-56 decreases sperm motility completely and almost instantaneously. It was also observed that SIF-56 at very high concentrations can even induce sperm death [46]. Sperm immobilization mediated by SIF-56 has been shown to depend on 115 kDa-receptor present in sperm [47]. Another *E. coli*

soluble factor described is the sperm agglutinating factor (SAF) of 71 kDa, which produces sperm agglutination, decreases ATPase activity and can cause sperm death [48]. A toxin candidate to be further investigated is HlyA, because this toxin is produced by *E. coli* strains most pathogenic to sperm both *in vitro* and *in vivo* [42].

## 6. Animal model research

*In vitro* investigations have the disadvantage of not necessarily representing what would happen *in vivo*. Hence, *in vivo* studies in animal models allow us to get closer to the reproductive reality of a man with accessory glands infected by *E. coli*. That is how the progressive reduction of testicular size with a consequent decrease in sperm count caused by the necrotic death of the testicular germ cell has been described in rats inoculated with *E. coli* [49]. After three days of injecting rats with HlyA producing *E. coli*, the epididymis had epithelial damage, leukocyte infiltration and edema and the sperm-fertilizing potential was lost, because despite being motile, the spermatozoa had a premature acrosome reaction [50]. The above-mentioned greater pathogenicity of the HlyA-producing *E. coli* strains was reported in the work of Lang [50], where the *E. coli* strains that did not produce HlyA induced only slight damage to the epididymis. As already stated, sperm recognize peptidoglycans and LPS through TLR-2 and TLR-4, and this recognition event induces sperm cell death. This was confirmed by using knockout mice for both TLR-2 and -4 and by observing that in these animals LPS or peptidoglycans did not induce sperm death [32].

Further evidence of the contribution of some *E. coli* strains to infertility was observed after inoculating the vaginal tract of rats with SAF-producing strains. Control rats were inoculated with *E. coli* non-SAF producers. It was observed that the rats inoculated with SAF-producing strains were incapable of pregnancy, demonstrating that these toxin-producing strains affect fertility profoundly [51].

## 7. Conclusion

It is clear that *E. coli* has an important role in causing male infertility associated with genital tract infections. The main mechanism postulated for male infertility by *E. coli* is the profound damage to different sperm processes and function, either by direct contact and/or through secreted toxins.

While today there is a consensus that *E. coli* is an important causal agent of MGTI that may actually cause infertility, from the latest evidence presented above, it is clear that this would not be completely true for all UPEC. Due to these differences among the various strains, it seems important to develop molecular studies that can clarify what specific features of the *E. coli* strains are associated with the pathogenic effects on sperm or with an aggressive inflammatory response this point should be followed.

To date, there have only been a few studies at the molecular level to try to explain how *E. coli* causes infertility.

Knowledge of the molecular mechanisms by which *E. coli* damages sperm will make it possible to take measures to avoid its consequences on the fertilizing potential of men.

## Author details

Juana V. Villegas<sup>1\*</sup>, Rodrigo Boguen<sup>1</sup> and Pamela Uribe<sup>2</sup>

\*Address all correspondence to: juana.villegas@ufrontera.cl

1 Department of Internal Medicine, Center of Biotechnology on Reproduction (CEBIOR-BIOREN), Medicine Faculty, University of La Frontera, Temuco, Chile

2 Center of Biotechnology on Reproduction (CEBIOR-BIOREN), Medicine Faculty, University of La Frontera, Temuco, Chile

## References

- [1] Practice Committee of American Society for Reproductive Medicine. Definitions of infertility and recurrent pregnancy loss: A committee opinion. *Fertility and Sterility*. 2013;**99**(1):63. DOI: 10.1016/j.fertnstert.2012.09.023
- [2] Farhi J, Ben-Haroush A. Distribution of causes of infertility in patients attending primary fertility clinics in Israel. *Israel Medical Association Journal*. 2011;**13**(1):51-54
- [3] Miyamoto T, Tsujimura A, Miyagawa Y, Koh E, Namiki M, Sengoku K. Male infertility and its causes in human. *Advances in Urology*. 2012;**2012**:384520. DOI: 10.1155/2012/384520
- [4] Weidner W, Krause W, Ludwig M. Relevance of male accessory gland infection for subsequent fertility with special focus on prostatitis. *Human Reproduction Update*. 1999;**5**:421-432. DOI: 10.1093/humupd/5.5.421
- [5] Diemer T, Ludwig M, Huwe P, Hales DB, Weidner W. Influence of urogenital infection on sperm function. *Current Opinion in Urology*. 2000;**10**:39-44
- [6] Wolf H, Joseph A. Leukocytospermia is associated with poor semen quality. *Fertility and Sterility*. 1990;**53**:528-536
- [7] Huwe P, Diemer T, Ludwig M, Liu J, Schiefer HG, Weidner W. Influence of different uropathogenic microorganisms on human sperm motility parameters in an in vitro experiment. *Andrologia*. 1998;**30**(Suppl 1):55-59. DOI: 10.1111/j.1439-0272.1998.tb02827.x
- [8] Sanocka-Maciejewska D, Ciupinska M, Kurpisz M. Bacterial infection and semen quality. *Journal of Reproductive Immunology*. 2005;**67**(1-2):51-56. DOI: 10.1016/j.jri.2005.06.003
- [9] Villegas J, Schulz M, Soto L, Sanchez R. Bacteria induce expression of apoptosis in human spermatozoa. *Apoptosis*. 2005;**10**(1):105-110. DOI: 10.1007/s10495-005-6065-8

- [10] Villegas J, Schulz M, Soto L, Iglesias T, Miska W, Sanchez R. Influence of reactive oxygen species produced by activated leukocytes at the level of apoptosis in mature human spermatozoa. *Fertility and Sterility*. 2005;**83**:808-810. DOI: 10.1016/j.fertnstert.2004.09.022
- [11] Merino G, Carranza-Lira S, Murrieta S, Rodriguez L, Cuevas E, Moran C. Bacterial infection and semen characteristic in infertile men. *Archives of Andrology*. 1995;**35**(1):43-47. DOI: 10.3109/01485019508987852
- [12] Weidner W, Jantos C, Schiefer HG, Haidl G, Friedrich HJ. Semen parameters in men with and without proven chronic prostatitis. *Archives of Andrology*. 1991;**26**(3):173-183. DOI: 10.3109/01485019108987640
- [13] Weidner W, Schiefer HG, Krauss H, Jantos C, Friedrich HJ, Altmannsberger M. Chronic prostatitis: A thorough search for etiologically involved microorganisms in 1,461 patients. *Infection*. 1991;**19**(suppl. 3):119-125. DOI: 10.1007/BF01643680
- [14] Lloyd AL, Rasko DA, Mobley HL. Defining genomic islands and uropathogen-specific genes in uropathogenic *Escherichia coli*. *Journal of Bacteriology*. 2007;**189**(9):3532-3546. DOI: 10.1128/JB.01744-06
- [15] Terai A, Yamamoto S, Mitsumori K, Okada Y, Kurazono H, Takeda Y, et al. *Escherichia coli* virulence factors and serotypes in acute bacterial prostatitis. *International Journal of Urology*. 1997;**4**(3):289-294. DOI: 10.1111/j.1442-2042.1997.tb00192.x
- [16] Bartoov B, Ozbonfil D, Maayan MC, Ohad E, Nitzan Y. Virulence characteristics of male genital tract *Escherichia coli* isolated from semen of suspected infertile men. *Andrologia*. 1991;**23**(5):387-394. DOI: 10.1111/j.1439-0272.1991.tb02586.x
- [17] World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th ed. Geneva, Switzerland: WHO Press; 2010. p. 271
- [18] Hill JA, Haimovici F, Politch JA, Anderson DJ. Effects of soluble products of activated lymphocytes and macrophages (lymphokines and monokines) on human sperm motion parameters. *Fertility and Sterility*. 1987;**47**:460-465
- [19] Hill JA, Cohen J, Anderson DJ. The effects of lymphokines and monokines on sperm-fertilizing ability in the zona-free hamster egg penetration test. *American Journal of Obstetrics & Gynecology*. 1989;**160**:1154-1160
- [20] Diemer T, Huwe P, Ludwig M, Schroeder-Printzen I, Michelmann H, Schiefer HG. Influence of autogenous leucocytes and *Escherichia coli* on sperm motility parameters in vitro. *Andrologia*. 2003;**23**:100-105. DOI: 10.1046/j.1439-0272.2003.00523.x
- [21] Fraczek M, Szumala-Kakol A, Jedrzejczak P, Kamieniczna M, Kurpiz M. Bacteria trigger oxygen radical release and sperm lipid peroxidation in in vitro model of semen inflammation. *Fertility and Sterility*. 2007;**88**:1076-1085. DOI: 10.1016/j.fertnstert.2006.12.025
- [22] Twigg J, Fulton N, Gomez E, Irvine DS, Aitken RJ. Analysis of the impact of intracellular reactive oxygen species generation on the structural and functional integrity of human spermatozoa: Lipid peroxidation, DNA fragmentation and effectiveness of antioxidants. *Human Reproduction*. 1998;**13**(6):1429-1436



- [23] Villegas J, Kehr K, Soto L, Henkel R, Miska W, Sanchez R. Reactive oxygen species induce reversible capacitation in human spermatozoa. *Andrologia*. 2003;**35**(4):227-232. 564 [pii]
- [24] Diemer T, Weidner W, Michelmann HW, Schiefer HG, Rován E, Mayer F. Influence of *Escherichia coli* on motility parameters of human spermatozoa in vitro. *International Journal of Andrology*. 1996;**19**(5):271-277. DOI: 10.1111/j.1365-2605.1996.tb00475.x
- [25] Berkast M, Aydin S, Yilmaz Y, Cecen K, Bozkurt H. Sperm motility changes after coincubation with various uropathogenic microorganisms: An in vitro experimental study. *International Urology and Nephrology*. 2008;**40**:383-389. DOI: 10.1007/s11255-007-9289-4
- [26] Ekhaïse FO, Richard FR. Common bacterial isolated associated with semen complaining of infertility in University of Benin Teaching Hospital (U.B.T.H.), Benin City, Nigeria. *World Journal of Medical Science*. 2008;**3**(1):28-33
- [27] Rosenthal L. Agglutinating properties of *Escherichia coli*: Agglutination of erythrocytes, leucocytes, thrombocytes, spermatozoa, spores of molds, and pollen by strains of *E. coli*. *Journal of Bacteriology*. 1943;**45**(6):545-550
- [28] Monga M, Roberts J. Spermagglutination by bacteria: Receptor-specific interactions. *Journal of Andrology*. 1994;**15**:151-156. DOI: 10.1002/j.1939-4640.1994.tb00423.x
- [29] Diemer T, Huwe P, Michelmann HW, Mayer F, Schiefer HG, Weidner W. *Escherichia coli*-induced alterations of human spermatozoa. An electron microscopy analysis. *International Journal of Andrology*. 2000;**23**:178-186. DOI: 10.1046/j.1365-2605.2000.00224.x
- [30] Galdiero F, Gorga F, Bentivoglio C, Mancuso R, Galdiero E, Tufano MA. The action of LPS porins and peptidoglycan fragments on human spermatozoa. *Infection*. 1988;**16**(6):349-353. DOI: 10.1007/BF01644545
- [31] Gorga F, Galdiero M, Buommino E, Galdiero E. Porins and lipopolysaccharide induce apoptosis in human spermatozoa. *Clinical and Diagnostic Laboratory Immunology*. 2001;**8**(11):206-208. DOI: 10.1128/CDLI.8.1.206-208.2001
- [32] Fujita Y, Mihara T, Okazaki T, Shitanaka M, Kushino R, Ikeda C, et al. Toll-like receptors (TLR) 2 and 4 on human sperm recognize bacterial endotoxins and mediate apoptosis. *Human Reproduction*. 2011;**26**(10):2799-2806. DOI: 10.1093/humrep/der234
- [33] Schulz M, Sanchez R, Soto L, Risopatron J, Villegas J. Effect of *Escherichia coli* and its soluble factors on mitochondrial membrane potential, phosphatidylserine translocation, viability, and motility of human spermatozoa. *Fertility and Sterility*. 2010;**94**(2):619-623. DOI: 10.1016/j.fertnstert.2009.01.140
- [34] Fraczek M, Piasecka M, Gaczarzewicz D, Szumala-Kakol A, Kazienko A, Lenart S, et al. Membrane stability and mitochondrial activity of human-ejaculated spermatozoa during in vitro experimental infection with *Escherichia coli*, *Staphylococcus haemolyticus* and *Bacteroides ureolyticus*. *Andrologia*. 2012;**44**(5):315-329. DOI: 10.1111/j.1439-0272.2012.01283.x

- [35] Espinoza JA, Schulz M, Sanchez R, Villegas J. Integrity of mitochondrial membrane potential reflects human sperm quality. *Andrologia*. 2009;**41**(1):51-54. DOI: 10.1111/j.1439-0272.2008.00878.x
- [36] Marchetti C, Jouy N, Leroy-Martin B, Deffosez A, Formstecher P, Marchetti P. Comparison of four fluorochromes for the detection of the inner mitochondrial membrane potential in human spermatozoa and their correlation with sperm motility. *Human Reproduction*. 2004;**19**(10):2267-2276. DOI: 10.1093/humrep/deh416
- [37] Boguen R, Uribe P, Treulen F, Villegas JV. Distinct isolates of uropathogenic *Escherichia coli* differentially affect human sperm parameters in vitro. *Andrologia*. 2014;**46**(8):943-947. DOI: 10.1111/and.12167
- [38] Wiles TJ, Kulesus RR, Mulvey MA. Origins and virulence mechanisms of uropathogenic *Escherichia coli*. *Experimental and Molecular Pathology*. 2008;**85**(1):11-19. DOI: 10.1016/j.yexmp.2008.03.007
- [39] Boguen R, Treulen F, Uribe P, Villegas JV. Ability of *Escherichia coli* to produce hemolysis leads to a greater pathogenic effect on human sperm. *Fertility and Sterility*. 2015;**103**(5):1155-1161. DOI: 10.1016/j.fertnstert.2015.01.044
- [40] Bhakdi S, Mackman N, Nicaud JM, Holland IB. *Escherichia coli* hemolysin may damage target cell membranes by generating transmembrane pores. *Infection and Immunity*. 1986;**52**(1):63-69
- [41] Wiles TJ, Dhakal BK, Eto DS, Mulvey MA. Inactivation of host Akt/protein kinase B signaling by bacterial pore-forming toxins. *Molecular Biology of the Cell*. 2008;**19**(4):1427-1438. DOI: 10.1091/mbc.E07-07-0638
- [42] Bhushan S, Hossain H, Lu Y, Geisler A, Tchatalbachev S, Mikulski Z, et al. Uropathogenic *E. coli* induce different immune response in testicular and peritoneal macrophages: implications for testicular immune privilege. *PLoS One*. 2011;**6**(12):e28452. DOI: 10.1371/journal.pone.0028452
- [43] Fraczek M, Wiland E, Piasecka M, Boksa M, Gaczarzewicz D, Szumala-Kakol A, et al. Fertilizing potential of ejaculated human spermatozoa during in vitro semen bacterial infection. *Fertility and Sterility*. 2014;**102**(3):711-719. e1. DOI: 10.1016/j.fertnstert.2014.06.002
- [44] Barbonetti A, Vassallo MR, Cinque B, Filipponi S, Mastromarino P, Cifone MG, et al. Soluble products of *Escherichia coli* induce mitochondrial dysfunction-related sperm membrane lipid peroxidation which is prevented by *Lactobacilli*. *PLoS One*. 2013;**8**(12):e83136. DOI: 10.1371/journal.pone.0083136
- [45] Paulson JD, Polakoski KL. Isolation of a spermatozoal immobilization factor from *Escherichia coli* filtrates. *Fertility and Sterility*. 1977;**28**(2):182-185.
- [46] Prabha V, Sandhu R, Kaur S, Kaur K, Sarwal A, Mavuduru RS, et al. Mechanism of sperm immobilization by *Escherichia coli*. *Advances in Urology*. 2010;**2010**:e240268. DOI: 10.1155/2010/240268

- [47] Kumar V, Prabha V, Kaur S, Kaur K, Singh SK. Receptor dependent immobilization of spermatozoa by sperm immobilization factor isolated from *Escherichia coli*: proof of evidence. International Journal of Urology. 2011;**18**(8):597-603. DOI: 10.1111/j.1442-2042.2011.02784.x
- [48] Kaur K, Prabha V. Sperm impairment by sperm agglutinating factor isolated from *Escherichia coli*: Receptor specific interactions. BioMed Research International. 2013;**2013**:548497. DOI: 10.1155/2013/548497
- [49] Lu Y, Bhushan S, Tchatalbachev S, Marconi M, Bergmann M, Weidner W, et al. Necrosis is the dominant cell death pathway in uropathogenic *Escherichia coli* elicited epididymo-orchitis and is responsible for damage of rat testis. PLoS One. 2013;**8**(1):e52919. DOI: 10.1371/journal.pone.0052919
- [50] Lang T, Dechant M, Sanchez V, Wistuba J, Boiani M, Pilatz A, et al. Structural and functional integrity of spermatozoa is compromised as a consequence of acute uropathogenic *E. coli*-associated epididymitis. Biology of Reproduction. 2013;**89**(3):59. DOI: 10.1095/biolreprod.113.110379
- [51] Kaur K, Prabha V. Spermagglutinating *Escherichia coli* and its role in infertility: In vivo study. Microbial Pathogenesis. 2014;**69-70**:33-38. DOI: 10.1016/j.micpath.2014.03.010