DE GRUYTER OPEN

Contemporary Agriculture
Vol. 66, No. 3 - 4, Pp. 22 - 31, 2017.

The Serbian Journal of Agricultural Sciences
ISSN (Online) 2466-4774 UDC: 63(497.1)(051)-"540.2"
www.contagri.info

Original scientific paper

UDC: 599.325.1
DOI:10.1515/contagri-2017-0015

IN VITRO EFFECTS OF ENTEROCOCCUS FAECALIS AND SELECTED BIOMOLECULES ON THE MOTILITY OF RABBIT SPERMATOZOA

Eva TVRDÁ♦, Michal ĎURAČKA, Marek HALENÁR, Attila KÁNTOR [1]

Summary: This study assessed the potential efficiency of selected biologically active substances on the motility behavior of rabbit spermatozoa subjected to in vitro induced E. faecalis contamination. Semen samples were collected from 10 male rabbits and the presence of E. faecalis was confirmed using MALDI-TOF Mass Spectrometry. For the in vitro experiments rabbit spermatozoa were resuspended in the presence of 0,3 McF E. faecalis and different concentrations of selected biomolecules (resveratrol - RES, quercetin - QUE, curcumin - CUR, epicatechin - EPI, isoquercitrin - IZO). Sperm motility was assessed using the computer-aided sperm analysis at 0h, 2h, 4h, 6h and 8h. The presence of E. faecalis significantly decreased the motility ($P<0.001$) when compared to the untreated Control starting at 2h and maintaining this negative impact throughout the entire in vitro culture. Meanwhile, the motility was significantly higher in the experimental samples subjected to E. faecalis together 5 µmol/L RES ($P<0.05$), 10 µmol/L QUE ($P<0.05$) as well as 1 µmol/L ($P<0.01$) and 10 µmol/L CUR ($P<0.05$) when compared to the Positive Control (4h). No biomolecule was able to maintain the motion comparable to the Negative Control, and none was effective against the rapid decline of sperm motility caused by the presence of E. faecalis during later stages of the in vitro experiment (6h and 8h). We may conclude that RES, QUE and CUR may provide a selective advantage to spermatozoa in the presence of E. faecalis, particularly during short-term rabbit semen handling.

Key words Enterococcus faecalis, spermatozoa, natural biomolecules, contamination, rabbit.

INTRODUCTION

Numerous reports have revealed that bacterial invasion could contribute to a decreased sperm quality visible in routine semen analysis (Villegas et al., 2005; Fraczek and Kurpisz, 2007; Ressing et al., 2007; Fraczek et al., 2012). Loss of sperm motility, morphological alterations, acrosome dysfunction, disruption of membrane integrity and oxidative stress have been repeatedly associated with the presence of bacteria in both in vivo and in vitro conditions.

Most data connected to bacterial contamination of ejaculates are focused on well-known causative agents of urogenital tract infections, such as Escherichia coli, Staphylococcus aureus, Ureaplasma urealyticum, Mycoplasma hominis and Chlamydia trachomatis. However, some authors have suggested that other bacteria, responsible for the colonization and contamination of the male urogenital tract, rather than infection, could also contribute to the decrease in sperm quality (Cottell et al., 2000; De Francesco et al., 2011; Fraczek et al., 2012).

The genus Enterococcus comprises Gram-positive, catalase-negative, non-spore-forming, facultative anaerobic bacteria that can occur both as single cocci or in chains. Enterococci belong to a group of microorganisms known as lactic acid bacteria (LAB) that produce bacteriocins. The origins of Enterococcus species may vary from environmental to animal or human sources. E. faecalis are the most common in the gastrointestinal tract, and may be found in human and animal feces (Fisher and Phillips, 2009). E. faecalis is the most common species associated with clinical urinary tract infections, hepatobiliary sepsis, endocarditis, surgical wound infection, bacteremia and neonatal sepsis (Poh et al., 2006). The ability of E. faecalis to survive a range of adverse environments allows multiple routes of cross-contamination of enterococci in causing disease, including those from food, environmental and hospital sources. Furthermore, E. faecalis has been shown to be resistant to a broad range of antibiotics including ampicillin, ciprofloxacin and imipenem (Billstrom et al., 2008).

[1]Eva Tvrdá, PhD, Junior Research Scientist; Michal Ďuračka, MS, PhD Student; Marek Halenár, PhD, Junior Research Scientist; Attila Kántor, PhD, Assistant Professor; Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Tr. A. Hlinku 2, 94976 Nitra, Slovakia.
♦Corresponding author: e-mail: evina.tvrda@gmail.com, Tel.: +412-37-641-4918.

Most ejaculates collected from otherwise healthy animals may be contaminated to a certain degree, as semen collection is not an entirely sterile process. Artificial vaginas often used in reproductive biotechnologies, followed by environmental conditions and human factors are among the most common reasons why microorganisms are frequently found in animal semen samples (Bielanski, 2007).

Antibiotics are added to semen extenders to be used for artificial insemination (AI) in livestock breeding to control bacterial contamination in semen arising during collection and processing. Since antibiotics may be toxic to spermatozoa, and because of an ever-increasing bacterial resistance to commonly used antibiotics, there is an urgent need to find alternatives to conventional antibiotics for use in animal reproduction science (Morrell and Wallgren, 2014).

Over the past years, the scientific society has witnessed a "reinassance" of naturally occurring compounds with potentially beneficial properties, rich chemical diversity, structural complexity and availability, lack of significant toxic effects and intrinsic biologic activity (Alarcón de la Rastra, 2008). Numerous flavonoid and polyphenolic compounds have been shown to possess anti-inflammatory, antibacterial and antioxidant properties which could potentially provide a selective advantage to male reproductive cells under stress conditions (Park et al., 2015; Tvrdá et al., 2016a;b).

In this study, we followed a systematic approach to assess the in vitro effects of a wide range of natural biologically active compounds (resveratrol, quercetin, curcumin, epicatechin, isoquercitrin) on the motility behavior of rabbit spermatozoa subjected to in vitro induced E. faecalis contamination.

MATERIAL AND METHODS

Sample collection. Ten male rabbits (New Zealand white broiler line) were used in the experiment. The animals were 4 months old, with a weight of 4.0±0.2 kg and kept at an experimental farm of the Animal Production Research Centre Nitra, Slovak Republic. The rabbits were housed in a partially air-conditioned rabbit house under a photoperiod of 16L:8D (a minimum light intensity of 80 lux), kept in individual cages and fed with a commercial diet. Water was provided ad libitum. The air temperature of 20-24 °C and relative humidity of 65% were maintained in the rabbit house. Institutional and national guidelines on the care and use of animals were followed, and all the experimental procedures were approved by the State Veterinary and Food Institute of Slovak Republic (no. 3398/11-221/3) and Ethics Committee. One ejaculate was collected from each rabbit using an artificial vagina. Immediately upon collection, the sperm concentration and motility were assessed in each ejaculate and samples were transferred to the laboratory.

Cultivation of microorganisms. Hundred µl of each semen sample were transferred into the MacConkey agar (Biomark, Pune) and MRS agar (Biolife, Italy). The cultures were maintained at 37°C during 24h for microorganisms which grew on the MacConkey agar and 37°C during 48-72h for microorganisms which grew on the MRS agar. Purification of all microorganisms was done by four ways streak plate method after the first cultivation. The Chromogenic colifrom agar (Oxoid, England) and the URI Select IV (Biolife, Italy) were subsequently used to purify those microorganisms which contaminated the MacConkey agar. Microorganisms which contaminated the MRS agar were repeatedly purified in the MRS agar. All steps of re-cultivation were done at the same conditions (Hleba et al., 2017).

Identification of microorganisms. Matrix-assisted laser desorption/ionization time-of-light (MALDI TOF MS) (Brucker Daltonics, Germany) was used for bacterial identification in the semen samples. Cells from a single colony of fresh overnight culture were used for each isolate to prepare samples according to the manufacturer's recommendations for microorganism profiling using the ethanol-formic acid extraction procedure. Each sample spot was overlaid with 2 µl of matrix solution (saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile with 2.5% trifluoroacetic acid) and again air-dried for 15 min. To identify the microorganisms, raw spectra obtained for each isolate were imported into the Biotyper software, version 2.0 (Brucker Daltonics, Germany), and analyzed without any user intervention (Hleba et al., 2017). E. faecalis was identified in all semen samples. The isolated E. faecalis was aseptically transferred to the culture medium selected for the in vitro experiments and cultured at 36°C for 24 to 48h. Following culture, E. faecalis concentration was adjusted to 0.3 McF using a densitometer (DEN–1 McFarland Densitometer, Grant-bio, UK). Such inoculum was suitable for the simulation of an in vivo environment under in vitro conditions taking into consideration an ideal environment for the sperm cells as well as the bacterium.

In vitro experiments. One ejaculate was collected from 10 male rabbits used for previous in vivo experiments on a regular collection schedule (twice a week for two consecutive weeks) using an artificial vagina. Immediately upon collection, the sperm concentration and motility were assessed in each ejaculate. Only samples with a minimum motility of 60% were used in the experiments. Individual ejaculates were mixed together in order to acquire a pooled sample of rabbit semen.

The resulting semen sample was centrifuged (300 x g) at 25°C for 5 min, seminal plasma was removed and the sperm pellet was washed twice with PBS (Dulbecco's phosphate-buffered saline without calcium chloride and magnesium chloride; Sigma-Aldrich, St. Louis, MO, USA), resuspended in a culture medium consisting of PBS, mineral supplements for semen cultures (Minitube, Tiefenbach, Germany), 5% glucose (Centralchem, Bratislava, Slovak Republic) and 4% BSA (bovine serum albumin, Sigma-Aldrich) using a dilution ratio of 1:20. Two controls were established – the Negative Control was resuspended in the culture medium exclusively, while the Positive Control contained the culture medium with 0,3 McF E. faecalis. Each experimental group was exposed to the bacterium and different concentrations of chosen biomolecules as follows:

- 50, 10 and 5 μmol/L resveratrol (RES; Sigma-Aldrich),
- 50, 10 and 5 μmol/L quercetin (QUE; Sigma-Aldrich),
- 10, 5 and 1 μmol/L curcumin (CUR; Sigma-Aldrich),
- 100, 50 and 10 μmol/L epicatechin (EPI; Sigma-Aldrich),
- 100, 50 and 10 μmol/L isoquercitrin (IZO; provided by the Center of Biocatalysis and Biotransformation, Czech Academy of Sciences).

At culture times of 0h, 2h, 4h, 6h and 8h, the spermatozoa motility (percentage of motile spermatozoa; motility > 5 μm/s; %) was assessed using the computer-aided sperm analysis (CASA; Version 14.0 TOX IVOS II.; Hamilton-Thorne Biosciences, Beverly, MA, USA). The samples were stained using the IDENT stain, a DNA-specific dye based on Hoechst bisbenzimide (Hamilton-Thorne Biosciences). The IDENT dye provided in Eppendorf tube was diluted with 1 ml of the culture medium and mixed with the sample using a ratio of 1:1. Following a 10 min incubation in the dark, the sample was analyzed under fluorescent illumination. The system was set up as follows: frame rate - 30 at 60 Hz, dark field; minimum contrast - 50; static head size - 0.28-4.30; static head intensity - 0.12-2.92; static elongation - 8-97; minimum cell size - 7 pixels; default cell intensity - 70, magnification - 1.75, illumination intensity - 2198. Ten μl of each sample were placed into the Makler counting chamber (depth 10 μm, 37 °C; Sefi Medical Instruments, Haifa, Israel) and immediately assessed. Ten microscopic fields were subjected to each analysis in order to include at least 300 cells.

Statistical analysis. All the data were subjected to statistical analysis using the GraphPad Prism program (3.02 version for Windows, GraphPad Software incorporated, San Diego, California, USA, http://www.graphpad.com/). The results are quoted as the arithmetic mean ± standard error of mean (SEM). The comparative analysis was carried out by a one-way ANOVA with the Dunnett's post test. The level of significance for the analysis was set at * $P<0.05$; ** $P<0.01$; *** $P<0.001$. The comparative analysis was performed as follows:

- Positive Control (PC) was compared to the Negative Control (NC),
- Experimental fractions exposed to E. faecalis and biomolecules were compared to both Controls.

RESULTS

The initial (Time 0h) CASA assessment is summarized in Figure 1. The initial (Time 0h) MOT was lower in the Positive Control (78.12±2.22%) when compared to the Negative Control (81.79±3.02%), although without any statistical significance ($P>0.05$). Furthermore, the initial MOT was insignificantly lower in groups exposed to E. faecalis together with 100 μmol/L RES (70.55±1.98%) or QUE (76.67±3.00%) as well as all selected concentrations of IZO (76.90±23% in case of 100 μmol/L IZO; 72.35±0.99% with respect to 50 μmol/L IZO; 69.70±2.18% in relation to 10 μmol/L IZO). In case of the remaining concentrations of biomolecules, the motility was similar to both Controls.

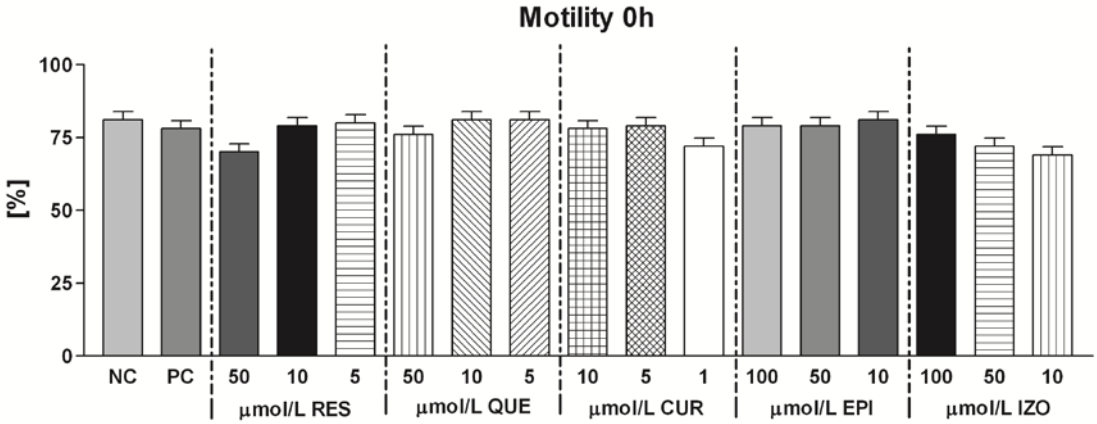

Figure 1. Immediate effects (Time 0h) of E. faecalis and selected biomolecules on rabbit spermatozoa motility [%]. Mean ± SEM. * P<0.05; ** P<0.01; *** P<0.001.

After 2h the CASA analysis showed that the presence of E. faecalis significantly decreased the rabbit sperm motility (36.07±1.40%; P<0.001) when compared to the untreated Control (72.27±3.05%). Meanwhile, the motility at Time 2h was significantly higher in the experimental samples subjected to E. faecalis together with 5 and 50 µmol/L RES (62.29±2.57% with respect to 5 µmol/L RES and 70.11±1.97% in case of 50 µmol/L RES; P<0.001), as well as 1 µmol/L (56.44±2.07%; P<0.001) and 10 µmol/L CUR (65.37±1.27%; P<0.01) in comparison to the Control exposed to the bacterium exclusively. Meanwhile, the MOT decreased abruptly in the experimental groups co-exposed to E. faecalis together with 50 µmol/L (5.77±0.17%; P<0.001) and 100 µmol/L IZO (10.13±0.39%; P<0.001) revealing toxic effects of IZO on the sperm behavior (Figure 2).

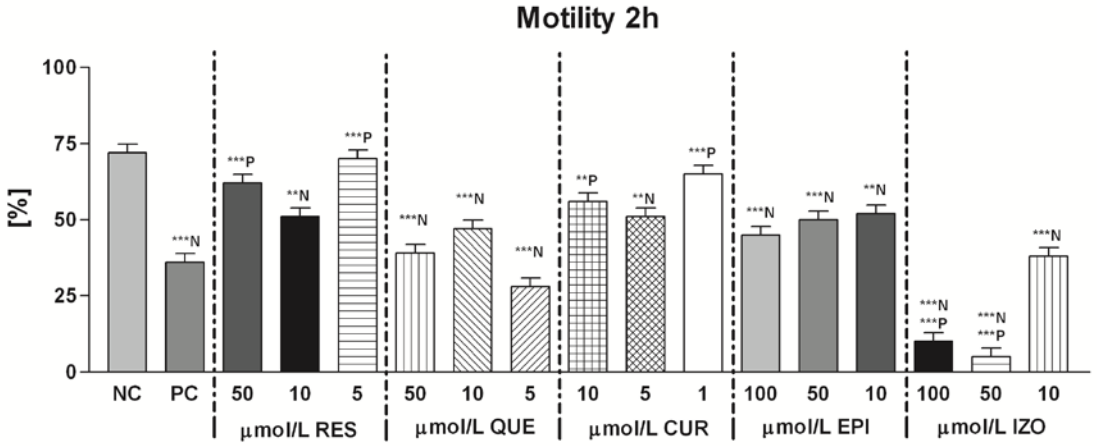

Figure 2. The effects of E. faecalis and selected biomolecules on rabbit spermatozoa motility following 2 hours of in vitro culture [%]. Mean ± SEM. Mean ± SEM. * P<0.05; ** P<0.01; *** P<0.001. N – vs. Negative (untreated) Control. P – vs. Positive Control (exposed to E. faecalis exclusively).

After 4h, the highest sperm motility was detected in the Negative Control (40.14±2.00%), while the MOT in the Positive Control was significantly lower in comparison with the untreated Control group (11.17±1.09%; P<0.001). While the motion in all experimental groups was lower when compared to the Negative Control, the parameter remained significantly higher in the groups co-treated with the inoculum and 5 µmol/L RES (27.88±2.02%; P<0.05), 10 µmol/L QUE (26.56±1.79%; P<0.05) as well as 1 µmol/L (27.97±2.10%; P<0.01) and 10 µmol/L CUR (30.34±3.00%; P<0.05) when compared to the Positive Control. In the meantime, the MOT was the lowest in the

experimental groups co-exposed to E. faecalis and 1 µmol/L QUE (1.23±0.12%; P<0.001) as well as all selected IZO concentrations (0.00±0.00% in case of 50 and 100 µmol/L IZO; 5.55±0.18% with respect to 10 µmol/L IZO; P<0.001) in comparison to the Negative Control (Figure 3).

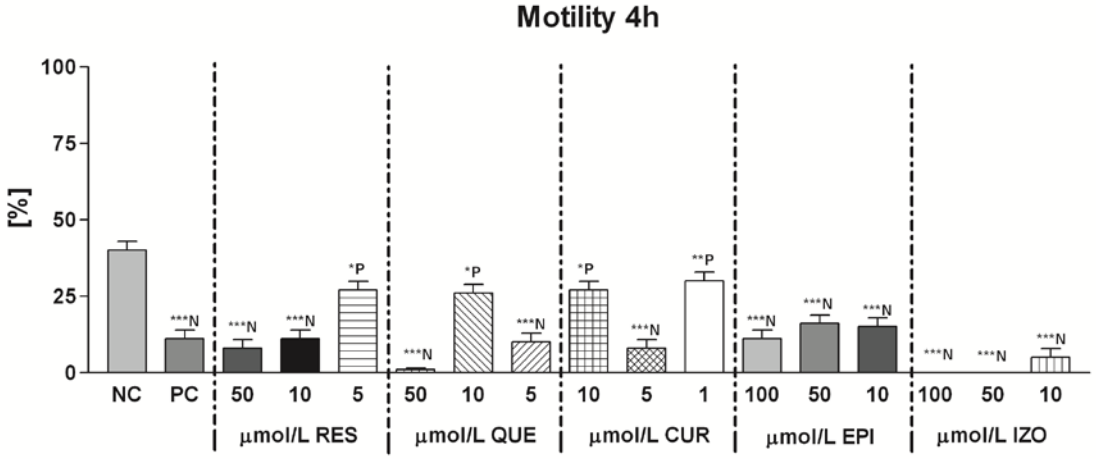

Figure 3. The effects of E. faecalis and selected biomolecules on rabbit spermatozoa motility following 4 hours of in vitro culture [%]. Mean ± SEM. Mean ± SEM. * P<0.05; ** P<0.01; *** P<0.001. N – vs. Negative (untreated) Control. P – vs. Positive Control (exposed to E. faecalis exclusively).

Following 6h, the highest spermatozoa motion was detected in the untreated Negative Control (20.34±1.90%). Spermatozoa exposed to the bacterium exclusively exhibited very little to no active movement (1.01±0.05%; P<0.001 when compared to the Negative control; Figure 4). At the same time, the sperm motility was significantly decreased in all experimental groups when compared to the Negative Control (P<0.001) while no significant differences were observed in comparison with the Positive Control (P>0.05). Although insignificant, a slightly increased MOT was observed in groups following co-incubation of spermatozoa, E. faecalis, 5 µmol/L (6.55±0.75%) and 10 µmol/L RES (7.02±0.90%) as well as 10 µmol/L QUE (3.39±0.71%) and 1 µmol/L CUR (3.92±0.60%; P>0.05; Figure 4).

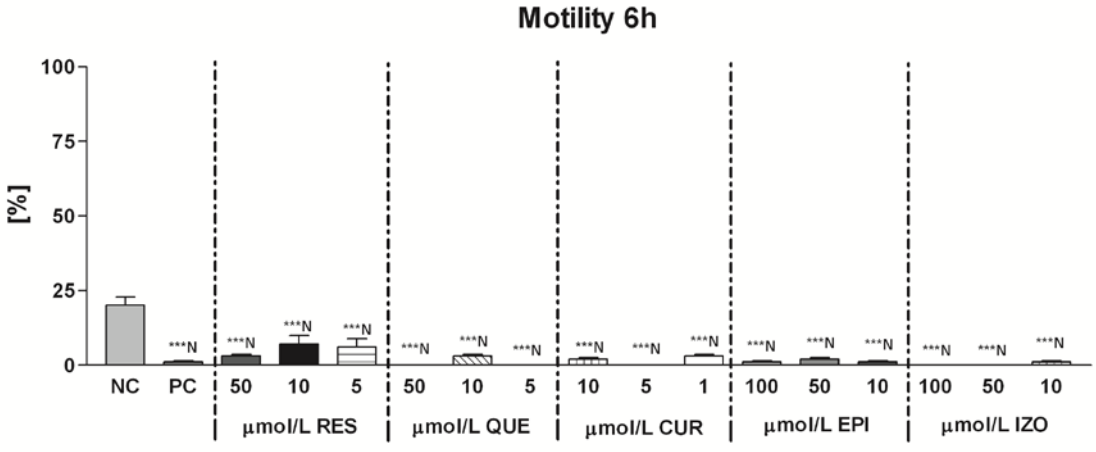

Figure 4. The effects of E. faecalis and selected biomolecules on rabbit spermatozoa motility following 6 hours of in vitro culture [%]. Mean ± SEM. Mean ± SEM. * P<0.05; ** P<0.01; *** P<0.001. N – vs. Negative (untreated) Control. P – vs. Positive Control (exposed to E. faecalis exclusively).

The final (Time 8h) assessment revealed that the only group containing actively moving spermatozoa was the Negative Control (6.77±0.90%). No moving spermatozoa were detected in the Positive Control (0.00±0.00%). At the same time, none of the concentrations of biomolecules applied was able to maintain the sperm motility comparable to the motion parameters detected in the Negative Control, and none was effective against the rapid decline of sperm motility caused by the presence of E. faecalis (0.00±0.00%; Figure 5).

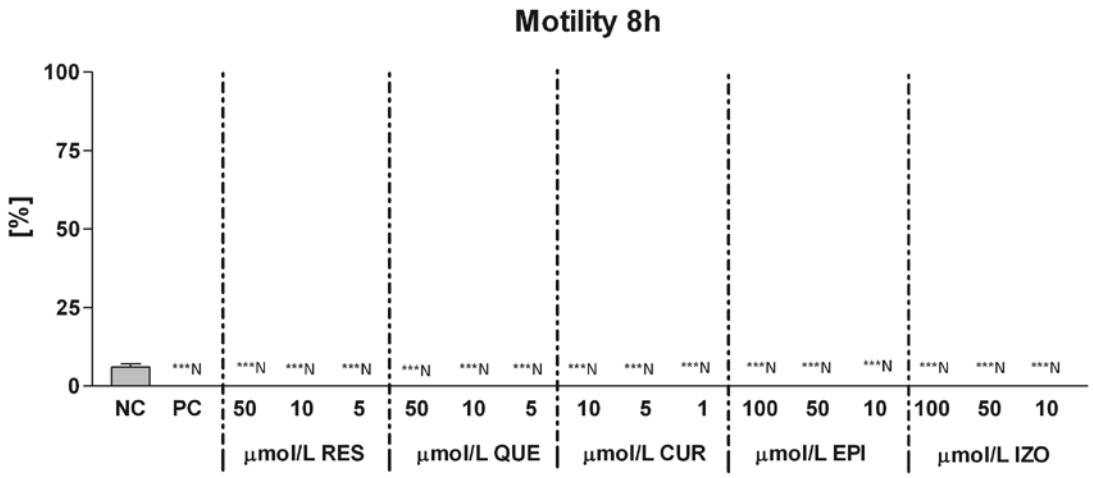

Figure 5. The effects of E. faecalis and selected biomolecules on rabbit spermatozoa motility following 8 hours of in vitro culture [%]. Mean ± SEM. Mean ± SEM. * P<0.05; ** P<0.01; *** P<0.001. N – vs. Negative (untreated) Control. P – vs. Positive Control (exposed to E. faecalis exclusively).

DISCUSSION

Microorganisms such as bacteria, mycoplasma and chlamydia have been repeatedly isolated from human and animal semen (Keck et al., 1998; Cottell et al., 2000; Mehta et al., 2002). Mycoplasma and chlamydia are well known to have detrimental effects on male reproductive performance (Shalika et al., 1996), while the presence of anaerobic bacteria is believed not to significantly affect male fertility (Eggert-Kruse et al., 1995). Meanwhile, the role of aerobic bacteria in semen remains unclear. Some reports suggest that the presence of bacteria in semen is mainly due to contamination (Cottell et al., 2000), while other have revealed an increased incidence of abnormal semen in the presence of bacteria (Mehta et al., 2002; Fraczek et al., 2012).

During bacterial contamination of semen, the loss of sperm motility and normal morphology may be resulting from adhesion and sperm agglutination. The sperm surface is rich in glycoproteins and hence susceptible to bacteria-spermatozoa interactions at the level of receptors and ligands (Monga and Roberts, 1994; Moretti et al., 2008). Harmful effects of microbial contamination may furthermore result from the expression of other surface virulent factors, such as lipopolysaccharides (LPS), cytotoxic necrotizing factor, α- or ß-haemolysins, or from soluble spermatotoxic factors such as sperm immobilization factor (Prabha et al., 2011; Agarwal et al., 2012). It is also well known that bacteria stimulate reactive oxygen species (ROS) production in semen (Wang et al., 1997). Lastly, the involvement of bacteria in apoptotic phenomena in ejaculated spermatozoa may be mediated through a direct contact of bacteria and their toxins with spermatozoa, which is considered to be an initial signal for sperm death (Villegas et al., 2005; Fraczek et al., 2015).

E. faecalis has been identified in normal intestinal flora, and it has also been isolated from the vagina and urethra. On the other hand, the bacterium can cause urinary tract infections, endocarditis and endopthalmitis (Billstrom et al., 2008). E. faecalis has recently emerged as an important microbial contaminant of ejaculates. Similarly to our MALDI TOF data, E. faecalis was isolated from semen by other investigators (Merino et al., 1995; Shalika et al., 1996; Villanueva-Diaz et al., 1999). Villanueva-Diaz et al. (1999) isolated E. faecalis from 43% of semen samples studied. Shalika et al. (1996) isolated enterococci from 73% of the infected semen samples. On the other hand, Mehta et al. (2002) showed that the presence of E. faecalis was associated with a high incidence of oligozoospermia and teratozoospermia. The mean sperm concentration, as well as the incidence of morphologically normal spermatozoa, was also lower in semen samples infected with E. faecalis when compared with subjects infected with micrococci or α-haemolytic streptococci as well as uninfected samples, suggesting a strong negative effect of E.

faecalis on the most important markers of semen quality in practical settings. Similarly to our in vitro results, Merino et al. (1995) observed that sperm motility and vitality were significantly compromised in the presence of bacteria. On the contrary, Huwe et al. (1997) studied the effect of different bacterial species on sperm motility parameters and reported that Enterococcus did not have any effect on the sperm motility. Possible associations between other microbial species isolated and semen quality were not evaluated, as these species were detected only in 6% of the infected semen samples.

Semen processing for reproductive biotechnologies routinely involves the use of culture medium, which contains antibiotics. Processing of infected semen with antibiotic-rich medium may effectively eliminate 95% of the present microorganisms (Cottell et al., 2000), however a large number of antibiotics have been shown to exhibit toxic effects on the sperm motility, viability and DNA integrity. As such, there is an urgent need to search for alternative substances preferably with antibacterial properties which could provide a selective advantage to male reproductive cells against the stress resulting from microbial contamination of semen (Morrell and Wallgren, 2014).

RES has been recently discovered to possess a wide range of cardiovascular, anticancer, antiinflamatory and protective effects (Calabrese et al., 2010). Our CASA results are contradictory to Collodel et al. (2010) who evaluated the effects of RES on human spermatozoa. Unlike our results, human sperm cells were more sensitive to the potentially toxic effect of RES with a LD50 between 30 and 50 μmol/L. Moreover, Tvrdá et al. (2015b) reported a significant decrease of bull sperm motility following exposure to 100 or 200 μmol/L RES during a 24 in vitro culture. The differences in our results may be explained by the donor species, as well as a shorter exposure to the biomolecules. On the contrary, protective effects of lower RES concentrations on spermatozoa are in agreement with Tvrdá et al. (2015a;b) who emphasize on the beneficial effects of a concentration range of 5-50 μmol/L RES on the motion behavior, mitochondrial activity and intracellular superoxide production by bovine sperm cells. Our data are in agreement with Mojica-Villegas et al. (2014) who reported that a pretreatment with 15 μmol/L RES 15 min prior to incubation with ferrous ascorbate (FeAA) showed an 8.0-fold increase in murine spermatozoa motility. Similar results were reported in studies focused on swim-up selected human spermatozoa where low RES doses led to a higher progressive motility (Collodel et al., 2010).

QUE is a common dietary flavonoid, reported to exhibit a broad variety of favorable biological effects (Aherne and O'Brien, 2000). Protective effects of QUE on the rabbit spermatozoa in our study disagrees with earlier reports, according to which QUE compromised human sperm motility (at 5–200 μmol/L QUE) and viability (50-100 μmol/L QUE), associated with Ca2+-ATPase downregulation, resulting in the loss of sperm motion (Khanduja et al., 2001). Talking in favor of our results, Tvrdá et al. (2014; 2016b) emphasized on the protective effects of QUE on bovine sperm motion activity when incubated over a period of 24 h (Tvrdá et al., 2014) or without the presence of seminal plasma (Tvrdá et al., 2016b), although we must take into consideration that QUE may act dose dependently as either a stimulant at low concentrations or as an inhibitor at high doses. Mazzi et al. (2011) and Moretti et al. (2012) compared the potential of QUE and RES on human sperm incubated with tert-butylhydroperoxide. Although QUE showed a higher toxicity with respect to the sperm motility and viability than RES, its antioxidant activity was stronger. QUE was particularly active in preserving sperm membranes, chromatin texture and acrosomes, which were compromised by TBHP. Similarly, Tvrdá et al. (2016b) found that QUE was capable to prevent the decline of spermatozoa vitality and functional activity as a consequence of FeAA-associated oxidative damage.

Previous reports on the impact of CUR on male fertility are controversial. Naz (2011) report that exposure of human and murine sperm to CUR caused a concentration-dependent decrease of sperm motility, capacitation and acrosome reaction. At high CUR concentrations, a complete inhibiton of spermatozoa motility and function was observed. Moreover Salashoor et al. (2012) reported that increasing doses of CUR significantly increased the sperm concentration, motility and testosterone content in rats. On the contrary, our results agree with Salashoor et al. (2012) and Tvrdá et al. (2016a) suggesting stimulating and protective effects of CUR on spermatozoa motility and antioxidant status. Motility parameters recorded by our IDENT CASA technique complement previous findings by Bucak et al. (2008; 2010) demonstrating a significant motion improvement of ram spermatozoa supplemented with CUR. Interestingly, their later study focused on CUR administration to bovine semen led to non-significant differences in the sperm motion (Bucak et al., 2012). Nevertheless, our results correlate with the report by Soleimanzadeh and Saberivand (2013) as well as Tvrda et al. (2016b), where CUR addition had a positive impact on both sperm motility and viability.

EPI is a flavonoid and antioxidant commonly found in green tea and cocoa (Katz et al., 2011). Jamalan et al. (2016) evaluated the effects of different flavonoids including EPI on the recovery of sperm motility and prevention of membrane damage from aluminum chloride, cadmium chloride, and lead chloride. Similarly to our study, the report revealed that EPI behaved in an unexpected manner as it did not protect spermatozoa from heavy metal-mediated damage, nor it did not exhibit any protective effects, rather, it showed inhibitory effects on the sperm MOT associated with a co-incubation with selected heavy metals. Following the administration of increased EPI concentrations from 0 to 1000 μmol/L, a gradual and dose-dependent decrease in sperm motility compared to the

untreated control group was observed. Similarly, Moretti et al. (2012) showed that none of the selected EPI concentrations selected (20, 30, 50, 100, 200, 400 μmol/L) was not effective as an antioxidant to protect swim-up selected human sperm against tert-butylhydroperoxide toxicity. On the contrary Purdy et al. (2004) revealed that EPI may aid in maintaining the motility of cooled goat sperm in a dose dependent manner.

IZO is found in foods such as apple and onion, as well as in a variety of medicinal plants, likely contributing to the pharmacological qualities of a large number of botanical medicines (Appleton, 2010). To our knowledge, no study is currently available on the impact of IZO on male reproduction. According to our data, although being structurally similar to QUE, the molecule did not exhibit beneficial effects on the sperm MOT. On the other hand, although experiments based on the administration of pure IZO have not been done yet, numerous animal studies emphasize on potential ameliorative and antioxidant effect of plant extracts containing IZO on the testicular structure and function, as well as sperm concentration, motility and morphology in sickness and health (Awoniyi et al., 2011; Ayeleso et al., 2014). As such, we may suggest that more specific experiments on the roles of IZO are to be designed in order to elucidate its beneficial and/or harmful roles in male reproduction.

CONCLUSION

Based on our preliminary CASA results we may conclude that resveratrol, quercetin and curcumin exhibit antibacterial properties providing a selective advantage to the male gametes in the presence of Enterococcus faecalis, particularly during short-term rabbit semen handling. On the other hand, epicatechin and isoquercitrin did not prove to possess significant protective or beneficial effects on the in vitro survival of rabbit spermatozoa in the presence of uropathogenic bacteria. Last, but not least more experiments will be necessary to unravel specific molecular mechanisms of action of E. faecalis and/or natural biomolecules on the structure and function of male reproductive cells.

ACKNOWLEDGEMENT

This research is a part of project supporting by the Research Center AgroBioTech built in accordance with the project Building Research Centre "AgroBioTech" ITMS 26220220180 and by the Slovak Research and Development Agency Grants no. APVV-0304-12 and APVV-15-0544. The authors are grateful to Prof. Dr. Vladimir Kren, FRSC from the Center of Biocatalysis and Biotransformation, Czech Academy of Sciences, for the provision of isoquercitrin.

REFERENCES

AGARWAL, J., SRIVASTAVA, S., SINGH, M.: Pathogenomics of uropathogenic Escherichia coli. Indian Journal of Medical Microbiology, 30:141-149, 2012.

AHERNE, SA., O'BRIEN, NM.: Mechanism of protection by the flavonoids, quercetin and rutin, against tert-butylhydroperoxide- and menadione-induced DNA single strand breaks in Caco-2 cells. Free Radical Biology and Medicine, 29:507-514, 2000.

ALARCÓN DE LA RASTRA C.: Curcumin: a promising spice for therapeutics. Molecular Nutrition & Food Research, 52:985, 2008.

APPLETON, J.: Evaluating the bioavailability of isoquercetin. Journal of Natural Medicines, 2:1-4, 2010.

AWONIYI, DO., ABOUA, YG., MARNEWICK, JL., DU PLESIS, SS., BROOKS, NL.: Protective effects of rooibos (Aspalathus linearis), green tea (Camellia sinensis) and commercial supplements on testicular tissue of oxidative stress induced rats. African Journal of Biotechnology, 10:17317-17322, 2011.

AYELESO, AO., OGUNTIBEJU, OO., ABOUA, YG., BROOKS, NL.: Effects of red palm oil and rooibos on sperm motility parameters in streptozotocin-induced diabetic rats. African Journal of Traditional, Complementary and Alternative Medicines, 11:8-15, 2014.

BIELANSKI, A.: Disinfection procedures for controlling microorganisms in the semen and embryos of humans and farm animals. Theriogenology, 68:1-22, 2007.

BILLSTROM, H., LUND, B., SULLIVAN, A., NORD, CE.: Virulence and antimicrobial resistance in clinical Enterococcus faecium. International Journal of Antimicrobial Agents, 32:374-377, 2008.

BUCAK, MN., ATESSAHIN, A., YUCE, A.: Effect of antioxidants and oxidative stress parameters on ram semen after the freeze-thawing process. Small Ruminant Research, 75:128-134, 2008.

BUCAK, MN., SARIOZKAN, S., TUNCER, PB., SAKIN, F., ATESSAHIN, A., KULAKSIZ, R., CEVIK, M.: The effect of antioxidants on post-thawed Angora goat (Capra hircus ancryrensis) sperm parameters, lipid peroxidation and antioxidant activities. Small Ruminant Research, 89:24-30, 2010.

BUCAK, MN., BASSPINAR, N., TUNCER, BP., COYAN, K., SARIOZKAN, S., AKALIN, PP., BUYUKLEBLEBICI, S., KUCUKGUNAY, S.: Effects of curcumin and dithioerythritol on frozen-thawed bovine semen. Andrologia, 44:102-109, 2012.

CALABRESE, EJ., MATTSON, MP., CALABRESE, V.: Dose response biology: the case of resveratrol. Human & Experimental Toxicology, 29:1034-1037, 2010.

COLLODEL, G., FEDERICO, MG., GEMINIANI, M., MARTINI, S., BONECHI, C., ROSSI, C., FIGURA, N., MORETTI, E.: Effect of trans-resveratrol on induced oxidative stress in human sperm and in rat germinal cells. Reproductive Toxicology, 31:239-246, 2010.
COTTELL, E., HARRISON, RF., MCCAFFREY, M., WALSH, T., MALLON, E., BARRY-KINSELLA, C.: Are seminal fluid microorganisms of significance or merely contaminants? Fertility and Sterility, 74:465-470, 2000.
DE FRANCESCO, MA., NEGRINI, R., RAVIZZOLA, G., GALLI, P., MANCA, N.: Bacterial species present in the lower male genital tract: a five-year retrospective study. European Journal of Contraception & Reproductive Health Care, 16:47-53, 2011.
EGGERT-KRUSE, W., ROHR, G., STROCK, W., POHL, S., SCHWALBACH, B., RUNNEBAUM, B.: Anaerobes in ejaculates of sub fertile men. Human Reproduction Update 1:462-478, 1995.
FISHER, K., PHILLIPS, C.: The ecology, epidemiology and virulence of Enterococcus. Microbiology, 155:1749-1757, 2009.
FRACZEK, M., HRYHOROWICZ, M., GACZARZEWICZ, D., SZUMALA-KAKOL, A., KOLANOWSKI, TJ., BEUTIN L., KURPISZC M.: Can apoptosis and necrosis coexist in ejaculated human spermatozoa during in vitro semen bacterial infection? Journal of Assisted Reproduction and Genetics, 32:771-779, 2015.
FRACZEK, M., KURPISZ, M.: Inflammatory mediators exert toxic effects of oxidative stress on human spermatozoa. Journal of Andrology, 28:325-333, 2007.
FRACZEK, M., PIASECKA, M., GACZARZEWICZ, D., SZUMALA-KAKOL, A., KAZIENKO, A., LENART, S., LASZCZYNSKA, M., KURPISZ, M.: Membrane stability and mitochondrial activity of human-ejaculated spermatozoa during in vitro experimental infection with Escherichia coli, Staphylococcus haemolyticus and Bacteroides ureolyticus. Andrologia, 44:315-329, 2012.
HLEBA, L., KMEŤ, V., TÓTH, T., KAČÁNIOVÁ M.: Resistance in bacteria and indirect beta-lactamase detection in E. coli isolated from Culex pipiens detected by matrix-assisted laser desorption ionization time of flight mass spectrometry. Journal of Environmental Science and Health B, 52:64-69, 2017.
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, 30: 55-59, 1998.
JAMALAN, M., GHAFFARI, MA., HOSEINZADEH, P., HASHEMITABAR, M., ZEINALI, M.: Human sperm quality and metal toxicants: protective effects of some flavonoids on male reproductive function. International Journal of Fertility and Sterility, 10:215-223, 2016.
KATZ, DL., DOUGHTY, K., ALI, A.: Cocoa and chocolate in human health and disease. Antioxidants & Redox Signaling, 15:2779-2811, 2011.
KECK, C., GERBER-SCHÄFER, C., CLAD, A., WILHELM, C., BRECKWOLDT, M.: Seminal tract infections: impact on male fertility and treatment options. Human Reproduction Update, 4:891-903, 1998.
KHANDUJA, KL., VERMA, A., BHARDWAJ, A.: Impairment of human sperm motility and viability by quercetin is independent of lipid peroxidation. Andrologia, 33:277-281, 2001.
KÖHN, FM., ERDMANN, I., OEDA, T., EL-MULLA, KF., SCHIEFER, HG., SCHILL, WB.: Influence of urogenital infections on sperm functions. Andrologia, 30:73-80, 1998.
MAZZI, L., GEMINIANI, M., COLLODEL, G., IACOPONI, G., MARTINI, S., BOENCHI, C., ROSSI, C., MORETTI, E.: Quercetin and rutin: effects of two flavonoids on induced oxidative stress in human ejaculated sperm. Journal of the Siena Academy of Sciences, 3:22-26, 2011.
MEHTA, RH., SRIDHAR, H., VIJAY KUMAR, BR., ANAND KUMAR, TC.: High incidence of oligozoospermia and teratozoospermia in human semen infected with the aerobic bacterium Streptococcus faecalis. Reproductive Biomedicine Online, 5:17-21, 2002.
MERINO, G., CARRANZA-LIRA, S., MURRIETA, S., RODRIGUEZ, L., CUEVAS, E., MORÁN, C.: Bacterial infection and semen characteristics in infertile men. Archives of Andrology, 35:43-47, 1995.
MOJICA-VILLEGAS, MA., IZQUIERDO-VEGA, JA., CHAMORRO CEVALLOS, G., SANCHEZ-GUITERREZ, M.: Protective effect of resveratrol on biomarkers of oxidative stress induced by iron/ascorbate in mouse spermatozoa. Nutrients, 6:489-503, 2014.
MONGA, M., ROBERTS, JA.: Sperm agglutination by bacteria: receptor-specific interactions. Journal of Andrology, 15:151-156, 1994.
MORRELL, JM., WALLGREN, M.: Alternatives to antibiotics in semen extenders: a review. Pathogens. 3, 934-946, 2014.
MORETTI, E., CAPITANI, S., FIGURA, N., PAMMOLLI, A., FEDERICO, M.G., GIANNERINI, V., COLLODEL, G.: The presence of bacteria species in semen and sperm quality. Journal of Assisted Reproduction and Genetics, 26:47-56, 2008.
MORETTI, E., MAZZI, L., TERZUOLI, G., BONECHI, C., IACOPONI, F., MARTINI, S., ROSSI, C., COLLODEL, G.: Effect of quercetin, rutin, naringenin and epicatechin on lipid peroxidation induced in human sperm. Reproductive Toxicology, 34, 651-657, 2012.
NAZ, RJ.: Can curcumin provide an ideal contraceptive? Molecular Reproduction and Development, 78:116-123, 2011.
PARK, S., CHA, SH., CHO, I., PARK, S., PARK, Y., CHO, S., PARK, Y.: Antibacterial nanocarriers of resveratrol with gold and silver nanoparticles. Materials Science and Engineering C: Materials for Biological Applications, 58:1160-1169, 2015.
POH, CH., OH, HML., TAN, AL.: Epidemiology and clinical outcome of enterococcal bacteraemia in an acute care hospital. Journal of Infection, 52:383-386, 2006.
PRABHA, V., CHAUDHARY, N., KAUR, S.: Molecular mimicry between spermatozoa and bacteria. Journal of Urology, 186:2442-2447, 2011.
PURDY, PH., ERICSSON, SA., DODSON, RE., STERNES, KL., GARNER, DL.: Effects of the flavonoids, silibinin and catechin, on the motility of extended cooled caprine sperm. Small Ruminant Research, 55:239-243, 2004.

RESSING, H., RESSING, M., DURANCE, T.: Modelling the mechanisms of dough puffing during vacuum microwave drying using the finite element method. Journal of Food Engineering, 82:498-508, 2007.
SALAHSHOOR, MR., JALILI, C., KHAZAEI, M., KHANI, F.: Effects of curcumin on reproductive parameters in male mice. Journal of Clinical Research in Paramedical Sciences, 1:1-3, 2012.
SHALIKA, S., DUGAN, K., SMITH, RD., PADILLA, SL.: The effect of positive semen bacterial and Ureaplasma cultures on in vitro fertilization success. Human Reproduction, 1:2789-2792, 1996.
SOLEIMANZADEH, A., SABERIVAND, A.: Effect of curcumin on rat sperm morphology after the freeze-thawing process. Veterinary Research Forum, 4:185-189, 2013.
TVRDÁ, E., LUKÁČ, N., LUKÁČOVÁ, J., JAMBOR, T., HASHIM, F., MASSÁNYI, P.: Dose-and time-dependent in vitro effects of quercetin on bovine spermatozoa activity and superoxide production. Folia Veterinaria, 58: 224-231, 2014.
TVRDÁ, E., KOVÁČIK, A., TUŠIMOVÁ, E., MASSÁNYI, P., LUKÁČ, N.: Resveratrol offers protection to oxidative stress induced by ferrous ascorbate in bovine spermatozoa. Journal of Environmental Science and Health Part A, 50:1440-1451, 2015a.
TVRDÁ, E., LUKÁČ, N., LUKÁČOVÁ, J., HASHIM, F., MASSÁNYI, P.: In vitro supplementation of resveratrol to bovine spermatozoa: effects on motility, viability and superoxide production. Journal of Microbiology, Biotehncology and Food Sciences, 4:336-341, 2015b.
TVRDÁ, E., TUŠIMOVÁ, E., KOVÁČIK, A., PAÁL, D., GREIFOVÁ, H., ABDRAMANOV, A., LUKÁČ, N.: Curcumin has protective and antioxidant properties on bull spermatozoa subjected to induced oxidative stress. Animal Reproduction Sciences, 172:10-20, 2016a.
TVRDÁ, E., TUŠIMOVÁ, E., KOVÁČIK, A., PAÁL, D., LIBOVÁ, Ľ., LUKÁČ, N.: Protective effects of quercetin on selected oxidative biomarkers in bovine spermatozoa subjected to ferrous ascorbate. Reproduction in Domestic Animals, 51:524-537, 2016b.
VILLANUEVA-DIAZ, CA., FLORES-REYES, GA., BELTRÁN-ZÚÑIGA, M., ECHAVARRÍA-SÁNCHEZ, M., ORTIZ-IBARRA, FJ., ARREDONDO-GARCÍA, JL.: Bacteriospermia and male infertility: a method for increasing the sensitivity of semen culture. International Journal of Fertility and Women's Medicine, 44:198-203, 1999.
VILLEGAS, J., SCHULZ, M., SOTO, L., SANCHEZ, R.: Bacteria induce expression of apoptosis in human spermatozoa. Apoptosis, 10:105-110, 2005.
WANG, A., FANNING, L., ANDERSON, DJ., LOUGHLIN, K.R.: Generation of reactive oxygen species by leukocytes and sperm following exposure to urogenital tract infection. Archives of Andrology, 39:11-17, 1997.

Received / Primljen: 23.08.2017.
Accepted / Prihvaćen: 24.12.2017.