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Human sperm DNA damage has an effect on immunological interaction between spermatozoa and fallopian tube

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ABSTRACT

Background: Toll-like receptors play a crucial role in the immunological interaction between the spermatozoa and fallopian tube and contribute to the ovulation, sperm capacitation, fertilization, and pregnancy.

Objectives: To investigate the expression of toll-like receptors and their adaptor molecules and cytokines under the effect of spermatozoa with high DNA fragmentation (high DF) in human fallopian tube cell line (OE-E6/E7) and compare to those in normal spermatozoa.

Materials and methods: Fresh semen samples were obtained from 10 unexplained infertile males with high DF (more than 20%) and from 10 healthy donors with a DF less than 3%. After sperm preparation, samples were co-cultured with OE-E6/E7. Toll-like receptors, myeloid differentiation factor 88 (MyD88), TIR domain-containing adapter protein (TIRAP), TIR domain-containing adapter-inducing IFN-b (TRIF), TRIF-related adapter molecule as well as IL-6, IL-8, IFN-β, and TNFα mRNA expression were evaluated by quantitative real-time PCR. Protein levels of these cytokines and chemokines were measured using ELISA method.

Results: TLR 1-6 mRNA expression in OE-E6/E7 was significantly higher under the effect of spermatozoa with high DF compared to the spermatozoa with low DF. Furthermore, significantly increased mRNA expression of MyD88, TIRAP, and TRIF was observed in the high DF group compared to the low DF group, except TRIF-related adapter molecule. Moreover, the expression of IL-6 and IL-8 in the high DF group was significantly higher than low DF group, although there was no significant difference in IFN-β and TNFα expression between the groups.

Discussion and conclusion: Damage-associated molecular patterns from DNA damage activate TLR signaling pathway in human fallopian tubes and result in the upregulation of inflammatory cytokines and chemokines. This situation may provide pathologic environment for capacitation, fertilization, embryo development, and implantation in female reproductive tract and can be one of the mechanisms of infertility in men with high DF.

INTRODUCTION

Spermatozoa have friendly interaction with female reproductive tract and influence the pregnancy outcome and early embryo development (Fazeli, 2008). Nearly 40–50% of all infertility are because of male factor and in consequence unwell sperm parameters (Kumar & Singh, 2015). So, evaluation of sperm interaction with female reproductive system has much worth in finding out the causes of infertility. Fallopian tube provides a suitable environment for oocyte maturation, sperm capacitation, fertilization, and transport of gametes and embryos (Growe *et al.*, 2013). The epithelial cells and stromal fibroblasts of the fallopian tube play a role in physiological and pathological events, as it identifies gametes and expresses pro-inflammatory cytokines and chemokines to initiate inflammatory responses against the microbial invasion (Growe *et al.*, 2013), which can lead to infertility and pregnancy complications (Ibrahim *et al.*, 2015).

Toll-like receptors (TLRs) as a major part of innate immune system and first line of defense are differentially expressed throughout the distinct parts of female reproductive tract and their expression change during different phases of menstrual cycle (Amirchaghmaghi *et al.*, 2013; De Nardo, 2015; Zandieh

et al., 2016). Eleven known functional TLRs have been found in humans that selectively recognize a wide range of microbial components (Amirchaghmaghi *et al.*, 2013; Frazao *et al.*, 2013; Saeidi *et al.*, 2014). TLRs react with a wide spectrum of pathogen-associated molecular patterns (PAMPs) derived from bacteria, viruses, fungi, and parasites, and non-pathogenic molecular patterns as observed with commensal flora as well as endogenous damage-associated molecular patterns (DAMPs) released from oxidative damage of the cellular and molecular structures and necrotic cells such as hyaluronan, fatty acids, b-defensins, eosinophil-derived neuro-toxin, reactive oxygen species (ROS), surfactant protein A, haem and heat-shock protein (Amirchaghmaghi *et al.*, 2013; Taghavi *et al.*, 2013; Zandieh *et al.*, 2015). The activation of TLRs signaling pathways results in the inflammation, recruitment of the immune cells, and secretion of the antimicrobial factors that eliminate pathogens and subsequently drive adaptive immune responses (Zandieh *et al.*, 2015; Sheldon *et al.*, 2017). Furthermore, TLRs play a role in the immunological interaction between spermatozoa and fallopian tube and contribute to the ovulation, sperm capacitation, fertilization, and pregnancy (Horne *et al.*, 2008; Saeidi *et al.*, 2014; Zandieh *et al.*, 2015).

Sperm DNA fragmentation (SDF) is a parameter in male infertility evaluation. The major contributors of sperm DNA damage are oxidative stress, apoptosis, and chromatin remodeling (Fraczek *et al.*, 2013; Leach *et al.*, 2015; Rex *et al.*, 2017). High SDF has been associated with infertility and recurrent pregnancy loss (Bisht *et al.*, 2017), but this mechanism is not well recognized. Therefore, in this study the effect of spermatozoa with high DF on TLRs and their adaptor molecules and cytokines expression in human fallopian tube cell line (OE-E6/E7) was evaluated.

MATERIALS AND METHODS

Experimental design and sample selection

This study was confirmed by Iran University of Medical Sciences and Royan Institute Ethics Committees and study has been performed according to the Declaration of Helsinki. Informed written consent was taken prior to the collection of samples. Semen analysis was performed for all participants, and a complete medical history including age, BMI, lifestyle, smoking, use of alcohol, and recreational drugs was also recorded. Males with undescended testis, varicocoele, atrophic testis, urogenital infection or chronic prostatitis and habits of alcohol consumption, smoking and illicit drug use were excluded from the study.

Ten unexplained infertile males attending the infertility clinic with a normal sperm analysis and a history of 12 months infertility were selected according to World Health Organization (WHO) criteria (Fifth edition) and considered as a high DF group because of having DF more than 20%. Furthermore, 10 normozoospermic healthy donors who had at least one child and referred to the infertility clinic for embryo donation or sex determination were selected as control group with a DF less than 3% (low DF group). Fresh semen samples were obtained by masturbation from participants. All semen samples had no leukocytes or bacterial infection. After sperm preparation, samples were co-cultured with human fallopian tube cell line (OE-E6/E7), simultaneously TUNEL assay was performed. After co-culturing, in unexplained group, samples with high DF and in healthy donors group, sample with low DF were selected and other samples were excluded from evaluation. In the next part, TLR1-6 (our previous published data showed that OE-E6/E7 expressed TLR1-6), myeloid differentiation factor 88 (MyD88), TIR domain-containing adapter protein (TIRAP), TIR domain-containing adapter-inducing IFN-b (TRIF) (TICAM 1), TRIF-related adapter molecule (TRAM) (TICAM 2) as well as IL-6, IL-8, interferon β (IFN-β), and TNF-α mRNA expression levels were compared between the groups by quantitative real-time PCR, and supernatant was used for the measurement of IL-6, IL-8, IFN-β, and TNFα by ELISA method.

Cell line (OE-E6/E7) and sperm preparation

OE-E6/E7 cell line (Lee *et al.*, 2001) was cultured in DMEM (F12) culture media (Invitrogen, Paisley, UK) at 37 °C with 1% penicillin and streptomycin (Sigma-Aldrich, Poole, UK), 10% fetal bovine serum (FBS) (Invitrogen) and L-glutamine (Invitrogen) in atmosphere of 5% CO_2. Semen analysis was performed according to WHO guidelines and spermatozoa were prepared with Cook-Sydney gradient and then swim up method.

TUNEL assay

Terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL) assay was performed to visualize fragmented DNA in spermatozoa using a Cell Death Detection kit (Roche, Mannheim, Germany) according to the manufacturer's instruction. In brief, semen samples were isolated from seminal plasma by low-speed centrifugation (600 ***g***; 5 min) and then smeared on the microscope slides, dried in the air, and fixed for 60 min in 4% paraformaldehyde in the phosphate-buffered saline solution. Afterward, specimens were permeabilized using 0.1% Triton X-100 in 0.1% sodium citrate for 10 min at 4 °C. Sperm cells were incubated in TUNEL reaction mixture in the dark at room temperature for 1 h and then were evaluated under the fluorescence microscope. The percentage of TUNEL-positive cells were calculated and two slides were used as negative (reaction mixture without terminal deoxynucleotidyl transferase) and positive (pre-treated with DNase I, 1 mg/mL for 20 min at room temperature) controls.

Spermatozoa co-culture with OE-E6/E7

To clarify the effect of spermatozoa on human fallopian tube cell line, cells were cultured in 6-well culture plates until near confluency. Prepared spermatozoa in both groups were counted after washing and about 1000 spermatozoa were added to each well as high DF group and low DF group, separately. Experiments were repeated three times. OE-E6/E7 cells with spermatozoa were incubated for 24 h. After that, supernatants were collected, centrifuged at 10,000 ***g*** for 5 min on the ice, transferred to fresh tubes, and stored at −70 °C. For genomic evaluation, OE-E6/E7 cells were washed with phosphate-buffered saline (PBS) free of Ca2+ and Mg2+ and then were harvested with trypsin-EDTA (Invitrogen) and pelleted by centrifugation at 300 g for 5 min. 1 mL TRI reagent (Sigma-Aldrich) was added onto the pellet (5×10^6 cells) for RNA isolation.

Quantitative real-time PCR for TLRs and their pathways

Total RNA was extracted from pelleted cells using a standard protocol. Both groups were treated three times with DNase I (Fermentas, Sankt Leon-Rot, Germany) to remove genomic DNA

contamination. First strand cDNA was synthesized using oligo dT primers (Fermentase kit) and reverse transcripted by Super-Script II (200 Ul; Invitrogen). Real-time qPCR was carried out on the cDNA. The cDNA was prepared from OE-E6/E7 cells after co-culturing with spermatozoa in high and low DNA fragmented groups and primers for TLRs, MyD88, TIRAP, TRIF (TICAM 1), TRAM (TICAM 2) as well as IL-6, IL-8, IFN-β, and TNFα. All primers were confirmed and used in our previous investigation (Zandieh *et al.*, 2015). Real-time qPCRs performed in triplicates with ABI Prism 7300 Sequence Detector (Applied Biosystems, Foster, CA, USA) in a 20 μl volume containing 250 ng cDNA, 5 pmol gene-specific primers, and SYBR Green reagent (Applied Biosystems) using ROX dye as passive reference dye for signal intensity. The thermal cycle profile was as follows: 50 cycles at 95° for 30 s, 60–63° for 30 s, and 72° for 30 s. Samples were run in triplicate. The specificity of the PCR products was checked with melting curve analysis. Standard curves were prepared using logarithmic dilution series of total RNA.

Cytokine measurement by ELISA

After 24 h of spermatozoa and fallopian tube cell line co-culturing, supernatants were collected and centrifuged at 10,000 ***g*** for 5 min at 4 °C. Then, it was used to determine concentration of IL-6, IL-8, TNFα, and IFN-β by commercially enzyme-linked immunosorbent assay (ELISA) kits: IL-6 (AviBion, IL06001, Helsinki, Finland), IL-8 (eBioscience, San Diego, CA, USA) TNFα (AviBion, TNFa021, Helsinki, Finland), and IFN-β (Bioassay Technology, Shanghai, China) according to the manufacturer's instruction.

Statistics

The results were expressed as mean ± SEM. Statistical analysis was performed using Mann–Whitney *U* test for non-parametric analyses and independent Student *t*-test for parametric tests to compare statistical differences between groups in SPSS 18 software. $p < 0.05$ was considered significant.

RESULTS

After 24 h of co-culturing, up to 90% of spermatozoa were alive and without any attachment to the OE-E6/EE7 cells. Therefore, spermatozoa were easily separated from the cell line using PBS washing.

TUNEL assay results

According to TUNEL results, semen samples were divided into high DF and low DF groups. TUNEL results in high DF group were 20–25% and in low DF group were 1–3%. Figure 1 shows images of positive (A) and negative (B) controls and a sample with DNA fragmented spermatozoa (C). Only complete spermatozoa were considered for percentage estimation; heads without tails were not considered.

Quantitative PCR results

Our last study showed that OE-E6/E7 express TLR1-6 on mRNA and protein levels (Zandieh *et al.*, 2015). The quantitative expression profiles of TLR1-6 genes in OE-E6/E7 cells co-cultured with high and low DF groups are shown in Fig. 2. The mean relative expression of TLR genes 1-6 was significantly higher in response to spermatozoa with high DF compared to spermatozoa with low DF. Furthermore, the mean relative expression of adaptor molecules in high DF group was significantly higher than low DF group, except in TRAM pathway (Fig. 3). Besides, the mean relative expression of IL-6 and IL-8 showed marked increase in high DF group, although expression of IFN-β and TNFα was not statistically significant between the groups (Fig. 4).

ELISA results

Measurement of IL-6, IL-8, IFN-β, and TNFα by ELISA assay showed elevated levels of IL-6 and IL-8 in response to spermatozoa with high DF compared to ones with low DFI (Fig. 5). Production of IFN-β was not statistically significant between the groups and TNFα levels in both groups were below the detection limit.

DISCUSSION

The results of present study showed that spermatozoa with high DF from unexplained infertile men caused significantly higher expression of TLR 1-6, MyD88, TIRAP, and TRIF adaptor molecules as well as pro-inflammatory cytokines IL-6 and IL-8 in human fallopian tube epithelial cell line than those with normal spermatozoa.

The innate immunity is involved in the early detection and elimination of invasive pathogens (De Nardo, 2015). Innate immunity through the TLRs responds to the pathogens and by activating signaling pathways, produces immune-mediators such as interferon (IFN), TNF, IL-6, IL-8, MIF, and IL-10 (Takeuchi & Akira, 2010; Frazao *et al.*, 2013; De Nardo, 2015; Gomes *et al.*, 2015).

The presence of endogenous ligands could be one of the reasons for upregulation of TLRs in the current study. TLRs1-10 can be activated by several endogenous ligands (Yu *et al.*, 2010). The major contributors of sperm DNA damage are oxidative stress, apoptosis, and chromatin remodeling (Fraczek *et al.*, 2013; Leach *et al.*, 2015; Rex *et al.*, 2017). Therefore, excessive ROS production resulting oxidative stress and apoptotic processes can stimulate TLR2 and TLR6 expression (Frantz *et al.*, 2001; Gill *et al.*, 2010). TLR2 and TLR6 form a heterodimer to recognize ROS (Frantz *et al.*, 2001).

Moreover, in the patients with endometriosis, oxidative stress activates TLR4 which could affect the signaling pathway and higher expression of pro-inflammatory cytokines and chemokines through the nuclear factor (NF)-KB activation (Khan *et al.*, 2013). Increased apoptosis and inflammation activated by oxidative stress leads to the release of self-derived nucleic acids with auto-antigenic potential. These single- or double-stranded RNAs could destroy the protective mechanisms and activate TLR 3, 7, and 8 (Papadimitraki *et al.*, 2007). In addition, activation of TLR 2, 3, and 4 through the Fas-associated death domain (FADD) pathway results in the apoptosis and further production of inflammatory cytokines (Kannaki *et al.*, 2011). However, there are several unknown DAMPs for TLRs except for the ROS which could be involved in the stimulation of other TLRs. Additionally, the synergistic relationship between TLRs is important and could be another reason for higher expression of TLRs under the effect of spermatozoa with high DF. Although synergistic TLR stimulation may often boost the immune responses, the order and time of TLRs stimulation can affect magnitude of the immune responses (Tan *et al.*, 2014). Moreover, increase in TLRs has been reported in other organs,

Figure 1 Terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL) assay. Positive (A) and negative (B) controls and a sample with DNA fragmented spermatozoa (C). Complete spermatozoa were considered for percentage estimation and heads without tails were not considered.

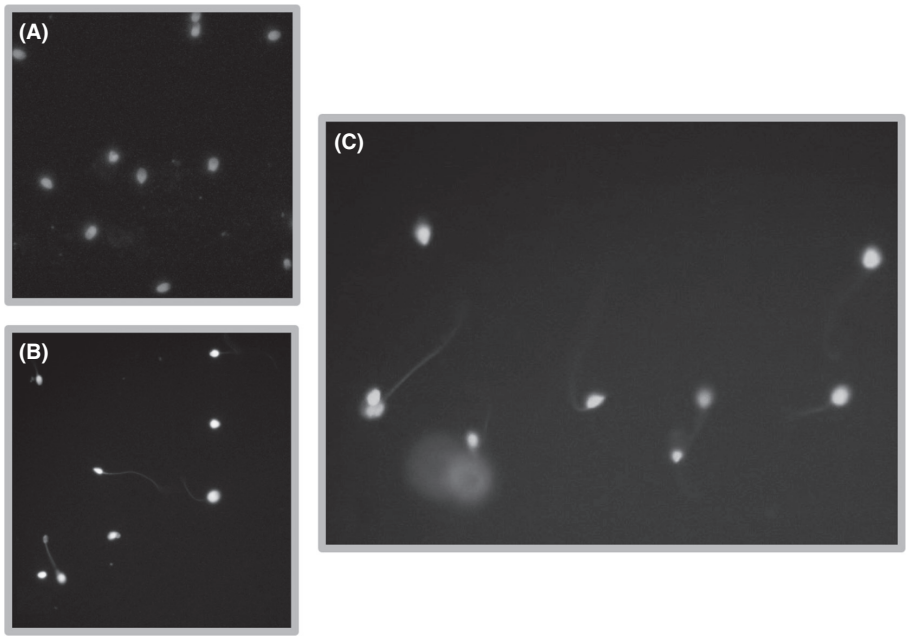
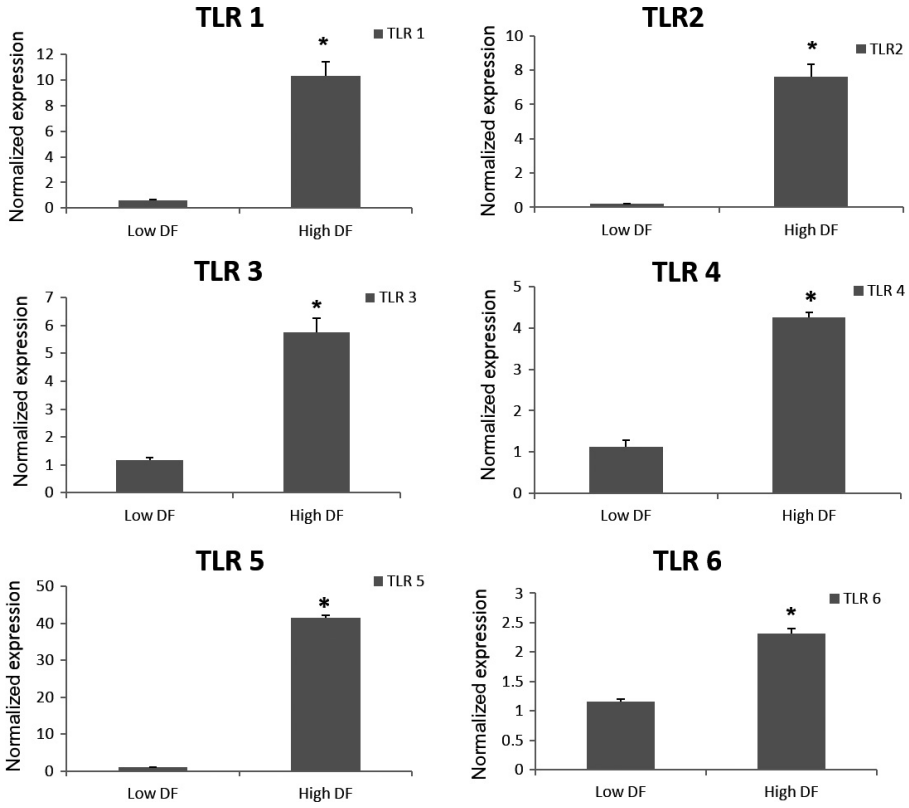


Figure 2 The mean relative expression of TLR genes 1-6 in OE-E6/E7 cells co-cultured with high and low DF groups. * shows the significant differences between groups.



although their DAMPs are still unknown (Brandao *et al.*, 2015 #24; Frantz *et al.*, 2001 #126).

For activation of TLR signaling pathway, intracellular domain of the TLRs, Toll-IL-1R (TIR) domain, binds to the TIR-containing cytosolic adapters MyD88, TRIF, TIRAP also known as MAL, and TRAM (Amirchaghmaghi *et al.*, 2013; Frazao *et al.*, 2013; Saeidi *et al.*, 2014).

TLR signaling activates through two main pathways mediated by either MyD88 or TRIF. Most of the TLRs except for TLR3 use MyD88-dependent signaling and TLR4 uses both pathways and

Figure 3 The mean relative expression of adaptor molecules MyD88, TIRAP, TRIF (TICAM 1), TRAM (TICAM 2) in high DNA fragmentation (DF) and low DF groups. * shows the significant differences between groups.

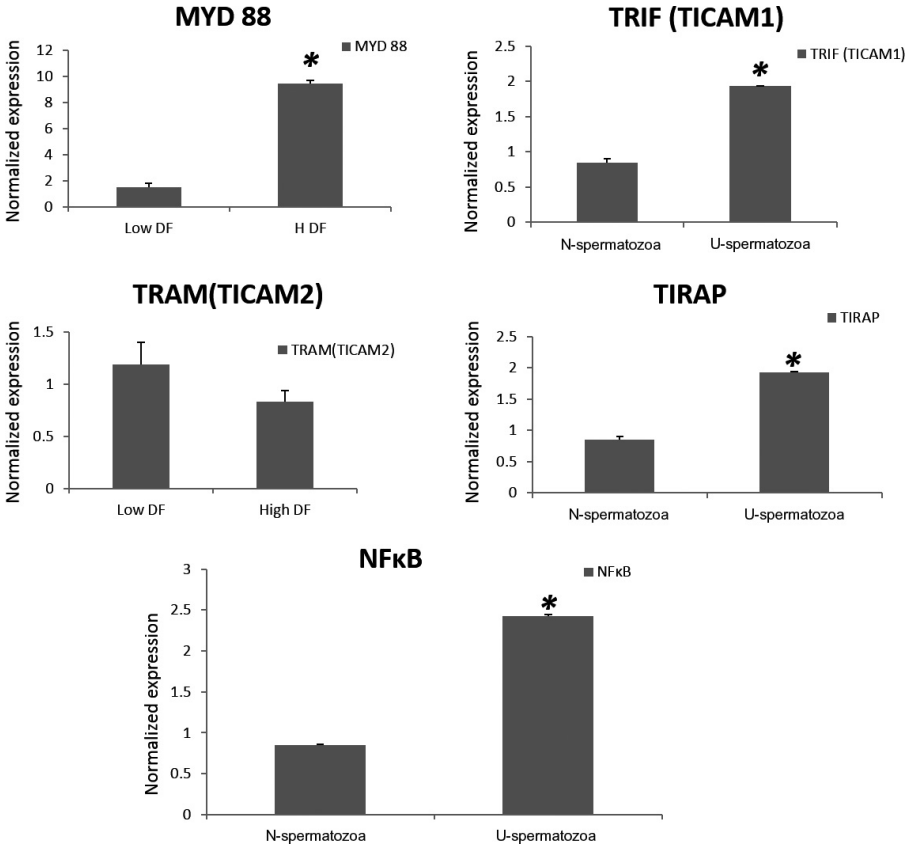
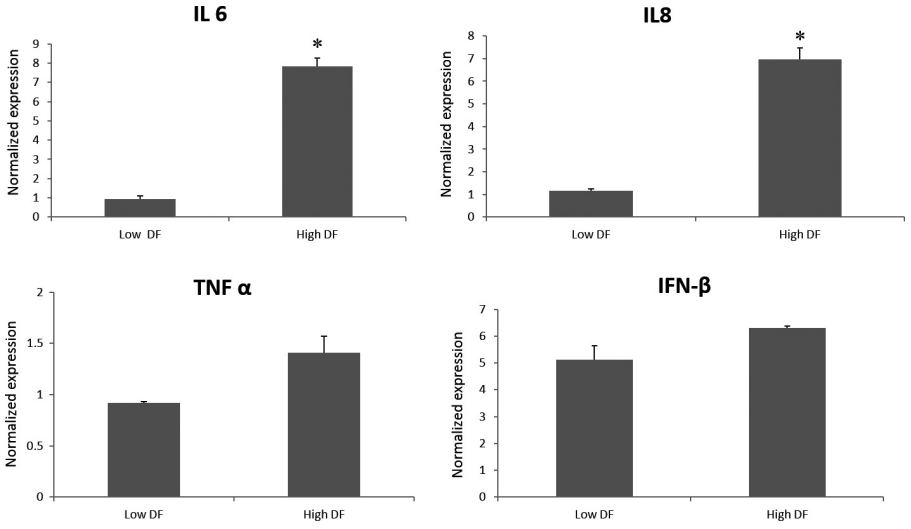


Figure 4 The mean relative expression of pro-inflammatory cytokines and chemokines IL-6, IL-8, IFN-β, and TNFα in high DNA fragmentation (DF) and low DF groups. * shows the significant differences between groups.

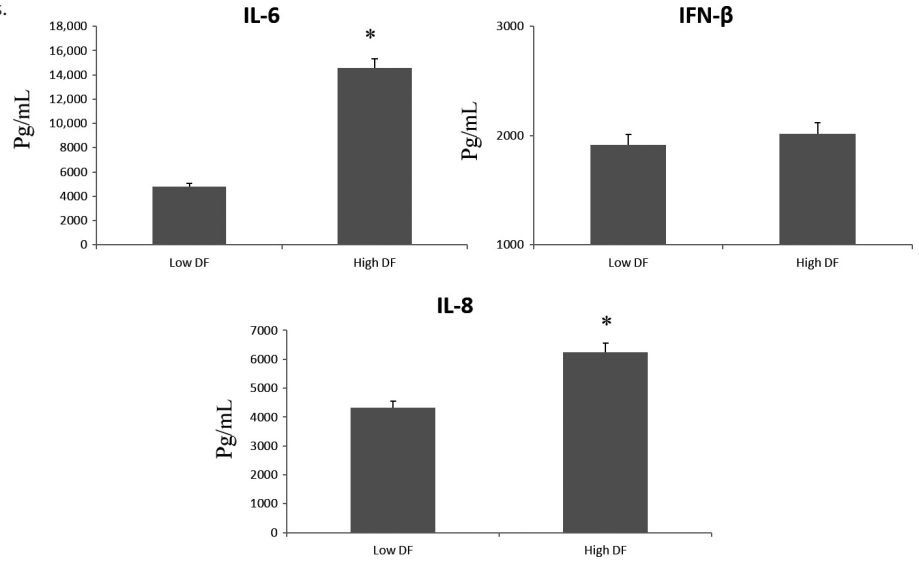


recruits all adaptor proteins (Hart *et al.*, 2009; Amirchaghmaghi *et al.*, 2013). MyD88-dependent signaling triggers transcription factor NF-KB and mitogen-activated protein kinases (MAPK) activator protein-1 which activates activating protein (AP-1), that results in the expression of numerous genes and production of pro-inflammatory cytokines and chemokines such IL-6 and IL-8 (Kawasaki & Kawai, 2014; Zandieh *et al.*, 2015).

Independent MyD88 signaling activated by the stimulation of TLR3 or TLR4 leads to the activation of interferon regulated factors (IRF) family of the transcription factors through the recruitment of TRIF and consequently end up to the production of IFN (Montazeri *et al.*, 2016; Sheldon *et al.*, 2017).

Therefore, as a result of TLR1-6 stimulation except for TLR3, we observed increased mRNA expression levels of

Figure 5 Concentration of IL-6, IL-8, and IFN-β measured by ELISA method in high DNA fragmentation (DF) and low DF groups. * shows the significant differences between groups.



MyD88-dependent signaling pathways and elevated mRNA expression levels of TRIF following TLR3 activation.

Moreover, as TLR5 uses MYD88 and TRIF in its signaling pathway (Brandao *et al.*, 2015; Funami *et al.*, 2017), higher expression levels of these adaptors could be explained by the upregulation of TLR5 under the effect of spermatozoa with high DF.

Furthermore, TIRAP is one of the adaptor molecules used by TLR2 and TLR4. Previous studies have demonstrated that TIRAP is essential in the recruitment of MyD88 to the cell surface of the TLR2 and TLR4 (Kawasaki & Kawai, 2014; Funami *et al.*, 2017). Therefore, increased mRNA expression levels of TIRAP in the present study could be explained by the elevated TLR1-6 expression levels under the effect of spermatozoa with high DF. TRAM is another adaptor which is only used by TLR4 for the recruitment of the TRIF (Ohnishi *et al.*, 2012; Kawasaki & Kawai, 2014). TLR4 and TLR3 both utilize TRIF, although in case of TLR4 recruitment of both TRAM and TRIF is needed to activate type I interferon signaling and to increase production of inflammatory cytokines (Hart *et al.*, 2009; Kawasaki & Kawai, 2014; Funami *et al.*, 2017). In the current study, there was no significant difference in TRAM expression levels between the groups which shows that in patients with high DF, TLR4 apart from TRAM uses other adaptor molecules such as MyD88 for its signaling pathway.

Activation of TLRs and their signaling pathway culminates in the releasing inflammatory cytokines and chemokines that initiate and promote inflammatory responses (Frazao *et al.*, 2013; Kawasaki & Kawai, 2014).

Cytokines and chemokines contribute to the endometrial growth, menstruation, and implantation and also have a pivotal role in the regulation of innate and adaptive immunity of the female reproductive tract (18). Fallopian tube epithelial cells secrete pro-inflammatory cytokines and chemokines such as IL-6, IL-8, and TNFα in the response of the TLR ligands (Balasubramaniam *et al.*, 2012; Growe *et al.*, 2013; Sheldon *et al.*, 2017).

IL-6 involves in the oocyte development, embryo implantation, regulation of endometrial function, and sperm capacitation (Markert *et al.*, 2011; Sheldon *et al.*, 2017).

Type I IFNs, including multiple subtypes of IFN-α and singular forms of IFN-β, IFN-ω, regulate inflammation and induce apoptosis in the virally infected cells (Takeuchi & Akira, 2010).

In the present study, production of inflammatory cytokines such as IL-6 and IL-8 showed a significant enhancement because of the presence of spermatozoa, albeit TNFα and IFN-β expression levels were higher but not significantly different between the groups.

As TRAM is required for the activation of TRIF pathway by TLR4 (Piccinini & Midwood, 2010) and no significant increase was observed in the TRAM expression levels in the presence of high DF, it can be concluded that here TLR4 stimulation may not lead to the synthesis of IFN-β and as a result TLR3 is the only source of IFN-β production.

Considering spermatozoa with high DF and also spermatozoa with low DF both activate (Zandieh *et al.*, 2015) TLR3 and TRIF pathway, which lead to the production of interferon, no significant increase was observed in the production of interferon between the groups.

Although IL-6 and IL-8 play pivotal roles such as sperm capacitation and implantation, excessive increase in these markers in the fallopian tube may not have good consequences (Balasubramaniam *et al.*, 2012). For example, upregulation of IL-6 will inactivate or reduce the ciliary activity of the tubal epithelium in the fallopian tube, which can cause severe tubal damage and indirectly encourage implantation of the fetus in the fallopian tube, which ultimately results in ectopic tubal gestation (Papathanasiou *et al.*, 2008). Furthermore, in another study increased IL-6 and IL-8 expression levels have been strongly associated with the pathophysiology of the tubal ectopic pregnancy (Balasubramaniam *et al.*, 2012).

CONCLUSIONS

DAMPS resulting from DNA damage activates TLR signaling pathway in human fallopian tubes and results in the upregulation of inflammatory cytokines and chemokines. This situation may provide pathologic environment for capacitation, fertilization, embryo development, and implantation in female reproductive tract. So, it can be one of the mechanisms of infertility in men with high sperm DNA damage.

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CONFLICT OF INTEREST

None declared.

AUTHORS' CONTRIBUTIONS

ZZ was responsible for the substantial contributions to the conception and analyses and writing the manuscript. KhA was responsible for cellular and molecular experiments. MA had scientific technical assistant and revised the manuscript critically. RA was responsible for design, interpretation of data, and final approval of the version to be published.

REFERENCES

Amirchaghmaghi E, Taghavi SA, Shapouri F, Saeidi S, Rezaei A & Aflatoonian R. (2013) The role of toll like receptors in pregnancy. *Int J Fertil Steril* 7, 147–154.

Balasubramaniam ES, Van Noorden S & El-Bahrawy M. (2012) The expression of interleukin (IL)-6, IL-8, and their receptors in fallopian tubes with ectopic tubal gestation. *Fertil Steril* 98, 898–904.

Bisht S, Faiq M, Tolahunase M & Dada R. (2017) Oxidative stress and male infertility. *Nat Rev Urol* 14, 470–485.

Brandao I, Hormann N, Jackel S & Reinhardt C. (2015) TLR5 expression in the small intestine depends on the adaptors MyD88 and TRIF, but is independent of the enteric microbiota. *Gut Microbes* 6, 202–206.

De Nardo D. (2015) Toll-like receptors: activation, signalling and transcriptional modulation. *Cytokine* 74, 181–189.

Fazeli A. (2008) Maternal communication with gametes and embryos. *Theriogenology* 70, 1182–1187.

Fraczek M, Szumala-Kakol A, Dworacki G, Sanocka D & Kurpisz M. (2013) In vitro reconstruction of inflammatory reaction in human semen: effect on sperm DNA fragmentation. *J Reprod Immunol* 100, 76–85.

Frantz S, Kelly RA & Bourcier T. (2001) Role of TLR-2 in the activation of nuclear factor kappaB by oxidative stress in cardiac myocytes. *J Biol Chem* 276, 5197–5203.

Frazao JB, Errante PR & Condino-Neto A. (2013) Toll-like receptors' pathway disturbances are associated with increased susceptibility to infections in humans. *Arch Immunol Ther Exp (Warsz)* 61, 427–443.

Funami K, Matsumoto M, Oshiumi H, Inagaki F & Seya T. (2017) Functional interfaces between TICAM-2/TRAM and TICAM-1/TRIF in TLR4 signaling. *Biochem Soc Trans* 45, 929–935.

Gill R, Tsung A & Billiar T. (2010) Linking oxidative stress to inflammation: toll-like receptors. *Free Radic Biol Med* 48, 1121–1132.

Gomes A, Miguel P, Alves D, Inoue V & Oliveira A. (2015) Pro-inflammatory cytokines in sepsis: biological studies and prospects from in silico research. *Biol Syst Open Access* 5, 1–7.

Growe RG, Luster MI, Fail PA & Lippes J. (2013) Quinacrine-induced occlusive fibrosis in the human fallopian tube is due to a unique inflammatory response and modification of repair mechanisms. *J Reprod Immunol* 97, 159–166.

Hart KM, Murphy AJ, Barrett KT, Wira CR, Guyre PM & Pioli PA. (2009) Functional expression of pattern recognition receptors in tissues of the human female reproductive tract. *J Reprod Immunol* 80, 33–40.

Horne AW, Stock SJ & King AE. (2008) Innate immunity and disorders of the female reproductive tract. *Reproduction* 135, 739–749.

Ibrahim S, Salilew-Wondim D, Rings F, Hoelker M, Neuhoff C, Tholen E, Looft C, Schellander K & Tesfaye D. (2015) Expression pattern of inflammatory response genes and their regulatory micrornas in bovine oviductal cells in response to lipopolysaccharide: implication for early embryonic development. *PLoS ONE* 10, e0119388.

Kannaki TR, Shanmugam M & Verma PC. (2011) Toll-like receptors and their role in animal reproduction. *Anim Reprod Sci* 125, 1–12.

Kawasaki T & Kawai T. (2014) Toll-like receptor signaling pathways. *Front Immunol* 5, 461.

Khan KN, Kitajima M, Fujishita A, Nakashima M & Masuzaki H. (2013) Toll-like receptor system and endometriosis. *J Obstet Gynaecol Res* 39, 1281–1292.

Kumar N & Singh AK. (2015) Trends of male factor infertility, an important cause of infertility: a review of literature. *J Hum Reprod Sci* 8, 191–196.

Leach M, Aitken RJ & Sacks G. (2015) Sperm DNA fragmentation abnormalities in men from couples with a history of recurrent miscarriage. *Aust N Z J Obstet Gynaecol* 55, 379–383.

Lee YL, Lee KF, Xu JS, Wang YL, Tsao SW, & Yeung WS. (2001) Establishment and characterization of an immortalized human oviductal cell line. *Mol Reprod Dev* 59, 400–409.

Markert UR, Morales-Prieto DM & Fitzgerald JS. (2011) Understanding the link between the IL-6 cytokine family and pregnancy: implications for future therapeutics. *Expert Rev Clin Immunol* 7, 603–609.

Montazeri M, Sanchez-Lopez JA, Caballero I, Maslehat Lay N, Elliott S & Fazeli A. (2016) Interleukin-1 receptor antagonist mediates toll-like receptor 3-induced inhibition of trophoblast adhesion to endometrial cells in vitro. *Hum Reprod* 31, 2098–2107.

Ohnishi H, Tochio H, Kato Z, Kawamoto N, Kimura T, Kubota K, Yamamoto T, Funasaka T, Nakano H, Wong RW, Shirakawa M & Kondo N. (2012) TRAM is involved in IL-18 signaling and functions as a sorting adaptor for MyD88. *PLoS ONE* 7, e38423.

Papadimitraki ED, Bertsias GK & Boumpas DT. (2007) Toll like receptors and autoimmunity: a critical appraisal. *J Autoimmun* 29, 310–318.

Papathanasiou A, Djahanbakhch O, Saridogan E & Lyons RA. (2008) The effect of interleukin-6 on ciliary beat frequency in the human fallopian tube. *Fertil Steril* 90, 391–394.

Piccinini AM & Midwood KS (2010) DAMPening inflammation by modulating TLR signalling. *Mediators Inflamm* 2010, pii: 672395.

Rex AS, Aagaard J & Fedder J. (2017) DNA fragmentation in spermatozoa: a historical review. *Andrology* 5, 622–630.

Saeidi S, Shapouri F, Amirchaghmaghi E, Hoseinifar H, Sabbaghian M, Sadighi Gilani MA, Pacey AA & Aflatoonian R. (2014) Sperm protection in the male reproductive tract by Toll-like receptors. *Andrologia* 46, 784–790.

Sheldon IM, Owens SE & Turner ML. (2017) Innate immunity and the sensing of infection, damage and danger in the female genital tract. *J Reprod Immunol* 119, 67–73.

Taghavi SAAM, Mehdizadeh M, Karimian L, Joghataie M & Aflatoonian R. (2013) Toll-like receptors expression in follicular cells of patients with poor ovarian response. *Int J Fertil Steril* 8, 183–192.

Takeuchi O & Akira S. (2010) Pattern recognition receptors and inflammation. *Cell* 140, 805–820.

Tan RS, Ho B, Leung BP & Ding JL. (2014) TLR cross-talk confers specificity to innate immunity. *Int Rev Immunol* 33, 443–453.

Yu L, Wang L & Chen S. (2010) Endogenous toll-like receptor ligands and their biological significance. *J Cell Mol Med* 14, 2592–2603.

Zandieh Z, Ashrafi M, Jameie B, Amanpour S, Mosaffa N, Salman Yazdi R, Pacey A & Aflatoonian R. (2015) Evaluation of immunological interaction between spermatozoa and fallopian tube epithelial cells. *Andrologia* 47, 1120–1130.

Zandieh Z, Amjadi F, Ashrafi M, Aflatoonian A, Fazeli A & Aflatoonian R (2016) The effect of estradiol and progesterone on toll like receptor gene expression in a human fallopian tube epithelial cell line. *Cell J (Yakhteh)* 17, 678.

