

Infliximab may reverse the toxic effects induced by tumor necrosis factor alpha in human spermatozoa: an in vitro model

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Objective: To examine the toxic effects of tumor necrosis factor alpha (TNF- α) on ejaculated spermatozoa and evaluate the ability of infliximab to reverse these effects.

Design: Prospective controlled study.

Setting: Cleveland Clinic Foundation, Cleveland, Ohio.

Patient(s): Thirty-one healthy sperm donors.

Intervention(s): Exposure of human spermatozoa to varying concentrations of TNF- α (100, 300, 400, 500 pg/mL, and 2.5 μ g/mL) and infliximab (400 μ g/mL).

Main Outcome Measure(s): Sperm motility, functional integrity of plasma membrane, and DNA fragmentation.

Result(s): Spermatozoa quality declined following incubation with TNF- α in a dose-dependent and time-dependent manner. Sperm motility and membrane integrity were higher in the samples incubated with TNF- α plus infliximab than in the samples treated with TNF- α only. These parameters improved significantly and were comparable with both controls and sperm incubated with infliximab alone. Similarly, the percentage of spermatozoa with DNA fragmentation improved significantly following incubation with TNF- α plus infliximab and again was comparable with both controls and sperm incubated with infliximab alone.

Conclusion(s): Spermatozoa may be exposed to abnormal levels of TNF- α in the male reproductive tract or during their passage into the female reproductive tract (in cases of endometriosis). Exposing spermatozoa to pathological concentrations of TNF- α can result in significant loss of their functional and genomic integrity. Infliximab could potentially be used to help treat female infertility caused by endometriosis in those with elevated levels of TNF- α in their peritoneal fluid. (*Fertil Steril*® 2005;83:1665–73. ©2005 by American Society for Reproductive Medicine.)

Key Words: Spermatozoa, TNF- α , infertility, DNA damage, endometriosis

Tumor necrosis factor alpha (TNF- α), a potent cytokine that exerts inflammatory, cytotoxic, angiogenic, and growth modulatory effects on different target cells (1), is mainly produced by neutrophils, activated lymphocytes, natural killer cells, and activated macrophages (2). Its presence within the female and male reproductive tracts suggests that it is involved in fertility modulation.

In a healthy male reproductive tract, TNF- α appears to be produced by the pachytene spermatocytes and round sper-

matids, which contain mRNA for TNF- α (3). Levels of TNF- α are usually low in seminal plasma (<10 pg/mL) (4) but tend to increase in conditions such as inflammation and male genital tract infections (4, 5). The increase may be attributed to testicular macrophages that release excessive amounts of TNF- α when exposed to endotoxins (6). In vitro studies document that human macrophage cell lines have elevated levels of TNF- α , which interfere with sperm binding to the zona pellucida (7).

In the female endometrium, TNF- α plays a role in the normal physiology of endometrial proliferation and shedding (8) and also in the pathogenesis of endometriosis, one of the most common gynecologic diseases associated with infertility (9, 10). Abnormally high levels of TNF- α have been reported in the peritoneal fluid of females with endometriosis (11). Thus, TNF- α has been proposed as a sensitive marker for the nonsurgical diagnosis of endometriosis (12, 13).

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The increase in TNF- α levels appears to correlate positively with the stage of the disease (14). Higher levels of TNF- α may also be released locally by activated peritoneal macrophages (15). Elevated TNF- α levels have also been reported in other conditions characterized by the presence of activated macrophages such as pelvic inflammatory disease (1, 16).

Endometriosis shares many similarities with autoimmune diseases where elevated cytokine levels are encountered (17). Therefore, therapies for autoimmune diseases (e.g., immunomodulators and inflammatory modulators) may prove useful in treating endometriosis. The concept of using TNF- α blockers in treating endometriosis has recently gained popularity (18, 19). Infliximab, a monoclonal antibody, binds with both soluble and membrane forms of TNF- α and neutralizes its toxic effects (20). Infliximab may improve the response to medical therapy for endometriosis (21).

Pathological levels of TNF- α may be present in the male reproductive tract and in the female reproductive tract in women with endometriosis. Exposing spermatozoa to high concentrations of TNF- α may affect their physiologic function and genomic integrity. Moreover, the central role of TNF- α in many reproductive disorders is unclear. The negative effects of TNF- α on fertility and the potential use of anti-TNF- α drugs in the management of some infertility disorders remains to be evaluated. The objectives of our study were [1] to evaluate the possible toxic effects of TNF- α on sperm functional and genomic integrity and [2] to assess the potential use of anti-TNF- α (infliximab) to reverse and/or protect spermatozoa against the toxic effects of TNF- α .

MATERIALS AND METHODS

Sample Collection and Preparation

This study was approved by the institutional review board of The Cleveland Clinic Foundation, and all participants gave written, informed consent. Semen samples were collected from 31 healthy donors with normal semen parameters according to the World Health Organization guidelines (22). Sperm concentration and motility (mean \pm standard error [SE]) in the raw semen samples included in our experiment were $61.42 \pm 27.32 \times 10^6/\text{mL}$ and $69.61\% \pm 11.08\%$, respectively. Specimens were collected by masturbation after 48 to 72 hours of abstinence. Following liquefaction at 37°C for 20 minutes, 5 μL of each specimen was loaded on a 20- μ Microcell chamber (Conception Technologies, San Diego, CA) and analyzed for sperm concentration and motility. All specimens were examined for white blood cell (WBC) contamination using myeloperoxidase (Endtz) staining (23).

The liquefied semen was loaded onto a 47% and 90% discontinuous ISolate gradient (Irvine Scientific, Santa Ana, CA) and centrifuged at $500 \times g$ for 20 minutes. The resulting 90% pellet was aspirated and resuspended in

Biggers, Whitten-Whittingham media (BWW, Irvine Scientific).

Experiment 1: Evaluation of TNF- α Toxicity

We prepared TNF- α (recombinant human TNF- α ; R&D Systems, Minneapolis, MN) in Dulbecco's phosphate buffered saline (PBS, pH 7.4) to give a final concentration of 100, 300, 400, 500 pg/mL , and 2.5 $\mu\text{g/mL}$. Five sperm aliquots were examined at each concentration. Each sample was incubated at 37°C, 5% CO_2 for 2, 6, 12, and 24 hours with or without TNF- α .

Experiment 2: Effect of Infliximab

Sperm suspensions ($n = 10$) were each divided into four aliquots and incubated for 6 hours at 37°C, 5% CO_2 . We added TNF- α (2.5 $\mu\text{g/mL}$) to aliquot 1, infliximab (400 $\mu\text{g/mL}$ of Remicade; Centocor, Malvern, PA) to aliquot 2, and both TNF- α and infliximab to aliquot 3. Aliquot 4 contained an equal volume of PBS and served as a control. We selected the concentration of infliximab based on results from our previous work (24).

After the aliquots in experiments 1 and 2 were incubated, spermatozoa in each aliquot were evaluated for sperm motility, functional integrity of plasma membrane (using hypoosmotic swelling test), and DNA fragmentation (using deoxynucleotidyl transferase-mediated fluorescein-dUTP nick end labeling assay).

Hypoosmotic Swelling Test

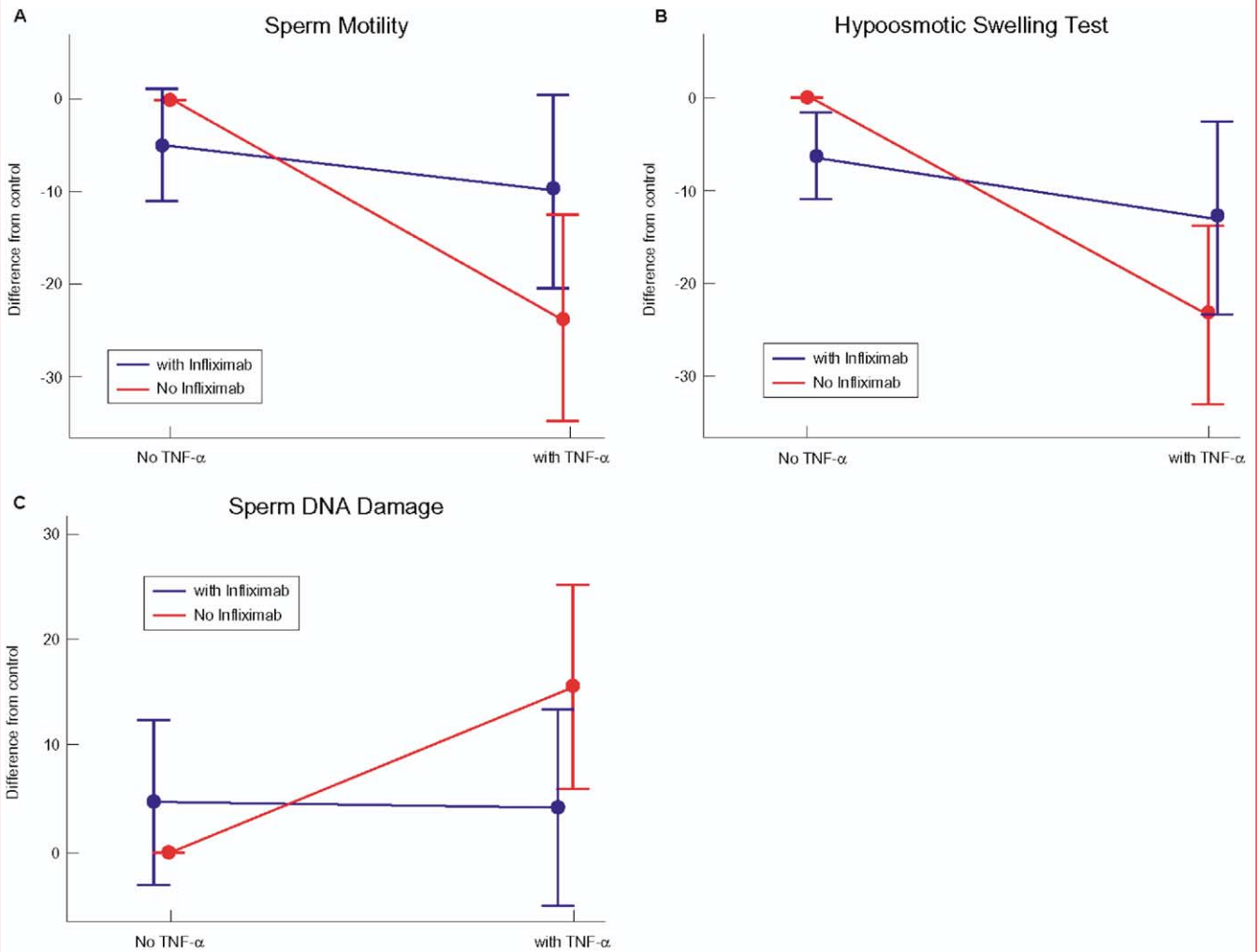
The hypoosmotic swelling (HOS) test was performed according to the methodology described by Jeyendran et al. (25). In brief, 1 mL of hypoosmotic solution (150 mOsmol/L) was mixed with 0.1 mL of sperm suspension and incubated for 30 minutes at 37°C. One drop of mixture was placed on a slide and examined at a magnification of $\times 400$ using phase contrast. A total of 100 spermatozoa were counted, and the percentage of spermatozoa with swollen tails was noted.

Evaluation of DNA Fragmentation

Sperm DNA strand breaks were evaluated using a flow cytometric terminal deoxynucleotidyl transferase-mediated fluorescein-dUTP nick end labeling (TUNEL) assay kit (Apo-Direct; BD Biosciences, Mississauga, ON, Canada) as described elsewhere (26). Briefly, spermatozoa were washed twice in PBS, resuspended in 1% paraformaldehyde at a concentration of $1-2 \times 10^6$ sperm/mL, and placed on ice for 30 to 60 minutes. These spermatozoa were again washed and resuspended in 70% ice-cold ethanol by centrifugation at $300 \times g$ for 5 minutes as per the kit instructions. After the ethanol was removed, the sperm pellets were washed twice in wash buffer and resuspended in 50 μL of the staining solution for 60 minutes at 37°C. The staining solution con-

FIGURE 1

Interaction between TNF- α and infliximab in terms of: (A) sperm motility, (B) hypoosmotic swelling test, and (C) DNA damage. The difference between each aliquot (TNF- α alone, infliximab alone, and TNF- α plus infliximab) and the control was calculated. The values plotted represent the mean and standard error of these differences. For A and B, negative values imply more toxicity; for C, positive values imply more DNA damage. The blue line connects the two points with infliximab, and the red line connects the two points without infliximab. The point of control was set at zero. Infliximab pulls the slopes toward zero, which is an indication of counteracting the toxicity of TNF- α .



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tained terminal deoxytransferase (TdT) enzyme, TdT reaction buffer; fluorescein-tagged deoxyuridine triphosphate nucleotides (FITC-dUTP), and distilled water. All cells were further washed in rinse buffer, resuspended in 0.5 mL of propidium iodide (PI)/RNase solution, and incubated for 30 minutes in the dark at room temperature.

Data were acquired within 3 hours using a flow cytometer equipped with a 488-nm argon laser as a light source (FAC-Scan; Becton Dickinson, San Jose, CA). A minimum of 10,000 spermatozoa were examined for each assay at a flow rate of <100 cells/second. The FITC (log green fluores-

cence) was measured on FL1 channel (Y-axis) and the PI (linear red fluorescence) on the FL2 channel (X-axis). Data were processed using FlowJo v4.4.4 software (Tree Star Inc., Ashland, OR).

Statistical Analysis

In experiment 1, the effect of TNF- α on sperm motility was quantified by taking the difference in sperm motility value between each sample (with TNF- α) and its control (without TNF- α) at each incubation time. These differences were then submitted to a two-way analysis of variance (ANOVA) with

TABLE 1

Effect of varying concentrations of TNF- α and incubation time on percentage of spermatozoa with normal motility.

TNF- α concentration	Time (hours)			
	2	6	12	24
100 pg/mL	1.6 \pm 1.9	-2.5 \pm 2.0	-6.3 \pm 2.7	-10.2 \pm 2.0
<i>P</i> value	.42	.23	.03	<.0001
300 pg/mL	3.4 \pm 1.9	-0.7 \pm 2.0	-4.5 \pm 2.7	-8.4 \pm 2.0
<i>P</i> value	.09	.74	.11	.0005
400 pg/mL	-5.4 \pm 1.9	-9.5 \pm 2.0	-13.3 \pm 2.7	-17.3 \pm 2.0
<i>P</i> value	.01	<.0001	<.0001	<.0001
500 pg/mL	-0.5 \pm 1.9	-4.6 \pm 2.0	-8.4 \pm 2.7	-12.4 \pm 2.0
<i>P</i> value	.79	.03	.0052	<.0001
2.5 μ g/mL	-9.9 \pm 1.9	-14.0 \pm 2.0	-17.8 \pm 2.7	-21.7 \pm 2.0
<i>P</i> value	<.0001	<.0001	<.0001	<.0001

Note: Values are estimated mean \pm standard error of the difference between sample (with TNF- α) and control (without TNF- α) at each combination of TNF concentration and time. $P < .025$ is considered statistically significantly different from zero as estimated from the two-way ANOVA model with repeated measures. A negative mean indicates the toxic effect of TNF- α on sperm motility.

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repeated measures, as each sample produced four such differences at incubation time of 2, 6, 12, and 24 hours. No statistically significant effect with TNF- α was seen at zero hours, and it was therefore excluded.

The toxic effect of TNF- α with incubation time and interaction were examined by *F*-tests. These tests provided evidence on whether different TNF- α concentrations or different incubation times resulted in different sperm motility and whether the effect of TNF- α was different at different time points.

Expected sperm motility (the mean differences from controls) was calculated from the two-way ANOVA model and tested against zero for each time by TNF- α concentration combination. A negative mean difference indicated that the sample has lower sperm motility compared with the control. We used a Bonferroni correction to adjust for false-positive inflation. Among these tests, $P < .0025$ was considered statistically significant. Similar analyses were done for HOS test and DNA damage.

In experiment 2, a two-way ANOVA model with repeated measures was applied to model the sperm motility, HOS test, and DNA damage data at 6 hours of incubation. The repeated measures analysis adjusted for the correlation among the four measurements (four aliquots) of the same sample. We used *F*-tests to show whether there was any difference with or without TNF- α (the main effect of TNF- α), with or without infliximab (the main effect of infliximab), and whether the effect of infliximab changed with the presence or absence of TNF- α (their interaction). The interaction was related to whether infliximab could counteract the toxic

effect of TNF- α (Fig. 1). All statistical tests were two-sided, and $P < .05$ was considered statistically significant (except the Bonferroni-corrected tests mentioned above). The statistical analysis was performed with SAS software 9.1 (SAS Institute, Cary, NC).

RESULTS

Experiment 1: Evaluation of TNF- α Toxicity

The model suggested that both TNF- α concentration and exposure time decrease sperm motility in a statistically significant manner ($P = .0005$, $P < .0001$, respectively) and their interaction does not ($P = .36$). These results implied that different concentrations of TNF- α and incubation times caused statistically significant, different decreases in sperm motility compared with the control but that the effect of TNF- α concentration did not depend on time. The model predicted that TNF- α in general would generate a statistically significant decrease in the sperm motility when compared with the control. By taking the average across TNF- α dosage levels, it was estimated that incubation with TNF- α caused sperm motility to drop by an average of 2.2% (SE = 1.2, $P = .09$) at hour 2, 6.3% (SE = 1.4, $P = .0002$) at hour 6, 10.1% (SE = 2.2, $P = .0002$) at hour 12, and 14.0% (SE = 1.4, $P < .0001$) at hour 24.

Pair-wise comparisons showed that a TNF- α concentration of 2.5 μ g/mL and 400 pg/mL had a greater effect than any of the other three concentrations ($P < .05$ for all comparisons); there were no statistically significant pair-wise differences between 2.5 μ g/mL and 400 pg/mL, or among 100 pg/mL, 300 pg/mL, and 500 pg/mL.

TABLE 2

Effect of TNF- α concentration and time on the percentage of spermatozoa with normal functional integrity of the sperm plasma membrane (hypoosmotic swelling test).

TNF- α concentration	Time (hours)			
	2	6	12	24
100 pg/mL	-5.4 \pm 3.6	-5.4 \pm 4.4	-5.8 \pm 3.7	-1.8 \pm 3.5
<i>P</i> value	.15	.24	.13	.61
300 pg/mL	-2.4 \pm 3.6	-2.2 \pm 4.4	1.6 \pm 3.7	-10.0 \pm 3.5
<i>P</i> value	.51	.62	.67	.0094
400 pg/mL	-10.6 \pm 3.6	-13.4 \pm 4.4	-3.4 \pm 3.7	-13.4 \pm 3.5
<i>P</i> value	.0008	.0066	.37	.001
500 pg/mL	-1.0 \pm 3.6	-4.0 \pm 4.4	-8.0 \pm 3.7	-10.2 \pm 3.5
<i>P</i> value	.78	.38	.04	.0082
2.5 μ g/mL	-19.2 \pm 3.6	-29.6 \pm 4.4	-35.6 \pm 3.7	-43.8 \pm 3.5
<i>P</i> value	<.0001	<.0001	<.0001	<.0001

Note: Values are estimated mean \pm standard error of the difference between sample (with TNF- α) and control (without TNF- α) at each combination of TNF concentration and time. $P < .025$ is considered statistically significantly different from zero as estimated from the two-way ANOVA model with repeated measures. A negative mean indicates the toxic effect of TNF- α on sperm plasma integrity.

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Table 1 displays the estimated mean differences from the control for each concentration by time combination. As a result of Bonferroni correction, $P < .0025$ was considered significantly different from zero; if the mean was negative, it implied a statistically significant detrimental effect. A negative effect was seen in almost all combinations, reconfirming the observation that TNF- α decreases sperm motility. We found that TNF- α exerted a toxic effect even at a lowest concentration of 100 pg/mL. Early effects of TNF- α were seen at 2 or 6 hours with TNF- α concentrations of 400 pg/mL and 2.5 μ g/mL. An *F*-test for the effect of time showed that sperm motility decreased in a statistically significant manner over time ($P < .01$), which agrees with Table 1 and our other results. Note that in Table 1 the SE estimates were identical across concentration levels but different over time, because the ANOVA model assumes that data from different sperm suspensions are independent with similar variations, but the repeated measurements (at time 2, 6, 12, and 24 hours) of the same sperm suspension are correlated, a common assumption of repeated measures analysis. The detrimental effect of TNF- α may have happened earlier in time or at lower concentrations than the *P* values indicate due to the conservative nature of the Bonferroni correction. In fact, the boundary very likely lies in between the result based on Bonferroni correction and the result with no corrections to the statistical significance level.

The model for HOS values included the statistically significant effects for both TNF- α concentrations ($P = .0001$) and incubation time ($P = .001$), implying that a different TNF- α concentration or incubation time led to different decreases in HOS values. The interaction was also statisti-

cally significant ($P = .003$), indicating that the change in HOS values due to a particular level of TNF- α depended on the length of incubation. For this reason, the effect of TNF- α levels is discussed in reference with the incubation time.

The mean differences for each TNF- α concentration and time for HOS are shown in Table 2. The results suggest that TNF- α is toxic because of the decrease in the values for HOS. Higher toxic effects were seen with increasing concentrations of TNF- α . Although a TNF- α concentration of 100 pg/mL did not seem to have any statistically significant toxicity, the TNF- α concentration of 300 pg/mL showed some delayed toxic effects at 24 hours ($-10.0\% \pm 3.5\%$, $P = .009$). A TNF- α concentration of 400 pg/mL had toxic effects, especially after 24 hours ($-13.4\% \pm 3.5\%$, $P = .001$), whereas a TNF- α concentration of 500 pg/mL had toxic effects after 12 hours ($-8.0\% \pm 3.7\%$, $P = .04$). A TNF- α concentration of 2.5 μ g/mL had the strongest toxic effects, which were apparent as early as 2 hours of incubation (see Table 2).

The results for DNA damage were similar to and consistent with those for HOS. Statistically significant effects for TNF- α concentration, time, and their interactions were identified ($P = .0001$, $.0001$, $.002$, respectively). A statistically significant increase in the percentage of sperm with DNA fragmentation was seen following incubation for 24 hours with 400 pg/mL ($16.9\% \pm 3.4\%$, $P < .0001$) and 500 pg/mL of TNF- α ($12.0\% \pm 3.4\%$, $P = .0023$) compared with the controls. The concentration had to be at least 400 pg/mL for TNF- α to have a statistically significant toxic effect. The minimum time for TNF- α to exert a toxic effect was 6 hours

TABLE 3

Effect of TNF- α concentration and time on percentage of spermatozoa with fragmented DNA.

TNF- α concentration	Time (hours)			
	2	6	12	24
100 pg/mL	-0.1 \pm 2.0	-1.8 \pm 3.0	0.9 \pm 3.8	2.5 \pm 3.4
<i>P</i> value	.96	.56	.81	.47
300 pg/mL	0.6 \pm 2.0	0.9 \pm 3.0	-0.5 \pm 3.8	4.6 \pm 3.4
<i>P</i> value	.78	.76	.90	.20
400 pg/mL	3.1 \pm 2.0	1.4 \pm 3.0	5.0 \pm 3.8	16.9 \pm 3.4
<i>P</i> value	.14	.65	.20	<.0001
500 pg/mL	-0.3 \pm 2.0	2.5 \pm 3.0	6.1 \pm 3.8	12.0 \pm 3.4
<i>P</i> value	.90	.42	.13	.0023
2.5 μ g/mL	2.6 \pm 2.0	10.4 \pm 3.0	22.5 \pm 3.8	39.3 \pm 3.4
<i>P</i> value	.20	.0025	<.0001	<.0001

Note: Values are estimated mean \pm standard error of the difference between sample (with TNF- α) and control (without TNF- α) at each combination of TNF concentration and time. $P < .025$ is considered statistically significantly different from zero as estimated from the two-way ANOVA model with repeated measures. A positive mean indicates the toxic effects of TNF- α on DNA damage.

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at the highest concentration (2.5 μ g/mL) (Table 3). Because most of these mean differences between samples and controls were positive, it is clear that TNF- α leads to DNA damage.

Experiment 2: Effect of Infliximab

In experiment 2, the two-way repeated measures ANOVA model showed that TNF- α exerted statistically significant toxic effects on motility, HOS, and DNA damage ($P = .0001$, $P = .0029$, and $P = .0003$, respectively), which agrees with our findings in experiment 1. Infliximab did not appear to statistically significantly decrease HOS or increase DNA damage ($P = .31$ and $P = .11$, respectively), though it appeared to statistically significantly increase sperm motility ($P = .02$). Therefore, there was no evidence to suggest that infliximab alone was toxic. The interactions between TNF- α and infliximab were statistically significant in all cases ($P = .0022$ for motility, $P = .0044$ for HOS, and $P = .0035$ for DNA damage). Specifically, in the presence of TNF- α , infliximab statistically significantly increased motility by 18.8% \pm 4.5% and HOS by 16.7% \pm 4.4% and decreased the DNA damage by 16.2% \pm 4.1%. The interactions between TNF- α and infliximab are shown in Figures 1 and 2.

DISCUSSION

In the context of human reproduction, TNF- α has gained wide interest in recent years due to its probable detrimental effects on gametes. Studies of embryo toxicity using a mouse model have suggested that the peritoneal fluid from women with endometriosis is toxic. This fluid containing TNF- α inhibits the cleavage of a two-cell embryo (27). Moreover,

follicular fluid TNF- α levels are associated with poor oocyte quality and outcome in IVF (28).

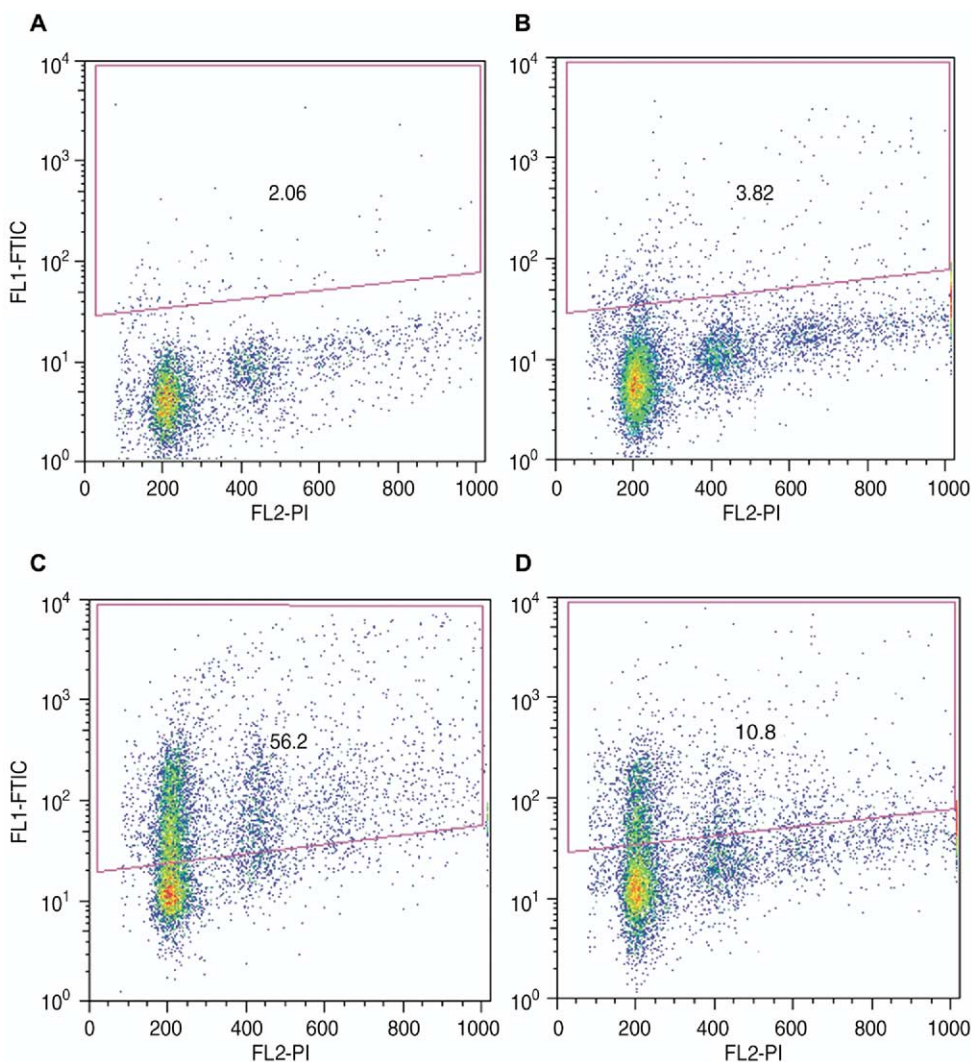
The correlation of TNF- α with male infertility and sperm parameters is controversial. Limited reports indicate that TNF- α has a negative correlation with sperm motility and morphology (4, 29). On the other hand, levels of TNF- α in seminal plasma detected in infertile patients were comparable with those of fertile donors (29, 30) and did not correlate with other semen parameters (31–33).

Inflammation, genital tract infections, and leukocytospermia are the only conditions that have been proven to correlate with TNF- α levels in seminal plasma (4, 29, 34–36). Soluble TNF- α receptor-1 is lower in infertile men with genital tract infection compared with fertile healthy men (30). Leukocytospermia leads to activation of macrophages in the male genital tract, releasing soluble products such as TNF- α and leading to initiation and regulation of the inflammatory response (37). Therefore, TNF- α appears to play a role in the mechanism of occurrence of sperm dysfunction in cases of leukocytospermia (36). Along with other inflammatory cytokines, TNF- α may serve as a sensitive marker for silent genital tract infection (30, 38).

The effect of TNF- α on human sperm motility was previously evaluated in vitro. Both total sperm motility and progressive sperm motility were significantly reduced following incubation for 4 and 21 hours in peritoneal fluid containing concentrations of TNF- α higher than 400 U/mL (39). In our present study, we were able to document subtle changes in sperm function and integrity with much lower concentrations. This discrepancy may be attributed to differences in the origin of the TNF- α used. In a study by Estrada

FIGURE 2

Flow cytometric analysis of FITC (Y-axis) and propidium iodide (X-axis) of (A) assay negative control sample used to set the gate, (B) experiment negative control aliquot consisting of sperm suspension with no additions, (C) sample with TNF- α only, and (D) sample with TNF- α plus infliximab.



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et al. (40), sperm motion characteristics, viability, and membrane integrity deteriorated within 60 to 180 minutes after incubation with 1 $\mu\text{g}/\text{mL}$ of TNF- α which agrees with our study.

The toxic effects that TNF- α exerts on spermatozoa could possibly be mediated by reactive oxygen species (ROS) because TNF- α has the potential to stimulate spermatozoa to generate ROS. In turn, ROS-related sperm membrane peroxidation may occur (41). Apoptosis may also be a potential mechanism for the occurrence of TNF- α toxic effects. Upon engagement with its receptor, TNF- α activates the TNF- α receptor associated factor, TNF-receptor-associated death domain, and receptor-interacting protein kinase 1. In turn, caspase-2 and caspase-8 become activated with the end

result of effector caspase-3 activation followed by cell death (42).

Tumor necrosis factor alpha is toxic not only to ejaculated spermatozoa but also to testicular somatic and germ cells. Continuous IV infusion of TNF- α in rats leads to a rapid decrease in testicular weight and plasma T and to an increase in levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The decrease in T and increase in gonadotropin levels may be attributed to TNF- α interfering with Leydig cell function; germ cell damage may have occurred either directly or secondarily through Leydig and/or Sertoli cell dysfunction (43). Moreover, TNF- α enhances the inhibitory effect of interleukin-1 β on Leydig cell steroidogenesis (44).

Infliximab, approved by the U.S. Food and Drug Administration in 1998, is a chimeric (part human, part mouse) monoclonal antibody that contains a murine binding site for TNF- α (45). Although a large portion of patients receiving infliximab are at risk of developing antibodies to the murine portion of the drug molecule (45), long-term therapy regimens are usually well tolerated (46). Infliximab binds to soluble TNF- α with high affinity and specificity, thus preventing its binding to its membrane-bound receptors on target cells (20).

Infliximab may also induce apoptosis via a caspase-dependent pathway. Treatment with infliximab at therapeutic concentrations induced apoptosis in monocytes by activating caspase-8, caspase-9, and caspase-3 (47). We find the apoptosis-inducing theory intriguing because we found that infliximab neutralizes TNF- α toxic effects, which may also be mediated by caspase-dependent apoptosis. Modulation in the kinetics of caspase activation may provide an explanation for the actual action of infliximab and whether it mediates proapoptotic or antiapoptotic signals. However, this hypothesis requires further validation.

Our results suggest that if infliximab can block the toxic effects of TNF- α , it potentially could be used in the treatment of selected infertile patients, especially as clinical trials are underway to develop a human anti-TNF- α antibody (D2E7) (48). In our research, we used an excessively high concentration of infliximab (400 $\mu\text{g}/\text{mL}$) to detect a statistically significant decreasing pattern in the toxicity induced by TNF- α . We did not observe any deterioration in spermatozoa quality as a result of using this concentration. However, similar concentrations have embryotoxic effects (24), and lower concentrations may be needed for its use in vivo. Future research may be directed toward optimizing the minimum concentration of infliximab required to neutralize the toxicity induced by TNF- α .

The fact that infliximab was able to reverse the TNF- α detrimental effects in our study lends further support to the newly emerging concept of using this monoclonal antibody for endometriosis therapy. The ideal drug in the treatment of endometriosis would cure pain and infertility without inhibiting ovulation or menstruation and without exerting detrimental side effects or teratologic effects. Such a drug would also allow women to become pregnant during treatment and change the management of this puzzling disorder from a surgical approach to a medical one. We strongly believe that infliximab has a considerable potential to be such a drug (49).

Depending on its concentration and the length of the exposure, TNF- α is detrimental to human spermatozoa. Elevated levels of TNF- α may be a potential cause of infertility in men with genital tract infection or in women with endometriosis. Infliximab is capable of reversing the toxic effects induced by TNF- α and of protecting spermatozoa against further insult. Therefore, infliximab may be a potential can-

didate in the management of infertile patients presenting with elevated levels of TNF- α .

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