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Effects of Ciprofloxacin on Male Fertility Parameters and Sperm DNA Integrity in Rats

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Abstract

Background The administration of antibiotics is of great value in the therapy of infections of male genital tract, which may have an effect on fertility. Antibiotics are generally prescribed for a variety of infections. A number of patients requiring assisted conception occasionally show evidence of reproductive tract infections. While fluoroquinolones being excessively prescribed in the treatment of male genital tract infections, too little information concerning their outcome on fertility are present.

Objective To estimate ciprofloxacin effects on sperm function parameters in addition to inspect whether ciprofloxacin can affect the integrity of sperm DNA.

Methods In the present study, 48 male adult rats were enrolled. The animals were randomly allocated into six groups; four ciprofloxacin treated groups, which were treated with either (40 mg/kg/day) or (80 mg/kg/day) of ciprofloxacin and 2 control groups. For each dose, the treatment maintained for either 14 days or 28 days. At the end of each duration of treatment, certain epididymal sperm function parameters: sperm morphological normality, sperm concentration and sperm motility were analyzed together with sperm DNA integrity analysis.

Results A significant reduction in sperm concentration, motility and percentage of morphologically normal sperm (in a dose dependent manner) was observed when ciprofloxacin administered for 28 days. The level of DNA fragmentation was significantly elevated with a significant reduction in sperm chromatin quality in ciprofloxacin treated groups whereas serum testosterone level was not significantly affected.

Conclusion Ciprofloxacin can adversely influence fertility parameters in male rat.

Keywords Fertility, Ciprofloxacin, Sperm DNA

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List of abbreviations: None

Introduction

Antibiotics are frequently prescribed in the treatment of different types of infections. Whereas some patients need assisted fertilization, in several cases, these patients display evidence of infection in male reproductive tract [(1)]. Consequently, the use of antibacterial agents is essential in the treatment of genital tract infections, which can probably influence fertility in male.

The antibiotic fluoroquinolones are frequently prescribed by fertility specialists in the therapy of numerous types of bacterial infections when high level of leukocytes is observed in the semen or before in vitro fertilization program, without taking consideration to bacterial evidence of infection [(2)].

Infertility can be defined as failure to attain pregnancy after 12 months of a usual

unprotected sexual intercourse [3]. Male infertility problem represents more than 45% of infertility problems. There are multiple factors, which can affect fertility in male; anatomical causes like varicocele or ductal obstructions represent some infertility factors in male [4]. Additionally, male infertility can be produced from abnormality in sperm; as it was predicted that defects in sperm production represent 36-75% of male infertility causes. Other factors that associated with infertility of male are reactive oxygen species, sperm antibodies, infection, cigarette smoking, radiation, heavy metals, hormonal causes, several therapeutic drugs and others [5].

Ciprofloxacin, which represent a second-generation fluoroquinolone, a broad-spectrum antibiotic used in the treatment of several gram-positive and gram-negative bacterial infections which affect the bones, joints, urinary and respiratory tracts. It generally acts by inhibition of type II topoisomerase, DNA gyrase, which is required for unwinding of replicated prokaryotic DNA. It is routinely prescribed by fertility specialists and urologists in the therapy of reproductive infections. Its side effects take place mostly in the central nervous system and gastrointestinal tract. Allergic and cardiovascular complications are other adverse effects observed during administration of ciprofloxacin [6].

In vitro and in vivo genotoxicity researches had recommended that this antibiotic is harmless for therapeutic use [7]. On the other hand, other studies have confirmed that ciprofloxacin could impair testicular structure and function [8]. Therefore, this study was intended to detect the effect of ciprofloxacin on sperm function parameters and integrity of sperm DNA.

Methods

This study involved the use of 48 male adult Albino-rats. Their weight was about (225±25) gm and the age of rats was ranged between 7-8 weeks old. The rats were housed in controlled temperature around 24 °C and 13±1 hour light-dark cycles. Rats were fed an ordinary commercial pellet. The experimental groups were equally allocated into six groups (4 ciprofloxacin treated groups and 2 control groups), ciprofloxacin was injected intraperitonially in a doses of (40 mg/kg/day) and (80 mg/kg/day) and each dose was administered in two periods, short duration of 14 days and long one (28 days) (the use of two doses was intended to detect the effect of increasing the dose on fertility parameters), in the control groups rats were injected intraperitonially with normal saline (in the same volume as ciprofloxacin): in the first control group rats were injected for 14 days and in the second control group they were injected for 28 days. At the end of each duration of treatment, rats from each group were anesthetized by diethyl ether, blood and epididymis were collected from each rat for the measurement of: sperm morphological normality, sperm concentration and sperm motility together with sperm DNA integrity analysis. The work on the animal was approved by the Institute Review Board in the College of Medicine of Al-Nahrain University.

Preparation of epididymal sperm

The caudal epididymis part of each rat was dissected and placed in (1 ml) of a previously warmed Hams F12 medium. Tearing of the tissue was made in order that the spermatozoa would swim out into the culture medium.

Microscopic examination

The microscopic observation was performed for each sample. one drop of sperm sample was put on a warm slide then covered by standard cover slip for scoring under light microscope of (40 X) objective.

Sperm function parameters analysis

Certain sperm function parameters were examined specifically; sperm motility, concentration and morphology. Motility was expressed as the fraction of progressive motility including speedy spermatozoa, grade A; slow spermatozoa, grade B; non-progressive sperm, grade C; and non-motile sperm, grade D [9].

Sperm DNA integrity (Acridine Orange Test)
Acridine orange represent a metachromatic fluorescence probe for evaluation of the degree of sperm nuclear DNA affinity for in-situ acid-induced denaturation by differentiation between native double-stranded DNA, which give green fluorescence and the red fluorescent which is produced by denatured single-stranded DNA [10]. Smears were fixed in glacial acetic acid-methanol (1:3), followed by staining with acridine orange {0.19 mg/ml, pH 2.5}.

Aniline Blue staining
Aniline blue distinctively stains histones rich in lysine and consequently detecting anomalies in sperm chromatin condensation [11]. In order to attain this, 3% buffered glutaraldehyde was used for fixation of sperm samples smears. Then stained with 5% aqueous aniline blue. Under light microscope, examination of spermatozoa was performed using a magnification of ×100 eyepiece [12].

Testosterone measurement
After centrifugation of whole blood, the serum was obtained and the concentrations of testosterone was measured by radioimmunoassay by means of a readymade kit (ichromaboditech).

Statistical analysis
The analysis of the study data was performed by SPSS software version 16. All results are expressed as mean±SE. The difference between the quantitative data was analyzed with one-way ANOVA, and followed by the Tukey test. P-value less than 0.05 were considered significant for all data in this study.

Results

Effects of ciprofloxacin on sperm concentration:
Administration of ciprofloxacin for 14 days (in a dose of 40 and 80 mg/kg/day) was not significantly affect sperm concentration, while significant decline in sperm concentration was observed when the drug was administered in a dose of (80 mg/kg/day) for 28 days as compared with the control group (Table 1).

Effects of ciprofloxacin on sperm motility
No significant alterations were seen in progressive motility or in the total motility of sperm (as compared with the control group) when the drug was administered (in 40 and 80 mg/kg/day) for 2 weeks, while a significant lessening in the total and in the progressive motility was observed when ciprofloxacin was injected for 28 days and the decline in sperm progressive motility was dose related (Table 1).

Effects of ciprofloxacin administration on sperm morphology
A significant reduction in sperm morphological normality was resulted when ciprofloxacin injected for 28 days and in high dose (80 mg/kg/day) as compared with the control group (Table 1).

Effects of ciprofloxacin on sperm DNA integrity and chromatin quality
Treatment with ciprofloxacin resulted in a significant elevation in the level of staining propensity of sperm DNA with acridine orange and this elevation was increase as the dose and /or the duration of the drug was increased, these differences as compared to the control groups (Table 2) and (Figure 1).
The fraction of sperm, which is positively stained with aniline blue was significantly elevated and in positive correlation with the dose and the duration of treatment with ciprofloxacin as compared with the control group (Table 2).

Effect of ciprofloxacin on serum testosterone level
In the present study, treatment with ciprofloxacin in two different doses and in different durations show no significant changes in the level of serum testosterone as compared with the control groups (Table 3).

Table 1. Effects of intraperitoneal injection of ciprofloxacin on sperm motility, concentration, and morphological normality in adult male rats

Treatment	Duration (days)	Progressive motility%	Total motility%	Immotile sperm%	Sperm concentration $^{\times}10^{6}$ (sperm/ml)	Morphologically normal sperm %
Control	14	49.0±1.41 (a)	80.71±0.92 (a)	19.29±0.92 (a)	40.14±1.32 (a)	91.71±0.42 (a)
	28	52.0±1.27 (a)	83.0±1.27 (a)	17.00±1.27 (a)	37.71±1.61 (a)	92.57±1.0 (a)
Ciprofloxacin (40 mg/kg/day)	14	47.86±1.92 (a)	79.43±1.84 (a)	20.57±1.84 (a)	38.0±0.8 $^{\times}10^{6}$ (a)	91.71±1.09 (a)
	28	36.57±.72 (b)	71.71±1.02 (b)	28.29±1.02 (b)	36± 0.48 $^{\times}10^{6}$ (ab)	90.71 ± 1.04 (ab)
Ciprofloxacin (80 mg/kg/day)	14	44.0±1.0 (a)	77.71±0.68 (a)	22.29±.68 (a)	37.2±0.84 $^{\times}10^{6}$ (a)	91.0±1.11 (a)
	28	29.57±0.53 (c)	68.29±1.30 (b)	31.71±1.30 (b)	32.7±0.42 $^{\times}10^{6}$ (b)	88.14± 0.63 (b)

Values are expressed as mean±standard error (n=8)
Values on the same column having the same letter (for example letter a) are not significantly different.

Table 2. Effects of intraperitoneal administration of ciprofloxacin on sperm DNA integrity and chromatin quality in adult male rats

Treatment	Duration (days)	Positive acridine orange staining %	Positive aniline blue staining %
Control	14	7.57±0.37 (a)	9.71±0.57 (a)
	28	6.29±0.42 (a)	10.14±0.94 (a)
Ciprofloxacin (40 mg /kg/day)	14	26.0±0.44 (b)	14.43±0.95 (b)
	28	42.57±0.37 (d)	20.14±0.51 (cd)
Ciprofloxacin (80mg/kg/day)	14	28.71±0.68 (c)	18.0±0.62 (c)
	28	47.0±0.53 (e)	22.14±0.8 (d)

Values are expressed as mean ± standard error (n=7)
Values on the same column having the same letter (for example letter a) are not significantly different.

Table 3. Effects of intraperitoneal administration of ciprofloxacin on serum testosterone level in adult male rats

	Serum Testosterone level (ng/ml)	
Treatment	14 days	28 days
Control	1.79±0.29 (a)	1.93±0.27 (a)
ciprofloxacin (40 mg/kg/day)	2.48±0.28 (a)	1.94±0.19 (a)
Ciprofloxacin (80 mg/kg/day)	2.25±0.25 (a)	2.06±0.28 (a)

Values are expressed as mean ± standard error (n=7)
Values on the same column having the same letter (for example letter a) are not significantly different.

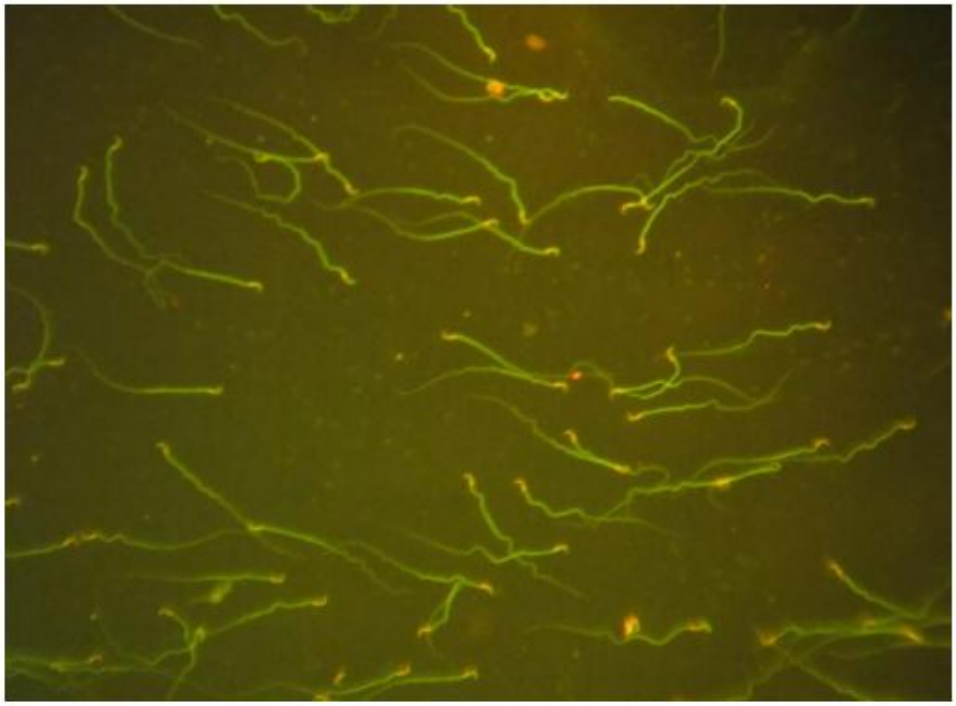

Figure 1. Fluorescence microscopic images of acridine orange stained cells, which present that spermatozoa generating green fluorescence are considered to contain normal DNA content, whereas sperms that display spectrum of yellow or orange to red fluorescence are considered to contain fragmented DNA

Discussion

In the present study, administration of ciprofloxacin resulted in significant decline in sperm concentration in a dose and time related manner, similar findings were observed by Demir et al. in 2007 as they had noticed a marked drop in sperm count after ten days of ciprofloxacin treatment [13]. Kaki et al. (2008), who recorded that treatment with ciprofloxacin for 60 days resulted in a significant reduce in the number of spermatogenic cells in seminiferous tubules [14]. There are a number of postulated mechanisms by which sperm concentration could negatively affected as direct toxicity of sperm or by suppression of cell growth or cellular production [15]. Eukaryotic cells apoptosis represents other mechanism and can be resulted from interference with the mitochondrial pathway [16], reduction in testosterone level [17], as well as, decline in chromatin quality or integrity of DNA can also have detrimental effect on sperm concentration [18]. In the current study, testosterone level was not considerably affected by ciprofloxacin, hence it cannot consider as a reason by which sperm concentration was unfavorably affected, however, when chromatin quality and sperm DNA integrity were tested, current results indicate a marked increase in the intensity of DNA fragmentation with reduction in sperm chromatin structure quality. As a result, it can be predicted that these detrimental effects on sperm genetic material can be considered as the mechanisms by which other sperm function parameters such as sperm concentration being negatively affected in the present study.

The motility of sperm depends mainly on Ca^{2+} influx and on mitochondrial oxidation process to obtain the energy necessary for hyperactivity and flagellum movement [19]. Thus, the adverse effect of ciprofloxacin on sperm motility propose that the drug may interfere with the function or the structure of Ca^{2+} channels by direct or indirect toxicity. The

other explanation is that; the drug may affect Cat Sper channels. As these channels are responsible for increasing Ca ions exhaustion which in turn stimulate sperm motility [20].

Evidences from previous reports in sperm morphology indicated that, alteration in the morphology of sperm might be resulted from modification in the compaction of chromatin [21]. These supposed mechanisms for abnormalities in sperm morphology are well-matched with the present findings as in the present study, it is founded that ciprofloxacin could adversely affect sperm chromatin structure.

The present findings showed that following ciprofloxacin administration, the level of sperms with a single stranded DNA (as indicated by acridine orange stain) and that of immature sperms (as indicated by aniline blue) were significantly elevated. Generally, the occurrence of elevated level of DNA nicks reflects cell necessity to unwind the torsional strain that produced from negative supercoiling which, on the other hand, associated with the protamines displacement as an alternative of nucleosomal histones and the modification in tertiary structure of the elongating spermatids. The presence of these nicks is not risky as they are continually ligated by topoisomerase II enzyme before spermiogenesis completion. Yet, these nicks cannot be correctly repaired if irregularity in topo II ligating activity are present or if its activity is blocked by the inhibitors of topo II enzyme [22]. Since ciprofloxacin is known as topoisomerase inhibitor [23], so it can be concluded that this drug could block the formation and ligation of DNA nicks which sequentially disturbs protamination and as a consequence stimulate internal damage in DNA by increasing its propensity to damage and prevention of its repair. This theory has been confirmed with elevation of DNA damage as indicated by the acridine orange and aniline blue staining in sperms after ciprofloxacin administration in the current study.

The current study concluded that ciprofloxacin could adversely affect the process of spermatogenesis in rats in time- and dose-dependent manner.

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Author Contribution

Al-Saray: Acquisition of data with participation. All authors participated in the conception and design of the study, analysis and interpretation of data.

Conflict of interest

The authors declare no conflict of interest.

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References

1. Pajovic B, Radojevic N, Vukovic M, et al. Semen analysis before and after antibiotic treatment of asymptomatic chlamydia-and ureaplasma-related pyospermia. Andrologia. 2013; 45(4): 266-71. doi: 10.1111/and.12004.
2. Calogero AE, Condorelli RA, Russo GI, el al. Conservative nonhormonal options for the treatment of male infertility: antibiotics, anti-inflammatory drugs, and antioxidants. Biomed Res Int. 2017; 2017: 4650182. doi: 10.1155/2017/4650182.
3. Gurunath S, Pandian Z, Anderson RA, et al. Defining infertility- a systematic review of prevalence studies. Hum Reprod Update. 2011; 17(5): 575-88. doi: 10.1093/humupd/dmr015.
4. Olayemi, FO. A review on some causes of male infertility. Afr J Biotechnol. 2010; 9(20): 2834-42.
5. Punab M, Poolamets O, Paju P, et al. Causes of male infertility: A 9-year prospective monocentre study on 1737 patients with reduced total sperm counts. Hum Reprod. 2017; 32(1): 18-31. doi: 10.1093/humrep/dew284.
6. Sharma PC, Jain A, Jain S, et al. Ciprofloxacin: review on developments in synthetic, analytical, and medicinal aspects. J Enzyme Inhib Med Chem. 2010; 25(4): 577-89. doi: 10.3109/14756360903373350.
7. Herbold BA, Brendler-Schwaab SY, Ahr HJ. Ciprofloxacin: in vivo genotoxicity studies. Mutat Res. 2001; 498(1-2): 193-205.
8. Khaki A. Assessment on the adverse effects of Aminoglycosides and Flouroquinolone on sperm parameters and male reproductive tissue: A systematic review. Iran J Reprod Med. 2015; 13(3): 125-34.
9. Vasan SS. Semen analysis and sperm function tests: How much to test? Indian J Urol. 2011; 27(1): 41-8. doi: 10.4103/0970-1591.78424.

10. Agarwal A. Said TM. Sperm chromatin assessment. In: Gardner DK, Weissman A, Howles CM, et al (eds). Textbook of assisted reproductive techniques Laboratory perspectives. 4th ed. Vol. 1. Informa; 2012. p. 75.

11. Zidi-Jrah I, Hajlaoui A, Mougou-Zerelli S, et al. Relationship between sperm aneuploidy, sperm DNA integrity, chromatin packaging, traditional semen parameters, and recurrent pregnancy loss. Fertil Steril. 2016; 105(1): 58-64. doi: 10.1016/j.fertnstert.2015.09.041.

12. Talebi AR, Vahidi S, Aflatoonian A, et al. Cytochemical evaluation of sperm chromatin and DNA integrity in couples with unexplained recurrent spontaneous abortions. Andrologia. 2012; 44 Suppl 1: 462-70. doi: 10.1111/j.1439-0272.2011.01206.x.

13. Demir A, Türker P, Önol FF, Sirvanci S, Findik A, and Tarcan T. Effect of experimentally induced Escherichia coli epididymo-orchitis and ciprofloxacin treatment on rat spermatogenesis. Int J Urol. 2007; 14(3): 268-72. doi: 10.1111/j.1442-2042.2007.01682.x

14. Khaki A, Heidari M, Novin MG, et al. Adverse effects of ciprofloxacin on testis apoptosis and sperm parameters in rats. Iran J Reprod Med. 2008; 6(2): 71-6.

15. Zobeiri F, Salam S, Sadrkhanlou R, et al. Role of mitochondria in ciprofloxacin-induced apoptosis in murine sperm cells. Reprod Sci. 2013; 20(9): 1090-5. doi: 10.1177/1933719113477482.

16. Abd-Allah AR, Aly HA, Moustafa AM, et al. Adverse testicular effects of some quinolone members in rats. Pharmacol Res. 2000; 41(2): 211-9. doi: 10.1006/phrs.1999.0580.

17. Elias A, Nelson B. Sub chronic evaluation of ciprofloxacin and perfloxacin on sperm parameters of male guinea pigs. Asian J Exp Biol Sci. 2012; 3: 595-601.

18. Zobeiri F, Sadrkhanlou RA, Salami S, et al. The effect of ciprofloxacin on sperm DNA damage, fertility potential and early embryonic development in NMRI mice. Vet Res Forum. 2012; 3(2): 131-5.

19. Correia J, Michelangeli F, Publicover S. Regulation and roles of Ca2+ stores in human sperm. Reproduction. 2015; 150(2): R65-76. doi: 10.1530/REP-15-0102.

20. Rahman MS, Kwon WS, and Pang MG. Calcium influx and male fertility in the context of the sperm proteome: an update. Biomed Res Int. 2014; 2014: 841615. doi: 10.1155/2014/841615.

21. Zini A, Phillips S, Courchesne A, et al. Sperm head morphology is related to high deoxyribonucleic acid stainability assessed by sperm chromatin structure assay. Fertil Steril. 2009; 91(6): 2495-500. doi: 10.1016/j.fertnstert.2008.03.032.

22. Evenson DP, Larson KL, Jost LK. Sperm chromatin structure assay: its clinical use for detecting sperm DNA fragmentation in male infertility and comparisons with other techniques. J Androl. 2002; 23(1): 25-43.

23. Mitscher LA. Bacterial topoisomerase inhibitors: quinolone and pyridone antibacterial agents. Chemical reviews. 2005; 105(2): 559-92.

24. Abdul Kahaleq MA, Abu-Raghif AR, Kadhim SR. Antibacterial activity of Fenugreek essential oil against Pseudomonas aeroginosa: In vitro and in vivo Studies. Iraqi J Med Sci. 2015; 13(3): 227-34.

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