VERSITA

© **2012** ILEX PUBLISHING HOUSE, Bucharest, Roumania
http://www.jrdiabet.ro
Rom J Diabetes Nutr Metab Dis. **19**(4):405-415
doi: 10.2478/v10255-012-0047-8

REPRODUCTIVE DISORDERS IN STREPTOZOTOCIN-TREATED MALE RATS FOLLOWING CO-ADMINISTRATION OF ETHAMBUTOL, RIFAMPICIN, ISONIAZID AND PYRAZINAMIDE

Ganna M. Shayakhmetova*✉*, Larysa B. Bondarenko, Anatoliy V. Matvienko, Valentina M. Kovalenko

SI "Institute of Pharmacology & Toxicology National Academy of Medical Sciences of Ukraine"

received: September 12, 2012 *accepted*: November 29, 2012
available online: December 15, 2012

Abstract

Objectives: *To evaluate the effect of anti-tuberculosis drugs (ATD) on indices of reproductive capability, DNA fragmentation and offspring development of male rats with testicular malfunction caused by experimental diabetes.* ***Materials and Methods:*** *Wistar albino male rats (body weight 160–200 g) were divided into three groups: I – control, II - streptozotocin diabetes, III – streptozotocin diabetes + ATD. The testis DNA fragmentation was determined electrophoretically; spermatogenetic indices, offspring antenatal and postnatal development indices - by standard procedures. Morphological analyses of gonadic structures were carried out by optic microscopy.* ***Results:*** *The study of the effects of diabetes and ATD administration on testis cells morphologic and morphometric parameters and spermatogenesis suggested the presence of specific diabetes- and anti tuberculosis drugs - mediated quantitative and qualitative changes in male rat reproductive organs, spermatogenetic epithelial cells, level and character of DNA fragmentation in comparison with normal rats. These changes were accompanied by alterations in processes of fertilisation (with intact females), embryogenesis and by lowering of offspring survival.* ***Conclusions:*** *Observed changes could hence affect the state and correct functioning of spermatogenetic epithelium and of other tissues of reproductive organs, as well as offspring development in diabetic rats.*

key words: *diabetes, anti-tuberculosis drugs, spermatogenesis, DNA fragmentation, rats*

Background

Diabetes and tuberculosis are two diseases characterized by a widespread prevalence among subjects of different age groups [1]. A particularly significant problem is the combination of these diseases. Several studies have suggested that diabetes mellitus increases the risk of active tuberculosis [2]. It is known that male and female reproductive function is often disrupted in diabetes [3]. Diabetes mellitus is thought to affect male reproductive

✉ Eugene Pottier 14, Kyiv, 03680, Ukraine, Phone: 38044 456 78 65, Fax: 38044 456 83 90;
corresponding author e-mail: anna_shayakhmetova@yahoo.com

function at multiple levels due to its effects on the endocrine control of spermatogenesis and spermatogenesis itself, as well as causing disorders of penile erection or ejaculation [4]. The disturbance of testosterone synthesis is caused by molecular changes at the level of Leydig cells. The close correlation between Leydig and Sertoli cells function, i.e., between production of testosterone and spermatogenesis, results in certain anomalies in diabetic patients' spermograms. Sexual dysfunction in all its forms (reduced erection, impotence, and other libido alterations) is an accompanying phenomenon of the diabetic disease [5]. On the other hand our previous results suggest that combined administration of ethambutol (EMB), isoniazid (INH), rifampicin (RMP) and pyrazinamide (PZA) therapeutic doses to male rats during the period of spermatogenesis causes an increase of lipoperoxidation in testes and epididymis, decrease of testes glutathione and protein SH-group contents, profound changes in DNA fragmentation, fatal lowering of male fertilizing capacity and fertility [6]. It must be stressed that the increase in the numbers of diabetics and tuberculosis diagnosed at a young age has coincided with worldwide concerns over male fertility [2,7]. We suggest that disturbances in male reproductive health in diabetes could be alleviated if the concomitant causes of infertility are correctly identified and treated.

The aim of the study was to evaluate effect of anti-tuberculosis drugs (ATD) co-administration on indices of reproductive capability, DNA fragmentation, and offspring development of male rats with testicular malfunction caused by experimental diabetes.

Material and methods

Wistar albino male and female rats, 4 months old, body weights 150–170 g, were purchased from Biomodel Service (Kyiv, Ukraine). The animals were kept at standard conditions of nutrition, water and light regimens. All animal studies were performed in accordance with the recommendations of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes and approved by the Institutional Animal Care and Use Committee.

In the experiments, we used streptozotocin (STZ) produced by Sigma-Aldrich, USA. Rats were kept in quarantine for 10 days, then diabetes was induced by a single intraperitoneal injection of freshly prepared solution of STZ in citrate buffer (pH 4.5) at a dose of 60 mg/kg of B.W. [8]. The control group (I) received only citrate buffer in corresponding volumes. During two weeks blood glucose levels were controlled by Smartest Optima (Biotest T Medical Corporation, Germany). A range for normal blood glucose value in Wistar male rats (3.9-11.56 mmol/l) was taken into consideration [9]. Severity of the induced diabetic state was evaluated and the rats with blood glucose level over 16.5±0.8 mmol/l were taken into experiment. They were randomized into two experimental groups: diabetes (II) and diabetes with ATD co-administration (III). Each group included 10 experimental males and 20 untreated females for mating. B.W. was measured weekly.

EMB, INH, PZA and RMP were supplied by the Private Corporation Scientific Production Enterprise "Borzhagovsky Chemical-Pharmaceutical Plant", Ukraine. ATD suspended in 1% starch gel were given

by gavage in DOTS (directly observed treatment, short-course) regimen at maximal doses used in clinic [10]: EMB – 155 mg/kg/day, RMP – 74.4 mg/kg/day, INH – 62 mg/kg/day, PZA – 217 mg/kg/day for 60 days (duration of spermatogenesis process and time of germ cell maturation in epididymis). The coefficient for conversion of human doses to animal equivalent doses based on body surface area was taken into account [11].

Following 46 days of ATD repeated administrations, the males from all groups were mated with intact females at the ratio 1 male: 2 females during 14 days (approximately 2–3 estrous cycles). The administration of ATD to male rats was continued during this period. According to generally accepted guidelines for the fertility study in laboratory rats [12] first day of pregnancy was established by vaginal cytology (first day of sperm detection in vagina). Most males were mated within the first 5 days of cohabitation (i.e. at the first available oestrus), but part of them (from groups II and III) demonstrated infertility. This fact was taken into account for evaluation of effects of ATD and diabetes on male fertilizing capacity, which was determined by the index:

$$\frac{Number\ of\ pregnant\ females}{Number\ of\ females\ mated\ with\ males} \times 100$$

Males were sacrificed under mild ether anesthesia via decapitation after 14 days of mating period. Their testes and epididymises were used for further evaluation of fertility indices and spermatogenetic epithelium parameters - by standard methods [12] and levels of DNA fragmentation – as previously described [6].

Testes were rated for its spermatogenetic potential (spermatogenetic index) on a standardized scale of 1 to 4. The index was based on the appearance of the spermatogenetic cells throughout the testis and included number of cell layers, types of cells, and the presence of late spermatids in the seminiferous tubules. The index and criteria were as follows: 1 - only spermatogonia present; 2 - spermatogonia and spermatocytes present; 3 - spermatogonia, spermatocytes and round (early) spermatids present with < 5 late spermatids per tubule; 4 - spermatogonia, spermatocytes, and round spermatids present with up to 25 late spermatids per tubule [12].

The pregnant females were sacrificed under mild ether anaesthesia via decapitation on day 20 of pregnancy for determination of foetal antenatal development indices. Females with negative vaginal cytology (sperm not detected) were sacrificed at the same conditions to confirm the absence of pregnancy.

The numbers of corpora lutea in ovaries, of implantation sites and of live and dead foetuses in each uterine horn were counted after laparotomy of pregnant females. Indices of embryonic death at pre- and post-implantation periods of development were calculated as it was previously described [6].

The obtained data were calculated by one-way analysis of variance (ANOVA) and expressed as the mean ± standard error of the mean (M±S.E.M.). Data were compared using Tukey test. Differences were considered to be statistically significant at $p < 0.05$.

Results

Testes of experimental animals were rated for their spermatogenetic potential (spermatogenetic index), which characterize different types of spermatogenetic epithelium maintenance, based on the appearance of the

spermatogenetic cells throughout the testis and included number of cell layers, types of cells, and the presence of late spermatids in the seminiferous tubules. According to Table 1 data, spermatogenetic index was lower in testes of diabetic rats in comparison with control. For the group with diabetes and ATD co-administration it didn't reach statistical significance because of the low number of animals (only 6). This was explained by the great morphological violations in some experimental animals (tubules with regions free of germ cells, deformed and empty tubules, germinative epithelium without signs of spermatogenesis, tubules dystrophy and necrosis – see Figure 1, 2) with no possibility to carry out quantitative analyses of spermatogenetic epithelium indices in these animals.

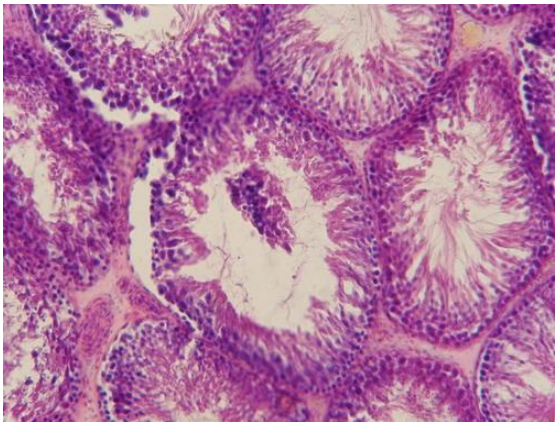

Figure 1. Focuses of spermatogenetic epithelium desquamation from basal membrane and interstitial edema in testis of rat with diabetes following ATD administration. Hematoxilin and eosin, x 400.

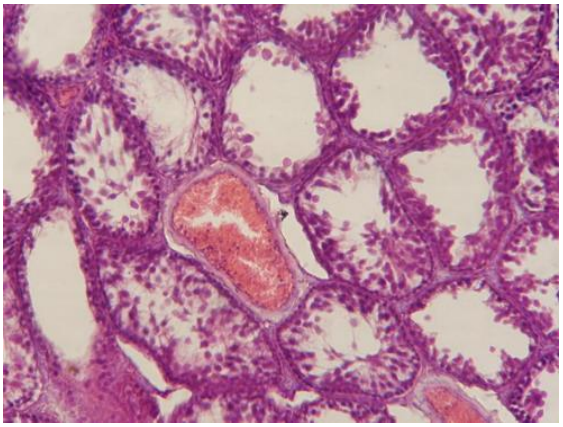

Figure 2. Focal loss of spermatogenetic epithelium, blood clots in vessels in testis of rat with diabetes following ATD administration. Hematoxilin and eosin, x 200.

Table 1. Rat spermatogenetic epithelium morphometric indices with diabetes and ATD administration (M±S.E.M., n = 10).

Indices	Group of males		
	Control	Diabetes	Diabetes+ATD
Spermatogenetic index (stages of spermatogenesis total / number of examined tubules)	3.61±0.01	3.49±0.01*	3.56±0.42 #
Number of spermatogonia (per tubular cross section)	80.03±0.68	65.99±0.71*	70.68±1.37 #
12-th stage of meiosis, %	5.21±0.44	2.02±0.39*	5.67±1.86 #
Desquamated epithelium, %	0.99±0.55	3.01±1.06	6.0±3.79 #
Exfoliation of epithelium, %	0	1.4±0.58*	6.67±2.91* #
Germ cell-free regions - "Windows",%	0	5.25±1.29*	4.67±1.45* #

Footnotes:
*- $p<0.05$ in comparison with control
- n=6 due to great morphological violations in some rats testes and impossibility to perform quantitative evaluation

Primary spermatogenesis cell population was also changed under the influence of the diabetic milieu. At these conditions, mitotic activity was inhibited and number of spermatogonia in testes tubules sections was lowered. Simultaneously, in the group of diabetic rats, the number of cells at 12-th stage of meiosis (characterizing meiotic division of primary spermatocytes) was lowered two times in comparison with controls (Table 1).

As for the animals in the group with diabetes and ATD administration, the above mentioned parameters had the same character (Table 1) but their quantitative evaluation was complicated by great morphological violations in some experimental animals as commented above.

Beside the previously mentioned quantitative indices of spermatogenetic epithelium cells damage, some qualitative changes in testes tubules were also investigated and the obtained results could be an evidence of degenerative changes in gonads. According to Table 1 data, both experimental groups exhibited increased levels of desquamated epithelium (shedding of epithelial elements), epithelium exfoliation (detachment from tubule basal membrane) and "windows" (germ cell-free regions), while in controls the percent of desquamated epithelium was insignificant and epithelium exfoliation and "windows" were absent.

The epididymal total sperm count in the experimental group with diabetes was 1.6 fold decreased as compared with controls (Figure 3).

At the same time in the ATD-exposed diabetic group, the total sperm count was 2.7 times lower as compared with control (Figure 3).

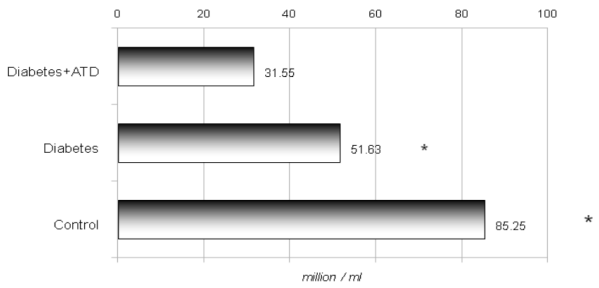

Figure 3. The total count of epididymal sperm in experimental rats.

Daily observation of animals with STZ diabetes, including rats with ATD co-administration, evidenced changes in their sexual behavior in comparison with the control group (lowering of motivation and mating activity). These data are in good correspondence with previously published data [13].

Table 2 data evidences fertility index lowering (- 25% and respectively 30%) in both experimental groups in comparison with control males.

Investigation of rats' testis DNA fragmentation demonstrated its increase in testes of diabetic rats with or without ATD co-administration in comparison with the control group (Figure 4). In the control group were present 7 fractions of DNA fragments, with weights over 1000 (1 fraction), 650, 600, 500, 480, 50 and 30 b.p. In diabetic rats there were present 19 fractions of DNA fragments, with weights over 1000 (6 different fractions), 1000, 950, 900, 800, 750, 650, 600, 550, 500, 480, 100, 50 and 40 b.p. In diabetic rats with ATD co-administration there were present 15 fractions of DNA fragments, with weights over 1000 (4 different fractions), 1000, 800, 700, 550, 500, 420, 400, 340, 80, 70 and 50 b.p.

Table 2. Fertility index of rats in norm and with diabetes and ATD administration.

Group of males	Number of mated females	Number of pregnant females	Fertility index, %
Control	20	20	100,0
Diabetes	20	15	75,0
Diabetes + ATD	20	14	70,0

Table 3. Embryologic indices at 20-th day of pregnancy of female rats coupled with male rats.

Group of males	Number of pregnant females	Total number of corpora lutea	Total number of implantation sites	Preimplanta-tional loss		Postimplanta-tional loss		Total embryo-nal loss % (3)	Total number of live conceptuses	
				abs	% (1)	abs	% (2)		abs	% (4)
Control	20	246	212	34	13.8	6	2.8	16.26	206	97.16
Diabetes	15	160	134	26	16.3	12	8.96	23.75	122	91.04
Diabetes + ATD	14	150	116	34	22.6	22	18.96	41.56	94	81.03

(1) % of preimplantational loss = [(number of corpora lutea - number of implantation sites) / number of corpora lutea)] x 100%;
(2) % of postimplantational loss = (number of lost fetuses / - number of implantation sites) x 100%;
(3) % of total embryonic loss = [(number of corpora lutea - number of live conceptuses) / number of corpora lutea] x 100%
(4) % of live conceptuses = (number of implantation sites / absolute number of live conceptuses) x 100%

Figure 4. DNA fragmentation in testes of normal rats in comparison with diabetic rats, with or without ATD administration (1 - controls; 2 - diabetes, 3 - diabetes + ATD).

Alterations at genomic level (DNA fragmentation) in our experiments were accompanied by changes of embryonic loss at different periods in diabetic rats with or without ATD administration (Table 3).

The results from Table 3 represent evidence for offspring embryonic development alterations such as preimlpantational and postimlpantational losses in the presence of the experimental conditions used in our study. Especially sensitive was stage of development after implantation. While pre-implantational losses in females mated with diabetic, including ATD-exposed, males were only some percents higher in comparison with control (2.2% and

8.8% respectively), simultaneously postimplantational losses in these groups were 3 and 7 times higher than in normal animals. Certainly, this led to a change in the level of total embryonic loss. The fact that this parameter was higher in the group of rats with diabetes + ATD in comparison with diabetic and control groups (2 and 2.5 times respectively) was of special interest.

Discussion

Our data showed abnormal spermatogenesis following STZ injection in male rats, result which is concordant with data of other authors [14]. Our observations illustrate depressed cellular activity of spermatogenetic cells. The number of spermatogonia cells decreased in diabetic testes and the number of primary spermatocytes decreased significantly in comparison with control rats. This finding suggests that the conversion of spermatogonia into primary spermatocytes is reduced in diabetic conditions. These alterations in cellular conversion and/or activity lead to a reduction in spermatozoid production. Following ATD administration in diabetic rats, the negative changes in the rats' testes spermatogenetic epithelium were aggravated, and the total sperm count was markedly reduced, leading to lower fertility of experimental animals.

Increased cellular oxidative stress and altered antioxidant pool have been implicated in the pathogenesis of diabetes chronic complications [15]. Hyperglycemia, auto-oxidation of glycated proteins, increased production of reactive oxygen species (ROS), decreased antioxidant defense, increased lipid peroxidation and associated membrane degeneration are implicated as main causes of cellular death (apoptosis or necrosis), a process common in diabetes complications [16,17]. Among the mechanisms of reproductive disorders in animals with experimental diabetes, oxidative processes in the testis and sperm could play an important role. It was shown that oxidative stress is persistent and associated with significant increases in testicular DNA damage and the frequency of abnormal sperm occurrence [18].

On the other hand ATD could induce the production of ROS, activation of lipid peroxidation and oxidative stress development [19] with consequent damage of major intracellular molecules (glutathione, DNA, RNA, proteins, lipids and ATP). Any changes in the level of these substances are of key importance for cell viability and great deviations cause cell damage and death [20,21].

For understanding the exact effect of oxidative stress on germ cells maturation and function, it is important to emphasize that some studies indicated the presence of high-inducible cytochrome P-4502E1 isoform (CYP2E1) in male gonads [22-24]. It is known that CYP2E1 expression is affected by a variety of pathophysiological situations including diabetes [25]. Elevated oxidative stress and ROS production in diabetes often parallels an increased expression of CYP2E1. The extent of oxidative stress increase, steady-state levels of CYP2E1 and activation of glutathione-dependent oxidant metabolism were different in different tissues [26]. CYP2E1 generates reactive oxygen intermediates, such as superoxide radicals, which in turn could rapidly react with organic molecules generating secondary free radicals

and ROS [27]. Such cascades may alter the antioxidant milieu of testis and epididymis, producing conditions for sperm oxidative damage. Deregulation of physiological germ cells apoptosis, which could cause male infertility [28], may be the result of pathologic processes (such as diabetes) and/or external factors (such as exposure to certain chemotherapeutic agents) [29]. It should be noted that the ATDs used in our experiments, at least INH and RMP may act as CYP2E1 inducers [30].

DNA is an important molecular target for toxic drugs [31], which induce endo-nucleases, leading to lethally DNA splitting. Such compounds could also inhibit processes of DNA repair by nuclear DNA-polymerases. Levels and character of DNA fragmentation are markers of apoptotic processes in organism [32]. Our results on DNA-fragmentation intensification in rat testis with diabetes and ATD co-administration are concordant with our previous data which demonstrated the presence of epigenetic effects following PZA administration in rats [31] and with the results of other authors indicating some negative ATD effects on chromosome structure and functioning [33]. Differences in DNA-fragmentation processes between diabetes with or without ATD co-administration could be caused by specific effects of these compounds on different nucleases activities [32]. Both diabetes and ATD treatment could cause qualitative changes in the length and structure of nucleic acid molecules [31].

Testes and sperm DNA quality are known to be associated with decreased embryo quality, low embryo implantation rates, higher miscarriage rates, and some serious childhood diseases, in particular some childhood cancers [29,34]. In our experiments, postimplan-tational lethality may have been caused by the genotoxic action of ATDs [35]. It is generally accepted that the cytotoxicity produced by STZ depends on DNA alkylation while several lines of evidence indicate that free radicals play an essential role in the mechanism of DNA damage and cytotoxicity by STZ [36,37]. First of all, it was found that STZ enhances O_2^- radical generation by the xanthine oxidase system of pancreatic cells [38], it stimulates H_2O_2 generation and causes DNA fragmentation in isolated rat pancreatic islets [39]. DNA lesions produced by STZ include double and single-strand breaks, covalent adducts and alkali-labile sites. Severe DNA damage by STZ results in cell death by apoptosis or necrosis. Furthermore, the DNA strand breaks resulting from the alkylating action of STZ can lead to chromosomal rearrangements [40]. We suggest that, in our experiment, the genotoxic effects of STZ added to the genotoxic effects of ATDs contributed to the increase of postimplantation embryo-lethality indices. This hypothesis is supported by the data of other authors' experiments on mice demonstrating weak genotoxicity of PZA at doses of 125, 250 and 500 mg/kg [33]. Moreover, other authors' *in vitro* experiments showed that an INH metabolite (monoacethylhydrazine) exhibited mutagenic actions [41]. The weak mutagenic effect of INH and its ability to cause liver DNA injury was also demonstrated [42,43]. Finally, RMP genotoxicity investigation showed increased frequency of sister chromatids exchanges in bone marrow cells at doses of 160, 240 and 310 mg/kg and a

number of chromosomal aberrations of spermatocytes at the dose of 80 mg/kg [44].

As to pre-implantational lethality, it must be stressed that mutagenic as well as non-mutagenic effects could be involved. Particularly in diabetes, a disrupted mitochondrial trans-membrane potential contributes to reduced sperm motility. Together with the increased presence of intracellular ROS and higher levels of sperm DNA fragmentation, several subcellular factors are now available to explain subfertility in diabetic males [45]. Finally, we suggest that ethambutol may have contributed to increase of pre-implantational lethality indices since it was established that at doses of 25 mg/kg and 250 mg/kg this compound caused spermatogenetic epithelium disturbances with further blocking of spermatogenesis in rats and chicken [46].

It should be noted that direct extrapolation of obtained results to humans cannot be done due to interspecies differences. Another limitation of our study is represented by the relatively small number of experimental animals. In our opinion a critical examination of the evidence, both epidemiological and laboratory animals' data, for simultaneous effects of diabetes mellitus and ATD treatment on human fertility should be done in order to reach a general conclusion.

Conclusion

Our study showed specific diabetes and ATD mediated quantitative and qualitative changes in male rat testes, spermatogenetic epithelial cells, levels and character of DNA fragmentation in comparison with normal animals as well as detrimental effects on offspring.

The obtained data possibly provide evidence for increased adverse effects of ATD on testes and reproductive capacity in STZ-treated diabetic rats. We hypothesize that probably CYP2E1 is an important initial direct contributor in generating ROS and other toxic metabolites in testes which subsequently cause DNA damage and altered male reproduction. These results could provide the framework for a hypothesis on how these xenobiotics may interact with diabetes in inducing male infertility. Future research is needed to expand our understanding of these relationships.

REFERENCES

1. **Mladovsky P, Allin S, Masseria C et al.** Health in the European Union. Trends and Analysis. Observatory Studies Series № 19, *Publications, World Health Organization Regional Office for Europe*, Copenhagen, pp 200, 2009. (http://www.euro.who.int/pubrequest).

2. **Jeon CY, Murray MB**. Diabetes mellitus increases the risk of active tuberculosis: a systematic review of 13 observational studies. *PLoS Med* 5: e152, 2008.

3. **Amaral S, Oliveira PJ, Ramalho-Santos J.** Diabetes and the impairment of reproductive function: possible role of mitochondria and reactive oxygen species. *Curr Diabetes Rev* 4: 46-54, 2008.

4. **Agbaje IM, Rogers DA, McVicar CM et al.** Insulin dependent diabetes mellitus: implications for male reproductive function. Hum Reprod *22: 1871-1877, 2007*.

5. **Dinulovic D, Radonjic G.** Diabetes mellitus/male infertility. *Arch Androl* 25: 277-293, 1990.

6. **Shayakhmetova GM, Bondarenko LB, Kovalenko VM.** Damage of testicular cell macromolecules and reproductive capacity of male rats

following co-administration of ethambutol, rifampicin, isoniazid and pyrazinamide. *Interdiscip Toxicol* 5: 9-14, 2012.

7. Stahl PJ, Stember DS, Goldstein M. Contemporary management of male infertility. *Annu Rev Med* 63: 525-540, 2012.

8. Erejuwa OO, Sulaiman SA, Wahab MS, Salam SK, Salleh MS, Gurtu S. Antioxidant protective effect of glibenclamide and metformin in combination with honey in pancreas of streptozotocin-induced diabetic rats. *Int J Mol Sci* 11: 2056- 2066, 2010.

9. Giknis MLA, Clifford CB. Clinical Laboratory Parameters for Crl:WI(Han) Rats. Charles River, 2008 (http://www.criver.com/SiteCollection Documents/rm_rm_r_Wistar_Han_clin_lab_parameters _08.pdf).

10. Donate JMB. Treatment of tuberculosis. In: *Business Briefing: European Pharmacotherapy* 2006. (http://www.touchbriefings.com/pdf/1692/Broquetas-donate.pdf).

11. Office of New Drug Evaluation and Research (CDER) at the Food and Drug Administration. Guidance for industry and reviewers estimating the safe starting dose in clinical trials for therapeutics in adult healthy volunteers. U.S. Department of Health and Human Services, FDA, CDER and CBER, 2002. (http://www.fda.gov/OHRMS/DOCKETS/98fr/02d-0492-gdl0001-vol1.pdf).

12. Boekelheide Kim, Chapin Robert. Male reproductive toxicology**.** In: *Current Protocols in Toxicology* Maines MD (Ed), John Wiley & Sons Inc, New York, pp. 16.0.1- 16.0.2, 2005.

13. El'tseva TV, Adamskaya EI, Peryshkova TA, Babichev VN. Disturbance of neuroendocrine regulation of sexual behavior of male rats with streptozotocin diabetes. *Neurosci Behav Physiol* 23: 538-544, 1993.

14. Rabbani SI, Devi K, Khanam S. Pioglitazone, a PPAR-γ ligand inhibited the nicotinamide-streptozotocin induced sperm abnormalities in type-2 diabetic Wistar rats. *Pak J Pharm Sci* 23: 326-331, 2010.

15. Vincent AM, Brownlee M, Russell JW. Oxidative stress and programmed cell death in diabetic neuropathy. *Ann N Y Acad Sci* 959: 368-383, 2002.

16. Desco MC, Asensi M, Marquez R et al. Xanthine oxidase is involved in free radical production in type-1 diabetes: protection by allopurinol. *Diabetes* 51 : 1118-1124, 2002.

17. Wolf SP. Diabetes mellitus and free radicals. Free radicals, transition metals and oxidative stress in the aetiology of diabetes mellitus and complications. *Br Med Bull* 49: 642-652, 1993.

18. Shrilatha B, Muralidhara A. Early oxidative stress in testis and epididymal sperm in streptozotocin-induced diabetic mice: its progression and genotoxic consequences. *Reprod Toxicol* 23: 578-587, 2007.

19. Tasduq SA, Peerzada K, Koul S, Bhat R, Johri RK. Biochemical manifestations of anti-tuberculosis drugs induced hepatotoxicity and the effect of silymarin. *Hepatol Res* 31: 132–135, 2005.

20. Cooke MS, Evans DM, Dizdaroglu M, Lunec J. Oxidative DNA damage: mechanisms, mutation, and disease *FASEB J* 17: 1195–1214, 2003.

21. Jones DP. Radical-free biology of oxidative stress. *Am J Physiol Cell Physiol* 295: C849-C868, 2008.

22. Healy LN, Puta LJ, Recio L. Expression and distribution of cytochrome P450 2E1 in B6C3F1 mouse liver and testes. *Chem Biol Interact* 121:199–207, 1999.

23. Oropeza-Hernandez LF, Quintanilla-Vega B, Reyes-Mejia RA et al. Trifluoroacetylated adducts in spermatozoa, testes, liver and plasma and CYP2E1 induction in rats after subchronic inhalatory exposure to halothane. *Toxicol Lett* 144: 105–116, 2003.

24. Quintans LN, Castro GD, Castro JA. Oxidation of ethanol to acetaldehyde and free radicals by rat testicular microsomes. *Arch Toxicol* 79: 25–30, 2005.

25. Knockaert L, Fromenty B., Robin MA. Mechanisms of mitochondrial targeting of cytochrome P4502E1: physiopathological role in liver injury and obesity. *FEBS J* 278: 4252-4260, 2011.

26. Raza H, Pradu S K, Robin MA, Avadhani NG. Elevated mitochondrial cytochrome P450 2E1 and glutathione S-transferase A4-4 in streptozotocin-

induced diabetic rats: tissue-specific variations and roles in oxidative stress. Diabetes 53: 185-194, 2004.

27. Lieber CS. Cytochrome P-4502E1: its physiological and pathological role. *Physiol Rev* 77: 517–544, 1997.

28. Erkkila K, Aito H, Aalto K et al. Lactate inhibits germ cell apoptosis in the human testis. *Mol Hum Reprod* 8: 109-117, 2002.

29. Aitken RJ, De Iuliis GN. Origins and consequences of DNA damage in male germ cells. *Reprod Biomed Online* 14: 727-733, 2007.

30. Madan A, Graham RA, Carroll KM et al. Effects of prototypical microsomal enzyme inducers on cytochrome P450 expression in cultured human hepatocytes. *Drug Metab Dispos* 31: 421–431, 2003.

31. Kovalenko VM, Bagnyukova TV, Sergienko OV et al. Epigenetic changes in the rat livers induced by pyrazinamide treatment *Toxicol Appl Pharm* 225: 293–299, 2007.

32. Wang X, Lu Y, Cederbaum AI. Induction of cytochrome P450 2E1 increases hepatotoxicity caused by Fas agonistic Jo2 antibody in mice. *Hepatology* 42: 400–410, 2005.

33. Anitha B, Sudha S, Gopinath PM, Durairaj G. Genotoxicity evaluation of pyrazinamide in mice *Mutat Res* 321: 1-5, 1994.

34. Shalaby T, Hiyama E., Grotzer MA. Telomere maintenance as therapeutic target in embryonal tumor. *Anticancer Agents Med Chem*10: 196-212, 2010.

35. Clegg E, Perreault S, Klinefelger G. Assessment of male reproductive toxicity. In: *Principles and Methods of Toxicology, 4th edition*, Hayes AW (Ed.), Taylor & Francis, Philadelphia, pp. 1263–1300, 2001.

36. Tjalve H. Streptozotocin: distribution, metabolism and mechanisms of action. *Uppsala J Med Sci* 39: 145–157, 1983.

37. Bennett RA, Pegg AE. Alkylation of DNA in rat tissues following administration of Streptozotocin. *Cancer Res* 41: 2786–2790, 1981.

38. Nukatsuka M, Sakurai H, Yoshimura Y, Nishida M, Kawada J. Enhancement by streptozotocin of O2− radical generation by the xanthine oxidase system of pancreatic cells. *FEBS Lett* 239: 295–298, 1988.

39. Takasu N, Komiya I, Asawa T, Nagasawa Y, Yamada T. Streptozotocin- and alloxan-induced H_2O_2 generation and DNA fragmentation in pancreatic islets. H_2O_2 as mediator for DNA fragmentation. *Diabetes* 40: 1141–1145, 1991.

40. Bolzan AD, Bianchi MS. Genotoxicity of streptozotocin. *Mutat Res* 512: 121–134, 2002.

41. Bhide SV, Bhalerao EB, Sarode AV, Maru GB. Mutagenicity and carcinogenicity of mono- and diacetyl hydrazine. *Cancer Lett* 23: 235–240, 1984.

42. Braun R, Jakel HP, Schoneich J. Genetic effects of isoniazid and the relationship to in vivo and in vitro biotransformation. *Mutat Res* 137:61–69, 1984.

43. Yue J, Dong G, He C, Chen J, Liu Y, Peng R. Protective effects of thiopronin against isoniazid-induced hepatotoxicity in rats. *Toxicology* 264: 185–191, 2009.

44. Aboul-Ela EI. Cytogenetic effects of rifampicin in somatic and germinal cells of the mouse. *J Appl Toxicol* 15: 325–326, 1995.

45. Roessner C, Paasch U, Kratzsch J, Glander HJ, Grunewald S. Sperm apoptosis signalling in diabetic men. *Reprod Biomed Online* 25: 292-299, 2012.

46. Asole A, Panu R, Palmieri G. Effects of ethambutol on the seminal epithelium of the rat and chicken. *Boll Soc Ital Biol Sper* 52: 846–848, 1976.