THE EFFECTS OF UNILATERAL TESTICULAR TORSION UPON IMMUNITY MODULATION AND APOPTOSIS OF GERMINAL CELLS IN THE CONTRALATERAL TESTIS
An Experimental Study in Rats

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ABSTRACT

Since the report of Krarup in 1978, deteriorating effects of unilateral testicular torsion to the contralateral testis are generally accepted, but the mechanism of the damage remains controversial. This study aims to explain the mechanism of spermatogenesis disorder of the contralateral testis after unilateral testicular torsion that underlies infertility. This study used 40 Wistar strain Rattus norwegicus *white rats divided into 4 groups of 10 rats each with a post test control group design. Two groups of white rats were subjected to left sided testicular torsion three times counter-clockwise for 4 hours and 24 hours respectively, and two other groups, which served as control, were subjected to sham operation. Subsequently, the determination of serum testosterone was carried out using radioimmunoassay and investigations on the proportion of apoptosis of the germinal epithelial cells, the percentage of IL-4 producing lymphocytes and the IgG-producing plasma cells in the contralateral testis was performed by means of an immunohistochemical method. The results of multivariate analysis showed that unilateral torsion in white rats for 4 hours induced significant increase in the percentage of IL-4 producing lymphocytes in the contralateral testis, but no significant change was demonstrated in serum testosterone level, the percentage of IgG-producing plasma cells, or the proportion of apoptotic germinal epithelial cells in the contralateral testis. Unilateral testicular torsion for 24 hours could induce significant decrease in serum testosterone level along with significant proportional increase of apoptosis of the germinal epithelial cells in the contralateral testis. No significant change in the percentage of IL-4 producing lymphocyte and the IgG-producing plasma cells in the contralateral testis could be measured. It can be concluded that the mechanism of spermatogenesis disorder post-unilateral testicular torsion can be explained by the immunopathobiology paradigm. Immunity modulation was found in the rats subjected to testicular torsion for 4 hours, as reflected by an increase in the percentage of IL-4 producing lymphocytes and pathobiological modulation occurred in rats subjected to testicular torsion for 24 hours as reflected by the reduction in serum testosterone level along with an increase in the proportion of apoptosis of the germinal epithelial cells in the contralateral testis.*

***Keywords**: testicular torsion, testosterone, immunity modulation, germ cell, apoptosis, white rats*

INTRODUCTION

Testicular torsion results in twisting of the spermatic cord and occlusion of the vascular supply to the testis. If not untwisted immediately complete infarction of the testis may results. If it lasts for 3 hours or less all the twisted testis remain viable after detorsion, if it lasts for 5 hours the testicular viability will decrease to 83 – 90% and if it last for 24 hours or longer the testis is rare viable (Sonda and Wang, 1988). The degree of torsion is also a critical factor in determining testicular viability and most of the cases had three times (1080°) torsion (Haynes et al, 1983). The frequency of testis torsion has been estimated at 1 an 4000 males younger than age 25 years, making it relatively common (Williamson, 1976, Barada 1989).

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In Surabaya at Emergency Department of Dr. Soetomo Hospital for 2 years 1989 and 1990 a number of 75 patients of testicular torsion has been treated (Alif, 1992). The majority of cases of testicular torsion occur in boys between ages 12 and 18 years (Scorer and Farrington, 1971; Williamson, 1976; Alif, 1991). Since the report of Krarup in 1978 that has proven the evidence of spermatogenesis disorder after unilateral torsion (Krarup, 1978) the deteriorating effects of unilateral testicular torsion to the contralateral testis are generally accepted(Bartsch et al, 1980; Harrison, 1981; Consentino et al, 1985; Handerson et al, 1985; Anderson dan Williamson, 1986; Awojobi and NKposong, 1986; Anderson and Williamson, 1990; Heindel et al, 1990; Tarmono, 1998). To date, however, the actual mechanism of contralateral damage remains uncertain and still debatable. Some data suggest that the damaged organ may allow immunological injury to the opposite un involved testis (Nugle and de Vere White, 1982 ; Henderson et al, 1985; Turek, 1997). It is also suggest that there is an increase of apoptosis in the contralateral testis after unilateral torsion

(Hadziselmovic et al, 1998 ; Sarioglu - Buke et al, 2001).

Twisting of the spermatic cord causes occlusion of the vascular supply to the testis and it makes the testis containing cells become hypoxia and anoxia. The anoxic Sertoli cells damage the blood testis barrier system and allow germinal epithelial cell releasing protein enter blood circulation and recoqnized by immunocompetent cells as an autoantigen and trigger the immune response.

Furthermore, the differentiation and activation of T cells may secrete various cytokines. The cytokine IL-4 induces activation, proliferation, and maturation of B lymphocytes, resulting in its differentiation to become plasma cells, which is producing antibody that may induce damage in contralateral testicular germinal epithelial cells. The complex of antigen antibody activates the complements, generating lysis in target cells and replaced with connective tissue (Golsdby et al, 2000 and Elyana 2001).

Testosterone level may reduce due to various factors, such as psychological, physical, and metabolic stress (Vasankari, 1983; Mc Grady, 1984; Bergendahl et al, 1989; Veldhuis, 1997). The reduction of testosterone level produced by Leydig cells increases apoptosis (Nandi et al, 1999). The reduction of testosterone level due to the ischemia in Leydig cells results in Fas expression at the surface of germinal epithelial cells as well as the expression of Fas L at the surface of Sertoli cells. The binding of Fas and Fas L brings about apoptosis in germinal epithelial cells (Lee et al, 1997; Nandi et al, 1999) through the activation of Fas Associated Protein with Death Domain (FADD), which subsequently activates caspase, and, thereafter, activates DNAase, resulting in chromosome fragmentation, DNA damage, and, finally, apoptosis occurs (Emari, 1998; Kuby, 2000). The purpose of this study was to explain the mechanism of spermatogenesis disorder of contralateral testis after unilateral testicular torsion that underlies infertility.

MATERIALS AND METHODS

This was an experimental study involving male Wistar strain *Rattus norwegicus* white rats as samples subjected to treatment of left testicular torsion for 4 and 24 hours, and as control of each treatment that were subjected to sham operation. This study used Posttest Control Group Design, variables measurement was carried out only after treatment. Samples were taken from breeding laboratory at Gadjah Mada University, Yogyakarta, with the following criteria: male, aged 10 - 12 weeks, bodyweight ranged 150 - 200 grams, healthy and without any disabilities.

According to the design, four groups of samples were required. Grouping was carried out in random using random number (Snedecor and Cochran, 1974). Sample size was determined by means of Danniel's formula (1987), which revealing 7.85 rats/group. As there was possibility of drop out, it was decided to use 10 rats in each group, so that there were totally 40 rats involved in this study. These rats were adapted from 2 weeks and divided into 4 groups, treatment groups E1 and E2, and their respective control groups C1 and C2. E1 was subjected to left testicular torsion by twisting spermatic cord counter-clockwise 3 x 360 degrees. After 4 hours, detorsion was undergone to the left testis and followed immediately with dextral orchidectomy for examining apoptosis in germinal epithelial cells, IL-4 producing lymphocytes, and IgG-producing plasma cells, and blood was taken by cardiac puncture for serum testosterone level examination. Similar procedure was also underwent in E2 group, with the difference that detorsion of the left testicle and right orchidectomy was performed after 24 hours. C1 and C2 groups were subjected only to sham operation, in which scrotal skin was incised, without carrying out testicular torsion and the wound closed.

Analysis unit and dependent variables in this study was serum for testosterone level examination and contralateral testicular tissue for examining germinal epithelial cell apoptosis, IL-4 producing lymphocytes, and IgG-producing plasma. The examination of serum testosterone level was done using radioimmunoassay (RIA), while apoptosis in germinal epithelial cell was examined by means of tunnel assay apoptag method, a combination of molecular reaction and immunohistochemistry. Apoptosis in germinal epithelial cells is marked by positive reaction in apoptag staining. The number of apoptotic germinal epithelial cells was counted using light microscope with 400 x magnification in 5 sections of seminiferous tubules.

IL-4 producing lymphocytes examination was done using indirect immunohistochemistry staining with monoclonal antibody (anti IL-4). The cytoplasma of IL-4 producing lymphocytes induces positive light reaction presenting in yellowish brown color. Counting was done using light microscope with 400 x magnification on 5 locations taken randomly from contralateral testicular sections. IgG- producing plasma cells examination was performed immunohistochemically using monoclonal antibody (anti IgG), and IgG-producing plasma cells reacted positively, showing yellowish brown cellular cytoplasma. The number of IgG-producing plasma cells was counted using light microscope with 400 x

magnification on 5 locations taken randomly from contralateral testicular sections.

Data obtained in this study were processed using several statistical tests with level of confidence of 95%. Statistical test and analysis were variance analysis (Anova) for homogenity test of rats bodyweight, Kolmogorov-Smirnov normality test to find the distribution of data obtained, and multivariate analysis (Manova) using Hotelling T test to examine the difference of all variables in treatment and control groups and to test the difference of changes (delta) in the variables in E1 and E2 groups. Experimental animals were handled from treatment to their sacrifice according to International Guiding Principal for Biomedical Research Involving Animals from the Council for International Organizations of Medical Sciences.

RESULTS

To confirm the internal validity, the homogeneity test for sample body weight was done using one way Anova, and the result showed that white rats bodyweight in those four groups were not significantly different ($p > 0.05$). The variables in this study included serum testosterone level, the apoptotic proportion of germinal epithelial cells, the percentage of IL-4 producing lymphocytes, and the percentage of IgG-producing plasma cells in contralateral testis.

The results of normal distribution test to the four variables in four groups using Kolmogorov Smirnov (K-S) test revealed that the variable of serum testosterone level and the proportion of apoptotic germinal epithelial cells in contralateral testis had normal distribution ($p > 0.05$). Therefore, both variables were further examined using multivariate analysis of variance (MANOVA) with Hotelling T statistical test. It was found that the percentage of IgG-producing plasma cells and IL-4 producing lymphocytes was not normally distributed ($p < 0.05$). Therefore, the data was further analyzed using non-parametric Kruskal Wallis or Mann Whitney tests.

Table 1. Combined results of discriminant test in 4 variables between 4 groups

Variables	Mean of variables in groups				F	Prob.
	C1	E1	C2	E2		
Serum testosterone level	236.30	115.65	212.95	63.60	5.520	0.003
The proportion of apoptotic contralateral testicular germinal epithelial cells	1.48	1.00	0.86	115.86	8.693	0.000
The percentage of IL-4 producing lymphocytes in contralateral testis	0.10	0.96	0.14	0.12	-	0.001
The percentage of IgG-producing plasma cells in contralateral testis	0.18	0.40	0.06	0.10	-	0.063

The results of analysis of the four variables between 2 control groups are shown in the following table :

Table 2. Results of discriminant test between C1 and C2

Variables	C1 group		C2 group		F	Prob
	Mean	SD	Mean	SD		
Serum Testosterone Level	236.30	176.48	212.95	101.63	0.131	0.721
The proportion of apoptotic contralateral testicular germinal epithelial cells	1.48	1.39	0.86	1.03	1.294	0.270
The percentage of IL-4 producing lymphocytes in contralateral testis	0.10	0.20	0.14	0.27	-	0.889
The percentage of IgG-producing plasma cells in contralateral testis	0.18	0.26	0.06	0.13	-	0.263

Hotelling's Trace = 0.075, p = 0.540

Above table indicates that the four observed variables between groups C1 and C2 are not significantly different. Table 3 below displays the results of analysis of four variables between groups C1 and E1 using MANOVA and Hotelling T statistical test for the level of serum testosterone and the proportion of apoptotic germinal epithelial cells using Mann Whitney test for the percentages of IgG-producing plasma cells and IL-4-producing lymphocytes.

Table 3. Results of discriminant test between C1 and E1

Variables	C1 Group		E1 Group		F	Prob
	Mean	SD	Mean	SD		
Serum Testosterone Level	236.30	176.48	115.65	70.70	4.027	0.060
The proportion of apoptotic germinal epithelial cells	1.48	1.39	1.00	0.59	1.012	0.328
The percentage of IL-4 producing lymphocytes in contralateral testis	0.10	0.20	0.96	0.78	-	0.002
The percentage of IgG-producing plasma cells in contralateral testis	0.18	0.26	0.40	0.42	-	0.203

Hotelling's Trace = 0.279, p = 0.124

Table 3 shows that there is significant difference between groups C1 and E1 only in the variable of IL-4 producing lymphocytes (p = 0.002), while other variables are not significantly different. Analysis was carried out using MANOVA with Hotelling T statistical test for serum testosterone level and the proportion of apoptotic germinal epithelial cells in contralateral testis as well as Mann Whitney test for the percentage of IgG-producing plasma cells and the percentage of IL-4-producing lymphocytes. The results are shown in the following table :

Table 4. Results of discriminant test between C2 and E2

Variables	C2 Group		E2 Group		F	Prob
	Mean	SD	Mean	SD		
Serum testosterone level	212.95	101.63	63.60	38.50	18.885	0.000
The proportion of apoptotic germinal epithelial cells	0.86	1.02	115.86	123.06	8.733	0.008
The percentage of IL-4 producing lymphocytes in contralateral testis	0.14	0.27	0.12	0.31	-	0.655
The percentage of IgG-producing plasma cells in contralateral testis	0.06	0.13	0.10	0.30	-	0.871

Hotelling's Trace = 1.692, p = 0.000

The results of analysis as seen in Table 4 indicate that there is significant difference in both groups for the variable of serum testosterone level ($p < 0.001$) and the proportion of apoptotic germinal epithelial cells ($p = 0.008$). However, significant difference was not found in the percentage of IL-4-producing lymphocytes ($p = 0.655$) and the percentage of IgG producing plasma cells ($p = 0.871$).

Table 5. Results of discriminant test between variables E1 and E2

Variables	E1 Group		E2 Group		F	Prob
	Mean	SD	Mean	SD		
Serum Testosterone Level	115.65	70.70	63.60	38.50	4.180	0.056
The proportion of apoptotic germinal epithelial cells	1.00	0.59	115.86	123.06	8.712	0.009
The percentage of IL-4 producing lymphocytes in contralateral testis	0.96	0.78	0.12	0.31	-	0.002
The percentage of IgG-producing plasma cells in contralateral testis	0.40	0.42	0.10	0.30	-	0.042

Hotelling's Trace = 0.817, p = 0.006

Table 5 shows that groups E1 and E2 have significant difference in the variable of the apoptotic germinal epithelial cells proportion ($p = 0.009$) and the percentage of IL-4 producing lymphocytes ($p = 0.006$). However, significant difference is not found in the variable of serum testosterone level ($p = 0.056$) and the percentage of IgG-producing plasma ($p = 0.063$).

Results of Immunohistochemical Staining

Immunohistochemical staining in this study was done to seminiferous tubule in contralateral testis. The results are as follows :

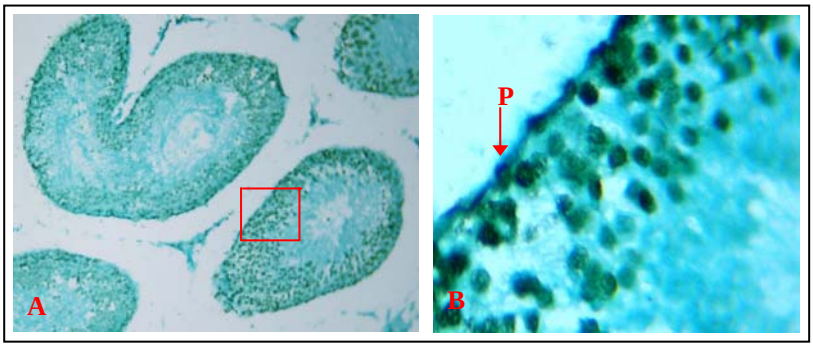

Figure 1. Incision of seminiferous tubule of contralateral testis from Wistar strain in treatment group (E2) using apoptag staining with tunel assay method.
A: magnification 100x B: magnification 400x P: apoptotic cells with dark brown nucleus.

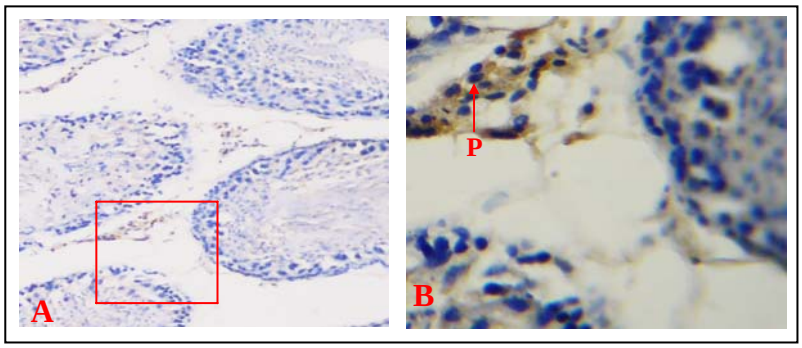

Figure 2. Incision of seminiferous tubule of contralateral testis from Wistar strain in treatment group (E2) using immunohistochemical staining with IgG monoclonal antibody.
A : magnification 100x, B: magnification 400x, P: IgG-producing cells (cytoplasma appears brown).

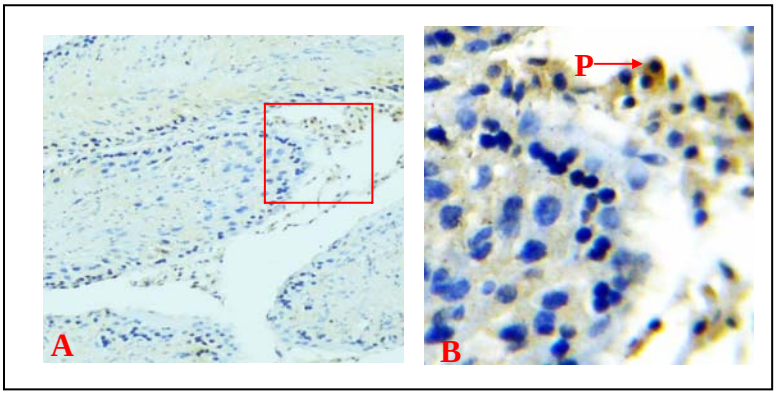

Figure 3. Incision of seminiferous tubule of contralateral testis from Wistar strain in treatment group (E2) using immunohistochemical staining with IL-4 monoclonal antibody.
A: magnification 100x, B: magnification 400x, P: IL-4-producing cells (cytoplasma appears brown).

DISCUSSION

It can be seen in Table 1 that serum testosterone level in four studied groups demonstrate significant difference ($p = 0.003$), while Table 2, comparing serum testosterone level between 2 control groups, shows no significant difference (Hotelling's Trace = 0.075, $p = 0.540$). Table 3, comparing serum testosterone level in control group (C1) to that in treatment group of testicular torsion for 4 hours (E1), also shows no significant difference ($p = 0.060$). Table 4 that compares serum testosterone level between groups C2 and E2 shows significant difference ($p < 0.001$). Conclusively, the decrease of testosterone level occurred in experimental animals subjected to unilateral testicular torsion, and the decrease was significant in testicular torsion for 24 hours. It is apparent that unilateral testicular torsion results in the decrease of serum testosterone level, and the decrease is more remarkable in the torsion for 24 hours. Savas also found similar findings in his study using Wistar strain rats subjected to testicular torsion (Savas et al, 2003). Bartsch et al (1980) investigated serum testosterone level in 30 patients who experienced testicular torsion at least for 2.5 years and found that the level was normal. This might be due to the compensatory function from the existing Leydig cells.

Table 1 shows that in four studied groups there are significant difference in the variable of apoptotic germinal epithelial cells proportion ($p < 0.001$). Table 2, comparing the proportion of apoptotic germinal epithelial cells in contralateral testis between 2 control groups, significant difference is not found using Hotelling's Trace value of 0.075 and $p = 0.540$. This was because both groups were not subjected to treatment. Table 3, which compares the proportion of apoptotic germinal epithelial cells in contralateral testis between control group (C1) and group receiving testicular torsion for 4 hours (E1), shows no significant difference ($p = 0.328$). Conversely, significant difference is found with Hotelling's Trace value of 1.692 and $p < 0.001$ in Table 4, where the proportion of apoptotic germinal epithelial cells in contralateral testis is compared between that in control group (C2) and that in treatment group receiving testicular torsion for 24 hours (E2) is compared. Table 5, comparing the proportion of apoptotic germinal epithelial cells in contralateral testis in treatment group receiving testicular torsion for 4 hours (E1) and that for 24 hours, demonstrated significant difference with $p = 0.009$.

Those data lead to the conclusion that there was an increase of apoptotic proportion in contralateral testis due to unilateral testicular torsion for 24 hours. Correlation between the reduction of testosterone and the increase of apoptotic germinal epithelial cells had been proved by Nandi et al (1999) who found that the administration of ethane 1,2 dimethanesulfonate (EDS), a toxic substance for Leydig cells, was followed by the increase of apoptosis in germinal epithelial cells. In contrast, apoptotic process can be inhibited by the administration of glucocorticoid (Jazawa, 2001). Additionally, apoptosis can also be prevented by providing Capsaicin, a blocker to the afferent nerve fiber, which works by preventing blood flow reduction in contralateral testis (Sarioglu-Buke, 2001).

Table 1 showed that the percentage of IL-4 producing lymphocyte in contralateral testis in four studied groups had significant difference ($p = 0.001$). However, significant difference was not found ($p = 0.889$) in Table 2 where the percentage of IL-4 producing lymphocyte in contralateral testis is compared in two control groups (C1 and C2). Table 3 demonstrated significant difference ($p = 0.002$) in the percentage of IL-4 producing lymphocyte in contralateral testis between control group (C1) and treatment group receiving testicular torsion for 4 hours (E1). No significant difference ($p = 0.655$) is shown in Table 4 that compares the percentage of IL-4 producing lymphocyte in contralateral testis between that in control group (C2) and that in group subjected to testicular torsion for 24 hours (E2).

It can be concluded that the increased percentage of IL-4 producing lymphocyte in contralateral testis only occurred in group treated with testicular torsion for 4 hours. Although the role of IL-4 in immune response has long been recognized, its correlation with infertility immunology remains unclear. Several types of cytokines that have been studied in relations with infertility immunology are IL-1, IL-2, Tumor Necrosis Factor (TNF) and interferon gamma (Anderson and Hill, 1988; Haney et al, 1992 ; Hill et al, 1989).

Table 1 shows that there is no significant difference ($p = 0.063$) in the percentage of IgG-producing plasma cells in contralateral testis in four studied groups. Similarly, significant difference ($p = 0.263$) is not shown in Table 2, in which the percentage of IgG-producing plasma cells in contralateral testis in C1 group is compared to that in control C2 group. Table 3, comparing the percentage of IgG-producing plasma cells in contralateral testis between that in C1 and E1 groups, indicates no significant difference ($p = 0.203$). Insignificant difference is also seen in Table 4 that compares the percentage of IgG-producing plasma cells in contralateral testis between that in C2 and that in E2 groups. It is apparent from these data that the treatment of testicular torsion for 3 and 24 hours not or not yet result in the change of IgG-producing plasma cells

percentage. From the occurrence of general response until the formation of immunoglobulin took time for 1 - 2 weeks. In relations with the infertility immunology, a subject that has been commonly studied is the antisperm antibody (ASA) (Hendry et al, 1977 ; Marthur, 1981; Cerasaro et al, 1984 ; Meinertz ,1992; Naz and Menge ,1994; Peters and Coulan, 1992). The antisperm antibody can be found in serum, semen, and binding with sperm (Turek, 1997). So far, there is no study has been performed to examine the presence of immunoglobulin in testicular tissue was negative.

CONCLUSIONS AND SUGGESTIONS

Unilateral testicular torsion for 4 hours may result in the increase of the percentage of IL-4 producing lymphocyte in contralateral testis, but not changing of the serum testosterone level, the percentage of IgG producing plasma cells, and the proportion of apoptosis in germinal epithelial cells in the contralateral cells testis. Unilateral testicular torsion for 24 hours may result in the reduction of serum testosterone level along with the increase of the proportion of apoptosis in germinal epithelial cells in the`contralateral testis, but no changing of the percentage of IL-4 producing lymphocyte and the percentage of IgG producing plasma cells in contralateral testis. Conclusively, immunity modulation occurs in experimental animals subjected to unilateral testicular torsion for 4 hours, as reflected by the increase of the proportion of apoptotic germinal epithelial cells in contralateral testis. It can also be suggested that the management of testicular torsion should consider the duration of torsion to provide rational treatment. Further studies are warranted to identify the role of immunosuppressant as an adjuvant therapy for patients with testicular torsion for 4 hours or less, and to disclose the role of apoptotic inhibitor in patients with testicular torsion for 4 hours or more.

REFERENCES

Alif S, 1991. Torsio Testis di RSUD Dr. Soetomo, Studi Prospektif Selama Dua Tahun. Surabaya: Laboratorium/UPF Ilmu Bedah Fakultas Kedokteran Universitas Airlangga. Laporan PPDS, p. 17,2.

Anderson DJ, Hill JA, 1988 ; Cell mediated immunity in infertility. Am. J. Reprod. Immunology Microbiology, 17 : 22 – 26.

Anderson JB, Williamson RCN, 1986. The Fate of The Human Testing Following Unilateral Torsion of The Spermatic Cord. Br Journal Of Urology 58: 698-704.

Anderson JB, Williamson RCN, 1990. Fertility After Torsion of The Spermatic Cord. Br Journal of Urology 65: 225-230.

Awojobi OA, Nkposong EO, 1986. Seminal Fluid Changes After Testicular Torsion. Urology 27 (2): 109-111.

Barada JH, Weingarten JL, Cromie WJ. 1989. Testicular Salvage and Age Related Delay In The Presentation of Testicular Torsion. J Urol 142: 746 - 748.

Bartsch G, Frank ST, Marberger H, Mikuz G, 1980. Testicular Torsion: Late Results With Special Regard to Fertility and Endocrine Function. The Journal of Urology 124: 375-378.

Bergendahl M, Perheentupa A, Huhtaniemi I, 1989. Effect of Short Term Starvation on Reproductive Hormone Gene Expression, Secretion and Receptors Levels in Male Rat. J. Endocrinol (121): 409-415.

Cerasaro TS, Daniel A, Nachttsheim, Fernando Otero, 1984. The Effect of Testiscular Torsion on Contralateral Testis and The Production of Antisperm Antibodies in Rabbits. The Journal of Urology 132: 577-579.

Consentino MJ, Nishida M, Robinowitz R, 1985. Histological Changes Occuring In The Contralateral Testis of Prepubertal Rats Subjected to Various Durations Of Unilateral Spermatic Cord Torsion. The Journal of Urology 133: 906-911.

DeJong FH, Robertson DM, 1985. Inhibin: Update on action and perification. Mol Cell. Endocrinol 42: 94 – 99.

Elyana Asnar STP, 2001. Peran Perubahan Limfosit Penghasil Sitokin dan Pepida Motilitas Usus Terhadap Modulasi Respon Imun Mukosal Tikus Yang Stres Akibat Stressor Renjatan Listrik. Disertasi Program Doktor. Program Pasca Sarjana. Universitas Airlangga Indonesia.

Emari M, Sakahira H, Yokoyama H, Okawa K, Iwamatsu A, Nagata S, 1998. A Caspase Activated Dnse That Degrades DNA During Apoptosis, and It's Inhibitor ICAD. Nature Japan. Reg. No 785998. England.

Goldsby RA, Kindt TJ, Osborne BA, 2000. The Complement System, In Kuby Immunology. 4th Edition. New York. Freeman and Company. pp: 329 - 350.

Hadziselimovic F, Geneto R, Emmons LR, 1998. Increased Apoptosis in The Contralateral Testes of Patients With Testicular Torsion as Factor For Infertility. J Urol Sep 160 : 1158-1160.

Haney AF, Hughes SF, Weinberg JB., 1992 : The lack of effect of tumor necrosis factor-alpha, interleukin 1-alpha and interferon – gamma on human sperm motility in vitro. J. Androl. 13 : 249 – 256.

Harrison RG, Jones DIL, Connally RC, 1981. Mechanism of Damage to The Contralateral Testis In Rats With an Ischemic Testis. The Lancet 3: 723-725.

Haynes BE, Bessen HA, Haynes VE, 1983. The Diagnosis of Testicular Torsion. JAMA 249: 2522-7.

Heindel RM, Pakyz RE, Reinking LN, 1990. The Effect of Various Degrees of Unilateral Spermatic Cord Torsion on Fertility in The Rat. The Journal of Urology 144: 366-369.

Henderson JA, Smey P, Cohen MS, Davis CP, Payer AF, Parkening TA, Warren MM, 1985. The Effect of Unilateral Testicular Torsion On The Contralateral Testicule In Prepubertal Chinese Hamster. J Pediatr Surg 20(6): 592-77

Hendry WF, Morgan H, Stedronska J, 1977. The Clinical Significance of Anti Sperm Antibodies in Male Subfertility. BJ Urol 49: 757 – 762.

Hill JA, Cohen J., Anderson DJ, 1989 : The effects of lymphokines and monokines on human fertilizing ability in the zona free hamster egg penetration test. Am. J. Obstet. Gynecol. 160 : 1154 – 1161.

Kramp T, 1978. The Testes After Torsion. Br J Urol 50: 43-6.

Kuby J, 2000. Cell mediated effector responses. In: Immunology. 4th ed. Goldsby RA, Kindt Th J. Osborne BA. Editors; USA, WH Freeman and Company pp: 351-369.

Lee J, Richburg JH, Younkin SC, Boekelheide K, 1997. The Fas System is A Key Regulator of Germ Cell Apoptosis in Testis. J Endocrinology 138 (5): 2081-8.

Marthur S, 1981. Clinical Significance of Sperm Antibodies in Infertility. Fertil Steril 36: 486-491.

McGrady AV, 1984. Effects of Psychological Stress on Male Reproduction: A Review. Arch Androl (13):1-6.

Meinertz H, 1992. Antisperm Antibodies in Male : Detector and Clinical Impact. Am J Reprod Immunol 28 : 110-116.

Nandi S, Banerjee PP, Zirkin BR, 1999. Germ Cell Apoptosis In Testis of Sprague Dawley Rats Following Testosteron Withdrawal By Ethene 1,2-dimethanesulfonate administration: Relationship to fast. J Biol Reprod 61 (1): 70-5.

Naz RK, Menge AC, 1994. Antisperm Antibodies : Origin, Regulation, and Sperm Reactivity in Human Infertility. Fertil Steril 61: 1001-1006.

Peters AJ, Coulan CB, 1992. Sperm Antibodies. Am J Reprod Immunol 27 : 156-160.

Savas C, Ozguner M, Ozguner F, Delibas N, 2003. The Effects of Human Chorinic Gonado Tropin Treatment On The Contralateral Side in Unilateral Testicular Torsion. International Urology and Nephrology 35 : 237-241.

Scorer CG, Farrington HG, 1971. Congenital Deformities of The Testis and Epididymis. London, Butterworths.

Snedecor GW, Cochran WG, 1974. Ten Thousand Randomly Assorted Digits. Statistical Methods. 6th edition. Ames Iowa. The Iowa State University Press. pp: 543

Sonda LP, Wang S, 1988. Evaluation of Male External Genital Disease in The Emergency Room Setting. Emerg Med Clin North Am 6: 473-86.

Tarmono, 1998. Pengaruh Torsio Testis Terhadap Spermatogenesis Testis Kontralateral. SMF Urologi/Lab Ilmu Bedah. RSUD Dr Soetomo/FK Unair. Surabaya.

Turek PJ, 1997. Immunopathology and Male Infertility. In Infertility in The male. Eds. Lipshultz LI and Howards SS, 3rd edition St Louis, Missuori Mosby. pp: 305-325.

Vasankari TJ, 1993. Effects of Endurance Training on Hormonal Responses to Prolonged Physical Exercise in Male : Acta Endocrinal 129: 109-115

Veldhuis JD, 1997. Male Hypothalamic - Pituitary - Gonadal Axis. Infertility in The Male 3rd edition. Editors Liphultz LI and Howards SS. St. Louis Missouri. Mosby. pp. 23-58.

Williamson RC, 1976. Torsion of The Testis and Allied Condition. Br J Surg 63: 465-76.

Yazawa H, Sasagawa I, Ishigooka M dan Nakada T, 1999. Effect of Immobilization Stress on Testicular Germ Cell Apoptosis in Rats. Human Reproduction 14 (7): 1806-1810.