# EFFECT OF IPSILATERAL TESTICULAR TORSION ON THE QUALITY OF SPERM IN CONTRALATERAL TESTIS OF RAT (Rattusnorvegicus) WISTAR STRAIN

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***ABSTRACT***

***Objective:*** *This study aims to identify the effect of testicular torsion on the quality of sperm which include: concentration, morphology, and motility of the contralateral testis Rattus norvegicus.* ***Material & Methods:*** *This study is an experimental laboratory study with a post-test only control and completely randomized design (CRD) and divided into 3 groups: control (KO) and treatment (P1 and P2). The treatment group induced testicular torsion of 360˚ to the left for 4 hours. Each group consisted of 9 rats were observed immediately after detorsi (rapid effects) and 9 rats were observed after 30 days do detorsi (slow effect). Data were analyzed by ANOVA - One way and followed by Tuckey HSD test.* ***Results:*** *The results showed a significant difference ( P< 0.05 ) the concentration and morphology of spermatozoa between the control and treatment groups but there is no real difference between P1 and P2 . As for sperm motility are no significant differences ( P < 0.05 ) for each treatment .* ***Conclusion:*** *Based on this it can be concluded that testicular torsion lead to changes in concentration , motility , and morphology of spermatozoa contralateral testis.*

***Keywords:****Testicular torsion, quality of sperm, Rattus norvegicus.*

***ABSTRAK***

***Tujuan:*** *Penelitian ini bertujuan untuk mengetahui pengaruh torsio testis terhadap kualitas sperma yang meliputi: konsentrasi, morfologi, dan motilitas testis kontralateral Rattus norvegicus.* ***Bahan& Cara:*** *Penelitian ini merupakan penelitian eksperimental laboratorium dengan rancangan post-test only control dan rancangan acak lengkap (RAL) dan dibagi menjadi 3 kelompok yaitu kontrol (KO) dan perlakuan (P1 dan P2). Kelompok perlakuan menginduksi torsi testis 360˚ kekiri selama 4 jam.Masing-masing kelompok terdiri dari 9 ekor tikus yang diamati segera setelah detorsi (efek cepat) dan 9 ekor tikus diamati setelah 30 hari melakukan detorsi (efek lambat). Data dianalisis dengan ANOVA - One way dan dilanjutkan dengan uji Tuckey HSD.* ***Hasil:*** *Hasil penelitian menunjukkan terdapat perbedaan signifikan (P<0.05) konsentrasi dan morfologi spermatozoa antara kelompok kontrol dan perlakuan namun tidak terdapat perbedaan nyata antara P1 dan P2. Sedangkan untuk motilitas spermatozoa tidak terdapat perbedaan nyata (P<0.05) pada setiap perlakuan.* ***Simpulan:*** *Berdasarkan hal tersebut dapat disimpulkan bahwa torsio testis menyebabkan perubahan konsentrasi, motilitas, dan morfologi testis kontralateral spermatozoa.*

***Kata kunci:*** *Torsio testis, kualitas sperma, Rattus norvegicus.*

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## INTRODUCTION

Testicular torsion is a urological emergency that occur when the funiculus spermaticus twists, leading to impaired blood supply to the rotated testis. This condition causes an obstruction of blood supply to the testes, which leads to ischemia.[1]

The ipsilateral obstruction or injury of the vas deferens can cause significant injury to the contralateral testis. Although various pathways have been suggested, the mechanism of contralateral testicular damage remains controversial. Some studies have shown that the damage can be repaired, but other studies suggest that the effects are irreversible. The most popular and proposed theory to explain the contralateral testicular damage in ipsilateral testicular torsion is the theory of the autoimmune respond and Reactive Oxygen Species (ROS) reaction.[2-4]

Researchers are interested in discussing the effect of ipsilateral testicular torsion on the quality of contralateral spermatozoa cells, particularly in the

decrease in concentration, morphologic changes, and motility of the spermatozoa cells immediately after detorsion and 30 days after detorsion because these cells are related to male infertility.

## OBJECTIVE

This study aims to identify the effect of testicular torsion on the quality of sperm which include: concentration, morphology, and motility of the contralateral testis Rattusnorvegicus.

## MATERIAL & METHODS

This study is an experimental laboratory study with a post-test only control and completely randomized design (CRD) methodology using 30 male white rats (Rattus norvegicus) Wistar strain, aged 3–4 months and weighing 150–200 grams. The samples were divided into three groups. The first group was the control group, consisting of 10 white rats who were not given any treatment for testicular torsion. The second and third groups, which were treatment groups, each consisting of 10 male Wistar white rats, will be observed for the quality of spermatozoa such as concentration, morphology, and motility. In first treatment group, detorsion was used after four hours of torsion. Immediately after detorsion, the spermatozoa are examined for concentration, morphology, and motility. In the third treatment group, 30 days after detorsion, the testes were removed to observe changes in the quality of the spermatozoa in the form of concentration, morphology and motility.

Thirty male white rats (Rattus norvegicus) Wistar strain, aged 3–4 months and weighing 150–200 grams were left for a week for the acclimatization process. During the research period, the rats were kept in husk-lined containers and fed pellets and water as their regular diet.

Thirty experimental animals were randomized and divided into three groups, each group consisting of nine rats. The control group was not performed testicular torsion. Meanwhile, both treatment groups were anesthetized by intramuscular injection using anesthetic drugs. After the experimental animals were anesthetized, the scrotum was incised and a 360° torsion performed medially to the ipsilateral testicle. It was then repositioned and fixed using siede 3/0 absorbable sutures. The incision was sutured, and the torsion was left for 4 hours. The scrotum was re-incised and repositioned laterally 360° after 4 hours. Nine rats from both the treatment and control groups had their testes removed, and histology preparations were made before being observed under a microscope (quick effect) on the contralateral testis. While the other nine rats from the third treatment group performed fixation with absorbable sutures, then the incision wound was closed, an antibacterial ointment was applied, and the rats were left for 30 days while the wound dressing was performed to prevent bias. After 30 days, a scrotal incision was made again, the testes were removed, and the concentration, morphology, and motility of spermatozoa were examined and then observed under a microscope (slow effect).

| Group | Test | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| K0 | $K0_1$ | $K0_2$ | $K0_3$ | $K0_4$ | $K0_5$ | $K0_6$ | $K0_7$ | $K0_8$ | $K0_9$ | $K0_{10}$ |
| P1 | $P1_1$ | $P1_2$ | $P1_3$ | $P1_4$ | $P1_5$ | $P1_6$ | $P1_7$ | $P1_8$ | $P1_9$ | $P1_{10}$ |
| P2 | $P2_1$ | $P2_2$ | $P2_3$ | $P2_4$ | $P2_5$ | $P2_6$ | $P2_7$ | $P2_8$ | $P2_9$ | $P2_{10}$ |

K0 (control group) = group that did not receive treatment
P1 (Treatment group 1) = group that received testicular torsion treatment for 4 hours and the spermatozoa immediately observed after being detorsi (quick effect)
P2 (Treatment group 2) = group that received testicular torsion treatment for 4 hours and the spermatozoa observed 30 days after detorsi (slow effect)

The research was conducted at the Reproductive Laboratory of the Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh. The time required from proposal preparation to research results seminars starts from July 2013 to March 2014.

Research Variable

1. Independent variable : Time duration variance after testicular detorsion
2. Dependent variable : Quality of spermatozoa (concentration, morphology and motility)
3. Control variable: Rattus norvegicus rats' gender, age, weight, environment, diet, health, torsion type, torsion implementation, and torsion duration.

The contralateral testis of experimental animals that have undergone ipsilateral testicular torsion was observed according to the time of observation (quick and slow effects). The specimens were taken from the contralateral cauda epididymis testis and placed in a petri dish. The specimen was then chopped to let the spermatozoa come out and then observed for concentration, morphology and motility.

Homogenous spermatozoa are drawn with an erythrocyte pipette up to the 0,5 mark, followed by NaCL 0.9%, up to the 101 mark. Then the erythrocyte pipette is shaken by rotating around the sagittal axis, so the spermatozoa become more homogeneous after being diluted.

Discard the first few drops from the erythrocyte pipette, then dripped the dilution of spermatozoa onto the Improved Neubauer counting chamber which had been covered with cover glass. The specimens were examined under a light microscope with a 10x eyepiece and 40x objective lens magnification. The procedure for examining the concentration of spermatozoa was carried out in each group. Spermatozoa concentration was calculated from the average of five visual fields. The normal concentration of spermatozoa in Winstar rats is above 800 x 106 spermatozoa. The spermatozoa concentration measurement scale is a numerical scale.

The findings of the calculation of the concentration of spermatozoa are then applied to the formula for determining the number of spermatozoa per milliliter of suspension of cauda epididymis secretion as follows:

One drop of cauda epididymis spermatozoa suspension is gently placed on top of the prepared glass object, dripped with 0.9% NaCl, and then immediately observed under a light microscope with a 40x objective lens magnification.

The observed spermatozoa movement was a progressive movement; the spermatozoa were moving forward. Calculations were made on the movement of spermatozoa in groups. Spermatozoa motility is defined as the ability of spermatozoa to move. Spermatozoa motility was measured by observing spermatozoa using a microscope. Spermatozoa motility is divided into three criteria: 0, if the spermatozoa are immotile or immobile; 1, if the spermatozoa move round in place; 2, if the spermatozoa move forward but slowly and do not form waves 3, if the spermatozoa move very progressively, forming a fast wave. Spermatozoa motility measurement scale is an ordinal scale.

To examine the morphology of the spermatozoa, drop one of the spermatozoa suspension was extracted from the cauda epididymis, smeared on an object glass, and dried. Then fixed by giving 2% eosin for 1 minute. After that rinsed with running water and dried. Then counted by the number of 100 spermatozoa with a light microscope, determined the percentage of normal and abnormal spermatozoa. The morphology includes the head, body and tail of the spermatozoa. Normal spermatozoa have straight and intact heads and tails. Abnormal spermatozoa are divided into nine categories: head without tail, tail without head, broken tail, curved tail, incomplete tail, broken body, curved body, coiled tail and the spermatozoa are

$$C = (N/n) \times (1/x) \times y$$

C = concentration of spermatozoa (spermatozoa/nL)
N = number of spermatozoa counted
n = number of square calculated
x = volume of liquid 1 box in nL
y = dilution (frequency of dilution)

$$\%\ abnormal\ spermatozoa = \frac{normal\ spermatozoa\ count}{normal + abnormal\ spermatozoa\ count} \times 100\%$$

circular. Spermatozoa morphological good if the abnormalities found are less than 20%. Spermatozoa morphology measurement scale is an numerical scale.

## RESULTS

The results of the research's observations on the concentration of spermatozoa cells are presented in the following table 1.

The results of the research's observations on the morphology of spermatozoa cells are presented in the following table 2.

The results of the research's observations on the motility of spermatozoa cells are presented in the following table 3.

## DISCUSSION

Testicular torsion, is pathological condition, in humans that results in ischemic conditions in the testes where surgery is required to restore blood flow. In the study of testicular torsion in rats, permanent disruption of spermatogenesis occurred after Leydig cells and Sertoli cells were disrupted. The primary factor causing the cessation of

**Table 1.** Distribution data for evaluation of spermatozoa concentrations of Rattus norvegicus rats.

| Group | Test | | | | | | | | | Mean ± SD |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| K0 ($x10^6$) | 700 | 750 | 983 | 810 | 750 | 1050 | 1056 | 1016 | 1057 | 908.04±151.82 |
| P1 ($x10^6$) | 520 | 530 | 470 | 510 | 260 | 850 | 740 | 580 | 340 | 533.33±180.97 |
| P2 ($x10^6$) | 340 | 650 | 500 | 120 | 230 | 300 | 460 | 690 | 480 | 418.89±270.97 |

K0 (control group) = group that did not receive treatment
P1 (Treatment group 1) = group that received testicular torsion treatment for 4 hours and the spermatozoa immediately observed after being detorsi (quick effect)
P2 (Treatment group 2) = group that received testicular torsion treatment for 4 hours and the spermatozoa observed 30 days after detorsi (slow effect)

**Table 2.** Distribution data for evaluation of spermatozoa morphology of Rattus norvegicus rats.

| Group | Test | | | | | | | | | Mean ± SD |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| K ( % ) | 4 | 1 | 1 | 0 | 0 | 5 | 8 | 2 | 2 | 2.56 ± 2.65 |
| P1 ( % ) | 46 | 56 | 44 | 33 | 48 | 37 | 31 | 52 | 44 | 43.44 ± 8.40 |
| P2 ( % ) | 54 | 36 | 27 | 42 | 42 | 65 | 40 | 47 | 25 | 29.44 ± 21.16 |

K0 (control group) = group that did not receive any treatment
P1 (Treatment group 1) = group that received testicular torsion treatment for 4 hours and the spermatozoa immediately observed after being detorsi (quick effect)
P2 (Treatment group 2) = group that received testicular torsion treatment for 4 hours and the spermatozoa observed 30 days afterdetorsi (slow effect)

**Table 3.** Distribution data for evaluation of spermatozoa motility of Rattus norvegicus rats.

| Group | Test | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| K ( + ) | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| P1 ( + ) | 3 | 3 | 2 | 3 | 2 | 2 | 3 | 2 | 2 |
| P2 ( + ) | 1 | 2 | 1 | 2 | 1 | 1 | 2 | 3 | 2 |

K0 (control group) = group that did not receive any treatment
P1 (Treatment group 1) = group that received testicular torsion treatment for 4 hours and the spermatozoa immediately observed after being detorsi (quick effect)
P2 (Treatment group 2) = group that received testicular torsion treatment for 4 hours and the spermatozoa observed 30 days after detorsi (slow effect)

spermatogenesis has been identified as cell apoptosis.[5]

The degree of infertility in a person with a torsion of the testicle also depends on the severity of the ischemia and damage to the contralateral testicle. Injury to the contralateral testis from ipsilateral testicular torsion is still controversial. Several studies have shown an ischemic effect on the contralateral testis in the setting of ipsilateral testicular torsion, but other studies do not support this concept. Several mechanisms have been proposed to explain the damage to the contralateral testis such as autoimmune theory against spermatogonia, decreased blood flow to the testis due to reflex sympathetic response, ROS reaction after detorsion, and overproduction of nitric oxide synthase.[6]

Apoptosis or programmed cell death is a continuous process of destruction of non-functioning cells. It is a physiological as well as pathological process in which the body gets rid of undesirable cells via cell destroyers and is also a crucial part of defense against damaged cells. Recent evidence suggests that apoptosis in the ischemic testes is significantly increased following the Ischemic/Reperfusion state, but the role of apoptosis in germ cell decline in the contralateral testis remains unknown. Preventing cell apoptosis may be crucial to the therapy of lowering or preventing infertility in testes that have been harmed by ischemia or reperfusion since increased apoptosis may be cause germ cell loss.[6]

Based on the study of Lysiak et al., which focused on variations in the degree and duration of testicular torsion, 720° torsion for 2 hours caused disruption of the seminiferous tubules, a significant decrease in testicular mass, and impaired spermatozoa production. This is followed by increased neutrophil adhesion, ROS, and the occurrence of apoptosis. The the testes that underwent treatment in the form of 360° and 720° for 1 hour, and 360° and 720° torsion for 2 hours, revealed the same outcomes such as reduced spermatogenesis.[5]

As a result of torsion, the testes experience oxidative stress, where the normal balance between the production of free radicals or reactive oxygen compounds and the body's natural antioxidant ability to eliminate them is disrupted, so that it disrupts the normal oxidation-reduction chain, and causes tissue oxidative damage, lipid peroxidation of cell membranes, and damage to cell membrane structure. The tissue damage also depends on several factors, including: the molecular target, the level of stress, the mechanisms involved, as well as the timing and nature of the system being attacked. Since this cell membrane is essential for receptor and enzyme action, the peroxidation of cell membrane lipids by free radicals may result a complete loss of cellular function. Oxidative stress is thought to be one of the potential reasons for a decrease in testosterone function in males.[7]

One of the mechanisms of testicular damage is increased NO. Increased NO is often associated with an increase in lipid peroxidase under various types of stress that cause a decrease in testosterone secretion. Testosterone is a hormone that plays an important role in spermatogenesis. The process of spermatogenesis can occur if there are adequate levels of the testicular testosterone hormone. Thus, a decrease in testosterone levels can affect the number of spermatozoa produced.[7-8]

Another mechanism, in the form of ischemia that occurs due to reduced blood flow in certain tissue blood vessels. Ischemia also compromises substrate delivery for glycolysis, in contrast to hypoxia, where glycolytic energy production can continue (although less effectively than the oxidative process). Therefore, anaerobic energy production also ceases in ischemic tissue when the potential substrate is depleted or if glycolysis is prevented by the buildup of metabolites that would otherwise be eliminated through the circulation. If hypoxia is left untreated, decreased mitochondrial performance and a consequent rise in membrane permeability result in poor spermatogenesis.[9]

The decrease in the average concentration of spermatozoa that occurred in the treatment group 2 indicates that there was probably no improvement after the detorsion process in the contralateral testicle, which result in worsen damage in group treatment 2 compared to group treatment 1.

Vigueras et al, showed that in 720˚unilateral testicular torsion there was a decrease for different time periods for 60 days, analyzed the spermatogenesis parameters of the contralateral testis, the results were degeneration, hypoplasia and germ cell loss, degeneration of the basement membrane tubules, and the presence of intraepithelial and intraepithelial vacuoles. all without quantitative evaluation. After 60 days' post torsion, the contralateral testis showed significant changes in all parameters, which indicated contralateral testicular damage in the process of spermatogenesis.[10]

Another study, Kosar et al, reported that in their study of unilateral testicular torsion for 12, 48 hours and 3 months, with or without orchidectomy on testicular torsion one month after detorsion of the contralateral testis in the first month, there was a

significant decrease in testicular weight, tubulus seminiferous diameter, and on the Jhonsen score with significant improvement at 3 months only in the group that received orchidectomy on testicular twisting, and concluded that orchidectomy was beneficial in preventing such damage.[11]

The mechanism of abnormalities in the morphology of spermatozoa is due to disturbances in the Sertoli cells that cause abnormalities in the maturation process of sperm cells that occur in the epididymis which can interfere with the process of spermatogenesis.[12-14]

The second mechanism, is the increase in the number of Reactive Oxygen Species (ROS) caused by an increase in the number of active granulocytes. Increased ROS can cause disturbances in the process of spermatogenesis and result abnormalities in the morphology of spermatozoa cells.[12-13]

The third mechanism is the presence of lipid peroxidase. The end result of lipid peroxidation in spermatozoa membranes is the breaking of unsaturated fatty acid chains, which produces Malondialdehyde (MDA), which known toxic to cells. MDA is a secondary product of lipid oxidation which becomes peroxide and causes damage to spermatozoa membranes and decreases the integrity of spermatozoa membranes, resulting in a decrease in the quality of spermatozoa. Measurement of MDA levels is an indirect method of measuring free radical activity, because what is assessed is the end result of free radical reactions rather than direct free radical measurements. There is a negative correlation between sperm MDA levels and spermatozoa membrane integrity. This correlation can be explained by high MDA levels will reduce cell membrane integrity and damage spermatozoa which causes a decrease in sperm quality, so that the higher the MDA level, the lower the percentage of normal integrity of the spermatozoa membrane.[15]

Normal spermatogenesis requires a synergistic interaction between testosterone and FSH. Spermatogenesis is also affected if testosterone and FSH output are suppressed, which causes a rise in primary abnormalities in spermatozoa. The maturation of spermatozoa in the epididymis is also compromised when testosterone secretion is inhibited. Spermatozoa maturation is one of the endogenous factors that affect spermatozoa motility so that disturbances in this process can reduce spermatozoa motility and increase secondary abnormalities in spermatozoa. Free radicals can cause cell damage through lipid peroxidation reactions and membrane cholesterol containing polyunsaturated fatty acids or polyunsaturated fatty acids (PUFAs).[16-17]

According to Hayati, lipid peroxidation in spermatozoa membranes can reduce membrane permeability for specific ions and reduce membrane flexibility. According to Sanocka and Kurpiz, damage to spermatozoa caused by ROS occurs because it can inhibit the acrosome reaction and damage the tail which greatly affects the motility of spermatozoa. According to Aryosetyo, high levels of ROS can damage the mitochondrial membrane, causing a loss of potential mitochondrial function which will disrupt the motility of spermatozoa because the energy for sperm motility is supplied in the form of adenosine triphosphate which is synthesized by the mitochondria in the tail body.[17]

Decreased motility of spermatozoa is thought caused by ischemia in testicular torsion. The first mechanism is the inhibition of oxidative phosphorylation. Free radicals inhibit the process of oxidative phosphorylation. Oxidative stress caused by increased production of ROS (reactive oxygen species) causes disturbances in the process of oxidative phosphorylation in spermatozoa. Oxidative phosphorylation is an energy-forming process involving an enzyme complex found in the inner mitochondrial membrane. Spermatozoa mitochondria are located in the middle of the spermatozoa, while the neck and tail function in the movement of spermatozoa. After being synthesized in the mitochondria, ATP is transported to the axoneme at the tail of the spermatozoa, then converted by dynein in the axoneme, which will decompose ATP into energy for the movement of spermatozoa. The inhibition of the release of ATP to the axoneme results in unfulfilled or reduced energy requirements to move the tail, further resulting in spermatozoa unable to move quickly or not moving at all.[15]

The mechanism of the decrease in sperm motility is also due to abnormalities in sperm morphology due to disturbances in the Sertoli cells that cause abnormal sperm morphology abnormalities in the maturation process of sperm cells that occur in the epididymis. The speed of forward movement and motility of spermatozoa are closely related to the morphology of spermatozoa. If the morphology of the spermatozoa is abnormal then the movement of the spermatozoa will be disturbed. The spermatozoa will function effectively, if the

morphology of the spermatozoa itself supports spermatozoa motility.[17-18]

## CONCLUSION

The results of the study on the effect of ipsilateral testicular torsion on the quality of the spermatozoa of the contralateral testis of rats (Rattus norvegicus) strain Wistar can be concluded as follows:

1. Ipsilateral testicular torsion has an effect on changes ($p < 0.05$) in the quality of spermatozoa, particularly in concentration, abnormal morphology, and motility in the contralateral cauda epididymis testis of Rattus norvegicus rats immediately after the distorsion (quick effect).
2. Ipsilateral testicular torsion has an effect on changes ($p < 0.05$) in the quality of spermatozoa, particularly in concentration, abnormal morphology, and motility in the contralateral cauda epididymis testis of Rattus norvegicus rats 30 days after the distorsion (slow effect).

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