

**SEXUAL ABSTINENCE AND IMMOBILISATION STRESS DECREASED  
NITRATE + NITRITE CONCENTRATION AND INCREASED NECROTIC  
OF PENILE CORPUS CAVERNOSUM SMOOTH MUSCLE AND  
ENDOTHELIAL CELLS OF ADULT MALE MICE**

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**ABSTRACT**

Sexual intercourse in adult people is not only related to reproduction, recreation and better relation but also for maintaining good health. Regular and frequent sexual intercourse has protective role for cardiovascular disease, malignancy and often related with longevity. On the contrary less frequent sexual intercourse is often correlated to early death and reproductive organ disorders. One main factor which involve in any process related to sexual intercourse is nitric oxide. This research aims to know the declination of total nitrate and nitrite concentration as the main oxidation product of nitric oxide and necrosis of the endothelial and smooth muscles of corpus cavernosum after three month sexual intercourse abstinence and stress immobilization.

By randomized pretest-posttest control group design, the research was conducted in adult male mice (*Mus musculus*) which were divided into four groups. each consisted of seven mice. One group as a control and the other three were exposed to sexual abstinence, immobilization stress and the combination of sexual abstinence and immobilization stress in three months period. The investigation was carried out to the penile total nitrate and nitrite concentration and the histological changes of the middle part of the penile shaft. To know the degree of lesion then the calculation of the histologic features was done in five view fields of every preparat. The result was compared to control group.

In this research, the mean of total nitrate and nitrite decline and the lowest was found in the combine intervention groups with significance level  $p < 0,005$ . The corpus cavernosum in the intervention groups showed multifocal necrosis on endothelial and smooth muscle cells of corpus cavernosum with picnotic nuclei, irregular form and dark colour. Some muscle cells are lysis. These features were found in three among seven samples in sexual abstinence group, two among seven samples in immobilization stress group and in all samples of combined intervention group.

Conclusions can be drawn from this research are that the sexual abstinence, immobilization stress and the combine interventions decline the nitrate and nitrite concentrations and induce endothelial and smooth muscle cells necrosis of the copus cavernosum.

Key words: *sexual intercourse abstinence, stress immobilization, total nitrate nitrite and necrosis.*

## INTRODUCTION

Recent studies revealed that sexual intercourse is related to health preservation, longevity and reduced risks for heart disease and cancer. Death risk decreased by 50% in the male group with two or more orgasms in a week as compared to only once or less in a month. Sexual activity apparently has a protective effect on male health

In a cohort of men with very low frequency of sexual intercourse, the adverse effects are not only limited to lower general health condition, cardiovascular disease such as heart disease, hypertension, stroke, but may have adverse effects on the reproductive organs such as prostate, testicle and penile disorders. Prolonged penile inactivity may cause atrophy of the penile muscles. Male erectile dysfunction after radical retropubic prostatectomy may lead to decreased penile length and diameter.

The relationship between frequency of sexual intercourse and health is thought to be linked to nitrogen monoxide (NO) production. Genital organ stimulation may lead to neural central and peripheral feedback mechanism inducing the production of NO at the motoric nerve terminals and in the endothelial lining of blood vessel walls. As a vasodilator, NO induces smooth muscle relaxation resulting in vasodilatation thus improving organ perfusion needed to preserve its integrity.

Nerve stimulation to the brain during sexual intercourse is also inducing the rising of testosterone and DHEA hormone production through hypothalamo-pituitary-testis axis and hypothalamo-pituitary-adrenal axis. These sex steroid hormones are anabolic in their nature and stimulate NO formation in penile tissue. During sexual intercourse, particularly sexual

experienced male animals, will increase the production of NOS, NO and also testosterone. In male testosterone acts as primary factor that determines the coital frequency, libido and sexual potential. In this case NO and testosterone are two interdependent substances and together preserve the tissue integrity.

Prolonged sexual intercourse abstinence may lead to reduced genital organ stimulation thus also reducing NO production through reduced nitric nerve feedback and hormonal mechanisms. Klabunde (2004) has hypothesized impairment of NO production and its reduced bioavailability may bring about vasoconstriction leading to coronary vasospasm, increase systemic vascular resistance and hypertension, thrombosis due to platelet aggregation and its adherence to the endothelium, and inflammation following increased leucocyte and endothelial adhesion molecules activity, vascular hypertrophy and stenosis. If this situation is prolonged, oxygen distribution will be reduced and stimulates reactive oxygen species (ROS) production from the mitochondria. ROS is a toxic substance that could induce necrosis (Schumacker, 2003). A decrease in testosterone production will induce structural and functional impairment. This situation induces necrosis.

In immobilization stress, the adaptive response of the body will activate hypothalamo-pituitary-adrenal axis to maintain homeostasis. Stress will suppress the reproductive axis at all levels and thus decreasing testosterone and NO production.

If the manipulations are mixed, i.e. sexual intercourse abstinence and immobilization stress are simultaneously applied, the impairment in the form of low NO

concentration and necrosis will become more obvious.

## **RESEARCH METHODS**

### **Research Design**

This is an experimental, randomized control group pretest and posttest design only. This was employed to obtain the effect of sexual abstinence and immobilization stress to the declining of nitrate and nitrite concentration and necrosis on endothelial and smooth muscle cells of penile corpus cavernosum.

### **Research procedure**

#### **Sample selection**

A total of one hundred and twelve 4-month-old male mice living freely with their females counterpart, was randomly divided into two groups: pretest and posttest (i.e. treatment) groups.

The pre-test group was randomly divided into two groups, each of which was divided again into 4 sub-group I, II, III, IV, each of which comprised of 7 male mice living freely with equal number of female mice. The posttest group was also divided with the same manner into 4 sub-groups depending on the kind of treatment.

#### **The pretest group**

All mice of pretest groups were sacrificed. Penile tissue were taken for nitrate and nitrite concentration examination at the Analytic Laboratory of Udayana University and for histological examination at the local Veterinary Pathology Laboratory.

Nitrate concentration examination was based on Bruin methode (1988) and nitrite concentration examination by Kramlich method (1973); histological specimens were obtained from the middle part shaft and cut 6 microns thickness and stained by hematoxylin-eosin, and

viewed through microscope with 30, 100 and 400 amplification.

#### **The posttest group**

Members of group I as control group were allowed to live freely with an equal number of their female counterparts in the cage for a period of three months. Total sexual abstinence by isolation from female mice was imposed on members of group II for a period of three months. Immobilization stress by placing in a 1.25 cm paralon tube was imposed on members of group III for 4 hours everyday; after which they were allowed to live freely with their female counterparts. This immobilization stress was applied for a period of three months. Members of group IV were treated with combined sexual and immobilization stress. The treatment would last for a period of three months.

#### **Nitrate nitrite concentration and histological examinations**

After three months all mice from all groups were sacrificed. Penile tissues were taken for nitrate and nitrite concentration and for evidence of smooth muscle and endothelial cell necrosis.

To estimate the severity of lesions resulting from the various treatments, the total number of lesions was counted in every slide. The counting was based on 5 view fields with 100 amplification; so there would be 35 view fields observation for every group. The total number of lesions was noted and analyzed statistically. Data were analyzed descriptively, normality test was done by Shapiro Wilk, homogeneity by Levene test, and for mean differences by ANOVA and Kruskal Wallis. To reduce bias, all examinations were carried out single-blinded.

## RESULTS

### Nitrate and nitrite concentrations

The mean concentrations of nitrate and nitrite for the control group differ significantly from those of the treatment groups ( $p < 0,05$ ). The mean concentrations of nitrate and nitrite among members of the treatment group also differ significantly ( $p < 0,05$ ),

except for members of group II (sexual abstinence) and group III (immobilization stress), with statistical differences ( $p > 0,05$ ). Post Hoc test results revealed that sexual abstinence, immobilization stress and combined treatment progressively decreased the nitrate and nitrite concentration in the penile tissues

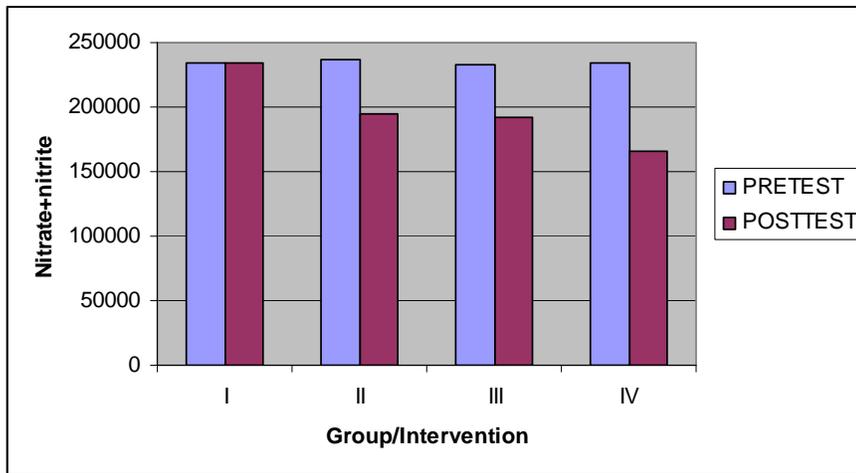


Figure 1 Mean difference pretest and posttest total nitrate and nitrite. Group I (control) pretest and post test with similar result ( $p > 0,05$ ), while the pretest and posttest of group II (sex abstinence), group III (immobilization stress) and group IV (combined treatment) were significantly difference ( $p < 0,05$ )

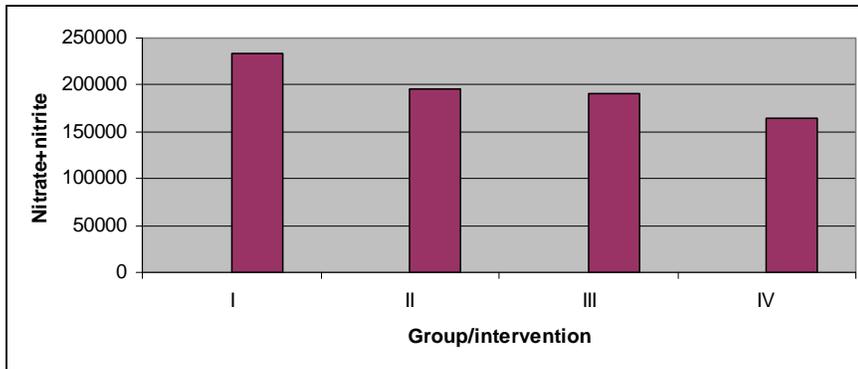


Figure 2 Mean diff. of total nitrate nitrite posttest. Diff. I-II  $p < 0,05$ ; diff. I-III  $p < 0,05$ ; diff. I-IV  $p < 0,05$ ; diff. II-III  $p > 0,05$ ; diff. II-IV  $p < 0,05$ ; diff. III-IV  $p < 0,05$ .

### Histological evidence of cellular necrosis

Multifocal necrosis with dark, irregular and piknotic nuclei of smooth muscle and endothelial cells in the corpus cavernosum were observed. In other parts of the tissue slices, lysis of the smooth muscle appearing as blank spaces were noted. The lesions appeared spotty and were not evenly distributed through the tissue slices.

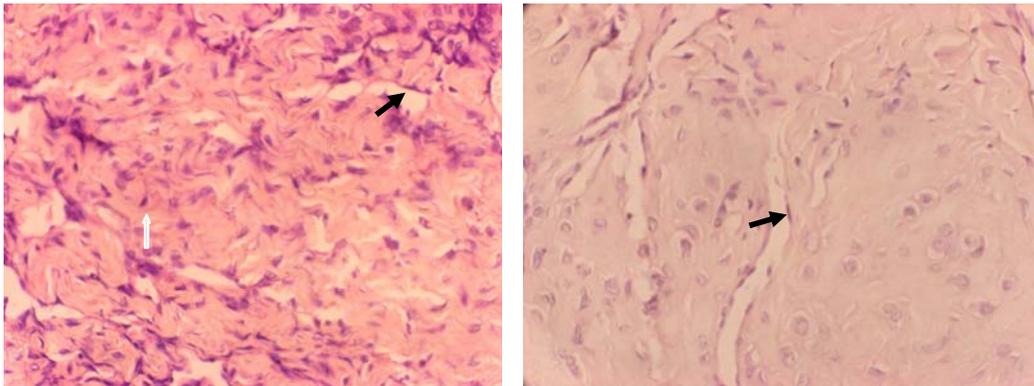
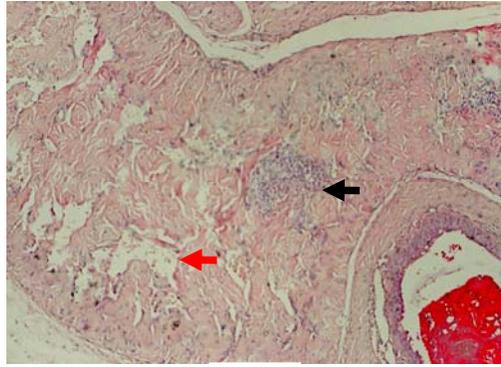
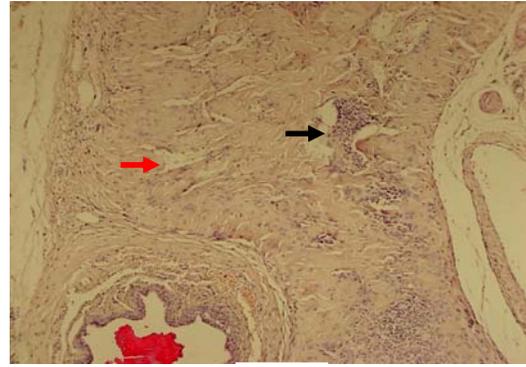


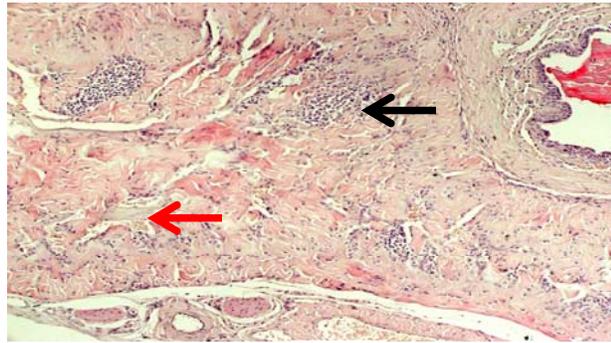
Figure 3 left: pretest corpus cavernosum. Its showed cavernous sinus lined by endothelial cell with oval nucleus (black arrow) and smooth muscle cell (white arrow). Right: posttest (control/free sexual life) penile with the same image. Corpus cavernosum consist cavernous sinus lined by endothelial cell with oval nucleus and smooth muscle cell (white arrow). (H & E 400)



A



B



C

Figure 4 A. Mice penile with treatment II: endothelial and smooth muscle cells piknotic nuclei, irregular form and dark colour (black arrow) lysis of smooth muscle necrosis appeared as blank space (red arrow) H&E; 100X.

Figure 4 B. Mice penile with treatment III also showed similar picture with endothelial and smooth muscle cells piknotic nuclei, irregular form and dark colour (black arrow) lysis of smooth muscle necrosis appeared as blank space (red arrow) H&E; 100X.

Figure 4 C. Mice penile with treatment IV endothelial and smooth muscle cells piknotic nuclei, irregular form and dark colour (black arrow) lysis of smooth muscle necrosis appeared as blank space (red arrow) H&E; 100X.

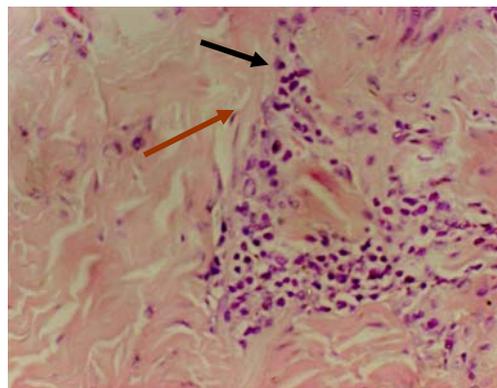


Figure 5. Endothelial and smooth muscle cell necrosis of mice corpus cavernosum, piknotic nuclei,irregular form and dark colour (black arrow), lysis of smooth muscle necrosis appeared as blank space (red arrow) H&E 400x.

### The total number of lesions

Group I (control): in the 7 tissue slices no lesions were observed; the same observation was true for the 35 view fields. Group II (sexual abstinence): out of the 7 slices examined, 3 slices showed lesions; in the 35 view fields there were 22 lesions. Group III (immobilization stress): out of the 7 slices examined, there were 2 slices with lesions; in the 35 view fields there were 10 lesions. Group IV (combined treatment): out of 7 slices examined, all showed lesions; in the 35 view fields 39 lesions were noted.

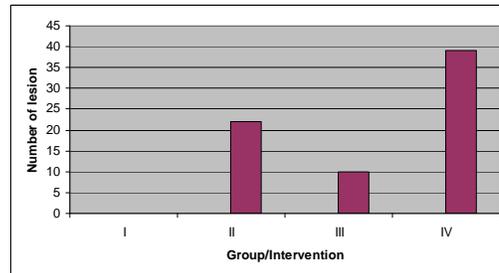


Figure 6 The comparison of total lesions posttest I (control), II (sex abstinence, III (immobilization stress and IV (combined sex abstinence and immobilization stress

The data were analyzed by Kruskal Wallis test, because of the data did not fulfil the normality criteria required for ANOVA. Significant statistical differences ( $p < 0,05$ ) were noted among members receiving different treatments. Median test revealed that at least one median for the 4 groups was unidentical ( $p < 0,05$ )

## DISCUSSION

### Free sexual intercourse

The total nitrate and nitrite concentrations as representations of tissue availability of NO and histological features of penile tissue in this study can be commented as follows: during free sexual life with the family counterparts, the male mice could have sexual intercourse ad libidum. Sexual intercourse increases spinal and central stimulation to the brain that assure the maintenance of supply of NO, testosterone, DHEA. NO, as a vasodilator, helps maintain normal blood perfusion to the tissue. Testosterone and DHEA, as an anabolic hormone, particularly are involved in protein metabolism, and with NO are involved in maintaining local tissue integrity.

### Sexual abstinence

Sexual abstinence lowers total nitrate and nitrite tissue concentration and could bring about multifocal necroses of the corpus cavernosum smooth muscle and endothelial cells through the decreased spinal and central stimulation of the brain. This sequence of events lead to declining NO, testosterone and DHEA production and tissue availability in the local tissue. Deficiency of NO leads to local vasoconstriction. If this condition persists over a prolonged period of time, local hypoxia will result. Declining levels of testosterone and DHEA may induce arteriosclerosis, vascular stenosis and vasculopathy. This condition induces hypoxia and necrosis. Karmaya (2007) reported the atrophy of the rat corpus cavernosum smooth muscle cells following a 3-month long sexual abstinence.

### **Immobilization stress**

Immobilization stress also lowers total nitrate and nitrite levels, and thus causing multifocal necroses of the corpus cavernosum smooth muscle and endothelial cells. The severity of the adverse effects of immobilization stress appears to be similar to those of sexual abstinence. Increase cortisol production following stress inhibits the activity of enzymes involved in steroidogenesis and also inhibit LH secretion and testosterone production. This accordance with the finding of Rai *et al* (2004).

Immobilization stress induces decrease in the frequency of sexual intercourse and non-contact erection, and decrease stimulation to the spinal cord and brain and ultimately leads to decrease testosterone and DHEA production and tends to lower NO production.

### **Combined treatment**

Sexual abstinence and immobilization stress as combined treatment caused the greatest declining of total nitrate and nitrite concentration and the greatest quantity of total lesion. These can be understood because the double load lead to extremely damage of penile tissue.

## **CONCLUSIONS AND FUTURE WORKS**

### **Conclusions**

1. Sexual abstinence over a period of 3 months decreased total nitrate and nitrite concentration in the penile tissue and induced necrosis of the smooth muscle and endothelial cells of the corpus cavernosum.
2. Immobilization stress over a period of 3 months also decreased the total nitrate and nitrite levels in the penile tissue.
3. The degree of decline in the total nitrate and nitrite levels following sexual abstinence and immobilization are statistically similar.
4. Treatment with combined sexual abstinence and immobilization stress produced the most severe tissue damage in the penile tissue.

### **Future works**

1. Further study would be needed to further explore the basic mechanisms underlying the emergence of tissue damages following various prolonged stimuli.
2. It is immature at this stage of our knowledge to try to extrapolate the initial results in this study to the human subjects.

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