Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Low sperm quality and increased testicular caspases in chronic stress mice induced by dexamethasone

Baixa qualidade espermática e aumento de caspases testiculares em camundongos com estresse crônico induzido por dexametasona

C. Chaimontri[a#], S. Arun[a#], T. Kamollerd[a], N. Lapyuneyong[a], C. Taoto[a], S. Innoi[a], T. Chawalchitiporn[a], T. Sawatpanich[a], N. Uabundit[a] and S. Iamsaard[a,b*]

[a]Khon Kaen University, Faculty of Medicine, Department of Anatomy, Khon Kaen, Thailand

[b]Khon Kaen University, Research Institute for Human High Performance and Health Promotion, Khon Kaen, Thailand

Abstract

Chronic stress (CS) from physical stressors and glucocorticoid administrations has been shown to induce germ cell apoptosis and low sperm quality. Among CS animal models, the effects of dexamethasone induced CS (DexCS) on testicular apoptosis and epididymal sperm parameters have not been fully demonstrated. This study aimed to investigate the changes of male reproductive system after CS induction by Dex. Adult male mice were divided into control and DexCS groups. Control mice were injected with sodium phosphate while DexCS mice were injected with DEX (4 mg/kg BW) for 21 consecutive days. The stress tests (sucrose preference, tail suspension, and forced swimming) were used to confirm CS behaviors. Sperm concentration, motility, morphology, viability, and acrosome status were assessed using computer-assisted semen analysis (CASA) and special staining. Histopathology of testis, epididymis, and penis was observed. Apoptotic protein expressions (Hsp70, caspases 3 and 9) in the testicular lysate were also determined. The results revealed that DexCS mice had significant increase of the immobility periods with decrease of total sucrose intake. DexCS significantly decreased sperm quality parameters particularly progressive motility. Testicular damages and decreased sperm mass in epididymis were obviously found in DexCS group. The expressions of testicular caspases 3 and 9, but not Hsp 70 were significantly increased in DexCS group compared to the control. It was concluded that DEX is a potential drug to induce chronic stress in mouse model, affecting male reproductive system via testicular histopathology and apoptotic pathway. Such effect may cause sperm physiology impairments like low progressive motile patterns.

Keywords: dexamethasone, chronic stress, sperm motility, testis, apoptosis.

Resumo

O estresse crônico (EC) causado por estressores físicos e administração de glicocorticoides demonstrou induzir apoptose de células germinativas e baixa qualidade espermática. Entre os modelos animais de EC, os efeitos do EC induzido por dexametasona (DexEC) na apoptose testicular e nos parâmetros espermáticos epididimais não foram totalmente demonstrados. Este estudo teve como objetivo investigar as alterações no sistema reprodutor masculino após a indução do EC por Dex. Camundongos machos adultos foram divididos em grupos controle e DexEC. Os camundongos controle foram injetados com fosfato de sódio, enquanto os camundongos DexEC receberam DEX (4 mg/kg de peso corporal) por 21 dias consecutivos. Os testes de estresse (preferência por sacarose, suspensão pela cauda e nado forçado) foram utilizados para confirmar os comportamentos de EC. A concentração, a motilidade, a morfologia, a viabilidade e o estado acrossômico dos espermatozoides foram avaliados por meio de Análise de Sêmen Assistida por Computador (CASA) e coloração especial. Foi observada histopatologia dos testículos, epidídimos e pênis. As expressões de proteínas apoptóticas (Hsp70, caspases 3 e 9) no lisado testicular também foram determinadas. Os resultados revelaram que camundongos DexCS apresentaram aumento significativo dos períodos de imobilidade com diminuição da ingestão total de sacarose. O DexCS reduziu significativamente os parâmetros de qualidade do esperma, particularmente a motilidade progressiva. Danos testiculares e diminuição da massa espermática no epidídimo foram obviamente encontrados no grupo DexCS. As expressões das caspases testiculares 3 e 9, mas não da Hsp 70, foram significativamente aumentadas no grupo DexCS em comparação ao controle. Concluiu-se que o DEX é um fármaco com potencial para induzir estresse crônico em modelos murinos, afetando o sistema reprodutor masculino por meio da histopatologia testicular e da via apoptótica. Tal efeito pode causar comprometimentos na fisiologia do esperma, como baixos padrões de motilidade progressiva.

Palavras-chave: dexametasona, estresse crônico, motilidade espermática, testículo, apoptose.

*e-mail: sittia@kku.ac.th
#Equal first authors
Received: August 29, 2024 – Accepted: April 26, 2025

1. Introduction

Chronic stress (CS) has been documented to increase the risks of strokes, heart attacks, peptic ulcer, and reproductive disorders particularly male infertility (López-Galindo et al., 2019; Everson-Rose et al., 2014). Physiologically, CS activates the hypothalamic–pituitary–adrenal axis (HPA) leading to increased secretion of the corticotropin-releasing hormone, adrenocorticotropin hormone, and cortisol levels, respectively.In humans, CS caused the erectile dysfunction and loss of libido (Carvalho and Nobre, 2011). Moreover, it could decrease sex hormone levels, resulting in decrease of the semen qualities (Díaz-Del Cerro et al., 2022; Hezavehei et al., 2022; Diao et al., 2019; Sengupta et al., 2018; Liang et al., 2016; Bhongade et al., 2015; Janevic et al., 2014; Malan et al., 2014). In stress animals, CS reduced sex hormone levels (FSH, LH, and testosterone), male sexual behaviors, and sperm parameter qualities (Choowong-In et al., 2021; Dadomo et al., 2020; Arun et al., 2016, 2018; Mohamadpour et al., 2017). Particularly, the expressions of major testicular proteins involved in androgen synthesis such as steroidogenic acute regulatory (StAR) and cytochrome P450 family 11 subfamily a member 1 (CYP11A1) have been demonstrated in the rat testis induced with CS (Arun et al., 2016, 2021; Choowong-In et al., 2021; Dallak, 2019). A previous study showed that the expressions of testicular caspase 3 and 9 (in both pro and cleaved forms) were increased under chronic unpredictable mild stress (Choowong-In et al., 2021). Moreover, the animals induced with CS have apoptosis in spermatogenic and Leydig cells (Fozooni et al., 2021; Shen et al., 2020; Demirci and Sahin, 2019; Kheirabad et al., 2016). Although many physical and mechanical stimuli have been used for CS induction in animal models, the mechanism of synthetic glucocorticoids, recently also applied in stress induction, on male reproductive biology still needs to be revealed. Such commercial glucocorticoids include hydrocortisone, prednisolone, and dexamethasone (Sánchez González et al., 2023; Kadir et al., 2020).

Dexamethasone (DEX) is a synthetic glucocorticoid commonly used in treating of anti-cancer, anti-inflammation, autoimmune diseases, allergies, and ocular disorders (Madamsetty et al., 2022; Krishnan, 2018). Currently, it has been used in animal stress induction with depressive-like behaviors (Wu et al., 2021; Souza et al., 2019). In CS animal models, the induction by DEX injection for 21 consecutive days has been proven in mice and rats (Delanogare et al., 2020; Skupio et al., 2015). Moreover, it could increase the body fats but decrease the body weight in mouse model (Wang et al., 2018; Skupio et al., 2015; Poggioli et al., 2013). Previous studies demonstrated that CS induced with DEX could decrease the testosterone levels, testicular StAR protein expression, seminiferous epithelium, and sperm quality (Khalil-Khalili et al., 2021; Kheradmand et al., 2019; Sadeghzadeh et al., 2019; Ing et al., 2015; Wanderley et al., 2013). However, the effects of CS induced with DEX on testicular apoptosis the apoptosis markers (Hsp70, caspase3, and caspase9 expressions) and systemic motility patterns of epididymal sperm analyzed by computer assisted sperm analysis (CASA) have never been documented. Therefore, this recent study was conducted to reveal the effects of dexamethasone induced-chronic stress (DexCS) on sperm quality and apoptotic protein expressions in mouse testis.

2. Materials and Methods

2.1. Animals and experimental design

Eight male ICR mice (35-40 g, 8 weeks) were purchased from the experimental animal unit, Faculty of Medicine, Khon Kaen University, Thailand. All animals were housed in plastic cages under the experimental room condition (12 hr. light/dark cycle, temperature 23 ± 2 °C, humidity 30-60% RH, sound < 85 decibels, and light intensity 350-400 lux) with providing commercial food pellet and water ad libitum. The recorded code number of animal ethic is IACUC-KKU-14/66. After acclimatization for 7 days, mice were divided into control and dexamethasone induced-chronic stress (DexCS) groups. To induce chronic stress, mice were injected with 4 mg/kg BW of dexamethasone (DEX) diluted in sodium phosphate (Na_3PO_4) via intraperitoneal injection (i.p.) and control animals were injected with Na_3PO_4 for 21 consecutive days as described in Delanogare et al. (2020). This study has been approved by the Animal Ethics Committee Research of Northeast Laboratory Animal Center, Khon Kaen University, based on the Ethics of Animal Experimentation of Nation Research Council of Thailand (recorded code number: IACUC-KKU 14/66).

2.2. Assessments of chronic stress

Three tests (sucrose preference, tail suspension, and forced swimming tests were used to estimate chronic stress behaviors in mice involved with DexCS. Each test is briefly described as following sections.

2.2.1. Sucrose preference test

The sucrose preference test (SPT) was used to evaluate the anhedonia caused from chronic stress in mice induced with DEX. It is defined as a reduction in sucrose preference ratio (Liu et al., 2018). Mice were presented to two identical bottles containing distilled water and 1% sucrose solution for every day. The total sucrose intake was determined as the total amount of solution that was consumed as described by Wu et al. (2021).

2.2.2. Tail suspension test

The tail suspension test (TST) was used to estimate the behavioral despair or hopelessness (Wu et al., 2021). Each mouse of both groups was put upside-down by fixing the tail. Within 6 min of a testing period, frequency of mobility and immobility was recorded using video camera.

2.2.3. Forced swimming test

Behavioral despair from stress mice was also assessed by using forced swimming test (FST) as previously described (Wu et al., 2021; Yankelevitch-Yahav et al., 2015). Animal was placed in the vertical transparent cylinder (30 cm in height and 12 cm in diameter) containing tap water (25 °C ± 1 °C and 20 cm in depth). Within 6 min of testing,

the immobility and mobility were recorded with a video camera. The results of the FST were expressed as the number of immobility times (second) within the last 4 min (Wu et al., 2021).

2.3. Organ collections

At the end of experiment, all mice were anesthetized by thiopental sodium at a dose of 80 mg/kg BW via intraperitoneal injection before euthanized by cervical dislocation. The testis, epididymis plus vas deferens, penis, seminal vesicle plus prostate gland, and other vital organs (liver and kidney) were collected and weighed. The weights of body, reproductive, and vital organs were calculated and expressed as the relative weights (100 x absolute organ weight divided by final body weight). The penis, seminal vesicle plus prostate gland, and right reproductive organs (testis, epididymis plus vas deferens) were fixed with 10% formalin fixative solution for further histological study. The left testis of each mouse was kept at -80 °C to the further investigation of apoptotic protein expressions.

2.4. Sperm analyses

The left caudal epididymis plus deferens were removed to collect sperm for quality evaluations including motility, concentration, morphology, viability, and acrosome status. Briefly, the luminal sperm masses from vas deferens and epididymis were squeezed and dipped into normal saline (37 °C). Then, the sperm suspension was aliquoted to analyze each sperm parameter as followings.

2.4.1. Sperm motility, concentrations of sperm and sperm abnormality

The sperm qualities consisting of motility and the concentrations of sperm and abnormality were performed and analyzed by using computer-assisted sperm analysis (CASA) (IVOS® II, Hamilton Thorne Inc., USA). To allow the temperature to stabilize, the loaded chamber (20 µm in depth) was placed on the thermal plate of the microscope (37 °C) for 2 min and warmed other slides on heating plate (37 °C) to wait to continue using before analysis. The sperm suspension (6 µl) was loaded into two-chamber slides (Leja Products B.V., Nieuw-Vennep, Netherlands). Sperm movements and non-movements were observed under negative phase contrast and recorded as videos for six fields per chamber. Finally, the sperm motility (progressive, slow, and static motility), total sperm concentration, and sperm abnormality concentration (sperm bent tail, coiled tail, distal droplet, distal midpiece reflex, and proximal droplet) were analyzed and reported as the million/ml.

2.4.2. Sperm viability

The sperm viability was evaluated by using sperm viability assay kit (1:1 ratio, eosin-nigrosin method, Baso diagnostics, Inc, Zhuhai) as previously described (Tangsrisakda et al., 2022). Sperm suspension (40 µl) was mixed with eosin-nigrosin dyes (40 µl) and incubated at 37 °C for 30 sec. Then, the stained sperm drop was smeared on glass slide to observe viability status of sperm under a light microscope: alive sperm (non-stained) and dead sperm (pink). Total 200 sperms from each mouse were counted and calculated to express as percentage of sperm viability.

2.4.3. Precocious acrosome reaction status

The 20 µl of sperm suspension of was fixed with 4% paraformaldehyde. and smeared on glass slides before drying overnight. Then smeared sperm were stained with 0.22% Coomassie Brilliant Blue G 250 (Bio-Rad Laboratories, Inc.) for 5 min and washed with distilled water (DW) before observation of acrosome status under light microscope (40x magnification). Two hundred sperms in total were counted to identify the sperm acrosome cap: positive stained with Coomassie blue acrosome-intact (AI) sperm while sperm head with no staining determined as acrosome-reacted (AR) sperm. The AR sperm percentage was calculated aspreviously described Chaimontri et al. (2021).

2.5. Histological analysis

The right the testis, cauda epididymis, and penis were fixed with 10% formalin in PBS (pH, 7.4) for 48 h before paraffin tissue processing. The paraffinised tissue blocks were sectioned (thickness, 7 µm) using the automatic microtome (ERM 3100, Hestion, Australia). The sections were deparaffinised, rehydrated, hydrated, and rountinely stained with haematoxylin and eosin dyes. All histophatologies were observed under light microscope (ZEISS Axio Imager.A2, Germany) and captured by using AxioCam ICc 5 digital camera (ZEISS, Germany) for represent pictures.

2.6. Protein extraction and western blot analysis

The testicular total proteins were extracted by homogenization using the radioimmuno-precipitation assay (RIPA) buffer (Cell Signaling Technology, Inc., USA) containing protease inhibitor cocktails (EDTA-Free,100X in DMSO, MedChemExpress (MCE), USA). The testicular lysate was then sonicated (20 Hz, 60 pulses) with the ultrasonic processor (Cole, Parmer, Vernon Hills, Illinois, USA). The homogenate was centrifuged to separate soluble total proteins from its pellet at 13,000 rpm, 4 ˚C for 10 min. Total protein concentration was measured at an absorbance of 280 nm by using a spectrophotometer (NanoDrop ND 1000 Technologies, Inc., USA). Then, total testicular proteins (150 µg) were separated on 10% SDS PAGE (sodium dodecyl-sulphate on a 10% polyacrylamide gel electrophoresis) before blotting onto nitrocellulose membranes (Cat. No. 10600004, GE Healthcare Life science, USA). Briefly, the non-specific proteins on membrane were blocked with 5% bovine serum albumin (BSA) in 0.1% TBST (for GAPDH, caspase 3, and caspase 9 antibodies) and 5% skim milk in 0.1% TBST (for Hsp70 antibody) for overnight. Subsequently, the membrane was washed and incubated with mouse anti-Hsp70 (1: 5000, Cat. No. MAB3516, Merck Millipore Co., USA), mouse anti-caspase-3 (1: 1000, Cat. No. sc-7272, Santa Cruz, USA), or mouse anti-caspase-9 (1:1000, Cat. No. sc-56076, Santa Cruz, USA) at 4 °C for overnight. Then, each membrane was incubated with a specific secondary antibody conjugated with horseradish peroxidase (HRP). The equal amount of total proteins was confirmed by glyceraldehyde-3-phosphate

dehydrogenase (GAPDH) expression intensity. Finally, the enhanced chemiluminescence (ECL) substrate kit (GE Healthcare Life science, USA) was used to detect specific Ab-Ag complexes on membrane visualized under Gel Documentation 4 (ImageQuant 600 GE Healthcare, USA).

2.7. Statistical analysis

All data were expressed as mean ± standard deviation (SD). The significant difference between DexCS and control groups was analysed by using independent t test. The p value was < 0.05 considered as a statistically significant difference. Statistical analysis was performed by using GraphPad Prism (Statistical Package for the Social Sciences, version 9.5.1, GraphPad Software Inc., San Diego, CA, USA).

3. Results

3.1. Effect of DexCS on vital and reproductive organ weights

The results in Figure 1 have confirmed that the mice induced with dexamethasone (DEX) have chronic stress condition revealed by significant increases of immobility and decrease of total sucrose intake (Table 1). Significantly, DEX induction decreased the final body but increased relative weight of epididymis plus vas deferens as compared to control mice. However, the weights of testis, penis, seminal vesicle plus prostate gland, liver, and kidney have no significant difference between control and DexCS groups (Table 1 and Figure 1).

3.2. Effect of DexCS on morphology of male reproductive organs

The morphology of testis, epididymis plus vas deferens, and penis of control and DexCS groups shown in Figure 2 was not obviously changed. However, the structure of seminal vesicle plus prostate gland of DexCS mice seemed to be distorted and smaller than control group (Figure 2).

3.3. Effect of DexCS on sperm quality and sperm motility patterns

Mice induced with DEX had significant decrease of the total sperm concentration, sperm viability, acrosome reaction, and sperm morphology of distal midpiece reflex concentration (Table 2 and Figure 3). In contrast, all sperm morphology parameters such as bent tail, coiled tail, distal droplet, and proximal droplet concentrations in DexCS mice did not affect (Table 2).

For sperm motility patterns revealed by CASA (Figure 4A-E), DexCS decreased the motility and progressive

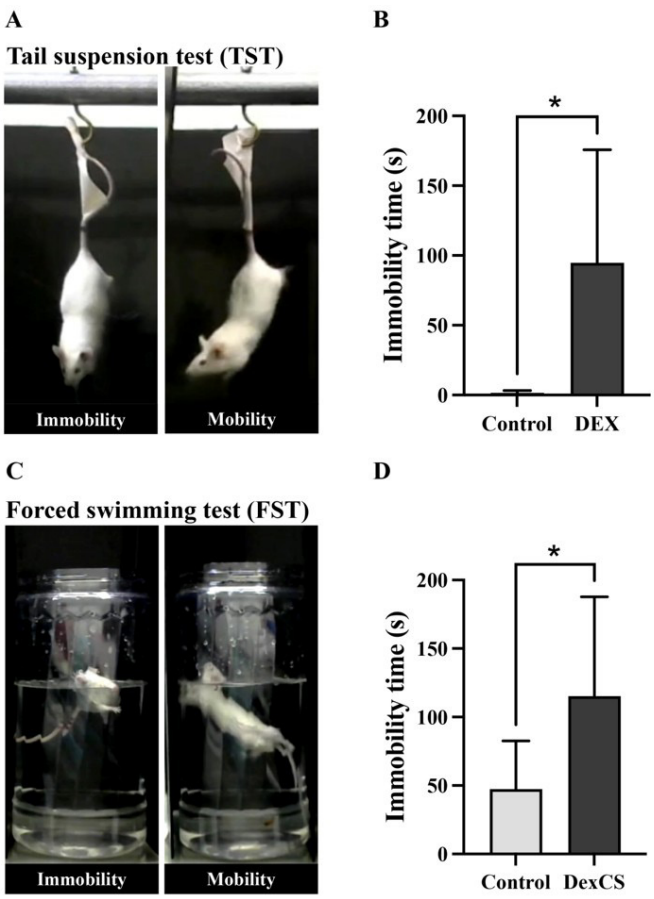

Figure 1. Showing (A) the tail suspension test (TST) with its immobility times (B) and (C) the forced swimming test (FST) with its immobility times (D) compared between control and DexCS groups. Significant difference (*P < 0.05) compared between groups.

Table 1. Comparisons of weights of body, vital organs, and reproductive organs weights between control and DexCS mice after 21 consecutive days of induction.

Parameters	Groups	
	Control	DexCS
Final body weight (g)	47.07 ± 1.69	41.34 ± 0.97*
Total sucrose intake/animal (ml)	170.00	140.00
Testis		
Absolute weight (g)	0.1820 ± 0.0380	0.1708 ± 0.0178
Relative weight (g/100 g)	0.3702 ± 0.0783	0.4103 ± 0.0427
Epididymis plus Vas deferens		
Absolute weight (g)	0.0821 ± 0.0036	0.0774 ± 0.0020
Relative weight (g/100 g)	0.1672 ± 0.0073	0.1859 ± 0.0048*
Penis		
Absolute weight (g)	0.0457 ± 0.0018	0.0435 ± 0.0021
Relative weight (g/100 g)	0.0931 ± 0.0037	0.1044 ± 0.0049
Seminal vesicle plus prostate gland		
Absolute weight (g)	0.5756 ± 0.0641	0.4717 ± 0.0650
Relative weight (g/100 g)	1.1723 ± 0.1305	1.1329 ± 0.1562
Kidney		
Absolute weight (g)	0.3451 ± 0.0266	0.3844 ± 0.1296
Relative weight (g/100 g)	0.7028 ± 0.0541	0.9233 ± 0.3113
Liver		
Absolute weight (g)	1.7940 ± 0.4277	2.0340 ± 0.2562
Relative weight (g/100 g)	3.6538 ± 0.8710	4.8856 ± 0.6155

Data represented as mean ± standard deviation (S.D.). DexCS = Dexamethasone induced-chronic stress. *Significant difference ($P < 0.05$) compared between groups.

Table 2. Comparisons of sperm parameters between control and DexCS treated mice after 21 days of chronic stress study.

Sperm parameters	Groups	
	Control	DexCS
Total sperm concentration (million/ml)	20.69 ± 3.15	10.23 ± 2.98*
Sperm viability (%)	95.25 ± 3.54	69.83 ± 14.57*
Acrosome reaction (%)	8.00 ± 1.41	45.00 ± 1.73*
Concentration of sperm abnormality		
Bent tail concentration (million/ml)	0.58 ± 0.23	0.70 ± 0.44
Coiled tail concentration (million/ml)	0.27 ± 0.16	0.29 ± 0.17
Distal droplet concentration (million/ml)	0.64 ± 0.45	0.38 ± 0.26
Distal midpiece reflex concentration (million/ml)	0.40 ± 0.26	0.23 ± 0.12*
Proximal droplet concentration (million/ml)	4.62 ± 2.56	3.57 ± 1.91

Data represented as mean ± standard deviation (S.D.). DexCS = Dexamethasone induced-chronic stress. *Significant difference ($P < 0.05$), compared between groups.

concentrations compared to control group. In addition, no significant difference in slow and static concentrations was found in DexCS animals as compared to those of control.

3.4. Effect of DexCS on histology of mice testis, cauda epididymis, and penis

The histology of mouse testis, cauda epididymis, and penis of control and DexCS groups was shown in Figure 5. As compared to control, the testicular damages including germ cell disorganization and interstitial space width were found in DexCS group. In addition, the sperm mass within the lumens of some cauda epididymis was decreased in DexCS group as compared to control group (Figure 5). In contrary, no histopathology of penis was found in DexCS mice when compared to control mice.

3.5 Effect of DexCS on expressions of Hsp70, caspase 3, and caspase 9 proteins in mouse testis

The results showed that the apoptotic protein expressions of caspase 3 and caspase 9 in the testicular lysate of DexCS group seemed to be increased as compared to control group (Figure 6A). In the same way, the relative intensities of both caspases 3 and 9 were significantly increased in DexCS group as compared to control group ($P < 0.05$, Figure 6B). In contrast, Hsp70 expression and its relative intensity in DexCS group have no difference as compared to those of the control group (Figure 6).

4. Discussion

This study demonstrated that the mice induced with DEX showed chronic stress conditions as confirmed by depressive-like behavior tests similar to previous reports (Wu et al., 2021; Delanogare et al., 2020; Skupio et al., 2015). The weight loss in DexCS mice may result from excess exogenous glucocorticoids (DEX), which play a role in suppression of appetite leading to anhedonia (Kuckuck et al., 2023). Recent study reported the increase in relative weight of epididymis plus vas deferens in DexCS mice for the first time. It could be explained that the ratio of low body versus organ weights was extremely lower as compared to that of control group. Previous studies have

Figure 2. Representative photographs showing the morphology of mouse reproductive organs compared between control and DexCS groups.

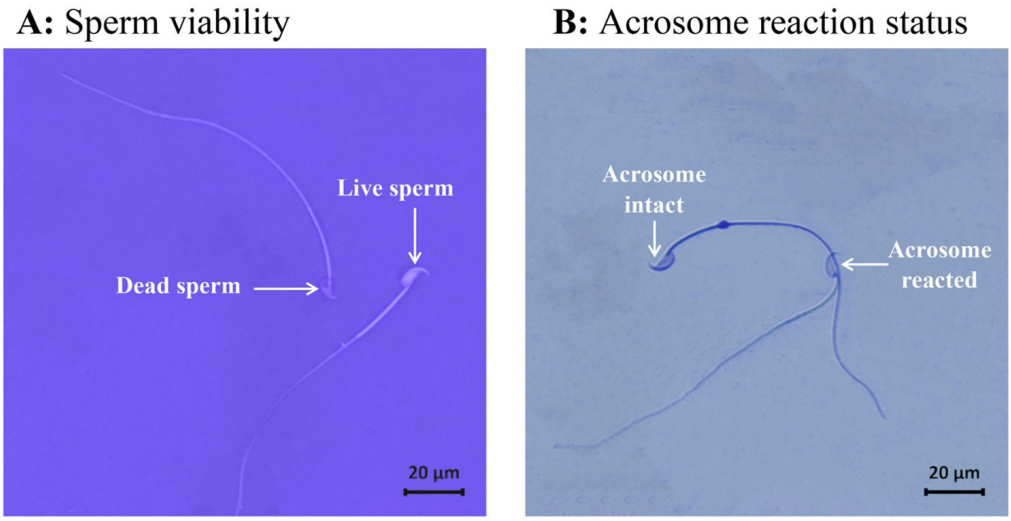

Figure 3. Photographs showing sperm viability revealed by eosin and nigrosin staining (A), acrosome status stained by Coomassie Blue (B).

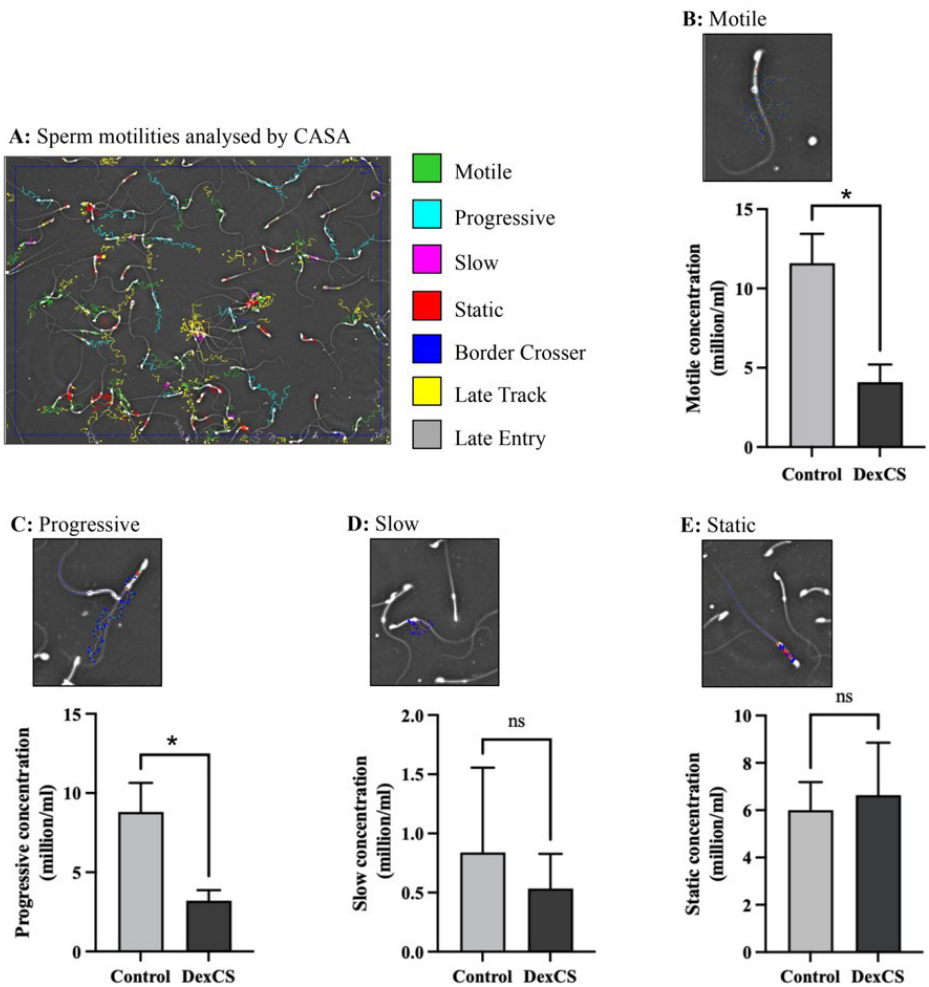

Figure 4. Photographs showing sperm motilities analyzed by computer assisted sperm analysis (CASA) (A), Motile (B), progressive (C), slow (D), and static (E) compared between control and DexCS groups. ns: no significant difference. Significant difference (*P < 0.05) compared between groups.

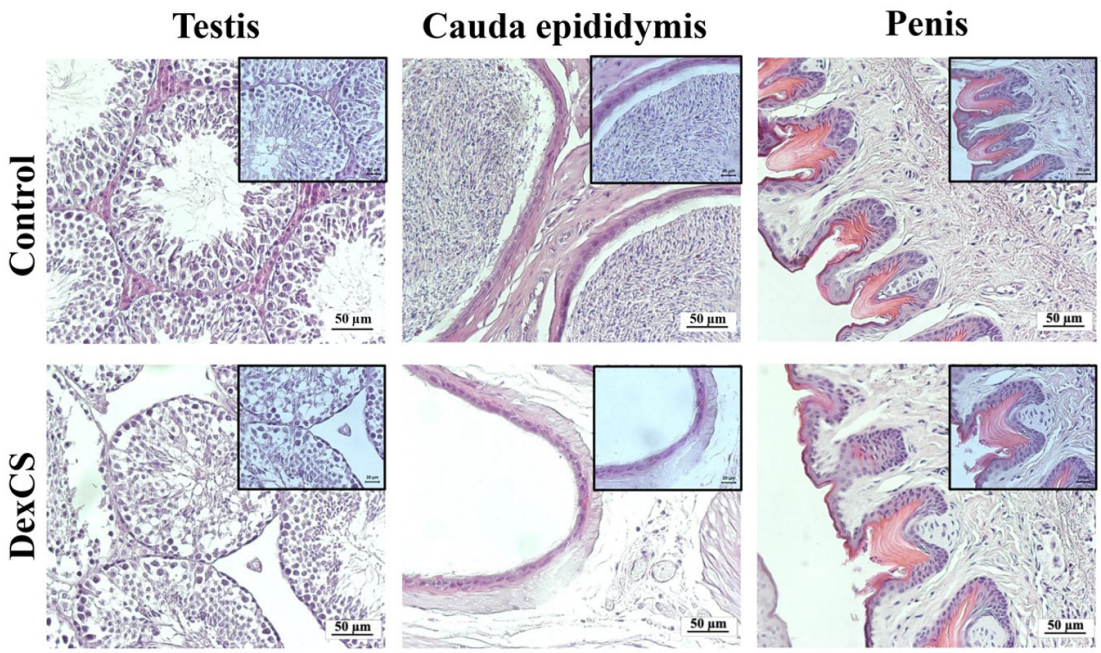

Figure 5. Representative photomicrographs showing the histology of testis, cauda epididymis, and penis of control and DexCS groups.

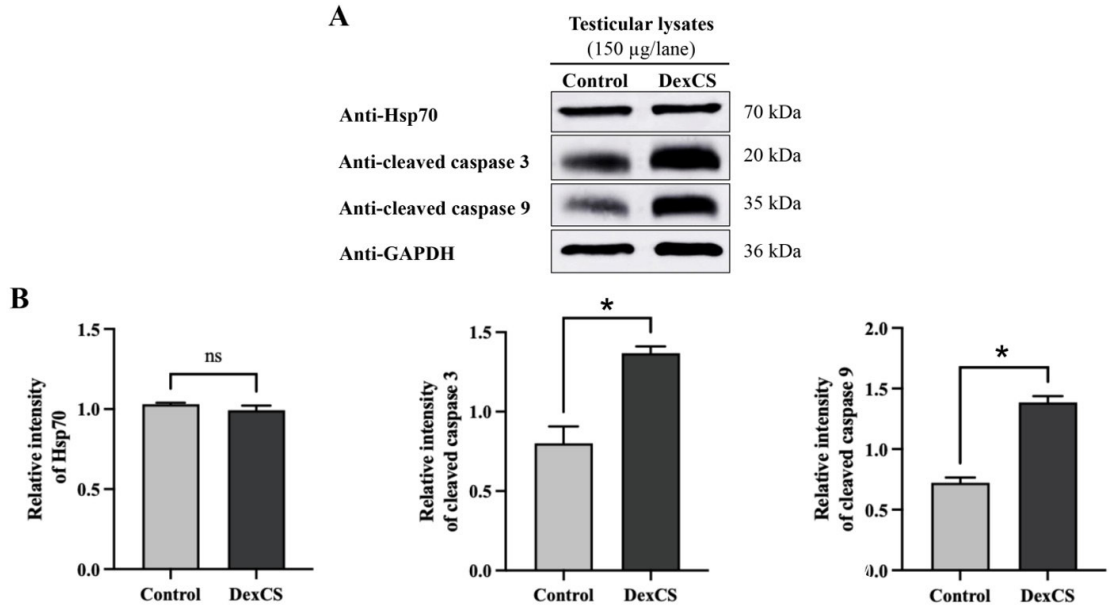

Figure 6. Immuno-Western blot analysis of heat shock protein 70 (Hsp70), caspase 3, and caspase 9 present in testicular lysate (A) and its relative intensities (B) compared between control and DexCS groups. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) used as an internal control. ns: no significant difference. Significant difference (*P < 0.05) compared between groups.

revealed that physical stressors also used to induce CS in male rats could decrease spermatogenesis and epididymal sperm qualities (Lapyuneyong et al., 2022; Meng et al., 2022; Arun et al., 2018). In male animal models, treatments with DEX for 7, 14, 28, and 42 days could significantly decrease sperm qualities including count, viability, motility, and normal morphology (Azimi Zangabad et al., 2023; Liu et al., 2023; Soleimani Mehranjani et al., 2022; Khalil-Khalili et al., 2021; Nassan et al., 2021; Ozer Kaya et al., 2020; Sadeghzadeh et al., 2019; Ing et al., 2015). Recent study has demonstrated the effects of CS induced by DEX on the reproductive system in male mice for the first time. Similar to a work from Ing et al. (2015) observing in adult stallion semen using CASA, significant decreases of total sperm number, motility, progressive motility, and normal morphology were also investigated in our study that sperm were retrieved from mouse epididymis. Moreover, we showed the increase of precocious acrosome reaction in DexCS sperm, indicating disability of sperm to penetrate zona pellucida before fertilizing the egg (Leung et al., 2023). All sperm impairments from DEX effect may be associated with the increased malondialdehyde (MDA) level as previously described (Khalil-Khalili et al., 2021; Nassan et al., 2021; Sadeghzadeh et al., 2019). In our study, DEX induced the testicular histopathologies which were consistent with previous studies showing effects of DEX on seminiferous epithelium (germ cells and Sertoli cells) and Leydig cells, leading to testicular apoptosis and decreased testosterone levels (Aziz et al., 2024; Soleimani Mehranjani et al., 2022; Sadeghzadeh et al., 2019; Khorsandi et al., 2013; Orazizadeh et al., 2010; Weber et al., 2000). For such reason, reduced production of testicular sperm caused the decrease of epididymal sperm numbers, consistent with a previous finding observed in unpredictable chronic stress (Choowong-In et al., 2021). Although GR and its functions via 11-hydroxysteroid dehydrogenase enzymes (11-HSD1 and 11-HSD2) have been found in penile erection of rats (Waddell et al., 2003; Penson et al., 1997), histopathology of DexCS penis was not obviously observed in our study. It is possible that the sensitivity of penile GR response is lesser than that of testis and cauda epididymis. Previous studies have reported that the apoptotic protein expressions such as caspase 3, caspase 9, and Hsp70 were corroborated with positive TUNEL staining in the stress testis and seminal vesicle induced by physical stressors (Lapyuneyong et al., 2022; Meng et al., 2022; Choowong-In et al., 2021; Fozooni et al., 2021; Iamsaard et al., 2021; Shen et al., 2020; Demirci and Sahin, 2019; Kheirabad et al., 2016). Additionally, several DEX doses have been shown to increase such apoptotic markers in both liver and testicular tissues (Nassan et al., 2021; Sadeghzadeh et al., 2019; Khosravanian et al., 2015; Khorsandi et al., 2013; Tiao et al., 2011; Orazizadeh et al., 2010). Indeed, our study showed the increase of caspase 3 and 9 expressions in the stress mouse testis induced with DEX for the first time, indicating that DexCS model can be also used as a potential chronic stress model which its pathophysiology is similar to those of physical stressor induction as previously reported (Lapyuneyong et al., 2022; Choowong-In et al., 2021; Nassan et al., 2021; Iamsaard et al., 2021; Khosravanian et al., 2015).

5. Conclusion

This study has demonstrated that dexamethasone (DEX) is a potential drug to be used for chronic stress induction, which its male reproductive damages on testis, epididymis, and sperm qualities were similar to those in physical stressor induction. These include changes of sperm motility patterns and testicular apoptosis particularly increasing of caspase 3 and 9 expressions.

5.1. Future prospects

Dexamethasone can be used to induce chronic stress status in mouse model to impair male reproductive system. Especially, it induced testicular histopathology and apoptosis involved in low sperm concentration and progressive motility.

Acknowledgements

Chadaporn Chaimontri and Therachon Kamollerd were supported by a Postgraduate Study Support Grant of Faculty of Medicine, Khon Kaen University. This research work was also financially supported by Invitation Research (Grant Number IN67006), Faculty of Medicine, Khon Kaen University, Thailand.

References

ARUN, S., BURAWAT, J., SUKHORUM, W., SAMPANNANG, A., UABUNDIT, N. and IAMSAARD, S., 2016. Changes of testicular phosphorylated proteins in response to restraint stress in male rats. *Journal of Zhejiang University. Science. B.*, vol. 17, no. 1, pp. 21-29. http://doi.org/10.1631/jzus.B1500174. PMid:26739523.

ARUN, S., BURAWAT, J., YANNASITHINON, S., SUKHORUM, W., LIMPONGSA, A. and IAMSAARD, S., 2018. *Phyllanthus emblica* leaf extract ameliorates testicular damage in rats with chronic stress. *Journal of Zhejiang University. Science. B.*, vol. 19, no. 12, pp. 948-959. http://doi.org/10.1631/jzus.B1800362. PMid:30507078.

ARUN, S., CHAIYAMOON, A., LAPYUNEYONG, N., BUNSUEB, S., WU, A.T. and IAMSAARD, S., 2021. Chronic stress affects tyrosine phosphorylated protein expression and secretion of male rat epididymis. *Andrologia*, vol. 53, no. 3, e13981. http://doi.org/10.1111/and.13981. PMid:33469986.

AZIMI ZANGABAD, E., SHOMALI, T. and ROSHANGAR, L., 2023. Effects of pharmacological doses of niacin on subacute glucocorticoid-induced testicular damage in rats. *Pharmacology Research & Perspectives*, vol. 11, no. 5, e01128. http://doi.org/10.1002/prp2.1128. PMid:37589322.

AZIZ, R.L.A., ABDEL-WAHAB, A., ABDEL-RAZIK, A.H., KAMEL, S., FARGHALI, A.A., SALEH, R., MAHMOUD, R., IBRAHIM, M.A., NABIL, T.M. and EL-ELA, F.I.A., 2024. Physiological roles of propolis and red ginseng nanoplatforms in alleviating dexamethasone-induced male reproductive challenges in a rat model. *Molecular Biology Reports*, vol. 51, no. 1, pp. 72. http://doi.org/10.1007/s11033-023-08991-4. PMid:38175282.

BHONGADE, M.B., PRASAD, S., JILOHA, R.C., RAY, P.C., MOHAPATRA, S. and KONER, B.C., 2015. Effect of psychological stress on fertility hormones and seminal quality in male partners of infertile couples. *Andrologia*, vol. 47, no. 3, pp. 336-342. http://doi.org/10.1111/and.12268. PMid:24673246.

CARVALHO, J. and NOBRE, P., 2011. Biopsychosocial determinants of men's sexual desire: testing an integrative model. *Journal of Sexual Medicine*, vol. 8, no. 3, pp. 754-763. http://doi.org/10.1111/j.1743-6109.2010.02156.x. PMid:21176114.

CHAIMONTRI, C., ARUN, S., SAWATPANICH, T., YANNASITHINON, S., TANGSRISAKDA, N., BUNSUEB, S., WU, A.T. and IAMSAARD, S., 2021. The effect of *Dolichandrone serrulata* (wall. ex DC.) Seem. flower extract containing antioxidant capacity and terpenoids on the male reproductive system. *Andrologia*, vol. 53, no. 3, e13966. http://doi.org/10.1111/and.13966. PMid:33427326.

CHOOWONG-IN, P., SATTAYASAI, J., BOONCHOONG, P., POODENDAEN, C., WU, A.T., TANGSRISAKDA, N., SAWATPANICH, T., ARUN, S., UABUNDIT, N. and IAMSAARD, S., 2021. Protective effects of Thai *Mucuna pruriens* (L.) DC. var. pruriens seeds on sexual behaviors and essential reproductive markers in chronic unpredictable mild stress mice. *Journal of Traditional and Complementary Medicine*, vol. 12, no. 4, pp. 402-413. http://doi.org/10.1016/j.jtcme.2021.12.001. PMid:35747354.

DADOMO, H., PONZI, D., NICOLINI, Y., VIGNALI, A., ABLONDI, F., CERESINI, G., MAGGIO, M., PALANZA, P., GOVONI, P., VOLPI, R. and PARMIGIANI, S., 2020. Behavioral and hormonal effects of prolonged Sildenafil treatment in a mouse model of chronic social stress. *Behavioural Brain Research*, vol. 392, pp. 112707. http://doi.org/10.1016/j.bbr.2020.112707. PMid:32461132.

DALLAK, M., 2019. Unacylated ghrelin stimulates steroidogenesis in lean rats and reverses reproductive dysfunction in high fat diet-fed rats. *Systems Biology in Reproductive Medicine*, vol. 65, no. 2, pp. 129-146. http://doi.org/10.1080/19396368.2018.1523971. PMid:30265152.

DELANOGARE, E., SOUZA, R.M., ROSA, G.K., GUANABARA, F.G., RAFACHO, A. and MOREIRA, E.L.G., 2020. Enriched environment ameliorates dexamethasone effects on emotional reactivity and metabolic parameters in mice. *Stress*, vol. 23, no. 4, pp. 466-473. http://doi.org/10.1080/10253890.2020.1735344. PMid:32107952.

DEMIRCI, T. and SAHIN, E., 2019. The effect of chronic stress and obesity on sperm quality and testis histology in male rats; a morphometric and immunohistochemical study. *Histology and Histopathology*, vol. 34, no. 3, pp. 287-302. http://doi.org/10.14670/HH-18-077. PMid:30566207.

DIAO, R., GAN, H., TIAN, F., CAI, X., ZHEN, W., SONG, X. and DUAN, Y.G., 2019. In vitro antioxidation effect of Quercetin on sperm function from the infertile patients with leukocytospermia. *American Journal of Reproductive Immunology*, vol. 82, no. 3, e13155. http://doi.org/10.1111/aji.13155. PMid:31166052.

DÍAZ-DEL CERRO, E., FÉLIX, J., TRESGUERRES, J. and DE LA FUENTE, M., 2022. Improvement of several stress response and sleep quality hormones in men and women after sleeping in a bed that protects against electromagnetic fields. *Environmental Health*, vol. 21, no. 1, pp. 72. http://doi.org/10.1186/s12940-022-00882-8. PMid:35864547.

EVERSON-ROSE, S.A., ROETKER, N.S., LUTSEY, P.L., KERSHAW, K.N., LONGSTRETH JUNIOR, W.T., SACCO, R.L., DIEZ ROUX, A.V. and ALONSO, A., 2014. Chronic stress, depressive symptoms, anger, hostility, and risk of stroke and transient ischemic attack in the multi-ethnic study of atherosclerosis. *Stroke*, vol. 45, no. 8, pp. 2318-2323. http://doi.org/10.1161/STROKEAHA.114.004815. PMid:25013018.

FOZOONI, R., JAFARZADEH SHIRAZI, M.R., SAEDI, S., NAMAVAR JAHROMI, B., KHORADMEHR, A., ANVARI, M., RAHMANIFAR, F., KHODABANDEH, Z. and TAMADON, A., 2021. Male subfertility effects of sub-chronic ethanol exposure during stress in a rat model. *Alcohol (Fayetteville, N.Y.)*, vol. 96, pp. 63-71. http://doi.org/10.1016/j.alcohol.2021.08.003. PMid:34461247.

HEZAVEHEI, M., MIRZAEI, M., SHARAFI, M., WU, Y., GUPTA, V., FITZHENRY, M., KOUCHESFAHANI, H.M., EFTEKHARI-YAZDI, P., BAHARVAND, H., DALMAN, A., HAYNES, P.A., SHAHVERDI, A. and SALEKDEH, G.H., 2022. Proteomics study reveals the molecular mechanisms underlying cryotolerance induced by mild sublethal stress in human sperm. *Cell and Tissue Research*, vol. 387, no. 1, pp. 143-157. http://doi.org/10.1007/s00441-021-03537-1. PMid:34729646.

IAMSAARD, S., TONGPAN, S., YANNASITHINON, S., ARUN, S., WU, A.T.H. and SUKHORUM, W., 2021. Effect of chronic stress on expression and secretion of seminal vesicle proteins in adult rats. *Andrologia*, vol. 53, no. 1, e13800. http://doi.org/10.1111/and.13800. PMid:32816406.

ING, N.H., BRINSKO, S.P., CURLEY JUNIOR, K.O., FORREST, D.W., LOVE, C.C., HINRICHS, K., VOGELSANG, M.M., VARNER, D.D. and WELSH JUNIOR, T.H., 2015. Dexamethasone acutely regulates endocrine parameters in stallions and subsequently affects gene expression in testicular germ cells. *Animal Reproduction Science*, vol. 152, pp. 47-54. http://doi.org/10.1016/j.anireprosci.2014.11.007. PMid:25487569.

JANEVIC, T., KAHN, L.G., LANDSBERGIS, P., CIRILLO, P.M., COHN, B.A., LIU, X. and FACTOR-LITVAK, P., 2014. Effects of work and life stress on semen quality. *Fertility and Sterility*, vol. 102, no. 2, pp. 530-538. http://doi.org/10.1016/j.fertnstert.2014.04.021. PMid:24856463.

KADIR, R.E., IBRAHIM, A., IBRAHIM, B.A., GWADABE, S.M., JAJI-SULAIMON, R., ADIGUN, M.F. and OYEWOPO, A.O., 2020. Low dose bitter leaf improves sperm quality disrupted in immunosuppressed Wistar rats: an experimental study. *International Journal of Reproductive Biomedicine*, vol. 18, no. 3, pp. 215-226. http://doi.org/10.18502/ijrm.v18i3.6720. PMid:32309771.

KHALIL-KHALILI, A.A., ZHANDI, M., ZAGHARI, M., MEHRABANI-YEGANEH, H., YOUSEFI, A.R. and TAVAKOLI-ALAMOOTI, M., 2021. The effect of dietary organic selenium on reproductive performance of broiler breeder roosters under dexamethasone induced stress. *Theriogenology*, vol. 161, pp. 16-25. http://doi.org/10.1016/j.theriogenology.2020.11.016. PMid:33278691.

KHEIRABAD, M.K., KHODABANDEH, Z., RAHMANIFAR, F., TAMADON, A., JAHROMI, B.N., OWJFARD, M. and KOOHI-HOSSEINABADI, O., 2016. Testicular germ cells apoptosis following exposure to chronic stress in rats. *Asian Pacific Journal of Reproduction*, vol. 5, no. 5, pp. 371-375. http://doi.org/10.1016/j.apjr.2016.07.005.

KHERADMAND, N., KAMKAR, R., MOSHAJJARI, M. and BAAZM, M., 2019. Effect of selenium and pentoxifylline on expression of CATSPER1 and 2 genes and FSH/LH levels in treated mice by dexamethasone. *Andrologia*, vol. 51, no. 6, e13279. http://doi.org/10.1111/and.13279. PMid:30983026.

KHORSANDI, L., MIRHOSEINI, M., MOHAMADPOUR, M., ORAZIZADEH, M. and KHAGHANI, S., 2013. Effect of curcumin on dexamethasone-induced testicular toxicity in mice. *Pharmaceutical Biology*, vol. 51, no. 2, pp. 206-212. http://doi.org/10.3109/13880209.2012.716854. PMid:23116244.

KHOSRAVANIAN, H., RAZI, M., FAROKHI, F. and KHOSRAVANIAN, N., 2015. Simultaneous administration of dexamethasone and vitamin E reversed experimental varicocele-induced impact in testicular tissue in rats; correlation with Hsp70-2 chaperone expression. *International Brazilian Journal of Urology*, vol. 41, no. 4, pp. 773-790. http://doi.org/10.1590/S1677-5538.IBJU.2013.0148. PMid:26401872.

KRISHNAN, K., 2018. Role of corticosteroids in oral and maxillofacial surgery. *Journal of Pharmaceutical Sciences and Research*, vol. 10, no. 1, pp. 208-210.

KUCKUCK, S., VAN DER VALK, E.S., SCHEURINK, A.J.W., VAN DER VOORN, B., IYER, A.M., VISSER, J.A., DELHANTY, P.J.D., VAN DEN BERG, S.A.A. and VAN ROSSUM, E.F.C., 2023. Glucocorticoids, stress and eating: the mediating role of appetite-regulating

hormones. *Obesity Reviews*, vol. 24, no. 3, e13539. http://doi.org/10.1111/obr.13539. PMid:36480471.

LAPYUNEYONG, N., TANGSRISAKDA, N., CHOOWONG-IN, P., CHAISIWAMONGKOL, K., UABUNDIT, N., SAWATPANICH, T., ARUN, S., WU, A.T. and IAMSAARD, S., 2022. Seed extract of Thai *Mucuna pruriens* reduced male reproductive damage in rats induced by chronic stress. *Pharmaceutical Biology*, vol. 60, no. 1, pp. 374-383. http://doi.org/10.1080/13880209.2022.2034896. PMid:35180038.

LEUNG, E.T.Y., LEE, B.K.M., LEE, C.L., TIAN, X., LAM, K.K.W., LI, R.H.W., NG, E.H.Y., YEUNG, W.S.B., OU, J.P. and CHIU, P.C.N., 2023. The role of spermatozoa-zona pellucida interaction in selecting fertilization-competent spermatozoa in humans. *Frontiers in Endocrinology*, vol. 14, pp. 1135973. http://doi.org/10.3389/fendo.2023.1135973. PMid:37020592.

LIANG, X., XIA, Z., YAN, J., WANG, Y., XUE, S. and ZHANG, X., 2016. Quercetin inhibits human sperm functions by reducing sperm [Ca^{2+}]i and tyrosine phosphorylation. *Pakistan Journal of Pharmaceutical Sciences*, vol. 29, no. 6, suppl., pp. 2391-2396. PMid:28167483.

LIU, M.Y., YIN, C.Y., ZHU, L.J., ZHU, X.H., XU, C., LUO, C.X., CHEN, H., ZHU, D.Y. and ZHOU, Q.G., 2018. Sucrose preference test for measurement of stress-induced anhedonia in mice. *Nature Protocols*, vol. 13, no. 7, pp. 1686-1698. http://doi.org/10.1038/s41596-018-0011-z. PMid:29988104.

LIU, Y., LIU, Y., CHEN, G. and WANG, H., 2023. Epigenetic programming of TBX2/CX43 mediates lower sperm quality in male offspring induced by prenatal dexamethasone exposure. *Toxicological Sciences*, vol. 192, no. 2, pp. 178-193. http://doi.org/10.1093/toxsci/kfad016. PMid:36790081.

LÓPEZ-GALINDO, L., JUÁREZ, O.E., LARIOS-SORIANO, E., DEL VECCHIO, G., VENTURA-LÓPEZ, C., LAGO-LESTÓN, A. and GALINDO-SÁNCHEZ, C., 2019. Transcriptomic analysis reveals insights on male infertility in *Octopus maya* under chronic thermal stress. *Frontiers in Physiology*, vol. 9, pp. 1920. http://doi.org/10.3389/fphys.2018.01920. PMid:30697164.

MADAMSETTY, V.S., MOHAMMADINEJAD, R., UZIELIENE, I., NABAVI, N., DEHSHAHRI, A., GARCÍA-COUCE, J., TAVAKOL, S., MOGHASSEMI, S., DADASHZADEH, A., MAKVANDI, P., PARDAKHTY, A., AGHAEI AFSHAR, A. and SEYFODDIN, A., 2022. Dexamethasone: insights into pharmacological aspects, therapeutic mechanisms, and delivery systems. *ACS Biomaterials Science & Engineering*, vol. 8, no. 5, pp. 1763-1790. http://doi.org/10.1021/acsbiomaterials.2c00026. PMid:35439408.

MALAN, N.T., STALDER, T., SCHLAICH, M.P., LAMBERT, G.W., HAMER, M., SCHUTTE, A.E., HUISMAN, H.W., SCHUTTE, R., SMITH, W., MELS, C.M., VAN ROOYEN, J.M. and MALAN, L., 2014. Chronic distress and acute vascular stress responses associated with ambulatory blood pressure in low-testosterone African men: the SABPA Study. *Journal of Human Hypertension*, vol. 28, no. 6, pp. 393-398. http://doi.org/10.1038/jhh.2013.124. PMid:24284381.

MENG, X., PENG, L., XU, J., GUO, D., CAO, W., XU, Y. and LI, S., 2022. Betaine attenuate chronic restraint stress-induced changes in testicular damage and oxidative stress in male mice. *Reproductive Biology and Endocrinology*, vol. 20, no. 1, pp. 80. http://doi.org/10.1186/s12958-022-00949-8. PMid:35597951.

MOHAMADPOUR, M., NOORAFSHAN, A., KARBALAY-DOUST, S., TALAEI-KHOZANI, T. and ALIABADI, E., 2017. Protective effects of curcumin co-treatment in rats with establishing chronic variable stress on testis and reproductive hormones. *International Journal of Reproductive Biomedicine*, vol. 15, no. 7, pp. 447-452. http://doi.org/10.29252/ijrm.15.7.447. PMid:29177247.

NASSAN, M.A., SOLIMAN, M.M., ALDHAHRANI, A., EL-SAWAY, H.B. and SWELUM, A.A., 2021. Ameliorative impacts of *Allium cepa Linnaeus* aqueous extract against testicular damage induced by dexamethasone. *Andrologia*, vol. 53, no. 4, e13955. http://doi.org/10.1111/and.13955. PMid:33682109.

ORAZIZADEH, M., KHORSANDI, L.S. and HASHEMITABAR, M., 2010. Toxic effects of dexamethasone on mouse testicular germ cells. *Andrologia*, vol. 42, no. 4, pp. 247-253. http://doi.org/10.1111/j.1439-0272.2009.00985.x. PMid:20629648.

OZER KAYA, S., ERISIR, M., GUR, S., KANDEMIR, F.M., BENZER, F., KAYA, E., SONMEZ, M. and TURK, G., 2020. The changes in semen quality, arginase activity and nitric oxide level in dexamethasone-treated rams. *Andrologia*, vol. 52, no. 2, e13464. http://doi.org/10.1111/and.13464. PMid:31721281.

PENSON, D.F., NG, C., RAJFER, J. and GONZALEZ-CADAVID, N.F., 1997. Adrenal control of erectile function and nitric oxide synthase in the rat penis. *Endocrinology*, vol. 138, no. 9, pp. 3925-3932. http://doi.org/10.1210/endo.138.9.5402. PMid:9275083.

POGGIOLI, R., UETA, C.B., DRIGO, R.A., CASTILLO, M., FONSECA, T.L. and BIANCO, A.C., 2013. Dexamethasone reduces energy expenditure and increases susceptibility to diet-induced obesity in mice. *Obesity*, vol. 21, no. 9, pp. E415-E420. http://doi.org/10.1002/oby.20338. PMid:23408649.

SADEGHZADEH, F., MEHRANJANI, M.S. and MAHMOODI, M., 2019. Vitamin C ameliorates the adverse effects of dexamethasone on sperm motility, testosterone level, and spermatogenesis indexes in mice. *Human and Experimental Toxicology*, vol. 38, no. 4, pp. 409-418. http://doi.org/10.1177/0960327118816137. PMid:30526067.

SÁNCHEZ GONZÁLEZ, S.R., MATA MARTÍNEZ, E., TORRES JUÁREZ, J.A., ARIAS, R.J., DE BLAS, G.A. and SÁNCHEZ TUSIE, A.A., 2023. Cortisol modulates Ca^{2+} signaling and acrosome reaction in human sperm. *Andrology*, vol. 11, no. 1, pp. 134-142. http://doi.org/10.1111/andr.13319. PMid:36251513.

SENGUPTA, P., BORGES JUNIOR , E., DUTTA, S. and KRAJEWSKA-KULAK, E., 2018. Decline in sperm count in European men during the past 50 years. *Human and Experimental Toxicology*, vol. 37, no. 3, pp. 247-255. http://doi.org/10.1177/0960327117703690. PMid:28413887.

SHEN, Y., HE, D., HE, L., BAI, Y., WANG, B., XUE, Y. and HOU, G., 2020. Chronic psychological stress, but not chronic pain stress, influences sexual motivation and induces testicular autophagy in male rats. *Frontiers in Psychology*, vol. 11, pp. 826. http://doi.org/10.3389/fpsyg.2020.00826. PMid:32425863.

SKUPIO, U., TERTIL, M., SIKORA, M., GOLDA, S., WAWRZCZAK-BARGIELA, A. and PRZEWLOCKI, R., 2015. Behavioral and molecular alterations in mice resulting from chronic treatment with dexamethasone: relevance to depression. *Neuroscience*, vol. 286, pp. 141-150. http://doi.org/10.1016/j.neuroscience.2014.11.035. PMid:25433240.

SOLEIMANI MEHRANJANI, M., AZIZI, M. and SADEGHZADEH, F., 2022. The effect of melatonin on testis histological changes and spermatogenesis indexes in mice following treatment with dexamethasone. *Drug and Chemical Toxicology*, vol. 45, no. 3, pp. 1140-1149. http://doi.org/10.1080/01480545.2020.1809672. PMid:33161762.

SOUZA, I.B.M.B., COSTA, L.R.F., TIAGO, P.R.F., CAGNI, F.C., LIMA, R.H., SILVA JUNIOR, E.D. and GAVIOLI, E.C., 2019. Venlafaxine and nortriptyline reverse acute dexamethasone-induced depressive-like behaviors in male and female mice. *Experimental and Clinical Psychopharmacology*, vol. 27, no. 5, pp. 433-442. http://doi.org/10.1037/pha0000263. PMid:30714753.

TANGSRISAKDA, N., KAMOLLERD, T., TAOTO, C., BUNSUEB, S., CHAIMONTRI, C., CHOOWONG-IN, P., LAPYUNEYONG, N., WU, A.T., THUKHAMMEE, W., WATTANATHORN, J., ARUN, S., SAWATPANICH, T. and IAMSAARD, S., 2022. Seed extract of Thai *Mucuna pruriens* (L.) DC. var. *pruriens* enhances sexual

performance and improves male reproductive damages in ethanol-induced rats. *Journal of Ethnopharmacology*, vol. 292, pp. 115219. http://doi.org/10.1016/j.jep.2022.115219. PMid:35339625.

TIAO, M.M., LIN, T.K., CHEN, J.B., LIOU, C.W., WANG, P.W., HUANG, C.C., CHOU, Y.M., HUANG, Y.H. and CHUANG, J.H., 2011. Dexamethasone decreases cholestatic liver injury via inhibition of intrinsic pathway with simultaneous enhancement of mitochondrial biogenesis. *Steroids*, vol. 76, no. 7, pp. 660-666. http://doi.org/10.1016/j.steroids.2011.03.002. PMid:21419148.

WADDELL, B.J., HISHEH, S., KROZOWSKI, Z.S. and BURTON, P.J., 2003. Localization of 11beta-hydroxysteroid dehydrogenase types 1 and 2 in the male reproductive tract. *Endocrinology*, vol. 144, no. 7, pp. 3101-3106. http://doi.org/10.1210/en.2003-0082. PMid:12810566.

WANDERLEY, M.I., SARAIVA, K.L., CÉSAR VIEIRA, J.S., PEIXOTO, C.A. and UDRISAR, D.P., 2013. Foetal exposure to Panax ginseng extract reverts the effects of prenatal dexamethasone in the synthesis of testosterone by Leydig cells of the adult rat. *International Journal of Experimental Pathology*, vol. 94, no. 3, pp. 230-240. http://doi.org/10.1111/iep.12026. PMid:23672767.

WANG, X., WANG, F., LU, Z., JIN, X. and ZHANG, Y., 2018. Semi-quantitative profiling of bile acids in serum and liver reveals the dosage-related effects of dexamethasone on bile acid metabolism in mice. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, vol. 1095, pp. 65-74. http://doi.org/10.1016/j.jchromb.2018.07.021. PMid:30055378.

WEBER, M., GROOS, S., HÖPFL, U., SPIELMANN, M., AUMÜLLER, G. and KONRAD, L., 2000. Glucocorticoid receptor distribution in rat testis during postnatal development and effects of dexamethasone on immature peritubular cells in vitro. *Andrologia*, vol. 32, no. 1, pp. 23-30. http://doi.org/10.1046/j.1439-0272.2000.00323.x.

WU, J., LI, J., GAURAV, C., MUHAMMAD, U., CHEN, Y., LI, X., CHEN, J. and WANG, Z., 2021. CUMS and dexamethasone induce depression-like phenotypes in mice by differentially altering gut microbiota and triggering macroglia activation. *General Psychiatry*, vol. 34, no. 6, e100529. http://doi.org/10.1136/gpsych-2021-100529. PMid:34970638.

YANKELEVITCH-YAHAV, R., FRANKO, M., HULY, A. and DORON, R., 2015. The forced swim test as a model of depressive-like behavior. *Journal of Visualized Experiments*, no. 97, pp. 52587. http://doi.org/10.3791/52587. PMid:25867960.