Tikrit Journal of Veterinary Sciences (2023) 1(2): 48 - 54

IRAQI Academic Scientific Journals

TJVS Tikrit Journal of Veterinary Sciences

Tikrit Journal of Veterinary Science

Hormonal and Histological effect of Nitric oxide on reproductive system of female mice

Najlaa A. Noori AL-bayati[1] Waseem Ali Hassan[1], Khalid Ahmed Hadi[1],

[1] *Dept. of Physiology, Pharmacology and Biochemistry, College of Veterinary medicine –Tikrit University, Tikrit , Iraq.*

[2] *Dept. of Basic Science ,College of Dentistry medicine , Tikrit University, Tikrit, Iraq*

ARTICLE INFO.

Article history:

-Received:

-Accepted:

-Available online:

Keywords:

Sodium nitroprusside, Follicles, hormones

Corresponding Author:

Name:

Najlaa A. Noori AL-bayati

E-mail:

najlaa_ali@tu.edu.iq

Tel:

ABSTRACT

The present study aimed to study the pharmacological effect of NO donor Sodium Nitroprusside on female reproductive system in mice through evaluate the levels of hormones (FSH , LH , Estrogen and progesterone) in blood serum and histology changes in ovaries tissue. The experiment was counducted on the Thirty female mice in good health, weighting ranged between (25-34) gm, were divided into six groups (five animals for each group) and treated by sodium nitroprusside (Intraperitoniolly) located in the animal house of veterinary medicine college/ Tikrit university were used in the current study. after 21 days of treatment by (SNP). The results of current study showed a significant increase ($P< 0.05$) in all treated groups, compared with control group, while it showed significant decrease ($P<0.05$) in progesterone hormone gradually with the increase in SPN dose in all groups compared with the control group. The histological evaluation results of average numbers of follicles per ovary in the treated groups, for determining the primordial follicles ,primary follicles and secondary follicles number showed a significant increased ($P<0.05$) in all treated groups when compared with control group , The G6 gradually decreased ($P< 0.05$) compared with other treated groups, The tertiary follicles number in all groups except the G2 showed a significant decrease ($P< 0.05$) compared with the control group, The results of ovaries histological changes of ovary showed cytoplasmic degeneration and vacuolation of germinal epithelium. also, certain Follicular cells were degenerated and surrounded by WBCs infiltration, Most of the primordial cells degenerated with the presence of inflammatory exudate between those cells and degeneration of oocyte of the tertiary follicle surrounded with many degenerated Follicular cells and infiltration of WBCs in the cortical tissue between the primordial follicle, the blood vessels of medulla were containing congested, hemolysis blood with increased of dosage, The study concluded the positive effect on the reproductive system in the female mice, but the effects appeared in the high doses of treatment.

Introduction

sodium Nitroprusside (SNP) is One of the safest and most effective drugs—, most efficient antihypertensive medications–, which result in blood NO mediated vascular blood pressure reduction, the vasodilation results from smooth muscles relaxation. SNP has long been utilized

to lessen hypertension risk symptoms, such as the Pulmonary hypertension, narrowing of aortic valve, myocardial infarction, and bleeding during operations[1], cyanide poisoning may be a problem. The earliest, most symptoms of cyanide toxicity in adults with the SNP treatment is acidosis, increased blood lactate levels are increasing along with mixed venous oxygen tension, and, symptoms of cyanide poisoning in CNS include seizures, headache, coma, lethargy, agitation, confusion and anxiety [2], NO is a gas that is soluble, unstable, and has a very short life (4 second), act as a powerful vasodilator, is released as part of the process of action, It is already apparent,that NO plays a crucial role in the reproduction, at all levels from the brain to the gonads and sex organs. This study reviews, the role of NO in the control of hypothalamic-pituitary function, and in the sexual behavior. It briefly describes its effects on the reproductive organs themselves[3], Sodium Nitroprusside (SNP), as an NO donor, could increase the NO concentration in cells [4], which is recognized, as a powerful vasodilator and Antigenic factor is crucial for ovarian angiogenesis during the development ovum and folliculogenesis. the evaluation of embryo developmental ability and oocyte quality, as well as prevention of atresia and apoptosis in follicles [5], Currently, a large amount of data shows that NO is related to ovulation, The NO levels increase with the development of Follicular, and this increase is associated with the Estrogen concentration. NO is speculated to be an important mediator of vascular changes and tissue remodeling during ovulation and luteinization[6], the current study were aimed to evaluate the effect of NO on serum hormones levels (FSH, LH, Estrogen and progesterone) and histological changed on the ovarian structures in female mice.

Materials and Methods

in the animal house of veterinary medicine college/ Tikrit university, Thirty albino female mice, weighing between (25 and 34 mg,) were housed in plastic cages and kept in the room until the experiment started. Commercial feed pellets made up the majority of the mice's diet, and water was always accessible. The temperature of the housing was kept at 28 2 °C, with a 24-hour light cycle ,Sodium Nitroprusside (SNP) was obtained from veterinary medicine college/ Baghdad university, and preparation of SNP solution by Dissolve 1 gm of SNP in 100 mL of distal water. 1ml of blood samples were collected directly from the heart from each animals and placed in plain tubes and centrifuged for 5 minutes at 3000 rpm for isolate the serum then put in eppendorf tubes and stored at -20 ^{o}C until analysis by special kits for each hormone using ELISA technique according to [7].

Study design

Thirty female mice were divided into 6 groups (5 animals per each group), IP daily dose

AII data denoted as (mean ± SE). One way analysis of variance (One-way ANOVA) and T-teat analysis by using SPSS, the significant differences among means of groups at the Level of statistical significant (P<0.05) [8, 9].

Results and Discussion

The results of current study showed significant increases (P<0 .05) in FSH, LH and Estrogen levels in all treated groups (G2, G3, G4, G5, G6), compared with control group (G1). but progesterone hormone showed significant decreases (P<0.05) in serum gradually with the increasing of SPN dose in all groups (G2, G3, G4, G5, G6) compared with control group (G1) (Table 1).

Table 1: Effect of SNP on serum hormones level in female mice (Mean ± SE)

Parameter / Groups	FSH (mlU/ml)	LH (mlU/ml)	Estrogen (Pg/ml)	Progesterone (µg/ml)
G1 (Control)	0 .35 ± 1 .913 d	0 .31 ± 0 .121 d	8 .4 ± 0 .761 c	46 .12 ± 2 .985 a
G2 0 .01 (mg/kg)	3 .12 ± 0 .721 c	2 .57 ± 0 .519 c	11 .32 ± 1 .125 b	37 .45±2 .509 b
G3 0 .02 (mg/kg)	6 .02 ± 0 .966 b	4 .03 ± 0 .570 b	14 .43 ± 1 .439 b	22 .67 ± 1 .864 c
G4 0 .03 (mg/kg)	7 .22 ± 0 .501 b	5 .16 ± 0 .598 b	13 .02 ± 1 .163 b	16 .64 ± 1 .767 d
G5 0 .04 (mg/kg)	10 .14 ± 0 .910 a	8 .41 ± 0 .716 a	16 .33 ± 2 .670 a	10 .37 ± 1 .970 e
G6 0 .05 (mg/kg)	8 .08 ± 0 .838 b	7 .32 ± 0 .820 ab	18 .40 ± 2 .620 a	7 .36 ± 0 .910 e

Small different letters vertically refer to presence of significant value at (P<0 .05) .

The present study showed decreased FSH and LH hormones in the group of take high dose, This result agreement with Canteros et al. (1996)[10] founded increasing amounts of SNP, the release of LHSH, such that the release increased with increasing concentrations of SNP up to a maximum at about 600 µM and then declined with higher concentrations, NO could increase the release of LH before ovulation , thus promoting the occurrence of a preovulatory LH peak, While LH acted on theca cells , promoting theca interna cell differentiation and androgen secretion, and stimulating follicle development, maturation and ovulation (McCosh et al., 2020) [11]. In mice, inhibin A produced by FSH - stimulate granulosa cells mediated the formation of the LH peak before ovulation (Watanobe and Schiöth 2001) [12, 13] stated that NO leads to activating guanylyl cyclase and generating c-GMP, and increases the release of FSH and LH, which in turn activates protein kinase C and causes an exocytosis of FSH and LH secretory granules. the results demonstrated a significantly higher LH level the results showed that G α q acts as a mediator for the detrimental effect of higher LH on Akt phosphorylation [14]. Our results showed increased in FSH and LH and Estrogen levels in serum, these results agree with many researchers, [15,16], the NO, known to be a potent vasodilator and antigenic factor, In addition, blood flow indexes in the early Follicular phase were negatively co-related with the number of follicles recruited and the number of oocytes retrieved, The results demonstrated a significant increase in estrogen hormone. This result agrees with that of [6] as an inhibitory effect of sodium nitroprusside was seen on steroid synthesis, specifically on the luteal cell cultures' production of estradiol . NO levels increased with Follicular development, and this increase was associated with Estrogen (E2) concentration. NO is associated with vascular remodeling.

The results of average numbers of follicles per ovary in the treated groups, for determining the primordial follicles number, showed a significant increase (P< 0.05) in all treated groups (G2, G3, G4, G5, G6) when compared with the control group (G1). and a significant increase (P<0.05) in primary folicles number in all groups (G2, G3, G4, G5, G6) compared with the control group (G1). The study showed a significant increase (P<0 .05) in secondary folicles number in all groups (G2, G3, G4, G5, G6) and the G6 gradually decreased (P<0 .05) compared with other treated groups. The tertiary folicles number in all groups. (G3, G4, G5, G6) except the G2 showed a significant decrease (P<0.05) compared with control group (G1), (Table 2).

Table 2: Effect of SNP on numbers of different types of Follicles in female mice (Mean ± SE).

Parameter / Groups	Primordial	Primary	Secondary	Tertiary
G1 (Control)	56 .8 ± 1 .913 e	5 .15 ± 0 .121 d	4 .20 ± 0 .761 d	4 .20 ± 2 .985 a
G2 0 .01 (mg/kg)	73 .3 ± 0 .721 c	6 .57 ± 0 .519 c	5 .32 ± 1 .125 c	4 .60 ± 2 .509 a
G3 0 .02 (mg/kg)	68 .5 ± 0 .966 d	7 .33 ± 0 .570 b	6 .82 ± 1 .439 ab	3 .00 ± 1 .864 c
G4 0 .03 (mg/kg)	86 .4 ± 0 .501 b	8 .46 ± 0 .598 a	7 .13 ± 1 .163 a	2 .80 ± 1 .767 c
G5 0 .04 (mg/kg)	94 .2 ± 0 .910 a	8 .24 ± 0 .716 a	6 .14 ± 2 .670 b	3 .65 ± 1 .970 b
G6 0 .05 (mg/kg)	75 .3 ± 0 .838 c	6 .65 ± 0 .820 c	6 .42 ± 2 .620 b	3 .40 ± 0 .910 b

Small different letters vertically refer to presence of significant value at (P<0 .05).

These results agree with those found by [17] It is possible that e-NOS expression in ovaries with fewer medium antral follicles is to blame for the resultant oocytes' diminished ability to mature. The results of this study agree with those found by [18] who observed that nuclear maturation was stimulated in a dose dependent manner when sodium nitroprusside (SNP), NO donor, was added during in vitro maturation, Sodium Nitroprusside has a short half-life, and co-injection of SNP with PMSG at the starting time of superovulation may increase the VEGFS expression in early follicle. VEGFS stimulates the number of primary and small secondary follicles and prenatal Follicular development . In particular, immunohistochemical staining showed that most graafian follicles were ovulated by human chorionic gonadotropin

(hCG) injection, and the remaining follicles were early immature follicles ,These results suggest that SNP treatment appears to enhance folliculogenesis rather than ovulatory capacity [19].

The results of histological examination for ovaries tissue, after 21 days from daily treatment for (control and treated groups with SNP) are as follows: There was a group of primordial cells surrounding each oocyte by a row of simple squamous cells, and delicate C.T. was present between those cells secondary follicle was also present (Fig. 1) and The cytoplasmic degeneration and vacuolation of germinal epithelium, as well as certain follicular cells were degenerated and surrounded by white blood cells (WBCs) infiltration, (Fig. 2), The medulla of ovary was infiltrated with great number of WBCs, The blood vessels of medulla were congested, hemolysis of blood (Fig. 3), Atretic follicle was present in the cortex of ovary, associated with degenerated, scattered follicular cells in small cavity of follicle, The follicle was surrounded by hypertrophic follicular cells of primordial (Fi. 4), Vacuolar degeneration was seen in certain luteal cells, with the presence of WBCs diffusion among luteal cells. The surface of cortical tissue had a germinal epithelium (Fig. 5), A degenerative change of germinal epithelium was seen on the surface of ovary, tertiary follicle contained spherical oocyte without it is nucleus (Fig. 6).

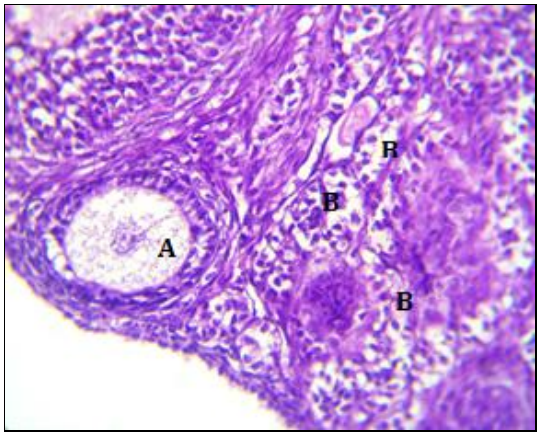

Fig. 1: (One group)(control) (A) Secondary follicle with oocyte,(B)Primordial cells(H&Ex40).

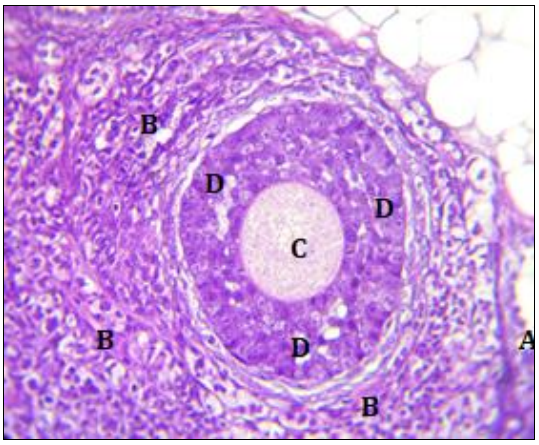

Fig. 2: (Two group) (A) Hyperplasia of germinal cells (B) Primordial Follicle, (C)Oocyte without nucleus, (D)Tertiary follicle, (H&Ex40)

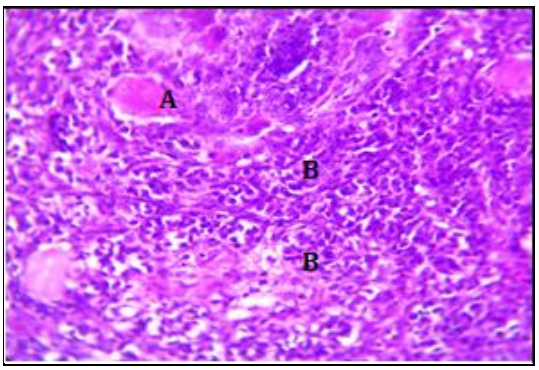

Fig. 3: (Three group)(A)Medulla of ovary, blood hemolysis, (B)WBCs infiltration (H& E x40).

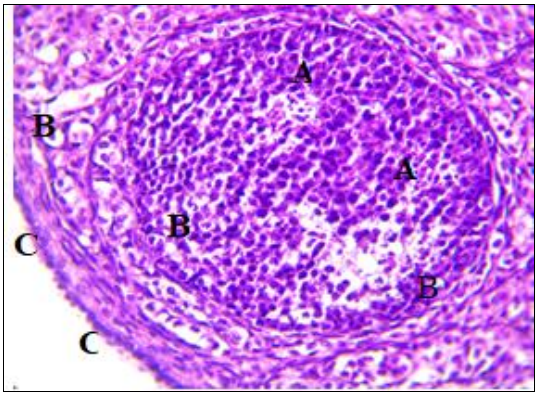

Fig.4:- (Four group)(A) Atretic follicle with degenerated follicular cells, (B)Vacuolar degeneration cells of primordial follicle, (C) Germinal epithelium (H&Ex40).

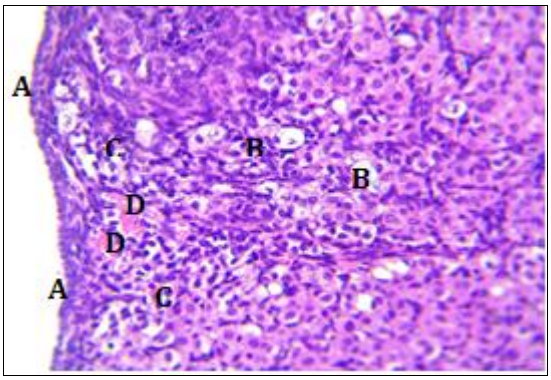

Fig. 5: (Five group)(A) Germinal epithelium , (B) Vacuolar degeneration of luteal cells, (C) WBCs infiltration, (D) Blood hemorrhage, (H&Ex40).

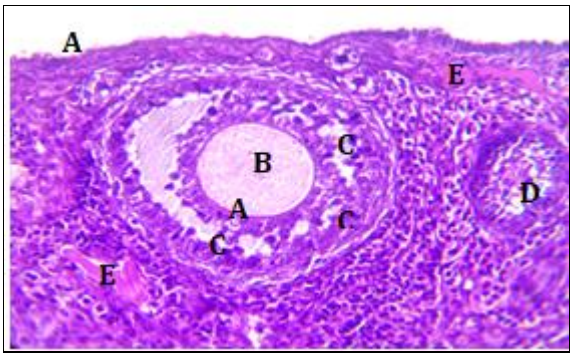

Fig.6: (Six group) (A) Germinal epithelium, (B) Tertiary follicle without nucleus, (C) Oocyte disorganization, (D) Atretic follicle, (E) Blood congestion, (F) WBCs infiltration, (H&E x40).

The results agree with those found by [20] At all phases of folicle development and expansion, atrecia, or the degeneration of ovarian folicles, occurs During the ovulatory stage, NO also influences the blood-folicle barrier, and gonadotrophins' stimulation of NO may be a factor in the development of hyperaemia, leukocyte migration, and enhanced vascular permeability. Our results agreed with [21,22] suggested that the oocytes treated to result in greater SNP concentrations could be undergoing a process cell degeneration, confirming the hypothesis that NO concentration can be a critical factor for cellular survival and function. The present study is in agreement with [23], concluding that the co-treatment of SNP with gonadotropins in ages female mice during the superovulation process increases ovarian response and oocyte developmental competence at a similar level to that of pubertal.

Conclusions: The study concluded the posative effect of SNP on the reproduction of female mice represented by inhacement of reproductive hormones and quality and quantity of follicles and the side effects appeared in the high doses of treatment .

References

[1] Zhang, D., Li, R., Chen, M., Vu, T., Sheng, H., Yang, W., ... and Yao, J. (2021).Photoacoustic imaging of in vivo hemodynamic responses to sodium nitroprusside. ***Journal of biophotonics***, *14*(7), e200478.

[2] Dawson, T. M., and Dawson V. L. (2018). Nitric Oxide Signaling in Neurodegeneration and Cell Death. ***Adv Pharmacol*** 82,57–83.

[3] Becerril, S., Rodríguez, A., Catalán, V., Ramírez, B., Unamuno, X., Portincasa, P., Gómez-Ambrosi, J. and Frühbeck, G. (2019). Functional Relationship between Leptin and Nitric Oxide in Metabolism. ***Nutrients,*** 11(2), E2129.

[4] Santana, P.D., Silva TV, da Costa NN, da Silva BB, Carter TF, Cordeiro Mda S, da Silva BJ, Santos Sdo S, Herculano AM, Adona PR, Ohashi OM and Miranda Mdos S.(2014). Supple-mentation of bovine embryo culture medium with L-arginine improves embryo quality via nitric oxide production. ***Mol Reprod Dev*** 81, 918-927.

[5] Lee, D. H., Joo, B. S., Suh, D. S., Park, J. H., Choi, Y. M., and Lee, K. S. (2008). Sodium nitroprusside treatment during the superovulation process improves ovarian response and ovarian expression of vascular endothelial growth factor in aged female mice. ***Fertility and sterility***, 89(5), 1514-1521.

[6] Kim, H., Moon C., Ahn M., Lee Y., Kim H., Kim S., (2005). Expression of Nitric Oxide Synthase Isoforms in the Porcine Ovary During Follicular Development. ***J Veterinary Sci*** 6(2),97–101.

[7] Coles, E. H. V. (1986). Veterinary Clinical Pathology, 4th ed. WB Philadelphia: ***Saunders Company***, *2*, 12-14.

[8] Duncan, D. B. (1955). Multiple range and multiple F tests. *Biometrics*, 11(1), 1-42.

[9] Joda, M. (2008). Statistical analysis spss. ***Amman for publishing and distribution***.

[10] Canteros, G., Rettori, V., Genaro, A., Suburo, A., Gimeno, M. and McCann, S. M. (1996). Nitric oxide synthase (NOS) content of hypothalamic explants: Increased by norepinephrine and inactivated by NO and cyclic GMP. ***Proceedings of the National Academy of Sciences, USA***, *93*, 4246-4250.

[11] McCosh, RB., Lopez JA, Szeligo BM, Bedenbaugh MN, Hileman SM, Coolen LM, et al(2020). Evidence That Nitric Oxide Is Critical for LH Surge Generation in Female Sheep. ***Endocrinology*** , 161(3),bqaa010.

[12] Watanobe, H. and Schiöth, H. B. (2001). Nitric Oxide Mediates Leptin-Induced Preovulatory Luteinizing Hormone and Prolactin Surges in Rats. ***Brain Res*** 923,193–7.

[13] Karanth, S, Yu WH, Walczewska A, Mastronardi CA, McCann SM(2001). Ascorbic acid stimulates gonadotropin release by autocrine action by means of NO. ***Proc Natl Acad Sci U S A.*** Sep 25,98(20),11783-8

[14] Meng, C(2019). Nitric oxide (NO) levels in patients with polycystic ovary syndrome (PCOS): *A meta-analysis. J. Int.* ***Med. Res***. 47, 4083–4094.

[15] Wulff, C, Wilson H, Wiegand SJ, Rudge JS, Fraser HM(2002). Prevention of thecal angiogenesis, antral follicular growth, and ovulation in primate by treatment with vascular endothelial growth factor trap R1R2. ***Endocrinology*** 43,2797–807.

[16] Merce, LT, Bau S, Bajo JM(2001). Doppler study of arterial and venous intraovarian blood flow in stimulated cycles. ***Ultrasound Obstet Gynecol*** ,18,505–10.

[17] Tessaro, I., Luciano, A. M., Franciosi, F., Lodde, V., Corbani, D., and Modina, S. C. (2011). The endothelial nitric oxide synthase/nitric oxide system is involved in the defective quality of bovine oocytes from low mid-antral follicle count ovaries. ***Journal of animal science***, 89(8), 2389-2396.

[18] Sengoku, K, Takuma N, Horikawa M, Tsuchiya K, Komori H, Sharifa D. (2011), Requirement of nitric oxide for murine oocyte maturation, embryo development, and trophoblast outgrowth in vitro**.** ***Mol Reprod Dev***., 58,262–8.

[19] Danforth, DR, Arbogast LK, Ghosh S, Dickerman A, Rofagha R, Friedman CI(2003). Vascular endothelial growth factor stimulates preantral follicle growth in the rat ovary. ***Biol Reprod*** ,68,1763–41.

[20] Bulbul, T., Akosman, M. S., Yilmaz, O., Ulutas, E. and Bulbul, A. (2015). Supplementary dietary nitric oxide donor (sodium nitroprusside) or inhibitor (NG-nitro-l-arginine methyl ester) depressed growth performance and ovarian primordial and primary follicles in Japanese quail (Coturnix japonica) in a dose-dependent manner. ***British poultry science,*** *56*(1): 113-120.

[21] Mitsube, K., Mikuni, M., Matousek, M., and Brännström, M. (1999). Effects of a nitric oxide donor and nitric oxide synthase inhibitors on luteinizing hormone-induced ovulation in the ex-vivo perfused rat ovary. ***Human Reproduction,*** 14(10), 2537-2543.

[22] Moncada, S., Erusalimsky, J.D., (2002). Does nitric oxide modulate mitochondrial energy generation and apoptosis? ***Nat. Rev. Mol. Cell. Biol***. 3, 214–220.

[23] Lee, D. H., Joo, B. S., Suh, D. S., Park, J. H., Choi, Y. M., and Lee, K. S.(2008). Sodium nitroprusside treatment during the superovulation process improves ovarian response and ovarian expression of vascular endothelial growth factor in aged female mice. ***Fertility and sterility***, 89(5), 1514-1521.

التأثير الهرموني والنسيجي لأكسيد النيتريك على الجهاز التناسلي في إناث الفئران

نجلاء علي نوري البياتي[1], وسيم علي حسين[2] ، خالد احمد هادي[1]

[1]فرع الفسلجة و الادوية والكيمياء الحيوية، كلية الطب البيطري – جامعة تكريت، تكريت، العراق

[2]فرع العلوم الأساسية، كلية طب الأسنان، جامعة تكريت، تكريت، العراق

الملخص

هدفت الدراسة الحالية إلى دراسة التأثير الدوائي لنيتروبروسايد الصوديوم NO المتبرع على الجهاز التناسلي الأنثوي في الفئران من خلال تقييم مستويات الهرمونات (FSH , LH , الاستروجين والبروجستيرون) في مصل الدم والتغيرات النسيجية في أنسجة المبيض. أجريت التجربة على ثلاثين فأراً بصحة جيدة تراوحت أوزانها ما بين (25–34) غم، قسمت إلى ستة مجاميع (خمسة حيوانات لكل مجموعة) وعوملت بمادة نيتروبروسايد الصوديوم (Intraperitoniolly) الموجودة في بيت الحيوان للطب البيطري. استخدمت في الدراسة الحالية كلية الطب / جامعة تكريت بعد 21 يوما من العلاج بـ (SNP)، وأظهرت نتائج الدراسة الحالية ارتفاعا معنويا ($P<0.05$) في جميع المجاميع المعالجة مقارنة مع مجموعة السيطرة، في حين أنها أظهر انخفاض معنوي ($P<0.05$) في هرمون البروجسترون تدريجيا مع زيادة جرعة SPN في جميع المجاميع مقارنة بمجموعة السيطرة. نتائج التقييم النسيجي لمتوسط أعداد الجريبات في المبيض الواحد في المجموعات المعالجة لتحديد الجريبات البدائية ,أظهر عدد البصيلات الأولية والثانوية ارتفاعا معنويا ($P<0.05$) في جميع المجاميع المعاملة بالمقارنة مع مجموعة السيطرة , كما انخفض عدد الجريبات G6 تدريجيا ($P<0.05$) مقارنة مع المجموعات المعاملة الأخرى , كما انخفض عدد البصيلات الثالثة في أظهرت جميع المجموعات باستثناء المجموعة G2 انخفاضاً معنوياً ($P<0.05$) مقارنة مع مجموعة السيطرة، كما أظهرت نتائج التغيرات النسيجية للمبيض تنكس السيتوبلازم وتفريغ الظهارة الجرثومية. كما تم تحلل بعض الخلايا الجريبة وإحاطتها بارتشاح كريات الدم البيضاء، وتحللت معظم الخلايا البدائية مع وجود إفرازات التهابية بين تلك الخلايا وتحلل البويضة من الجريب الثالث محاطة بالعديد من الخلايا الجريبية المتحللة وارتشاح كريات الدم البيضاء في الأنسجة القشرية. بين الجريب البدائي كانت الأوعية الدموية للنخاع تحتوي على دم محتقن وانحلالي مع زيادة الجرعة، وخلصت الدراسة إلى التأثير الإيجابي على الجهاز التناسلي في إناث الفئران، ولكن التأثيرات ظهرت في الجرعات العالية من العلاج.