

## Investigation Excitatory and Inhibitory Effects of L-Arginine and L-NAME on Ovarian Follicles Development Process in a Pregnant Rat

Kameli SM<sup>1</sup>, Khanehzad M<sup>2</sup>, Bagheri SA<sup>2</sup>, Noori Mugahi SMH<sup>1</sup>

1. Department of Histology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, I. R. Iran.

2. Department of Anatomy, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, I. R. Iran.

Corresponding Author email: [noorimoo@sina.tums.ac.ir](mailto:noorimoo@sina.tums.ac.ir)

**ABSTRACT:** Introduction: Nitric Oxide (NO) interfere to control some of physiologic interactions such as cell growth, apoptosis, regulating blood pressure, defense mechanism and especially in reproductive process as one of the smallest active products. The purpose of this study is investigating effect of L-Arginine and L-NAME on the development of ovarian follicles in pregnant rats according to important role of NO in reproductive. Methods & Materials: In this study 40 Wistar pregnant rats with 200- 250 grams weight and eight weeks old were used. Pregnant mice were divided into five groups, based on observation of vaginal plug. Except of control group other groups were received 1<sub>mg/kg</sub> Normal saline solution, 200 mg/kg L-Arginine, 20 mg/kg L-NAME and a mixture of the same doses L-Arginine & L-NAME respectively at 3<sup>th</sup>, 4<sup>th</sup> and 5<sup>th</sup> gestational days through intraperitoneal injection. At 18<sup>th</sup> gestational days ovarian was removed, and studied by Light microscopy after fixation and tissue preparation by H&E staining routine. Results: In comparison investigation with ANOVA test, in the group L-Arginine there was meaningful difference about decline number of primary follicles and increasing atretic follicles. Also the most histological changes observed in group L-Arginine. Conclusion: This study showed, Nitric oxide has impairing effects during pregnancy has damaging effects on ovaries and has advised, must carefully be used during pregnancy.

**Keywords:** Ovarian follicles, Nitric oxide, L-Arginine, L-NAME

implantation, embryo development and contraction of the uterus [7-9].

In this study we evaluated effects of L-Arginine and L-NAME on the development of ovarian follicles in pregnant rat with considering decreased quality of fertility, using assisted reproductive techniques (ART), and importance of used substances by mothers during pregnancy and role of Nitric oxide in ovulation and reproduction.

### Materials and Methods

In this experimental-interventional study, 40 female Wistar rats with 200- 250 g weighting and at average 8 weeks olds have taken from Razii Institute, have used. Every five rat (1 male and 4 females) based on National Institute of Health, maintained in the animal room of Tehran University of Medical Sciences under standard conditions in 20 C° and 12 hours of light and 12 hours dark alternatively. After mating, was considered as first day when vaginal plug observed and the pregnant rats were randomly divided into five groups including control, Normal saline, mixture of L-NAME and L-Arginine, L-NAME (obtained from Sigma, Germany). Except of the control group other groups intraperitoneal assay were received normal saline 2<sub>ml/kg</sub>, L-Arginine 200<sub>mg/kg</sub>, L-NAME 20<sub>mg/kg</sub> and mixture of two L-Arginine and L-NAME on same doses respectively in the third, fourth and fifth gestation days.

### Introduction

Today, humans by drugs and new compounds have attempted to increase their health level and quality of life, bidirectional effects of these compounds and their long-term effects human generation is noticeable. Many of these substances are affected physiological processes, and their normal amounts causes correct and balanced development, while more or less amount of them than normal can due to damage the vital organs even threatened individual life. The Arginine its derivatives are as nutritional supplements that is using by a large portion of population. L-Arginine as a precursor, by the enzyme Nitric oxide synthase (NOS) in the presence of oxygen and a compound called (Nicotinamide Adenine Dinucleotide Phosphate, NADPH) has converted to Nitric oxide (NO). Nitric Oxide (NO) is one of smallest active products in many organs of the body [1] and has interference in control of physiological processes including cell growth, apoptosis, nerve impulses transmission, blood pressure regulating and defense mechanism specially in reproductive process [2-4]. This enzyme has three isoforms, Neural (Neural ENOS = nNOS), inducible (Inducible NOS = iNOS) and endothelial (Endothelial NOS = eNOS) [5]. Among them, iNOS is the main isoforms in ovulation [3, 6]. Many researchers have pointed about NO has played its role in the producing of ovarian steroids, ovulation, follicle development,

± SD). For comparison among groups, the one-way ANOVA was used and  $P < 0.01$  was considered as the level of significance [11].

**The Quantitative findings**

In L-Arginine group, number of primary follicles had meaningful reduce than control group, while the number of Atretic Follicles has shown meaningful increase. In other groups, this difference was not meaningful than Control group (Diagram1).

On the eighteenth day of pregnancy, rats were anesthetized by pentobarbital and ovaries were removed. After Fixation samples in 10% Formalin and preparation of tissue, slices were prepared with 5 to 6 microns thickness. Finally after Hematoxylin-Eosin (H&E) staining specimens were investigated with Olympus optical Microscope (Olympus CX31) made in Japan and Software Image tools III [10].

**Results**

Data analyzed by SPSS software. Results have presented by Mean ± Standard deviation (Mean

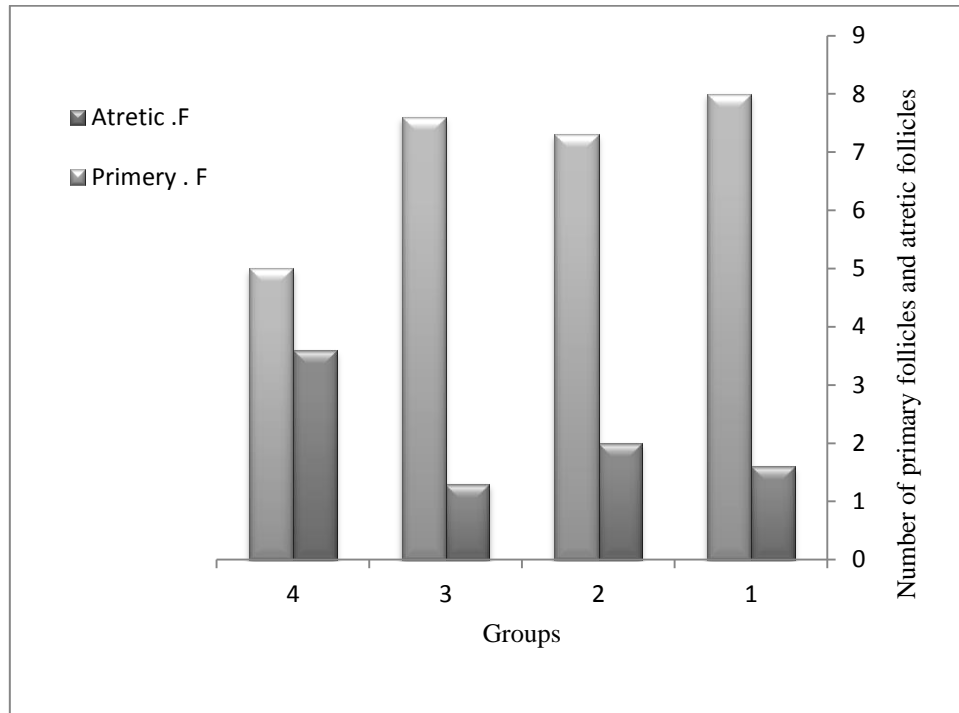
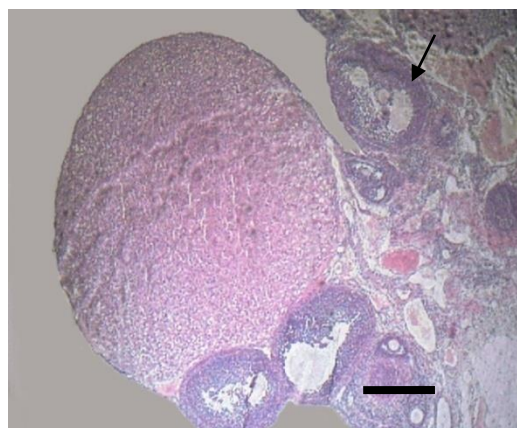


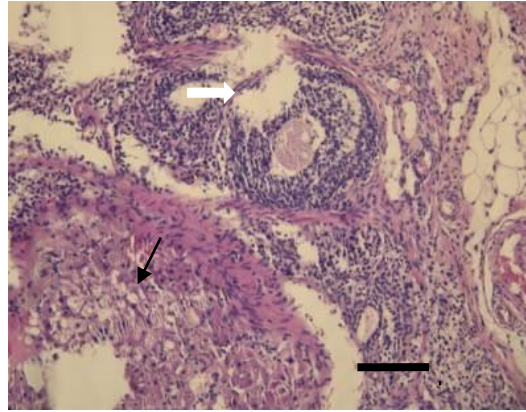
Diagram1: Comparison of the number of follicles in each group (n = 8,  $P < 0.01$ ).  
1=Control, 2= L-NAME, 3= L-Arginine and L-NAME, 4= L-Arginine

**The Qualitative findings**

The most changes including damage to follicle rim, Corpus Luteum of and ovary have detected by observing L-Arginine group photomicrograph. Other groups photomicrograph had no significant difference with the control group (Photomicrograph 1, 2).



Photomicrograph 1: Control group, Follicle (White arrow), Corpus Luteum (Black Arrow), (400 x, H&E).



Photomicrograph 2: L-Arginine group, Follicle (White arrow), Corpus Luteum (Black Arrow), (400 x, H&E).

Oocyte development can from follicle environment factors specially reduced follicular vessels and Nitric oxide deficiency concluded its deficiency is associated with reduced follicular vessels Oocyte and can affected Oocyte quality and causes decreasing in fertility[16]. Some studies refer to pivotal role of Nitric oxide in process of follicular maturation and ovulation [17-18]. Based on extensive researches on Nitric oxide's role in various body tissues [19-22] and different results about positive and negative effects of Nitric oxide on the organ's Histological structure, can be stated, Nitric oxide as a sword double-edged acts and has devastating effects in lesser or higher concentrations.

### Conclusion

In considering Corpus Luteum and follicle development during pregnancy and divesting effects of L-Arginine on these elements, Therefore during pregnancy must pay attention to medications and supplements and must be apply under with physician administration.

### References

- Abbasi M, Akbari M, Amidi F, Ragerdi Kashani I, Mahmoudi R, Sobhani A, Takzare N, Pasbakhsh P, Barbarestani M, Abolhassani F, Sato E. Nitric oxide acts through different signaling pathways in maturation of cumulus cell-enclosed mouse oocytes. *DARU*. 2009; 17 (1); 48-52
- Adhikari NK, Burns KE, Friedrich JO, Granton JT, Cook DJ, Meade MO. Effect of Nitric oxide on oxygenation and mortality in acute lung injury: Systematic review and meta-analysis. *BMJ*. 2007; 14; 334(7597):779.
- Bahmanzadeh M., Abolhassani F., Amidi F., Ejtemaiemehr Sh., Salehi M., Abbasi M. The Effects of Nitric oxide synthase inhibitor (L-NAME) on epididymal sperm count, motility, and morphology in Varicocele rat. *DARU Journal of Pharmaceutical Sciences*. 2008; 16(1):23-28.

### Discussion

L-NAME as Nitric oxide inhibitor had no damaging effect on follicle structure, results have shown. Related to it, role of L-NAME in reducing tissue damage resulting from NO, some studies have mentioned [12]. While L-Arginine causes decrease in number of primary follicles and increase in number of Atretic follicles than control group. In addition, in L-Arginine group, most histological changes including damage to follicle rim, Corpus Luteum and ovarian was observed. Bodis in 2010 reported, there is a meaningful inversely relation between follicular fluid's L-Arginine concentration and Oocytes and accepted embryos in women undergoing by IVF. Also they noted to increases the level L-Arginine of follicular fluid has destructive effects on fertility process [13]. Another study also has shown, edible L-Arginine supplementation during IVF pregnancy causes increasing NO concentration at follicle due to decreasing quality of implant embryos [14]. The findings of this study are same to other studies results. Based on normal follicular fluid has multiple Biological active substances such as growth factors, enzymes, Nitric oxide and regulator vascular factor, it has effects on controlling ovarian follicle maturation, creating uncondusive environment for Oocyte development and quality of pregnancy product. Probably L-Arginine followed by stimulating Nitric oxide production, causes its concentration in ovarian tissue has increased and due to distribute follicles and Corpus Luteum development. Since existence and proper function of Corpus Luteum is one of the most important factors in pregnancy maintaining and continuing and also, appropriate follicles development have feedback effects on sexual hormones secretion, so that damage to follicles and Corpus Luteum possibly can influences the course and outcome of pregnancy. In addition, about Nitric oxide effects on increasing quality of Oocyte, ovarian and follicle multiple reports have presented. In 2014 Mohanad have shown, edible L-Arginine increases placenta and fetus weighting, blood volume and placenta volume and level in pregnant rats [15]. Another study was applied for investigate weak

- Mohanad A Al-Bayati, Marawan A Ahmad, Wael Khamas. The Potential Effect of L-arginine on Mice Placenta. *Adv Pharmacoepidemiol Drug Saf*. 2014; 3 (2).
- Noori SMH, Mahmmoudzadeh Sagheb HR, Heidari Z. [Applied Methods and Terminology of Histotechnology, Stereology & Morphology]. 3rd ed. Tehran; Tehran University of Medical Sciences Publications, 2009; 71: 95-8. (Persian).
- Pautz A, Art J, Hahn S, Nowag S, Voss C, Kleinert H. Regulation of the expression of inducible nitric oxide synthase. *Nitric Oxide*. 2010; 23(2):75-93.
- Peck RL, Olsen C, and Devore J L. Introduction to statistics and data analysis. 2007; 148-195.
- Rodebaugh J, Sekulic M, Davies W, Montgomery S, Khraibi A, Solhaug MJ, Ratliff BB. Neuronal nitric oxide synthase, nNOS, regulates renal hemodynamics in the postnatal developing piglet. 2012; 71(2):144-9.
- Rostoker G. Colloids in Dialytic Refractory Hypotension. 2013; Chapter 14: 260 -267.
- Seham El-Berry, Mohamed Abdel Razik. Nitric oxide donor's increases pregnancy rate in clomiphene citrate treated polycystic ovary infertile patients. *Middle East Fertility Society Journal*. 2010; 15: 106-109.
- Sombrero A; Nobel A; Bruonton TI; A short history of nitroglycerin and nitric oxide in pharmacology and physiology. 2000; 27(4):313-9.
- Tessaro I, Luciano AM, Franciosi F, Lodde V, Corbani D, Modena SC. The Endothelial Nitric oxide synthase/Nitric oxide system is involved in the defective quality of bovine Oocytes from low mid-antral follicle count ovaries. *J Anim Sci*. 2011; 89(8):2389-96.
- Zadeh BS. Assesment of Endothelial Nitric Oxide Synthase Expression in Fallopian Tube at the Time of Term Pregnancy. *Medical Journal of Tabriz University of Medical Sciences and Health Services*. 2014; 35(6):80-7.
- Battaglia C, Regnani G, Marsella T, Facchinetti F, Volpe A, Venturoli S, Flamigni C. Adjuvant L-arginine treatment in controlled ovarian hyperstimulation: a double blind, randomized study. *Hum Reprod*. 2002; 17:659-665.
- Bódis J, Várnagy A, Sulyok E, Kovács GL, Martens-Lobenhoffer J, Bode-Böger SM. Negative association of L-arginine methylation products with Oocyte numbers. *Hum Reprod*. 2010; 25(12):3095-100.
- Fıçıoğlu C, Kumbak B, Akcin O, Attar R, Yıldırım G, Tecelioglu N, Yesildaglar N. Cytokine and Nitric oxide concentrations in follicular fluid and blood serum of patients undergoing assisted reproductive treatment: relationship to outcome. *J Turk Ger Gynecol Assoc*. 2009; 10(3): 132-136.
- Friden, B. E., Runesson, E., Hahlin, M. et al., Evidence for nitric oxide acting as a luteolytic factor in the human corpus luteum. *Mol. Hum. Reprod*. 2000; 6(5):397-403.
- Grazul-Bilska AT, Navanukraw C, Johnson ML, Vonnahme KA, Ford SP, Reynolds LP, Redmer DA. Vascularity and expression of angiogenic factors in bovine dominant follicles of the first follicular wave. *J Anim Sci*. 2007; 85(8):1914-22.
- Krause BJ, Hanson MA, Casanello P. Role of Nitric oxide in placental vascular development and function. *Placenta*, 2011; 32(11): 797-805.
- Lakzaei F, Karami M, Jalali NMR, Hassani F. Opposite Effect of Naloxone on Infertility Induced By Nitric Oxide System in an Animal Model of Polycystic Ovary. *Daneshvar Medicine*. 2014; 21.
- Levinsson A, Olin A-C, Björck L, Rosengren A, Nyberg F. Nitric oxide synthase (NOS) single nucleotide polymorphisms are associated with coronary heart disease and hypertension in the intergene study. *Nitric Oxide*. 2014; 39:1-7.
- Liu Y, Lu X, Feng Q. Deficiency in endothelial Nitric oxide synthase impairs fetal coronary artery development in mic. *Journal of Molecular and Cellular Cardiology*, 2010; 48, S94.