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Effects of L-Arginine and L-Omega-Nitro-L-Arginine Methyl Ester on Fertility of Female Rats

Homa Darmani[1]* Ahmed M. Elbetieha[1] Nabil Bashir[2]

[1]Department of Applied Biology, Faculty of Science, [2]Department of Biochemistry, Molecular Biology and Clinical Laboratory Sciences, Faculty of Medicine, Jordan University of Science and Technology, P.O. Box 3030, Irbid 22110, Jordan.

*Corresponding Author
E-mail: darmani@just.edu.jo

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Abstract
Nitric oxice (NO) is produced by the action of nitric oxide synthase (NOS) on L-arginine. This study examined the effect of stimulating and inhibiting NOS activity with L-arginine and L-omega-nitro-L-arginine methyl ester (L-NAME), respectively, on female rat fertility. Female rats received peritoneal injections of L-arginine, L-NAME or saline for two weeks and the effect on fertility was evaluated by determining the number of pregnancies, implantation sites per dam, fetal viability and embryonic resorptions. Stimulation of NOS activity significantly decreased pregnancy rates and increased embryolethality, with significant decreases in the weights of the ovaries and uteri. Inhibition of NOS activity had no signifcant effect on the pregnancy outcomes, but increased embryolethality relative to the implantation sites per dam. Furthermore, ovary weights were significantly greater and uterus weights significantly less than the controls. Thus, optimal levels of NO are important for gravidity, proper functioning of the endometrium, as well as, embryonic implantation.

Keywords: Nitric oxide synthase, Fertility, Reproductive effects, L-NAME, L-arginine.

INTRODUCTION

For more than three decades nitric oxide (NO) was just a pollutant that induced acid rain and ozone layer depletion. Today, NO is regarded as a major paracrine mediator of diverse biological roles in immunity, as well as, the circulatory and the nervous systems [1, 2]. NO is synthesized from L-arginine through the action of nitric oxide synthases (NOS). NOS include neuronal, incucible and endothelial isoforms (nNOS, iNOS and eNOS, respectively) [3, 4].

During the past decades, it has become clear that NO is important in the control of several functions in female reproduction, including the growth and development of follicles [5, 6]. NOS are present in the endometrial tissue of different animals and are key mediators in the function of the endometrium [7, 8]. The localisation of eNOS and iNOS in humans is reported to be the glandular and luminal epithelium, vascular endothelium and stroma of the non-pregnant uterus [7, 9, 10]. Furthermore, NOS is thought to be important in maintaining a quiescent state in human myometrium during pregnancy [11, 12]. Various studies have indicated the importance of NO in the receptivity of the uterine lining as well as the process of implantation and menstruation. In mice after embryonic implantation, the elevated concentrations of iNOS and eNOS at the site of implantation indicate that NO is important in the preparatory events for pregnancy including remodelling of the uterine tissues as well as suppression of the immune response and vasoregulation. NO can thus be considered an important player in the events needed for embryonic implantation and gestation [13, 14]. NOS inhibitors are also reported to show synergistic effects with antiprogestins in inhibiting gestation [9]. Furthermore, inadequate NO production is thought to impede placental perfusion as well as endometrial receptivity, which may lead to pregnancy loss [15].

NOS is present in the stroma of the ovaries and in the follicular cells of different species of animals, and is thought to control ovulation as well as steroidogenesis [16-20]. Other studies have reported an abnormal ovulatory process in impaired ovulation in mice with defects in nNOS [21]. Additionally, eNOS-deficiency in mice resulted in reduced ovulation with a lengthened estrous cycle and decreased embryonic viability as well as decreased implantation during pregnancy [22, 23].

Thus, NO is involved in the regulation of female reproduction, but studies investigating whether NO is required for female fertility are limited. To this aim, we examined the effects of L-arginine and L-NAME on the fertility of female rats.

MATERIALS AND METHODS

Ethics Statement

All animal handling and experimental protocols were in accordance with the Institutional and National Guidelines and Regulations. The Institutional Review Board of Jordan University of Science and Technology at King Abdulla University Hospital approved the study.

Animals

Adult female Sprague Dawley rats were allowed food and water *ad libitum* and kept at a temperature of 21°C on a light-dark cycle (12-12). They were divided randomly into three groups. Control rats received intraperitoneal injections of normal saline (0.9% sodium chloride). The second group were injected with 40 mg/Kg L-arginine (98.5% pure, Aldrich Chemical Company, Milwaukie, WI, USA) and the third group with 40 mg/Kg L-NAME (98% pure, Aldrich Chemical Company, Milwaukie, WI, USA) every day for 2 weeks, following which the effects on reproduction were examined.

Effects on Reproduction

The control and treated female rats were placed in cages with a sexually mature unexposed male at a ratio of 2 females per male for ten days which covers the duration of at least two estrus cycles [24]. After another ten days,

the female rats were placed under light ether anesthesia and sacrificed using cervical dislocation. To assess the effects on fertility, the number of pregnancies, implantation sites per dam, embryonic resorptions and viability of the fetuses were determined. In addition, the maternal and embryonic body weights were determined, together with the maternal uterine and ovarian weights.

Statistical Analysis

Minitab statistical package (Minitab Release 17, Minitab Inc., State College, PA, USA) was used to analyze the data.

RESULTS AND DISCUSSION

We examined the effect of stimulation and inhibition of NOS activity with L-arginine or L-NAME, respectively, for 2 weeks on female rat fertility, using an animal model that has been used previously to determine the effects of different substances on fertility.

Effect of L-arginine and L-NAME on fertility

Table 1 shows the effect of stimulating and inhibiting NOS activity with L-arginine and L-NAME, respectively, on female rat fertility. We found that exposure of adult female rats to L-arginine had a significant effect on their fertility with a significant reduction in the rate of pregnancies from 90% in controls to 30% in the L-arginine group ($p<0.01$). Our findings agree with former studies that have reported that high levels of NO has adverse effects on *in vitro* embryonic development, as well as*, in vivo* implantation in rodents [25]. Furthermore, Ota *et al.* [26] reported that ideal concentrations of NO are necessary for endometrial receptivity and embryonic implantation since exposure of pregnant mice to the NO donor molsidomine during the implantation period resulted in decreased pregnancy rates of 41% compared to the 100% rate for the control counterparts. Thus, our results also reiterate that although important for normal development of preimplantation embryos, exposure of female rats before gestation to an excess of NO may also inhibit this process [27].

In female rats exposed to L-arginine, no significant differences were observed in the number of implantation sites per dam, viable fetuses, nor embryolethality relative to the implantation sites, in comparsion to the controls. However, an increased percentage of females with embryonic resorptions were observed but due to the low incidence of pregnancies in the L-arginine group, the results were not statistically significant.

Table 1 also shows the effect of inhibition of NOS activity on the fertility of female rats. Treatment with L-NAME did not have any significant effect on the number of animals becoming pregnant. The number of implantation sites and viable fetuses were reduced in comparison to controls, but the decrease was not statistically significant. The percentage of animals with resorptions was also higher but the results were not statistically significant.

However, females exposed to L-NAME showed significantly increased embryolethality relative to the implantation sites per dam, in comparison to the controls ($p<0.01$). This is in agreement with the study of Miller *et al.* [28] who showed that administration of L-NAME to pregnant rats impeded fetal growth as a result of apoptosis probably due to formation of peroxynitrite. Other studies have found that exposure of embryos *in vitro* to NOS inhibitors caused abnormal development [27, 29]. eNOS gene knockout mice exhibited impaired ovulatory function, conception and embryonic survival [23]. In another study, eNOS gene knockout mice had disturbed placental function with subsequent disturbance in fetal oxygenation [30]. Thus, placental insufficiency may be one of the underlying reasons for the observed increases in fetal resorptions observed in this study in female rats subjected to L-NAME treatment.

Effect of L-arginine and L-NAME on organ weights

Table 2 shows that stimulation of NO production with L-arginine caused a significant reduction of absolute and relative ovarian weights, in comparison to the controls ($p<0.05$ and $p<0.01$, respectively). Nitric oxide is involved in many normal ovarian functions. NO has been reported to prevent apoptosis of rat preovulatory follicles *in vitro* through the stimulation of HSP70 as well as the supression of the expression of Bax [31]. NO also has been reported to be an important modulator of ovulatory efficiency in mice [23]. The decrease in the weights of the ovaries of females exposed to L-arginine requires histological assessment to determine whether it is due to cellular atrophy or involution of a particular part of this organ.

Table 2 also shows that inhibition of NO production with L-NAME resulted in significantly increased relative ovarian weights in comparison to the controls ($p<0.05$). The increase in the relative ovary weights of rats exposed to L-NAME is hormonally controlled. Our results suggest that exposure to L-NAME caused an imbalance in the conceptive endocrinal functions, feasibly with manifold sites of toxicity throughout the hypothalamic-pituitary-ovarian-uterine axis.

In the current study, we also observed significantly decreased relative uterine weights in both L-arginine and L-NAME treated animals in comparison to controls ($p<0.05$). In the course of gestation NO production in the uterus is controlled by estrogen, and it is believed that NO might be a crucial moderator of steroid hormone activity in the uterus [32, 33]. For implantation to occur and for adequate fetal development, there must be sufficient uterine perfusion as well as placental sufficiency to ensure optimum extraction of oxygen and nutrients by the fetus. Since NO is the major feto-placental vasodilatatory agent, it is not surprising that excess or insufficient levels can interfere with the normal physiological aspects of uncomplicated pregnancies in rats.

Table 1. The effect of stimulating and inhibiting NOS activity with L-arginine and L-NAME (respectively) on female rat fertility

Treatment Group Dose	No. (%) of pregnant females	No. implantation sites[a]	No. of viable fetuses[a]	Total no. of resorptions/ total no. of implantation site	No. (%) of animals with resorptions
Control Saline	9/10 (90)	8.44 ± 2.74	8.44 ± 2.74	0/79	0/9 (0)
L-Arginine	3/10* (30)	9.00 ± 1.00	8.33 ± 0.58	2/27	1/3 (33.3)
L-NAME	8/10 (80.0)	6.87 ± 3.48	6.12 ± 3.83	6/57*	3/8 (37.5)

[a] Results are expressed as mean ± S.D. * $P<0.01$ (Fishers exact test).

Table 2. Effect of stimulating and inhibiting NOS activity with L-arginine and L-NAME (respectively) on reproductive organ weights.

Treatment Group	Body weight (B.wt.) (g)[a]	Absolute weight of ovaries (g)[a] (mg/10 g B.wt.®)	Absolute weight of uterus (g)[a] (mg/10 g B.wt.®)
Control Saline	298 ± 32	0.239 ± 0.02 (81 ± 4.5)	3.379 ± 0.62 (1154 ± 180)
L-Arginine	309 ± 34	0.180 ± 0.03* (71 ± 1.15)**	2.99 ± 0.33 (968 ± 18.6)*
L-NAME	303 ± 38	0.250 ± 0.02 (89 ± 6.9)*	2.91 ± 0.40 (967 ± 111)*

[a] Results are expressed as mean ± S.D.
® Relative weights.
* P<0.05, ** P<0.01 Student t-test).

CONCLUSIONS

Based on the results of the current study, stimulation and inhibition of NOS activity results in the impairement of female rat fertility and reproduction.

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