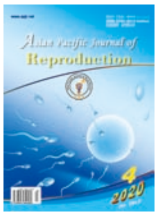

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Effects of nitric oxide on reproductive organs and related physiological processes

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ABSTRACT

Nitric oxide (NO), a member of the reactive nitrogen species family, plays a role in several physiologic processes, including vasculogenesis and angiogenesis, growth and puberty, and senescence and apoptosis. NO plays an important role in the production of ovarian steroids, ovulation, and follicular apoptosis. In other words, increased activity of nitric oxide synthase (NOS) leads to an increased amount of NO, which triggers production of prostaglandins and inflammatory cascades which facilitate follicular rupture and atresia. NO concentration elevation inhibits steroid synthesis in luteal and granulosa cells. Since NO is a major paracrine mediator of various biological processes, as well as a key factor in both the reproductive cycle and embryo implantation, oversynthesis of NO in the uterus results in toxicity and inflammation in epithelial cells and immunorejection of implantation. In the male physiological system, NO synthesized by NOS plays a major role in erectile function and androgen secretion, as well as semen parameters, and oocyte junction to the sperm. Furthermore, this supposedly simple molecule is involved in a number of other functions, such as germ cell evolution, connections between sertoli cells and germ cells in the blood-testis barrier, homodynamic contraction, and germ cell apoptosis. Moreover, NO is considered a key factor in male fertility due to its widespread distribution in both normal and diseased testis tissue. The difference of expression level of NOS in normal and pathological states is a probable cause of fertility destructive processes.

KEYWORDS: Reproduction; Nitric oxide; Nitric oxide synthase; Cell signaling; Oxidants

1. Introduction

Nitric oxide (NO) is a short half-time gas (about a few seconds) which has diverse biochemical and physiological potentials. This molecule was initially discovered in 1978 and nominated as the molecule of the year in the 1992[1,2]. This molecule is an inner cell and intra-cell messenger which plays a key role in the maintenance of body hemostasis[2]. NO usually accomplishes its purpose through synthesizing cyclic guanosine monophosphate. In the production of NO from the *L*-arginine, its synthesis is mediated by nitric oxide synthase (NOS). This enzyme exists in three isoforms: nervous, endothelial, and induction[1]. NO cascade activates different pathways in the various tissues, *e.g.*, they emerge as a vasodilator factor and a known endothelium-derived relaxing factor in the cardiovascular system[2]. However, in the nervous system, NO is considered as a neurotransmitter. Also, in some cases, it is involved in neutrophil-induced cell toxicity, platelet aggregation, blood flow, synaptic transmission, and long-term memory loss[3,4]. In addition to aforementioned functions, NO involves in ovulation, menstruation, and sperm capacity and motility[5,6]. NO is an important paracrine messenger, which participates in several physiological and pathophysiological events in the elementary and endocrine organs[7]. Furthermore, NO has some roles in immune system, such as antiviral and antimicrobial effects, excitation and suppression of the immune system, and cytoprotection[8].

2. NO sources in the body

2.1. NO sources

In mammals, NO can be generated by three isoforms of NO synthase including nervous NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). Mitochondrial NOS has been

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discovered recently, which is specifically found in mitochondrion[9]. Furthermore, iNOS and eNOS are localized in different reproductive tissues such as granulosa, theca cells and cytoplasm of oocytes. Most apoptotic cascades result in producing reactive nitrogen species and reactive oxygen species[9].

2.2. Mechanisms of nitric oxide synthesis

All isoforms of NOS utilize *L*-arginine as the substrate, and molecular oxygen and reduced nicotinamide-adenine-dinucleotide phosphate (NADPH) as co-substrates. A functional NOS transfers electrons from NADPH to the haem in the amino-terminal oxygenase domain[4,10]. At the haem site, the electrons are used to reduce and activate oxygen (O_2) and to oxidize *L*-arginine to *L*-citrulline and NO[11]. To synthesize nitric oxide, the NOS enzyme must pass two key steps, including hydroxylating *L*-arginine to *Nv*-hydroxy-*L*-arginine and also oxidizing *Nv*-hydroxy-*L*-arginine to *L*-citrulline and NO[12-14]. Binding to calcium via calmodulin in nNOS and eNOS is done by using an increase in intracellular calcium ion (Ca^{2+}). When calmodulin affinity to NOS increases, it triggers the transport of electrons from NADPH in the reductase domain to the haem in the oxygenase domain. Due to the presence of different amino acids in the iNOS structure, calmodulin is able to bind at extremely low intracellular Ca^{2+} concentrations approximately 40 nM[15,16].

The ovary is an organ that undergoes major structural and functional changes during the reproductive cycle[17]. Luteolysis is the structural and functional degradation of the corpus luteum which occurs at this cycle and indicates a decrease in cellular function. Analysis of corpus luteum in healthy ovaries is accompanied by increased production of reactive oxygen species such as O_2 and H_2O_2. One of the results of the free radical production in ovarian tissue has been the lipid peroxidation in the plasma membrane of the corpus luteum, which may lead to loss of gonadotropin receptors, reduce adenylyl cyclase–cyclic AMP cyclic adenosine monophosphate (cAMP) formation, and finally reduce steroidization of the corpus luteum following its destruction[18].

Previous studies have shown that NO plays an important role in maintaining the physiological balance of organs including the ovary[19]. Motta *et al*[20] reported a direct link between a destruction of the corpus luteum and an increase in ovarian prostaglandin F2. They stated that corpus luteum depletion was directly related to decreased ovarian glutathione production. Also, their study showed that increased NO through the mechanism of ovarian glutathione depletion could increase the oxidizing effect of oxidase substances and lead to destruction of the corpus luteum[21]. Another study from the same group showed the effect of NO on the ovary and stated that corpus luteum depletion may increase as a result of increased lipid oxidation by using *L*-NG-nitro-arginine methyl ester hydrochloride (*L*-NAME) to prevent the production of intracellular NO in the ovary[21,22].

3. NO in the female genital system

3.1. Oocyte

Full comprehension of the meiosis cell division program has not yet been accomplished. However, more evidence indicates NO involvement in meiosis administration. NO possesses an essential physiologic role in oocyte maturation and fertilization, embryo development, and fetal implantation[23-25]. eNOS and iNOS are reported to be synthesized in the mammalian oocyte, and their presence has been confirmed during follicular maturity. NO synthesis inhibition in the *in vivo* maturation of oocytes causes a reduction in the number of blastocysts and increases apoptosis in the fetus. Alternatively, high levels of NO cause disruption in meiosis development and fetus development in the cow, along with a delay in the restart (or resumption) of meiosis[25]. Goud *et al* demonstrated NO is a crucial factor in the maintenance of oocyte quality[26]. An investigation of earlier literature reveals the binary role of NO in oocyte maturity. Moreover, Bu *et al* described how the concentration dependence of NO has effects on oocyte maturity in the rat. For instance, NO produced from eNOS activity triggers oocyte maturity in the cumulus cells. Nevertheless, a higher concentration of NO causes meiosis arrest of oocyte. Before ovulation, NO reduction following the sudden increase in luteinizing hormone production (luteinizing hormone surge) can be act as a key agent in the restart (or resumption) of meiosis[27]. Recent evidence has validated that oocytes are able to block meiosis in the diplotene phase by expressing iNOS, which produces a sufficient amount of NO[28]. Abbasi *et al* reported that a cAMP cascade is the inducer platform of NO which starts meiosis in the rat oocyte, though cyclic guanosine monophosphate cascades support the inhibitory effort of NO[29] (Figure 1).

3.2. Follicular growth and maturation

Pituitary gonadotropin is a key regulator of the final steps of follicular evolution, and current data emphasize the balance between autocrine and/or paracrine factors in normal follicular growth. NO presence in the follicular liquid has been proven in several animal species. NOS expression in the follicles exhibits an inner ovarian scheme to synthesize NO and to manage follicular growth. NO is synthesized by several ovarian cell types, as well as ovarian arteries. The ovarian macrophages remove apoptotic cells during specific phases of tissue remodeling, as opposed to the removal of foreign debris macrophages, which is another source of NO in ovarian tissue[6]. These macrophage-rich regions of the ovary, such as the theca layer, corpus luteum, and atretic follicles, are involved in the phagocytosis of atretic granulosa cells and apoptotic luteal cells[30,31].

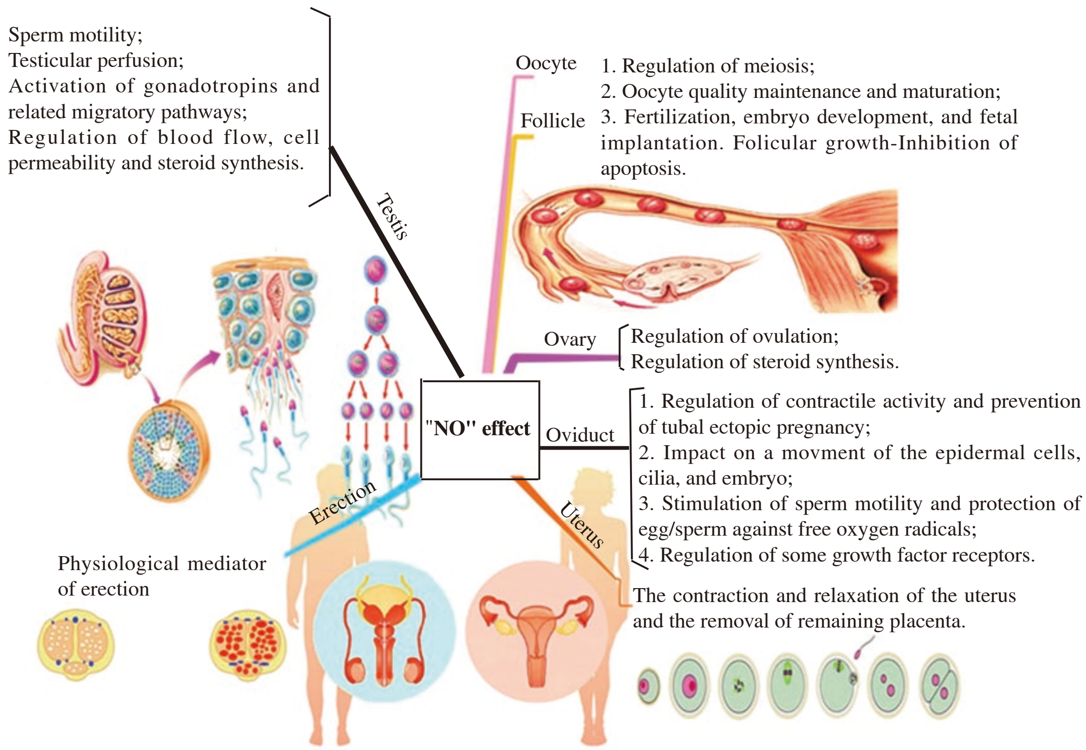

Figure 1. Role of nitric oxide (NO) in different parts of male (left side) and female (right side) genital system.

3.3. Ovary

The iNOS and eNOS have several roles in the ovulation process[6]. NO synthesis increases with follicle growth, and this NO increase is related to increased estrogen. There are similar changes in the amount of NO in circulation with the growth of follicles in women undergoing *in vitro* fertilization. NO is continuously treated with the gonadotropin releasing hormone and human chorionic gonadotropic hormone, and other hormones, such as luteinizing hormone, follicle stimulating hormone, and progesterone have been observed[5]. The use of NOS inhibitors intraperitoneally inhibits ovulation in rats, which is evidence of the role of NO in ovulation processes[30]. These findings suggest that NO is involved in the regulation of ovarian functions[31]. Stimulation of the ovary with gonadotropins increases the expression of both *iNOS* and *eNOS* genes and this suggests that both NO isoforms play a role in the ovulation process. Inhibition of iNOS by using specific inhibitors of NG-Methyl-*L*-arginine and aminoguanidine inhibitors leads to dose-dependent inhibition of ovulation in rats, which indicates the role of iNOS in the ovulation process[30]. During follicular growth, eNOS is expressed in theca cells and in the granulosa cells of the follicle wall, and after ovulation, eNOS is expressed in the yellow body. In the immature ovary and during follicular development, iNOS expression occurs in the theca cells and stroma cells, and after ovulation, iNOS is expressed in the outer layers of the yellow body. Estimating the quantity of iNOS suggests that unlike eNOS, iNOS concentration does not change during follicular growth[6]. Since theca cells, luteal granulosic cells, and yellow body cells are involved in steroidogenesis, it can be concluded that NO also plays a role in regulating steroid synthesis. In most organs of the body, iNOS is expressed only in response to an immune stimulation, such as infection or trauma, and the physiological association of iNOS expression in the natural ovaries at all stages is still uncertain. It is possible that the reduction of expression or non-expression is mainly due to the presence of macrophages and interleukin-1β in the ovary, and it is also possible that NO derived from iNOS acts as a monitoring/growth support molecule. Interleukin-1β is a stimulant for NO synthesis in humans and cows' fallopian tubes[6]. On the other hand, glucose increases in the middle of the menstrual cycle, and glucose stimulates NO synthesis. Therefore, it is possible that NO and glucose can interact as follicles or inducers and facilitate follicular growth pathways[6]. Sugino *et al* reported a relationship between the concentration of NO in follicular fluid and apoptosis, where small follicles show more apoptosis, in contrast to large and moderate-sized follicles. However, the concentration of NO (nitrite/nitrate), arginine, and citrulline is not different in these follicles[32]. Furthermore, the concentration of NO is increased in human follicular fluid, and this increase is directly related to follicle volume and estradiol concentration. Taken together, these observations suggest that the local production of NO causes follicular growth and inhibits apoptosis[33].

3.4. Oviduct (fallopian tube)

In the uterine tube, increased contraction due to endothelin in the presence of *L*-NAME, an NO synthesis inhibitor, was the first evidence of the role of NO in the regulation of the functions of the uterine tube[5]. NO regulates contractile activity in the human fallopian tube by using prostaglandins, prostacyclin and cAMP, so it even prevents tubal ectopic pregnancy[34]. Various studies confirm the presence of calcium-dependent, as well as NOS-dependent, calcium-shaped forms in the rat, cow, and human fallopian tube, and immunohistochemical studies confirm the presence of eNOS in the epithelial cells of the uterine tube[35,36]. Although the activity of NOS in the uterus during the proestrus phase is relatively less than in the other stages of the estrous cycle, the distribution of calcium-dependent NOS in the isthmus, fimbriae, and ampulla of uterine tube is the same[36]. It appears that the release of NO could stimulate sperm motility and protect the egg and sperm from damage caused by free oxygen radicals[6]. Also, NO may affect the movements of the epidermal cells of the uterine tube. NO has been shown to regulate some growth factor receptors, such as the epidermal growth factor, binding proteins and integrin[37]. In contrast to the physiological state, NO synthesis in the uterine tube can be increased under certain pathological conditions, such as infection or endometriosis that leads to reduced fertility through a destructive or toxic effect on sperm cells as well as oocytes. In addition, the increased production of NO may affect the movements of cilia and thus embryo transfer, and the final result can be miscarriage[6].

3.5. Uterus

NO regulates the contraction of smooth muscle cells and uterus dilation during pregnancy; therefore, the role of NO in the regulation of pathophysiology and uterine biology has been considered to be significant[38]. The presence of NOS in the glandular epithelium, endometrial stromal cells, smooth muscle cells, and mast cells indicates the role of NO in the control of the functions of the uterus. In addition, local NO synthesis in the uterus may be important for regulating myometrial activity, such as contraction and relaxation of the uterus[39]. Although smooth muscle cells express eNOS[40], the myometrium is one of the rare parts of uterine tissue which iNOS expresses in non-provocative implications[41], including 1) facilitating the uterine traction for removal of the remaining placenta of the uterus after parturition with nitroglycerin, 2) preventing preterm delivery and prolonging pregnancy with nitroglycerin, and 3) reducing the power of uterine contractions induced by oxytocin with amyl nitrate that this issue indicates the role of NO in regulating and controlling uterine contractions during pregnancy[6]. A study conducted by Bansal *et al* showed that the expression of iNOS rather than eNOS and nNOS in humans in preterm labor was the highest. It is possible that the increase in NOS activity during pregnancy due to positive regulation of cytokines and reduction afterwards during childbirth is highly related to inhibitory cytokines[42]. Interaction between cyclooxygenase, NO, and cytokine has been proven in the uterus of the mouse, and these factors may regulate the function of the uterus during pregnancy[43]. Ovarian hormones also induce iNOS expression in the uterus and may regulate the function of the uterus. However, the role of eNOS in epithelial cells and endometrial stroma is still unclear. But, it is possible that continuous production of NO through the synthesis of prostaglandin and through binding proteins facilitates processes such as menstruation and implantation. A NO derivative of eNOS which is the result of activating the production of guaniline cyclase solution or the decomposition of cyclooxygenase functions as an inhibitor of endometrial platelet aggregation[6]. Buhimschi *et al* showed that the cervix expresses all three NOS isoforms. In addition, the expression of iNOS increases during normal and preterm labor in the cervix and decreases in the uterus, and nNOS, which is not expressed in the uterus during pregnancy, increases during childbirth in the cervix[41]. During childbirth, regardless of the presence of iNOS and nNOS, there is no significant change in the expression of the *eNOS* gene. These findings indicate that NOS activity in the uterus and cervix has a different function and concentration during childbirth and may play a role in reconstructing the connective tissue during cavity preparation. The physiological and biological relevance of NO during pregnancy and childbirth shows that the NO synthesis inhibitor (meaning *L*-NAME) prolongs the delivery time and also reduces the opening of external orifice of the uterus (external os) and vagina[41,44].

3.6. Placenta and preeclampsia

Both *eNOS* and *iNOS* genes are expressed in the placenta, and eNOS expression in the fetal and placental vessels occurs in patients whose preeclampsia increases[45]. Placental vessels are more important in the pathophysiology of preeclampsia, and it seems eNOS is important in conditions such as preeclampsia. NO biosynthesis increases with advancing gestation during normal pregnancy and decreases in preeclampsia, and eNOS is expressed in human placental syncytiotrophoblasts and extravillous trophoblasts[43,45]. In fact, it may increase *eNOS* gene expression in the fetal and placental vessels in patients with preeclampsia who have an adaptive response to low perfusion and hypoxia. The study conducted by Buhimschi *et al* indicated that administration of *L*-NAME to pregnant mice leads to a condition similar to preeclampsia[41].

4. NO in the male reproductive system

4.1. Testis

NO has also been identified in the testicular vasculature endothelium, and its mechanism of action has been partially specified. Accordingly, it is seen that NO can be effective in testicular perfusion, activation of gonadotropins and its migratory pathway to the Leydig cells of the testes, and affect androgen displacement[39]. NO in the testes is involved in regulating blood flow, cell permeability, and contractile function of myofibroblasts, as well as in regulating steroid synthesis. Also, NO regulates sperm motility, as in a low NO concentration increases sperm motility and a medium (or high) concentration reduces sperm motility. In human semen (seminal fluid), a positive correlation between the concentration of NO and the percentage of sperm without mobility was observed[46]. Under physiological conditions, NO is produced in a small amount, and it causes the free radicals to scavenge, which is one of the main causes of decreasing sperm motility. In contrast, excessive NO production under pathological conditions, such as infection, varicocele, or diabetes mellitus, can cause sperm toxicity and also reduce sperm motility through the formation of peroxynitrite. It is likely that the sperm ejaculated into the female reproductive tract can lead to an immune response, which induces iNOS activity and produces a large amount of NO, which can cause the non-maturation and capacity of sperm[46]. Hence, the presence of endogenous NO inhibitor in seminal plasma can play a physiological role in inhibiting NOS activity and maintaining NO in low concentrations to prevent toxic injuries to sperm and Leydig cells or to suppress hypermotility of the sperm associated with the capacity-building process[47] (Figure 1).

4.2. Erection

In humans, NOS activity in the pelvic mesh, cavernous sinus nerves in penile tissue, dorsal branches of the penis, and deep arteries of the sinus cavernous is observed[6]. NOS activity in the rat penile neurons (which autonomically innervated corpus cavernosum and entered the glans cavernous tissue) and in the neural network in the adventitia layer of the penile vessels, shows that NO is a physiological mediator of erectile function. Apart from expression in the nerves, eNOS is abundantly expressed in the penile endothelium and the corpus cavernosum of the endothelium sinusoidum[40]. Different findings indicate that all three *iNOS*, *nNOS*, and *eNOS* genes are expressed in smooth muscle cells of the cavernous sinus of the penis. Administration of anti-androgenic drug to healthy rats reduces the expression of *nNOS* and *eNOS* genes and reduces erection[48]. Ignarro *et al* also showed that the electrical stimulation of the isolated cell line from the Corpus cavernosum of the rabbit secretes NO into the intestine. Based on these findings, they suggested that erection is caused by NO and occurs in response to a nonadrenergic-noncholinergic neurotransmitter. In addition, direct injection of *L*-NAME into periventricular nucleuses leads to inhibition of apomorphine and oxytocin (as an erection inducer)[6].

5. Conclusion and future perspectives

NO is a lipophilic molecule with a short half-life and is synthesized by many organs in the body. It is also introduced as an intra-cell messenger directing many physiologic cascades. Among other things, NO associates with cell growth, apoptosis, and reproduction signal transduction, as well as regulation of blood flow in sexual organs, regulation of vascular tonicity, genital tracts formation, and defense mechanisms. NO reacts with oxygen-active species, thiol groups, and proteins. Considering its concentration and site of action, it is able to protect or poison cells. NO is a nitrogen-active species which plays a role in most physiologic processes, such as vasculogenesis and angiogenesis, growth and puberty, senescence and apoptosis. In the male physiologic system, NO, which is synthesized by NOS, plays a significant role in physiological cascades, such as erectile function and androgen secretion, as well as sperm motility, maturity, quality, and capacity, and oocyte binding to the sperm. Furthermore, this supposedly simple molecule is involved in other functions, such as germ cell evolution, connections between Sertoli cells and germ cells in the blood-testis barrier, homodynamic contraction, and germ cell apoptosis. Moreover, NO is considered as a key factor in male fertility due to its widespread distribution in both normal and diseased testis tissue. The expression levels of eNOS and iNOS are different in the normal or pathological state, and overexpression of these two isoforms is a probable cause of fertility destructive processes, including low sperm motility and viability, disruption of testis tissue, induction of apoptosis in the germ cells, and, literally, perturbation of spermatogenesis. NO is an important factor in fabricating ovarian steroids, ovulation, and follicular apoptosis. iNOS is the major isoform in the ovulation process. In other words, increased activity of iNOS leads to an increased amount of NO, which triggers production of prostaglandins and runs inflammatory cascades which can cause follicular rupture and atresia. NO concentration elevation avoids steroid synthesis in the luteal and granulosa cells. Since NO is a major paracrine mediator of various biological processes and plays a key role in both the reproductive cycle and embryo implantation, oversynthesis of NO in the uterus results in toxicity and inflammation in epithelial cells and immunorejection of implantation.

Currently, the number of infertile couples has increased dramatically as different forms include decreased fertilization rates, increased levels of abortion and high level of morbidity. Increased NO level is one of the main chemical and physiopathological factors in this regard.

Due to different roles of NO and a variety of functions in the molecular signaling of male and female reproductive system, scientific community needs new technology and synthetic materials to detect, recognize, and control its level. Paradoxical role of NO in both pathologic and physiologic processes depends on general state of body and oxidant-antioxidant balance system, thus using the antioxidants acts to improve levels of NO.

Conflict of interest statement

There is no conflict of interest to declare.

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Authors' contributions

Ayoob Rostamzadeh designed this study and Amir Raoofi drafted the manuscript. Reza Ahmadi and Mahdi Heydari helped to draft the manuscript. All authors read and approved the final manuscript.

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