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# Physiological Effects of NO-cGMP Pathway on Ovarian Steroidogenesis in Rat

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**Abstract:** The present study was undertaken to determine whether NO involved in the regulation of ovarian steroidogenesis in female rats. The purpose of the present study was to investigate whether NO might inhibits ovarian steroidogenesis. Female rats were divided of five rats including, receiving, N-omega-nitro-L-arginine methyle ester (L-NAME), an inhibitor of NO synthesis, Trinitroglycerin (TNG), an NO donor, L-arginine, normal saline. After 21 days, concenteration of progesterone and oesteradiol were measured by Electerochemiluminescence (ECL). Result showed that: TNG significantly decreased concentration of progesterone (50%) and L-NAME partially increased ovarian oesteradiol, but not differ effect from that rats treated with trinitroglycerin.

**Key words:** Nitric oxide, nitroglycerin, progesterone, oesteradiol, ovary, ECL

## INTRODUCTION

Nitric Oxide (NO) is a free radical gas, with a half-life of only a few second (Ekerhovd *et al*., 2003). NO is a potent mediator with diverse roles in regulating cellular functions, including nitrosylation of proteins involved in signaling pathways (McCauly *et al*., 2005). In female reproductive organs NO has been recognized as an important regulatore of parturition, pregnancy, implantation, oviduct function and steroidogenesis. Moreover correlation between circulating NO and follicular development, implicates Luteinizing Hormone-Releasing Hormone (LHRH) in the regulation of NO synthesis and folliculogenesis, thereby functionally linking hypothalamic steructures with ovarian NO function (Fig. 1) (Calka, 2006).

NO is a multifunctional signal and important of cellular responses in a variety of tissues including those involved in human reproduction (Fig. 2) (AL-Hijji *et al*., 2003).

NO an active radical synthesized by Nitric Oxide Synthase (NOS) with the oxidation of amino acid L-arginine (Alderton *et al*., 2001). So far three different isoform of NOS have been identified, cloned and caracterised. While two of three (type I or neuronal and III or endothelial) are constitutively expressed in a variety of tissues, the expression of the third isoform (type II or inducible) can be induced by cytokines and some other agents (Moncada *et al*., 1991; Ignarro, 1991). This family enzymes are generally classified as constitutive, calcium dependent (Neuronal NOS, NOS 1 and endothelial NOS, NOS3) calcium independent (inducible NOS, NOS2) (McCauly *et al*., 2005). The three isoformes of NOS are products of separate genes that hare 50-60% amino acid homology. All NOS isoforms require Nicotinamid Adenine Dinucleotide Phosphate (NADPH) as an electron donor, as well as Flavin Adenine Dinucleotide (FAD) Flavin Mononucleotide (FMN)and Tetrahydrobiopterin (THB) for efficient generation of NO (Fig. 3) (Calka, 2006).

More recently, NOS has been found rat and rabbit ovary, where it has been shown to regulate steroidogenesis (Gobbetti *et al*., 1999). NO has been show to inhibit steroidogenesise in the rat testes (Adams *et al*., 1992). In both shown to be involved in the process and rate of ovulation (Gobbetti *et al*., 1992). NO is involved ovulation, but it is uncertain wether the mechanisms responsible are systemic or local. *In vivo* and in perfused ovaries, inhibition of NOS reduced valuation (Mitchell *et al*., 2004). All these finding, therefore, suggest that NO may have important implication in the physiology of the ovary by controlling several functions such as steroidogenesis ovulation and luteolysis (Gobbetti *et al*., 1992). In the present study we examined relations between NO and ovarian steroidogenesis. This study suggested that NO as an important factor, inhibited ovarian steroidogenesis.

## MATERIALS AND METHODS

A total 20 immature females Sprague-dawley rats, weighting 210±10 g, were used in the present study. They were cared for according to the guidelines of the animal

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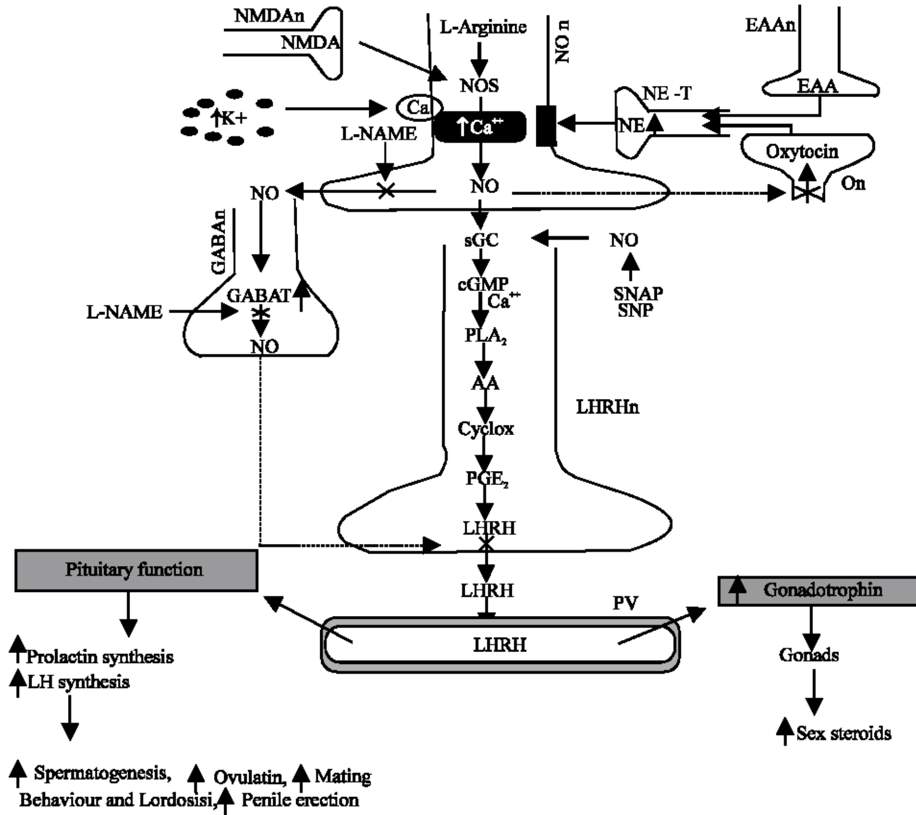


Fig. 1: Multiple mechanisms/pathways by which NO can regulate steroidogenesis. NO regulates the release of Luteinizing Hormone-releasing Hormone (LHRH) in response to oxytocin, Norepinephrine (NE) Excitatory Amino Acids (EAA) such as Glutamic Acid, γ-amino Butyric Acid (GABA), N-Methyl-D-Aspartate (NMDA) and extracellular potassium ($K^+$). LHRH in turn stimulates the pituitary and triggers the synthesis of prolactin and Luteinizing Hormone (LH) which subsequently regulate multiple reproductive functions. LHRH also stimulates the gonads via gonadotrophin release and this regulates the synthesis of sex steroids

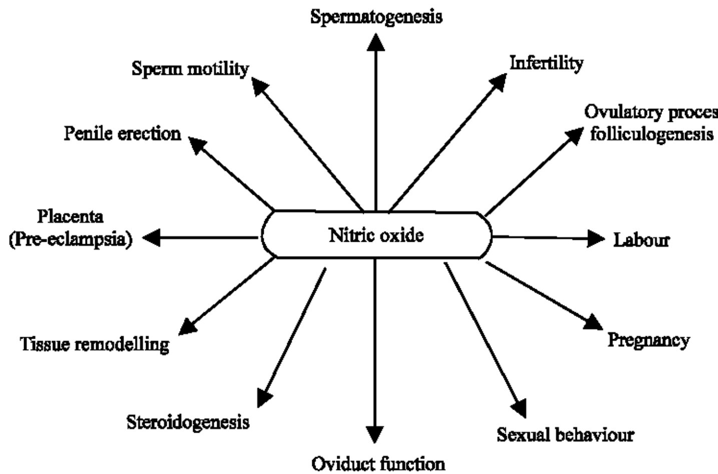


Fig. 2: Various reproductive processes regulated by nitric oxide

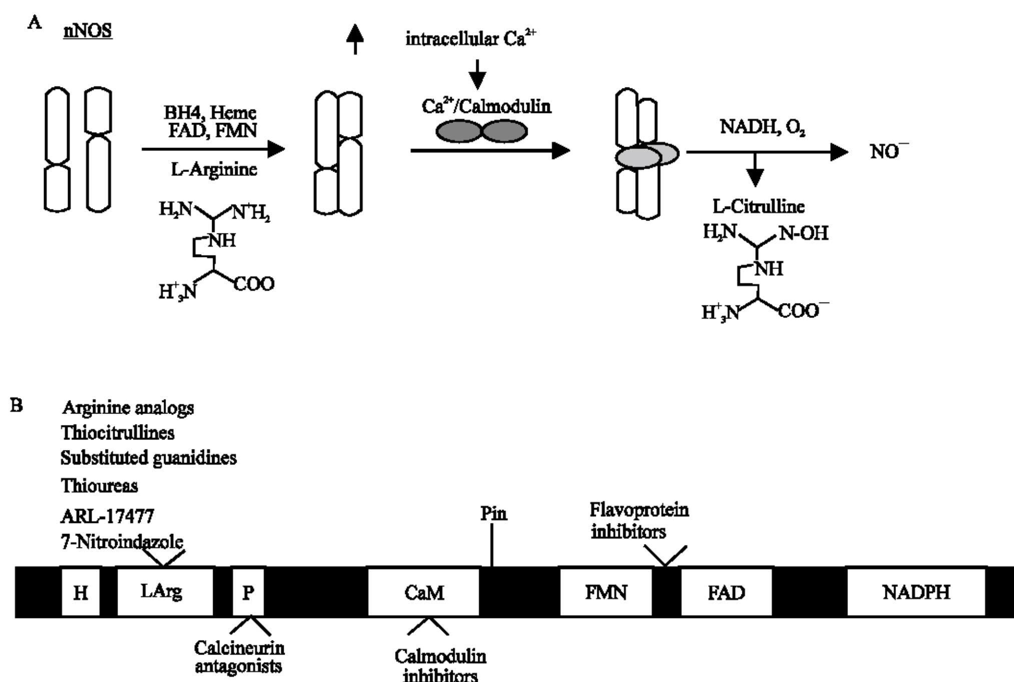


Fig. 3: Nitric oxide synthase. (A) NOS monomers loosely dimerize in the presence of tetrahydrobiopterin (BH4) heme, the flavoproteins, FMN and FAD and L-arginine. Rapid increase in intracellular calcium activates calmodulin. The calcium/calmodulin complex associates with the NOS dimers promoting tight dimerization and permitting the flow of electrons from the reductase moiety to the catalytic site where L-arginine is converted to L-citrulline and NO in the presence of NADPH and oxygen. (B) Nitric oxide synthase converts L-arginine to L-citrulline in the presence of tetrahydrobiopterin (BH4), oxygen, NADPH, heme and calmodulin. There are multiple regulatory sites on NOS that can be exploited for regulating enzyme activity

care. The rats were isolated in individual cages for a 2 weeks before the experimental procedure and allowed free access food and water. The animals were maintained on a 12 h light and 12 h dark at 28°C

Female rats divided in to 4 groups as follows:

Group A: 21 days usage of nitroglycerin (SC) 2.5 mg $kg^{-1}$ bw. per/day
Group B: 21 days usage of L-arginine (SC) 10 mg $kg^{-1}$ bw. per/day
Group C: 21 days usage of L-NAME (SC) 1 mg $kg^{-1}$ bw/per day
Group D: 21 days usage of normal saline (SC)

The rats receiving SC injection at 8:00 and 16:00 per day. After 21 days of treatment animals were anesthetized with diethy ether, heparnized and exanguinated. After concenteration of progesterone and oesteradiol were measured by Electerochemiluminescence (ECL).

**Statistics:** Differences were tested for significance using one-way analysis of variance (ANOVA) or students-test for paired or unpaired data where appropriate. A p-value of <0.05 was treated as significant.

**Electerochemiluminescence:** Immunossay for the *in vitro* quantitative of progesterone in human serum and plasma. The electerochemiluminescence immunoassay ECLIA is intended for use on the Roche Elecsys 1010/2010 and MODULAR ANALYTICS E170 (Elecsys module) immunoassay analyzers.

**Measurement of progesterone:** The analyzer automatically calculates the analyte concenteration of each sample (either in nmol L, ng $mL^{-1}$ or in μg $L^{-1}$).

**Conversion factors:** nmol L×0.314 = ng $mL^{-1}$ (μg $k^{-1}L^{-1}$) ng $mL^{-1}$×3.18 = nmol $L^{-1}$.

**Measuring range:** 0.095-191 nm p $L^{-1}$ or 0.030-60.00 ng $mL^{-1}$ (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as <0.095 nmol $L^{-1}$ or <0.030 ng $mL^{-1}$ values above the measuring range

are reported as 191 nmol $L^{-1}$ or 60.00 ng $mL^{-1}$ (or up to 1910 n mol $L^{-1}$ or 600 ng $mL^{-1}$ for 10-fold diluted samples).

**Measuring of oesteradiol:** The analyzer automatically calculates the analyte concenteration of each sample (either in pmol $L^{-1}$ pg m $L^{-1}$, ng $L^{-1}$ or with MODULAR ANALYTICS E170 analyzer).

**Conversion factor:** pmol $L^{-1}$×0.273 = pg m $L^{-1}$ (ng $L^{-1}$) pg $mL^{-1}$×3.67 = pmol $L^{-1}$.

**Measuring range:** 18.4-15, 781 pmol $L^{-1}$ (5.00-4300 pg $mL^{-1}$) ((defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as <18.4 pmol $L^{-1}$ or <5.00 p $mL^{-1}$. values above the measuring range are reported as >15, 781 pmol $L^{-1}$ or >4300 pg $mL^{-1}$ (or up to 78, 905 or 21, 500 pg $mL^{-1}$ for 5-fold diluted samples).

## RESULTS

**Effect of nitric oxide on ovarian progesterone synthesis:** Nitroglycerin, a donor of NO decreased the ovarian progesterone secretion, showing (50%). Wherease L-NAME and L-arginine did not showing effect from that rats treated with normal saline. Indicating that TNG inhibits progesterone secretion by ovary (Fig. 4). NO induces prostaglandin $E_2$ release that activates exocytpsis of LHRH secretory granules in to the portal vessels to induce pulsatile LH release, through a calcum-dependent, cGMP-independent mechanism (Rettori *et al*., 1993; Pinilla *et al*., 1998). LHRH induces progesterone release, probably progesterone by negative feed back mechanism effects on hypothalamic and reduses LH-RH release that lower progesterone secretion.

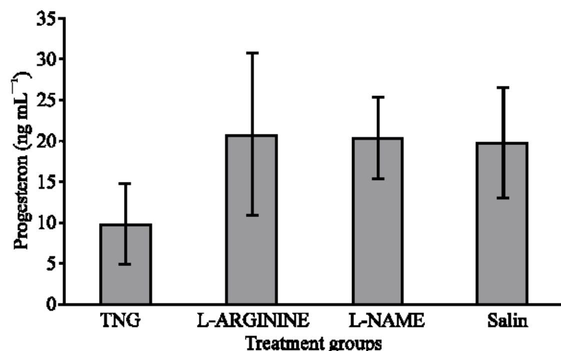


Fig. 4: Effect of nitric oxide on ovarian progesterone synthesis

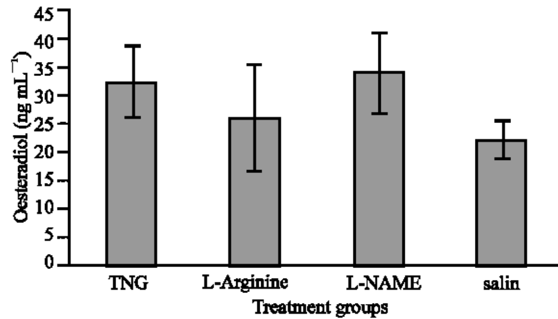


Fig. 5: Effect of nitric oxide on ovarian oesteradiol synthesis

**Effect of nitric oxide on ovarian oesteradiol synthesis:** The oesteradiol secretion by ovaries was lowely increased by L-NAME something like trinitroglycerin and L-Arginine. Indicating that L-NAME stimuluses oesteradiol secretion by ovary (Fig. 5). L-NAME led elevation of the production of oesteradiol, but not of progesterone.

## DISCUSSION

We investigated the effect of NO on ovarian steroidogenesis. TNG reduced the progesterone production by rat ovary, suggesting that NO inhibits the ovarian synthesis of progesterone. TNG had significant effect on oesteradiol production by rat ovary. NO as a local modulature of steroidogenesis (Savlemini *et al*., 1993). Modulation of NOS in these has also been shown to alter ovarian steroidogenesis (Olson *et al*., 1996). NO has been shown to inhibit steroidogenesis in the rat testes (Adams *et al*., 1992) femal rat decreases progesterone synthesis by the ovarian (Dong *et al*., 1999) and inhibit ovarian steroidogenesis from immature cultured rat granulose cells (Ishimaru *et al*., 2001). L-NAME led to elevation of the production oesteradiol, but not of progesterone. The observation in this study are consistent with previou studies in which NO has been shown to reduce oesteradiol synthesis in human granulose-luteal cells (Voorhis *et al*., 1994b) rat luteinized ovarian cells (Olson *et al*., 1996) and rat leydig cells (Punta *et al*., 1996). In the present study, it was found that NO regulated progesterone and oesteradiol production by rat ovaries, suggesting that NO may contribute in part to the regulatory influence on steoid hormone synthesis. In conclusion, this study revealed that NO inhibits progesterone secretion by rat ovaries. these finding may have important significance in the understanding of normal physiological function of the ovary, including the control of ovarian steroidogenesis.

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