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Role of Nitric Oxide in Animal Reproduction: A Review

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Abstract

Nitric oxide has emerged as an important regulator of reproductive bio-physiological system and plays a crucial role in reproduction. NO is synthesized from L-arginine by enzyme nitric oxide synthase, which exists in multiple isoforms in a wide range of mammalian cells and implicated in the control of gonadotrophin secretion at both hypothalamic and hypophyseal levels. NO is hypothesized to play a role in steroidogenesis, follicular development and growth, follicular apoptosis, maturation of oocytes, ovulation and luteolysis, and maintenance of pregnancy as well as control of male reproductive functions like spermatogenesis, penile erection, sexual behavior, sperm motility. While considerable work lies ahead in unraveling the role of NO at the peripheral, cellular and molecular level in the domestic animal reproduction, ûndings presented in this review provide a general overview of growing appreciation of NO as a vital molecule controlling hypothalamic–pituitary–gonadal (HPG) axis. Further investigations are warranted to explore the role and modulations of NO under normal and abnormal reproductive conditions in domestic animals.

Keywords: Estrous cycle, follicle, nitric oxide (NO), pregnancy, reproduction.

Introduction

The free radical gas, nitric oxide (NO) is now known to be an important biological messenger in animal reproduction and control many physiological processes within vasculature. NO is endogenously synthesized from L-arginine by the action of NO Synthase (NOS) forming citrulline as a co-product. NO induces vasodilation, inhibits platelets aggregation, inhibits smooth muscle proliferation and migration, regulates apoptosis and maintains endothelial cell barrier function. Apart from this NO is an important regulator of the biology and physiology of the reproductive system. Indeed, in the past 10 years, NO has established itself as a polyvalent molecule, which plays a decisive role in regulating multiple functions within the female as well as the male reproductive system (Rosselli *et al*., 1998). So far, studies have been conducted mainly on lab models and human beings and *in vitro* studies to investigate the role of NO

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in reproductive function. Estradiol has been reported to induce eNOS (endothelial NO synthase) in cultured endothelial cells (McNeill *et al*., 1999) and estrogen induced vasodilation and increased uterine blood flow is mediated via NO generation (Van-Buren *et al*., 1992). Stable metabolites in blood are nitrite/nitrate as indicators of total NO production in body fluids. NO generated by neurons acts as a neurotransmitter whereas NO generated by macrophages in response to invading microbes acts as antimicrobial agent. Since, neurons, blood vessels and cells of immune system are integral parts of reproductive organs and in view of the important functional role that NO plays in those systems.

Types of the NOS

There are three forms of the NOS found in variety of cell types (Van Voorhis *et al*., 1994). Brain NOS (b NOS) or neuronal NOS (n NOS or NOS1) and endothelial NOS (e NOS or NOS3) also referred as constitutive NOS (cNOS) are responsible for continuous basal release of NO and both require calcium/ calmodulin for activation (Griffith and Stuehr, 1995). A third isoform, an inducible calcium independent form (iNOS or NOS2) is not normally expressed unless stimulated by inflammatory cytokines and lipopolysaccharides (Morris and Billar, 1994). The three isoforms of NOS are the products of separate genes which share 50-60% amino acid homology (Nathan and Xie, 1994).

Nitric Oxide as a messenger at hypothalamic-pituitary-gonads axis:

Hypothalamus

The neurons containing NOS activity are in close proximity to GnRH/LHRH neurons in hypothalamus suggesting that NO play an important role in GnRH secretion (Bhat *et al.,* 1995). NO stimulates GnRH secretion by activation of heme containing enzyme, guanylate cyclase and through the activation of neuropeptide Y (Bonavera and Kalra, 1996). An interaction between NO and opioids has also been proposed. The administration of naloxone (an opioid antagonist) enhances NOS activity, whereas naltrexone blocks the inhibitory effect of ß-endorphin on LHRH release and NOS activity in the rat (Faletti *et al.,* 1999). It is postulated that the NO released from the NOergic neurons, near the LHRH neuronal terminals, increases the intracellular free calcium required to activate phospholypase A_2 (PLA_2) which subsequently causes the conversion of membrane phospholipids in the LHRH terminal to arachidonate, which then can be converted to PGE_2 á via the activated cyclooxygenase. The released PGE_2 á activates adenyl cyclase causing an increase in cAMP release, which activates protein kinase-A, leading to exocytosis of LHRH secretory granules into the hypophyseal portal capillaries for transmission to the anterior pituitary gland (Canteros *et al.,* 1996).

Norepinephrine (NE) has been shown to be a powerful releaser of LHRH. NE stimulates the release of NO from the

NOergic neurons by $á_1$ adrenergic receptors. Activation of the $á_1$ receptors is postulated to increase intracellular (Ca^{2+}) that combines with calmodulin to activate NOS leading to generation of NO. Glutamic acid (GA), acting at least in part through n-methyl-d-aspartate (NMDA) receptors, also plays a physiologically significant role in controlling the release of GnRH. GA acts by stimulation of the noradrenergic terminals in the medial basal hypothalamus to release NE which then initiates NO release leading stimulation of GnRH release (Kamat *et al.,* 1995). One of the few receptors to be identified on LHRH/GnRH neurons is the gamma amino butyric acid a ($GABA_a$) receptor. GABA suppressed LHRH release by blocking the response of LHRH neuronal terminals to NO. Additional experiments showed that NO stimulated the release of GABA, thereby providing an inhibitory feed-forward pathway to inhibit the pulsatile release of LHRH initiated by NE (Seilicovich *et al.,* 1995).

Pituitary gland

NOS is localized in certain pituitary cells, principally the folliculostellate cells, which are modified glial cells that bear a resemblance to macrophages, and also in the LH gonadotropes as revealed by immunocytochemistry. Though LHRH has been known to be candidate in the release of LH and FSH from pituitary and play a critical role in control of gonadotropin secretion. Presumably, NO activates guanylyl cyclase (GC) causing the release of cGMP, which can release both gonadotropins, presumably by acting on protein kinase-G (Yu *et al.,* 1997). Pinilla *et al*. (1998) has noticed that the NO acting at the pituitary level stimulates gonadotrophin secretion through a specific calcium-dependent, C-GMP-independent mechanism.

Ovarian activity

NO is synthesized by ovary and is hypothesized to play a role in follicular development, steroidogenesis, ovulation and luteolysis (Van Voorhis *et aI*., 1994). The role of NO in regulating ovarian function was evident from the observation that NO synthesis increases with follicular development concurrent with estradiol 17-â and progesterone concentrations during spontaneous menstrual cycles (Rosselli *et al*., 1994). A direct effect of NO on production of sex steroids is however still unclear.

Rosselli *et al*. (1994) first reported the circulating NO2/NO3 levels, indicators of total NO generated in body fluids increase with development of follicle and decrease at ovulation. In goats the plasma NO level ranged from 10.6 to 11.8 µM (Chandra, 2004), however higher concentrations have been reported in other species, viz., 50.36 µM in cattle, 40.88 µM in buffaloes and 39.5 µM in chicken (Sastry *et al*., 2002). NO has also been reported to play a role in luteolysis in vitro in the rabbit (Gobbetti *et al*., 1999) and cow (Skarzynski and Okuda, 2000). Skarzynski *et al*. (2003)

concluded that NO enhanced PGF2á-induced luteolysis, since NOS inhibitor prevents a PGF2á-induced luteolysis by dissociated bovine luteal cells *in vitro.* Estradiol has been reported to induce eNOS in cultured endothelial cells (McNeill *et al*., 1999) and shown that estrogen induced vasodilation and increased uterine blood flow is mediated via NO generation (Van-Buren *et al*., 1992; Rosselli *et al*., 1998). Keator *et al*. (2008) concluded that NO can either stimulate or inhibit luteal function in sheep, depending on the concentration of NO reaching the CL. Role of NO in the CL is dualistic and that the luteotropic and luteolytic actions of NO are dose dependent in sheep.

Testis

The testicular cells are equipped with NO-cGMP pathway that participates in different testicular functions, such as spermatogenesis and steroidogenesis. Both sertoli and leydig cells express eNOS at all stages of spermatogenesis. eNOS activity is absent in normal germ cells but present in degenerating or apoptotic intra-epithelial germ cells. It is also expressed in prematurely shed spermatocytes and spermatids. This suggests eNOS is important for spermatogenesis and sperm cell degeneration (Zini *et al.,* 1995). Most of the studies indicated that NO is a negative regulator of testosterone synthesis.

Uterus

The role of NO in regulating the pathophysiology and biology of uterus has recently gained considerable attention (Rosselli *et al*., 1998). In the endometrium, where changes in vascular function occur throughout the estrous cycle and pregnancy, this molecule is therefore likely to play an important role (Cameron and Campbell, 1998). Regulation of NO in pregnancy and labour suggest that iNOS, but not eNOS, is key player in regulating contractile function (Rosselli *et al*., 1998) and factor regulating iNOS activity is still unclear. In this aspect role of cytokines is of utmost importance as they are simultaneously increased during pregnancy. NO may also regulate PG secretion for implantation, since NOS inhibitors and NOS gene knockout also prevent implantation in rodents (Saxena *et al*., 2000). NOS is regulated by steroids and uterine NOS and NO decrease just prior to parturition (Zhang *et al*., 2000), NOS activity increases in rat uterus during pregnancy and falls at term (Natuzzi *et al*., 1993). Similar up regulation of NOS activity in the uterus during pregnancy has been demonstrated in rabbits also (Sladek *et al*., 1993). Furthermore, NO may be involved in embryo hatching to establish pregnancy, since embryo hatching occur on day 8 in cows as endometrial NOS increases at this time indicating the role of NO in embryo hatching (Welter *et al*., 2004). Thus, NO is important for implantation (Cheon *et al*., 2002), the increase in uterine arterial blood flow during pregnancy (Lowe, 2000) and subsequently the maintenance of pregnancy (Saxena *et al*., 2000).

As gestation advances serum NO profiles increases, indicating a vital role of NO during pregnancy in buffalo (Kumar, 2011) and goat (Sarath *et al*., 2010). NO causes vasodilatation and support the increase in uterine blood flow (Van-Buren *et al*., 1992) and also acts as a uterine relaxant contributing to uterine quiescence during pregnancy (Sladek *et al*., 1997) which is essential to maintain pregnancy. Arginine, the main source of NO (Lowe, 2000), increases in uterine fluids during gestation (Kweon *et al*., 2003). Placental NO synthase increases during early pregnancy in pigs (Self *et al*., 2003) which is responsible for endogenous production of NO. NO participates in vascular tone regulation, cellular respiration, proliferation, angiogenesis, apoptosis and gene expression in placenta. Indeed NO actively participates in trophoblast invasion, placental and embryonic development and represents the main vasodilator in this tissue. NO has important role in vasculogenesis and angiogenesis which depends on the expression of several signaling molecules such as Vascular endothelial growth factor (VEGF) and its receptors VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1), transforming growth factor â-1 (TGF â-1), angiopoietin (Ang-) 1 and 2 by exerting their effects in part through NO synthesis (Krause *et al.,* 2011).

Nitric Oxide and other reproductive functions

Penile erection

NO is a major physiological player of erectile function. NOS activity is localized in the rat penile neurons innervating the corpora cavernosa and to neuronal plexus in the adventitial layer of penile arteries (Burnett *et al.,* 1992). Endothelium of penile vasculature and sinusoidal epithelium within corpora cavernosa is also rich in eNOS. Neuronal NOS together with isoform eNOS seems to play a decisive role in penile erection.

Sexual behavior

NO appears to be present in all parts of the male reproductive system as well, and would undoubtedly play a role in testicular, epididymal and vas deferens function. LHRH controls lordosis behavior in the female rat and is also involved in mediating male sex behavior. Studies *in vivo* have shown that NO stimulates the release of LHRH that induces sex behavior. This behavior can be stimulated by injection of NP and is blocked by inhibitors of NOS. Apparently, there are two LHRH neuronal systems: one, with axons terminating on the hypophyseal portal vessels, the other with axons terminating on neurons which mediate sex behavior (Mani *et al.,* 1994).

Role of NO in sperm motility

Low concentrations of NO cause a significant increase in capacitation (Zini *et al.,* 1995) and zona pellucida binding (Sengoku *et al.,* 1998). Previously it was thought that NO reduces sperm motility, possibly by a mechanism involving inhibition of cellular respiration

independent of an elevation of intracellular cGMP. However, recently Miraglia *et al.* (2011) reported that it stimulates human sperm motility via the activation of sGC, the subsequent synthesis of cGMP, and the activation of cGMP-dependent protein kinases.

Conclusion

Studies cited in this review provide evidence that diffusible free radical gas NO functions as an important mediator in action of hormones and neurotransmitters which are vital for the regulation of animal reproduction like folliculogenesis, steroidogenesis, ovulation, oviduct function, sexual behavior, pregnancy, placental function, labor, infertility etc. NO is involved in control of LH surge and thus ovulation. A number of stimulatory and inhibitory neurotransmitters impinge on NOS neurons in hypothalamus, controlling the release of NO. Despite a large number of studies addressing the role of NO in human and rat reproduction, further investigations are warranted to explore the role and modulations of NO under normal and abnormal reproductive conditions in domestic animals as well which may offer not only the better understanding of reproductive events but certain promising corrective therapies too.

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