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Review Article

The Nitric Oxide System in Equine Reproduction: Current Status and Future Directions

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ABSTRACT

The nitric oxide (NO) system is fairly ubiquitous and is involved in a wide range of physiological and pathologic processes. Besides its well-established roles in vasodilation, platelet aggregation, neurotransmission, and cytotoxicity, it has been shown to regulate reproduction in several animal species. In horses, the NO system in reproduction is a relatively recent research area. Information available in the mare is limited to the presence of the NO system in the ovaries and uterus, a possible role of the system in the regulation of normal ovarian and uterine functions, and its association with various reproductive abnormalities. Little is known about the NO system in the stallion, except for expression of nitric oxide synthase isoforms in the testis and epididymis and the effects of NO on cryopreserved semen. However, there are clear indications from research to date that NO may be an important regulator of reproduction in both stallions and mares. This review is aimed at summarizing the available information on the NO system in equine reproduction and identifying gaps in the literature that need to be addressed in the future.

1. Introduction

Nitric oxide (NO) is synthesized in biological systems from L-arginine [1]. The reaction is catalyzed by nitric oxide synthase (NOS), an enzyme that exists in multiple isoforms in nature. Three of these isoforms have been identified in mammals, namely neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). The former two are constitutively expressed and collectively referred to as constitutive NOS [2]. The enzyme activities of nNOS and eNOS are regulated by calcium (Ca^{2+}) and calmodulin, whereas the enzyme activity of iNOS is largely or completely Ca^{2+} independent [3]. Nitric oxide is a versatile molecule with an established role in a wide range of biological processes including, but not limited to, vasodilation [4], platelet aggregation [5], neurotransmission [6], and cytotoxicity [7]. The biological actions of NO are mediated either through the guanosine cyclic 3′,5′-monophosphate pathway or occur as a result of the direct effect of NO [8]. Since the initial implication of NO in regulation of penile erection in rabbits by Ignarro et al [9], the molecule has been shown to be involved in regulation of several reproductive processes in different species [10,11]. In horses, NO in reproduction is a relatively recent research area, in which NO synthesis and regulation have not been fully elucidated (Fig. 1). Studies so far have mainly focused on investigating the presence of the NO system and its role in the ovaries and uterus in the female and the expression of the NO system in the testis and epididymis and its effects on cryopreserved semen in the male. To date, a review of the available literature on the NO system in equine reproduction is lacking. This review is aimed at summarizing the literature and identifying important gaps that need to be addressed in the future.

2. The NO System in Female Reproduction

The role of NO as a regulator of female reproduction has been investigated extensively in several laboratory animal

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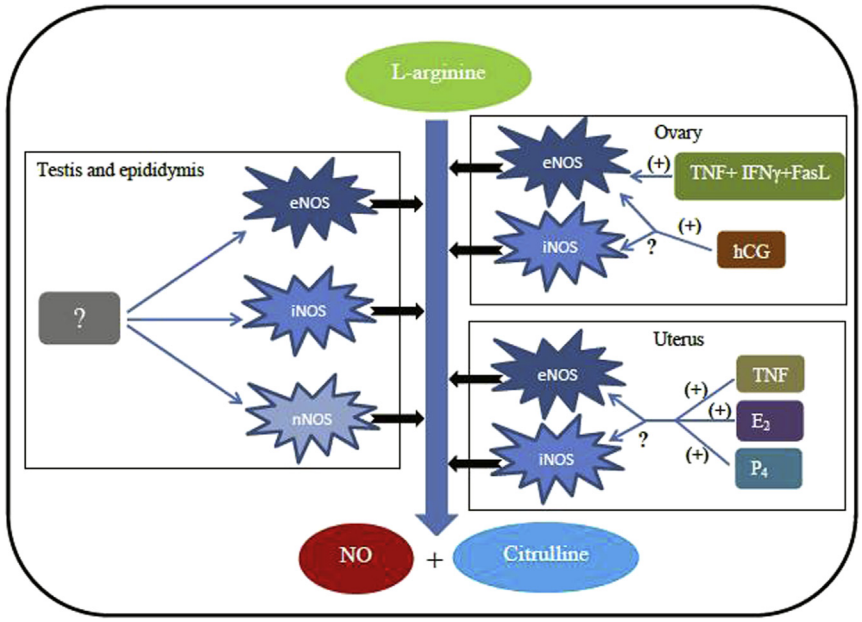

Fig. 1. A schematic diagram depicting the potential pathways and regulation of NO synthesis in the equine reproductive system (based on references [12,32,33,38,41–43,58] and [59]). Nitric oxide (NO) is synthesized in the equine reproductive tissues under the action of one or more of the nitric oxide synthase (NOS) isoforms. In the ovary, a combination of TNF, IFNγ, and FasL has been shown to upregulate luteal eNOS messenger RNA expression. Human chorionic gonadotropin (hCG) has been demonstrated to increase follicular NO production, but its target NOS isoform remains unknown. In the uterus, TNF, E_2, and P_4 have been shown to increase NO synthesis, but the target isoform(s) have not yet been identified. In the testis and epididymis, all the three NOS isoforms are expressed, but there is no information on the regulation of NO synthesis. E_2, estradiol; eNOS, endothelial nitric oxide synthase; FasL, Fas ligand; IFNγ, interferon gamma; iNOS, inducible nitric oxide synthase; nNOS, neuronal nitric oxide synthase; P_4, progesterone; TNF, tumor necrosis factor.

species and humans. There is abundant evidence supporting a multifaceted physiological role of NO in the regulation of gonadotropin secretion, steroidogenesis, follicular development, ovulation, luteal development and regression, pregnancy, parturition or labor, and oviductal, cervical, and vaginal function in these species [10,11]. However, it is a fairly new area of research in farm animal species in general and horses in particular. In mares, studies so far have focused on investigating the presence of the NO system in the ovary and uterus and its possible role in the modulation of ovarian and uterine function (Table 1).

2.1. The NO System in the Ovary

The role of NO in the regulation of ovarian function in mares is not completely understood. However, research findings from studies so far are suggestive of its involvement in various reproductive processes that occur within the ovary.

Table 1
Reproductive processes potentially modulated by NO in mares.

Organ	Process	References
Ovary	Follicular growth and ovulation	[12,14]
	Follicular steroidogenesis (E_2 and P_4)	[12,18]
	Follicular blood flow	[24]
	Luteal P_4 production	[32]
	Luteal angiogenesis	[32,36]
Uterus	Angiogenesis and blood flow	[39,43,44,46]
	PGE2 and PGF2α production	[47]
	Postmating-induced endometritis	[48–50]

Abbreviations: E_2, estradiol; NO, nitric oxide; P_4, progesterone; PGE2, prostaglandin E2; PGF2α, prostaglandin F2alpha.

2.1.1. The Role of NO in Follicular Development and Function

Although definitive proof of an intrafollicular NO synthase system in the mare is still lacking, there is some information on the concentration of NO in the preovulatory follicle and how it is influenced by treatments that affect follicular growth and ovulation. Administration of human chorionic gonadotropin (hCG) resulted in an increase in intrafollicular NO concentration [12], whereas that of equine pituitary extract had no effect [13]. It has been demonstrated that treatment of mares with L-NG-nitroarginine methyl ester (L-NAME), a nonspecific inhibitor of NOS, or aminoguanidine (AG), a relatively specific inhibitor of iNOS, delayed ovulation after induction with hCG. The median intervals from hCG administration to ovulation were 84 and 54 hours in L-NAME- and AG-treated mares, respectively, which were significantly longer than the interval (42 hours) in saline-treated control mares [14]. This response is, however, different from the complete blockage of ovulation reported with the use of NOS inhibitors in rats [15] and rabbits [16] or in NOS knockout mouse models [17].

Nitric oxide has also been shown to modulate follicular steroidogenesis in various species [10,11]. In mares, there are indications that NO may regulate follicular progesterone and estradiol production. Ovulation induction with hCG was associated with a concurrent increase in NO and