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# 소 자궁에서 endothelial nitric oxide synthase(NOS) 및 inducible NOS의 발현

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# Expression and localization of endothelial and inducible nitric oxide synthase in bovine uterus

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**Abstract :** Nitric oxide synthase (NOS) has been reported in uterus. We examined the expression of the NOS isoforms, constitutive endothelial (eNOS) and inducible NOS (iNOS), in bovine uterus by immunohistochemistry. eNOS immunoreactivity was localized predominantly to the endothelial cells that line uterine microvessels and to endometrial glandular epithelial cells, but was barely detectable in endometrial stromal cells. iNOS immunostaining was detected in glandular epithelial and stromal cells in the endometrium and in the endothelial cells of myometrial blood vessels. These findings suggest that both eNOS and iNOS may play important roles in the physiology of the uterus, possibly by generating NO.

**Key words :** bovine, nitric oxide synthase, uterus

## Introduction

Nitric oxide (NO) is a reactive free radical gas that is derived from L-arginine by the action of NO synthase (NOS) [20]. NO has diverse roles, including intracellular signaling and vasoregulation [1] and exists in a variety of isoforms. A constitutive, calcium-dependent isoform (cNOS) is activated rapidly by agonists that elevate intracellular free calcium, and is found in endothelial cells (eNOS) and brain (nNOS) [11]. A calcium-independent inducible isoform (iNOS) can be induced after several hours of immunological stimulation and is detectable in macrophages, neutrophils, and endothelial cells [7].

Several studies have identified different isoforms of NOS in several reproductive tissues. Uterine expression of NOS is influenced by both endogenous and exogenous sex steroids [2, 21] and eNOS expression was found to be elevated in the uterine tissue of rats during proestrus and estrus [2]. In estrogen-treated ovariectomized sheep, increased expression of eNOS was detected in myometrium, but not in the endometrium [3]. Treatment of mice with a combination of estrogen and progesterone enhanced eNOS expression in myometrium and iNOS expression in uterine glands, the stroma, and myometrium, relative to mice treated only with estrogen [10]. In addition, estrogen and progesterone can induce iNOS expression in different cell populations. Specifically, estrogen induces iNOS expression in myometrial mast cells, whereas progesterone induces iNOS expression in epithelial cells [4]. In rat, several studies have failed to demonstrate that estrogen administration affects uterine NOS activity or NOS-related gene expression [22]. In humans, several investigators have reported on the

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uterine expression of NOS. Studies examining uterine expression of NOS have focused on the identify of the predominant NOS isoforms present and their cellular localization [16, 17, 18, 19]. In ruminants, including bovids, the expression of NOS isoforms, particularly in the endometrium and myometrium during the secretory phase, has not been well described.

The objective of this study was to describe the immunohistochemical localization of eNOS and iNOS in the endometrium and myometrium of bovine uterus during the secretory phase.

## Materials and Methods

Samples of bovine uterus from 2- to 3-year-old cows were collected in a local slaughterhouse. Among these samples, the uteri of three animals that were killed during the secretory phase were selected for histological examination.

Immunostaining for eNOS and iNOS was performed as described previously [9]. Briefly, paraffin-embedded sections (5 μm) of bovine uterus were deparaffinized and treated with 0.3% hydrogen peroxide in methyl alcohol for 30 minutes to block endogenous peroxidase activity. After three washes in phosphate-buffered saline (PBS), the sections were incubated with 10% normal goat serum and thereafter incubated with rabbit anti-eNOS or rabbit anti-iNOS antisera (1:200 dilution; Transduction Laboratories, Lexington, KY) for 1 hour at room temperature. After three washes in PBS, the appropriate biotinylated secondary antibody and avidinbiotin peroxidase complex (Vector Elite, Vector Labs, Burlingame, CA) were added sequentially. The peroxidase was reacted with diaminobenzidine as a substrate. Before being mounted, the sections were counterstained with hematoxylin.

## Results

eNOS immunoreactivity was detected predominantly

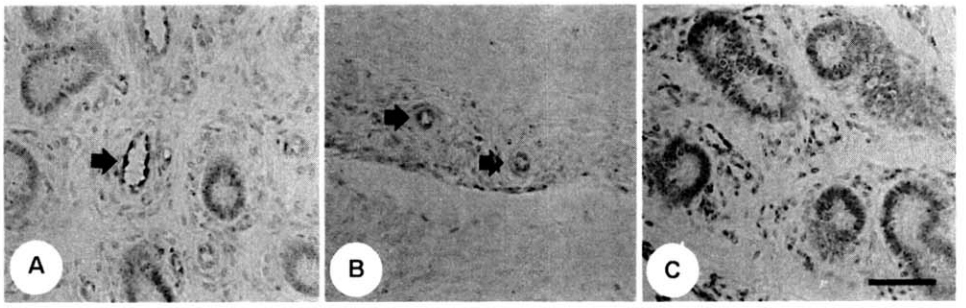


**Fig. 1.** eNOS immunostaining in bovine uterus. eNOS immunoreactivity was detected in vascular endothelial cells in endometrium (A, arrow) and myometrium (B, arrows) and in endothelial glandular cells (C), but not in smooth muscle cells (B). Sections counterstained with hematoxylin. Scale bar represents 30 μm.

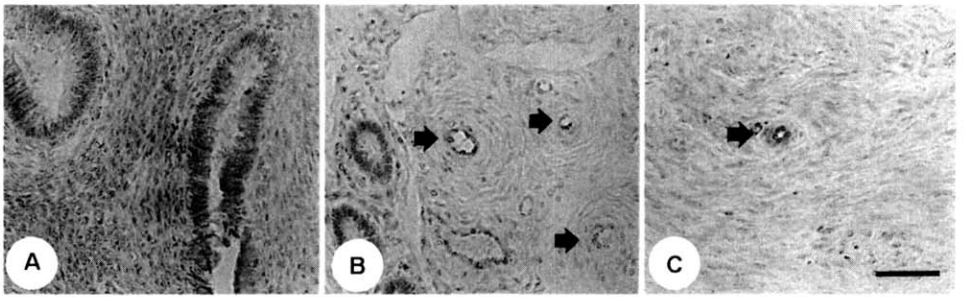


**Fig. 2.** iNOS immunostaining in bovine uterus. iNOS immunoreactivity was detected in glandular epithelial (A) and vascular endothelial cells (B, arrows) in the endometrium. In myometrium, iNOS immunoreactivity in the epithelium of blood vessels was intense (C, arrow). Sections counterstained with hematoxylin. Scale bar represents 30 μm.

**Table 1.** Immunohistochemical localization of endothelial (eNOS) and inducible (iNOS) isoforms of nitric oxide synthase (NOS) in the bovine uterus during the secretory phase. Immunoreactivity was categorized as either absent (−), moderately intense (+), or highly intense (++)

| | Cell type | eNOS | iNOS |
|---|---|---|---|
| Endometrium | Glandular epithelial cells | + | + |
| | Endothelium of blood vessels | ++ | ++ |
| | Basal layer | + | + |
| Myometrium | Smooth muscle cells | − | − |
| | Endothelium of blood vessels | ++ | ++ |

in the endothelial cells that line the microvessels in the endometrium (Fig. 1A, arrow) and myometrium (Fig. 1B, arrows). Some glandular epithelial cells in the endometrium strongly expressed eNOS during the secretory phase (Fig. 1C). There were a few, weakly stained eNOS-immunopositive cells in endometrial stroma cells.

iNOS immunoreactivity was localized predominantly to the glandular and stromal cells of the endometrium (Fig. 2A and B), and was intense in the epithelium of blood vessels in endometrium (Fig. 2B, arrows) and myometrium (Fig. 2C, arrow). All glandular cells in the endometrium were immunopositive for iNOS and the pattern of iNOS immunostaining in these cells was uniform.

The results of the immunostaining for eNOS and iNOS are summarized in Table 1.

## Discussion

This study demonstrated that the eNOS and iNOS isoforms of NOS are both expressed in the bovine uterus during the secretory phase. Several investigators have reported that different NOS isoforms are expressed in human endometrium and myometrium [16, 17, 18, 19]. For example, Telfer *et al.* [17] found eNOS immunoreactivity in endothelial, myometrial, and endometrial cells in paraffin-embedded sections obtained after hysterectomy. Specifically, eNOS immunoreactivity was found in the endothelial cells of blood vessels but not in epithelial cells. In addition, Tabibzadeh *et al.* [15] have suggested that iNOS-activated cytokines may play a role in the necrosis within endometrial glands that occurs during menstruation.

The activational effects on the endometrium of steroid hormones such as progesterone are due in part to the paracrine actions of cytokines. Such cytokines are produced by lymphoid cells within the stroma and modulate the functions of epithelial cells [12]. Cytokines such as tumor necrosis factor alpha (TNFα) and interleukin-1 (IL-1) can induce iNOS expression [13] and induce iNOS expression in the secretory endometrium immediately prior to menstruation (premenstrual period) [5, 14].

During the secretory phase, NOS is expressed in the glandular and stromal cells of human and mouse endometrium [4, 6, 8, 17]. In myometrium, iNOS immunoreactivity is intense in the epithelium of blood vessels [6]. In this study, iNOS immunoreactivity was not found in the smooth muscle cells of bovine myometrium during the secretory phase. The pattern of iNOS immunostaining in the glandular cells was the same as that reported for other mammals. Telfer *et al.* [16] reported that eNOS immunoreactivity was localized to the endometrial stroma and myometrial blood vessels, with weak immunostaining in glandular epithelial cells in the secretory endometrium. In this study, however, eNOS immunoreactivity was intense in the glandular epithelial cells of endometrium. In endometrial stroma cells, eNOS immunostaining was barely detectable. The evidence of NOS-like immunoreactivity in the secretory phase uterus is supported by the detection of NOS protein by immunoblot analysis. In addition, iNOS mRNA has been detected in human glandular epithelial cells by RT-PCR [17] and Northern blot analysis [18]. These findings support the hypothesis that eNOS and iNOS regulate the function of the endometrium in the late luteal phase under normal physiological conditions.

In conclusion, this study revealed that NOS is detectable in various types of cell in the myometrium and endometrium of the bovine uterus. The exact role that NOS plays in reproductive tissues such as uterus remains to be determined.

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