ANATOMIA HISTOLOGIA EMBRYOLOGIA
JOURNAL OF VETERINARY MEDICINE
Anatomia, Histologia, Embryologia

ORIGINAL ARTICLE

Immunohistochemical Expression of Nitric Oxide Synthase Enzymes (iNOS, eNOS, nNOS) in the Estrual and Luteal Phases of the Sexual Cycle in the Cow Oviduct

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With 12 figures and 3 tables

Received August 2012; accepted for publication December 2012

doi: 10.1111/ahe.12027

Summary

This study was aimed to determine staining intensity, cellular localization and distribution of the nitric oxide synthase (NOS) enzymes during the sexual cycle in the cow oviduct. Oviduct samples belonging to 20 cows, 10 of which were in the estrual phase and 10 in the luteal phase of the sexual cycle, were examined by an immunohistochemical procedure to determine the presence of the NOS enzymes. In the epithelial cells of the isthmus, endothelial NOS (eNOS) expression showed a strong positive reaction during the estrual phase and a weak positive reaction during the luteal phase in the endothelium and smooth muscle of the blood vessels found in the serosa and lamina propria. eNOS expression was not observed in the epithelium of either the ampulla or the fimbria in the two particular phases of the sexual cycle. The eNOS reactions observed in the blood vessel wall in these regions were stronger during the estrual phase. eNOS activity was not observed in the tunica muscularis in any of the regions of the oviduct. During the estrual phase, it was observed that inducible NOS expression showed a stronger positive reaction in the epithelium and muscle layer of the isthmus and ampulla and in the epithelium of the fimbria, compared to the luteal phase. Neuronal NOS immunoreactivity was observed in the epithelial cells of all oviduct regions and in the muscle layer of the isthmus and ampulla and did not display any significant difference between the estrual and luteal phases.

Introduction

Nitric oxide (NO), found extensively in the organism, is a molecule naturally synthesized in several cells and tissues. This molecule, which plays an important role in the regulation of physiological processes and the development of certain pathological processes, is synthesized endogenously from the amino acid L-arginine by certain cofactors and a group of enzymes referred to as nitric oxide synthase (NOS). The oxidation products of L-arginine are L-citrulline and NO (Knowles and Moncada, 1994; Sessa, 1994; Moncada et al., 1997; Gawronska et al., 2000; Alderton et al., 2001; Ulbrich et al., 2006).

NO is an unstable substance with a short half-life. Therefore, it is rather difficult to detect NO by direct measurements. However, it has been determined that NOS, the activity of which can be assessed by various techniques (Rosselli et al., 1996), has three isoforms, namely neuronal NOS (nNOS or NOS-I), inducible NOS (iNOS or NOS-II) and endothelial NOS (eNOS or NOS-III). nNOS and eNOS are structural enzymes, which are found in many cells (Sessa, 1994; Norman and Cameron, 1996; Lapointe et al., 2006).

The site of fertilization, the oviduct, is an important organ, which is involved in the transport of the ovum and embryo to the uterus by the contractions of its smooth

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muscles. Disorders of tubal contractile activity may cause the immobility of the fertilized ovum. This, in return, may result in infertility or tubal gestation (Uher et al., 1990). It is known that there are multiple factors that influence the functions of the oviduct (Bennett et al., 1988). As reported by several researchers (Rosselli et al., 1994, 1998; Bryant et al., 1995; Ekerhovd et al., 1999; Gawronska et al., 2000), NO plays a physiological role in the regulation of oviduct functions by means of its inhibitory effect on tubal contractions. NO induces this effect by ensuring smooth muscle relaxation (Ekerhovd et al., 1997).

The content of the oviduct lumen is defined by the secretion of epithelial cells and the blood–oviduct barrier (Garcia-Pascual et al., 1996). NO, synthesized by blood vessel endothelial cells, regulates blood circulation (Änggärd, 1994). NO synthesized by the blood vessel endothelium and epithelium of the oviduct may have a regulatory role in the production of the intratubal fluid (Ekerhovd et al., 1999).

The present study was aimed at determining immunohistochemically the NOS isoforms in different regions of the oviduct and investigating the correlation between these isoforms and the sexual cycle in cows.

Materials and Methods

Oviduct samples (fimbria, ampulla, isthmus) taken from cows slaughtered at a slaughterhouse constituted the material of the study. Oviduct samples were taken from 10 cows in the estrual phase and 10 cows in the luteal phase of the sexual cycle. The particular stage of the sexual cycle the animals were in at the time of the study was determined macroscopically according to the size of follicles and corpus luteum in the ovary as described by Rosenberger (1979) and by measuring progesterone levels in blood samples. Serum progesterone (P4) concentrations were determined by radioimmunoassay (RIA) using a gamma counter (Mini-Assay type 6-20; Mini Instruments Ltd., London, England) (International Atomic Energy Agency, 2010). A commercial progesterone RIA kit (Immunotech®, Marseille Cedex, France) was used as described by the manufacturer. Progesterone levels were measured only once.

The streptavidin–biotin complex (Strept-ABC) immunoperoxidase technique was employed for immunohistochemical examination. Part of the oviduct samples taken were fixed in 10% formaldehyde (for eNOS). The remaining part was frozen in liquid nitrogen (for nNOS and iNOS). Tissues fixed in formalin were passed through graded series of alcohol, methyl benzoate and benzole, and embedded in paraplast (Sigma-Aldrich, St. Louis, MO, USA). Five-micron-thick sections were cut from the blocks and affixed to glass slides coated with poly-L lysine (Menzel GmbH & Co. KG, Braunschweig, Germany). Following deparaffinization and dehydration, the slides were rinsed in phosphate-buffered saline (PBS, pH 7.4) for 10 min. After being washed in distilled water for 1–2 min, the slides were subjected to antigen retrieval in citrate buffer inside a microwave oven set to 800 W for 20 min. After being cooled for 20 min, the sections were passed once or twice through distilled water. Subsequently, the slides were washed in PBS-T (0.3%) for 3×5 min.

The 5-μm-thick cryostat sections taken from the oviduct samples frozen in liquid nitrogen were dried at room temperature for half an hour, and after being fixed in pure acetone for 10 min, they were washed in PBS-T (0.3%) for 15 min. The other processes were employed as described later, for all slides, including paraffin sections.

Endogenous peroxidase inhibition was performed by maintaining the sections in 30% H_2O_2 for 20 min. After being washed in PBS, the sections were incubated with 10% normal goat serum (Ultra V block-LABVISION TR-125-HL, Fremont, Canada) for 10 min. Subsequently, the sections were treated with primary antibodies at the appropriate dilutions (Table 1).

Later, the sections were washed in PBS-T (0.1%) for 5 min three times and incubated with biotinylated secondary antibodies (LABVISION TR-125-HL) for 30 min at room temperature, washed another three times, each for 5 min in PBS-T (0.1%) and treated with enzyme-conjugated streptavidin (Streptavidin-horseradish peroxidase-LABVISION TR-125-HL) for 30 min. To visualize the antigen–antibody reaction, the sections that were washed three times in PBS-T (0.1%) and were maintained in a 3-amino 9-ethyl carbazole (AEC) chromogen solution (Ultra vision detect system/AEC LABVISION TA-125-HA) for 5–10 min (the colour reaction was observed under a microscope). After being washed in distilled water, the sections were employed Gill's haematoxylin for 20–25 s for background staining. Under running tap water, the tissues were 'blued'. Later, using an adhesive (aqueous mounting medium-LABVISION), the tissue sections were affixed to the slide and covered with a coverslip.

Table 1. Antibody features, incubation periods and dilution rates

Primary antibody	Dilution	Incubation	Manufacturer (catalogue number)
Rabbit anti eNOS/NOS-III	1/50	At +4°C for 18 h	LABVISION RB9279-P
Rabbit anti iNOS/NOS-II	1/250	At +4°C for 18 h	CHEMICON 06-573
Rabbit anti nNOS/NOS-I	1/100	At +4°C for 18 h	CHEMICON AB1632

NOS, nitric oxide synthase; iNOS, inducible NOS; eNOS, endothelial NOS; nNOS, neuronal NOS.

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Table 2. Semiquantitative scoring of staining intensities for nitric oxide synthase (NOS) enzymes during the estrual and luteal phases

Oestrual phase								
	Isthmus			Ampulla			Fimbria	
	Epithelium	Blood vessel endothelium/muscle	Muscle	Epithelium	Blood vessel endothelium/muscle	Muscle	Epithelium	Blood vessel endothelium/muscle
iNOS	+++	–	++	+++	–	++	++	–
eNOS	++	+++	–	–	++	–	–	++
nNOS	++	–	+	++	–	+	++	–
Luteal phase								
	Isthmus			Ampulla			Fimbria	
	Epithelium	Blood vessel endothelium/muscle	Muscle	Epithelium	Blood vessel endothelium/muscle	Muscle	Epithelium	Blood vessel endothelium/muscle
iNOS	++	–	+	++	–	+	+	–
eNOS	+	++	–	–	+	–	–	+
nNOS	++	–	+	++	–	+	++	–

Semiquantitative scoring of immunostaining intensities: –, negative; +, weak; ++, moderate; +++, strong.
iNOS, inducible NOS; eNOS, endothelial NOS; nNOS, neuronal NOS.

All incubations with primary and secondary antibodies took place in a humidity chamber. For the staining of negative controls, primary antibodies were replaced by heat-inactivated 10% normal goat serum.

Staining intensity was assigned a semiquantitative score using a light microscope (Leica DM 2500): negative (−), weak reaction (+), moderate reaction (++) and strong reaction (+++) (Chatterjee et al., 1996). Two independent observers assessed the immunohistochemical reaction (Table 2).

Results

It was determined that the morphological appearance of the ovaries was consistent with the hormone levels measured. Blood progesterone levels below 1 ng/ml were considered as an indicator of the prevail of the estrual stage of the sexual cycle, while levels above 2 ng/ml were considered as an indicator of the prevail of the luteal stage of the sexual cycle (Table 3).

Table 3. Blood progesterone levels of the cows

Estrual phase (ng/ml)		Luteal phase (ng/ml)	
Case 1	0.55	Case 11	7.95
Case 2	0.30	Case 12	14.12
Case 3	0.62	Case 13	11.30
Case 4	0.78	Case 14	2.90
Case 5	0.35	Case 15	19.13
Case 6	0.85	Case 16	16.24
Case 7	0.30	Case 17	11.09
Case 8	0.55	Case 18	2.37
Case 9	0.67	Case 19	26.32
Case 10	0.83	Case 20	2.16

Endothelial NOS

During the estrual phase, which is characterized by rather low progesterone levels (below 1 ng/ml), immunohistochemically, eNOS expression was observed in the epithelial cells (Fig. 1a) and the endothelium and smooth muscle of blood vessels (Fig. 1b) of the isthmus. The intensity of staining was stronger in the epithelial cells located at the top of the mucosal folds (Fig. 1a). During the luteal stage, eNOS expression was weak in the epithelium (Fig. 2a) and blood vessels (Fig. 2b).

During both stages, no reaction was observed either in the epithelium of the ampulla or in the epithelium of the fimbria. The reactions in the blood vessels of the ampulla (Fig. 3a) and fimbria (Fig. 3b) were stronger during the estrual phase, compared with the luteal phase (Fig. 3c,d).

The tunica muscularis of the oviduct did not produce any reaction during any of the phases of the sexual cycle. The reaction observed in the veins of the oviduct was weaker than that observed in the arteries (Fig. 4). No reaction was observed in the lymph vessels. Staining performed using non-immune serum demonstrated no reaction in the epithelium and blood vessels.

Inducible NOS

iNOS expression was observed in the epithelium and muscles of the isthmus and ampulla and in the epithelium of the fimbria. The iNOS expression observed in the

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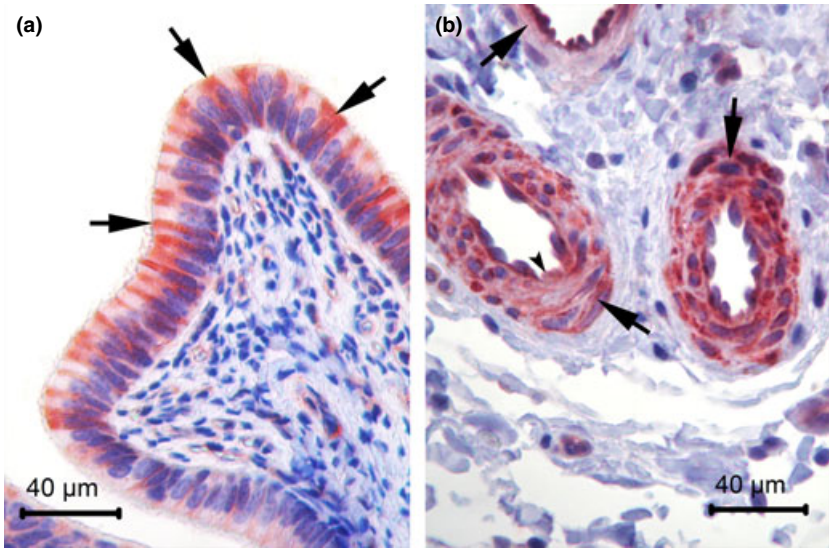


Fig. 1. (a) Strong eNOS expression in the isthmus epithelium (arrows), estrual phase, (b) strong eNOS expression in the endothelium (arrow head) and muscles (arrows) of the blood vessels in the connective tissue of the isthmus, estrual phase. Bar: 40 μm.

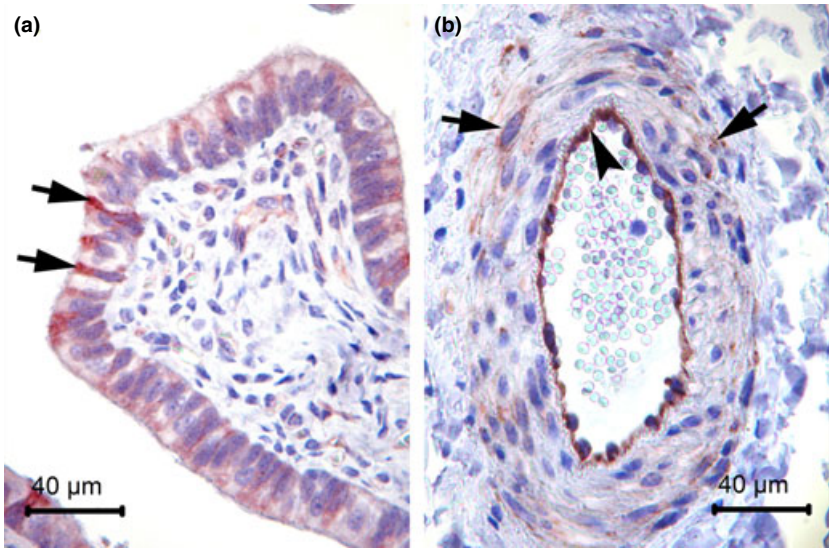


Fig. 2. (a) Weak eNOS expression in the isthmus epithelium (arrows), luteal phase, (b) Weak eNOS expression in the endothelium (arrow head) and muscles (arrows) of the blood vessels in the connective tissue of the isthmus, luteal phase. Bar: 40 μm.

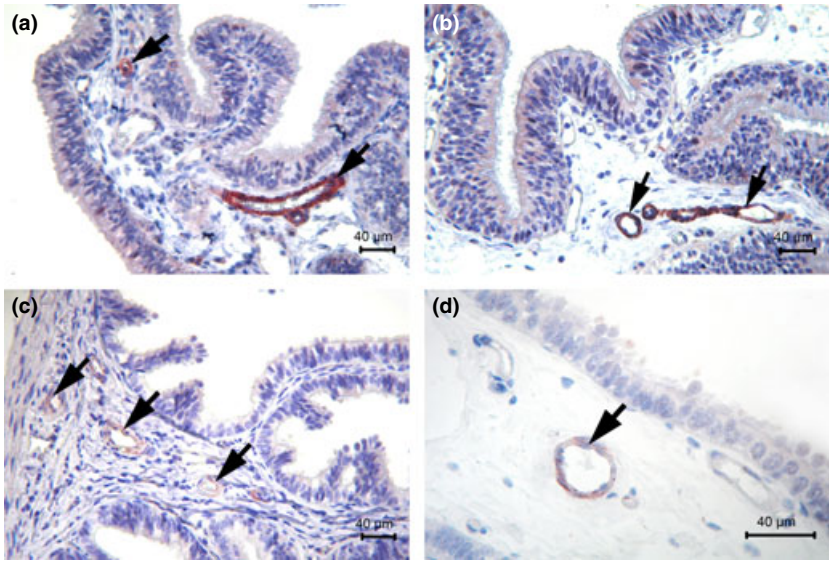


Fig. 3. (a) Strong eNOS expression in the blood vessels in the connective tissue of the ampulla (arrows), estrual phase. (b) Strong eNOS expression in the blood vessels in the connective tissue of the fimbria (arrows), estrual phase. (c) Weak eNOS expression in the blood vessels in the connective tissue of the ampulla (arrows), luteal phase. (d) Weak eNOS expression in the blood vessels in the connective tissue of the fimbria (arrow), luteal phase. Bar: 40 μm.

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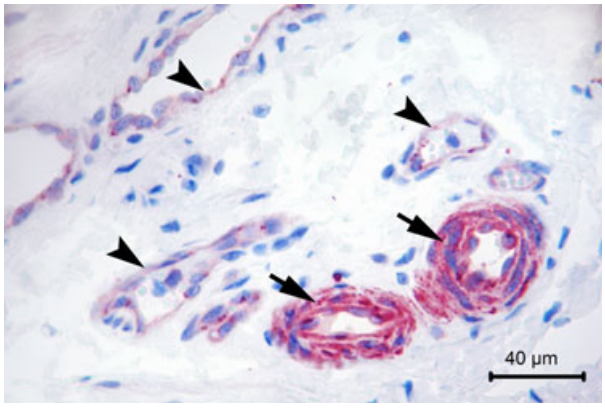


Fig. 4. Strong eNOS expression in the arteries (arrows) and weak eNOS expression in the veins (arrow heads) of the oviduct, estrual phase. Bar: 40 μm.

epithelium and muscles of the isthmus during the estrual phase (Fig. 5a) was stronger than that observed during the luteal phase (Fig. 5b). During both phases, the immunoreactive iNOS protein found in the epithelial layer of the isthmus was active in the part of the epithelial cells starting from the contact site with the lamina propria and extending to the lumen of the cell.

In the epithelium of the ampulla, the iNOS expression observed during the estrual phase (Fig. 6a) was stronger than that observed during the luteal phase (Fig. 6b). Furthermore, the reaction in the oviduct ampullary epithelium during the luteal phase was observed to have concentrated in the apical region of the cells (Fig. 6a). Ciliated cells also presented with positive iNOS immunoreactivity (Fig. 7).

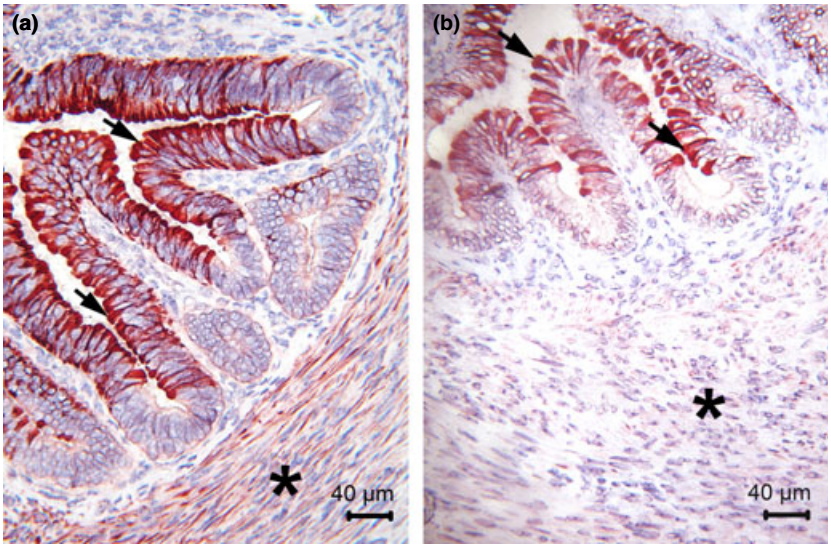


Fig. 5. (a) Strong inducible nitric oxide synthase (iNOS) expression in the epithelium (arrow) and muscle layer (*) of the isthmus, estrual phase. (b) Weaker iNOS expression in the epithelium (arrow) and muscle layer (*) of the isthmus, luteal phase. Bar: 40 μm.

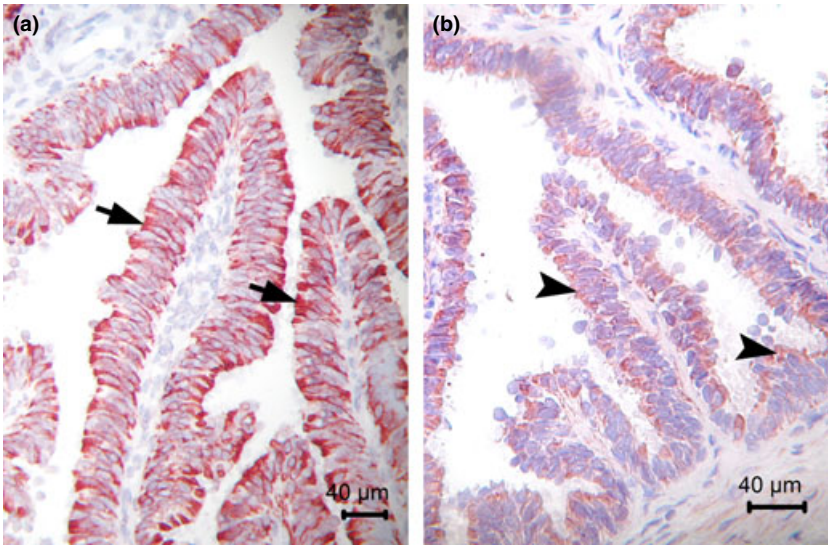


Fig. 6. (a) Strong inducible nitric oxide synthase (iNOS) expression in the epithelium of the ampulla (arrows), estrual phase. (b) Weak iNOS expression in the epithelium of the ampulla (arrow heads), luteal phase. Bar: 40 μm.

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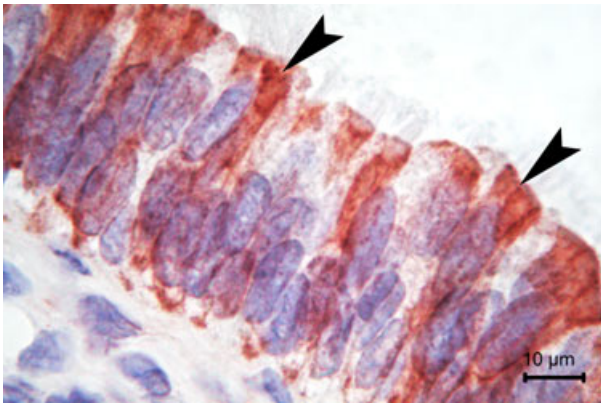


Fig. 7. Inducible nitric oxide synthase expression in the ciliated cells of the ampulla (arrow heads), oestrual phase. Bar: 10 μm.

In the fimbrial region, the iNOS expression observed during the estrual phase (Fig. 8a) was stronger than that observed during the luteal phase (Fig. 8b). The positive iNOS immunoreactivity produced in the isthmus was stronger than that produced in the ampulla and fimbria.

Neuronal NOS

nNOS immunoreactivity was observed in the epithelium and muscle layer of the isthmus (Fig. 9) and ampulla (Fig. 10), and in the epithelial cells of the fimbria (Fig. 11). The intensity of the reactions during the estrual and luteal phases in all three regions was the same. These reactions were concentrated particularly in the apical region of ciliated cells. Positive reactions were also observed in the nerve fibrils of the serosa, muscle layer and lamina propria of the isthmus (Fig. 12).

Negative staining employed to the cryostat sections did not demonstrate any reaction in the isthmus, ampulla and fimbria.

Discussion

NO has been reported to be involved in the regulation of multiple reproductive processes occurring in the oviduct (Jablonka-Shariff et al., 1999; Chen et al., 2001; Dixit and Parvizi, 2001; Ekerhovd and Norstrom, 2004; Manser et al., 2004; Wu et al., 2004; Lapointe et al., 2006) and to play a significant role in fertilization through autocrine and paracrine mechanisms (Lapointe et al., 2006).

The oviduct is a highly specialized organ, which provides a special microenvironment for fertilization and the early stages of embryonic development, and plays a key role in the transport of the gamete and embryo to the uterus. Therefore, the local synthesis of NOS plays a paracrine role in this tissue (Ignarro et al., 2001; Sayegh and Mastroianni, 1991; Lapointe et al., 2006). The present study demonstrated the expression of the NOS enzymes (iNOS, eNOS ve nNOS) in the cow oviduct, and it was observed that the oviduct presented with the activity of all three NOS enzymes.

The localization of NOS in the oviduct varies among animal species (Ulbrich et al., 2006). In a study conducted on the pig oviduct, Gawronska et al. (2000) reported the presence of eNOS in mucosal epithelial cells and blood vessel endothelium during all phases of the sexual cycle. These researchers indicated that the localization of positive immunoreactivity for iNOS was limited to the endothelium of lymph vessels and some blood vessels. No positive reaction for iNOS was observed in the epithelium. Based on these results, it was concluded that

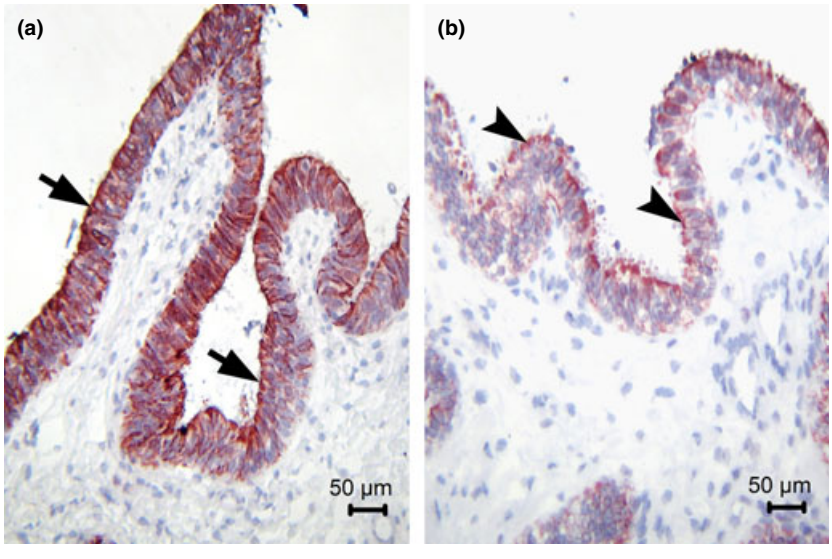


Fig. 8. (a) Strong inducible nitric oxide synthase (iNOS) expression in the epithelium of the fimbria (arrows), estrual phase. (b) Weak iNOS expression in the epithelium of the fimbria (arrow heads), luteal phase. Bar: 50 μm.

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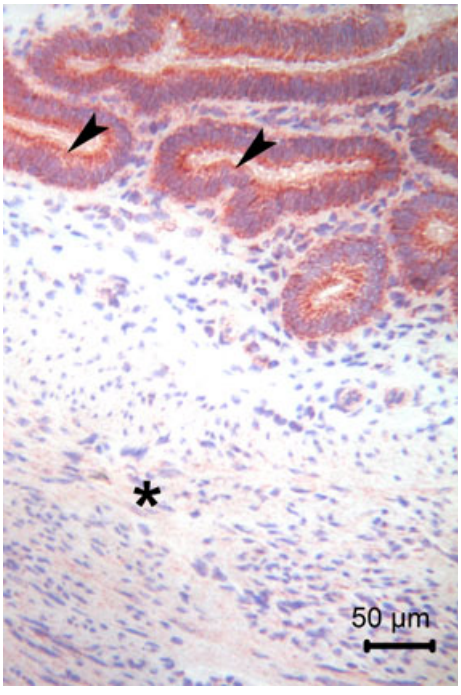


Fig. 9. Neuronal nitric oxide synthase expression in the epithelium (arrow heads) and muscle layer (*) of the isthmus, luteal phase. Bar: 50 μm.

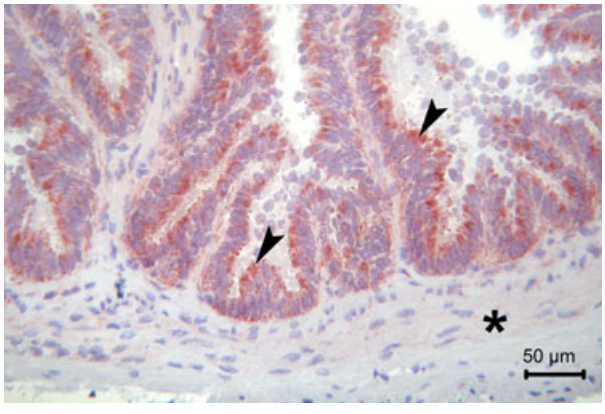


Fig. 10. Neuronal nitric oxide synthase expression in the epithelium (arrow heads) and muscle layer (*) of the ampulla, luteal phase. Bar: 50 μm.

the predominant isoform in pigs was eNOS. On the other hand, Ekerhovd et al. (1997) reported to have observed positive immune reactions for iNOS in the epithelium, smooth muscle layer, vessel endothelium and connective tissue of the oviduct in humans. It was indicated by these researchers that the intensity of these reactions was moderate in smooth muscle cells and weak in the epithelium and the wall of blood vessels. The same researchers were

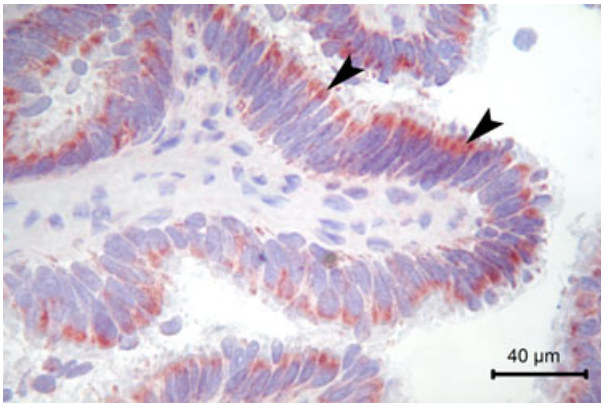


Fig. 11. Neuronal nitric oxide synthase expression in the epithelial cells of the fimbria (arrow heads), luteal phase. Bar: 40 μm.

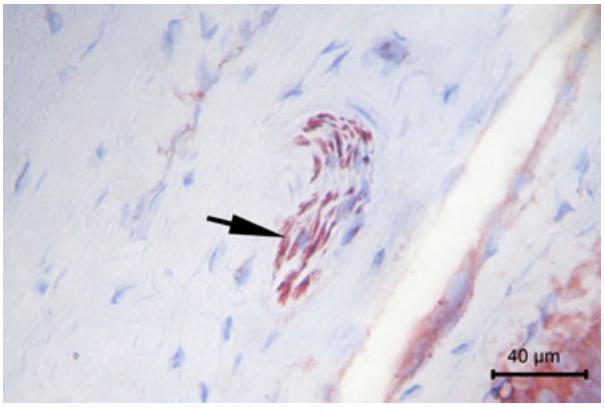


Fig. 12. Neuronal nitric oxide synthase expression in a nerve fibril in the lamina propria of the isthmus (arrow), luteal phase. Bar: 40 μm.

not able to detect the nNOS isoform immunohistochemically. Different NOS isoforms were also detected in the rat oviduct, and it was reported that these isoforms were involved in motility (Martinez et al., 1997). In the rat oviduct, immunopositivity for iNOS was limited to the epithelium (Bryant et al., 1995; Martinez et al., 1997). In a study carried out in rats, Chatterjee et al. (1996) detected positive immune reactions for eNOS in the epithelium and smooth muscle layer of the oviduct mucosa. Ulbrich et al. (2006) reported that, in cows, eNOS activity was present in the endothelium of blood vessels. Furthermore, they reported supranuclear staining in the secretory cells of the oviduct epithelium, and nuclear and cytoplasmic staining in the lamina muscularis and endothelium of blood vessels of the oviduct during the follicular phase. These researchers observed the supranuclear staining of secretory epithelial cells for iNOS. This kind of staining was located particularly in the apical region and did not exist in the basal region of the luminal folds.

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Furthermore, they reported that while the lamina muscularis presented with marked nuclear staining, the stroma lacked the iNOS protein. Lapointe et al. (2006) demonstrated that in the cow oviduct iNOS immunoreactivity was observed in the epithelium and muscle layer of the isthmus and ampulla with the reaction being weak in the lamina propria of the isthmus and reported that the nNOS protein existed in the epithelial layer, lamina propria and smooth muscle layer of the isthmus and ampulla. In the present study, it was demonstrated immunohistochemically that during both phases of the sexual cycle, eNOS activity existed in the serosa and blood vessel endothelium and muscle of the lamina propria in the fimbria, ampulla and isthmus and the epithelium of the isthmus, while positive staining for nNOS was observed in the epithelium, muscle layer and nerve fibrils and positive staining for iNOS was observed in the epithelium and muscle layer.

Ulbrich et al. (2006) reported that in the cow oviduct ciliated epithelial cells lacked the iNOS protein. However, in the present study, iNOS and nNOS immunoreactivity was also observed in ciliated cells. This suggests that, in the cow oviduct, NO is involved in the regulation of ciliary activity. The authors of the present study are in agreement with the report of Lapointe et al. (2006), which suggests that iNOS aids in the transport of the oocyte by increasing ciliary activity.

In agreement with the report of Hafez (1972), the present study demonstrated that secretory cells predominated in the isthmus, while ciliated cells predominated in the fimbria and ampulla. NOS expression having been determined to be concentrated particularly in the isthmus suggested that NO was also involved in the regulation of secretory activity. It has been indicated that the isthmus region of the oviduct acts as a reserve for sperm in certain mammalian species (Suarez, 2002). In the present study, in agreement with the report of Lapointe et al. (2006), it was observed that the epithelial cells of this specialized region presented with the expression of all three NOS enzymes. These findings are in support of previous literature reports (Weinberg et al., 1995; Herrero and Gagnon, 2001), indicating that the sperm adheres to the isthmus epithelium and that NO production plays a major role in maintaining the vitality of sperm.

Rosselli et al. (1998) suggested that the activity and expression of NOS in the uterus and oviduct were related to the level of steroid hormones (oestrogen and progesterone). Accordingly, these researchers determined that low oestrogen levels were associated with low levels of NOS enzymes (Rosselli et al., 1994). In a study conducted by Gawronska et al. (2000) in the pig and in another study carried out by Bryant et al. (1995) in the rat, the lowest staining intensity for NADPH diaphorase, as a histochemical indicator of NOS activity, was observed during the estrual phase. Gawronska et al. (2000) reported the highest level of iNOS expression during the luteal phase. Lapointe et al. (2006) suggested that NO plays a particularly significant role in the physiological processes that occur in the oviduct during the preovulatory period. In the present study, in agreement with the findings of Lapointe et al. (2006), it was determined that the expression of eNOS and iNOS was greater during the estrual phase. iNOS expression having been determined to be greater during the follicular phase, when the progesterone level was minimum (below 1 ng/ml), is in support of the claim of some researchers (Lapointe et al., 2006) that oestrogen increases iNOS expression during the preovulatory period. Furthermore, the differences observed in the reactions produced by NO enzymes during the estrual and luteal phases in the different regions of the oviduct show that NO is involved in the regulation of oviduct functions.

The amount of fluid in the lumen of the mammalian oviduct is greatest during oestrus and is formed as a result of the passage of blood serum (Lapointe et al., 2006). The increase in the amount of luminal fluid is related to the dilatation of blood vessels. NO is the strongest vessel dilatator known. Therefore, for the formation of the oviduct fluid, NOS expression increases during the preovulatory period. The dilatation of blood and lymph vessels accelerates the development of oedema, particularly in the isthmus mucosa, and leads to the constriction of the lumen. Thereby, NO, produced by eNOS, contributes to the development of oedema (Lapointe et al., 2006). Hisomoto et al. (2001) reported that oestrogens increase eNOS activity. Lapointe et al. (2006) observed the highest eNOS expression during the follicular phase. Gawronska et al. (2000) indicated that, in the pig oviduct, eNOS activity in the epithelium was lowest during oestrus. In the present study, it was observed that eNOS, contrary to the report in pigs, was expressed at a higher level during the estrual phase.

It has been reported that the capacity of the endothelium to synthesize and secrete NO is greater in arteries, compared to veins (Kayaalp, 1990). The findings obtained in the present study are in support of this report.

In some previously conducted studies, it has been suggested that NO is involved in the regulation of the contraction of the smooth muscles of the oviduct (Ekerhovd et al., 1999; Martinez et al., 2000). According to these researches, NO plays the role of a dilative agent in the mammalian oviduct, and the inhibition of NO accelerates the transport of the ovum via the increase of tubal motility. According to some researchers (Rosselli et al., 1994; Martinez et al., 1997, 2000), before the ovum leaves the oviduct, both the activity and expression of NOS

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decrease, thereby enabling the increase in the motility of the oviduct by contractile molecules such as endothelin-1 and PGF2α. This hypothesis has been supported by Chatterjee et al. (1996). Chatterjee et al. (1996) reported that eNOS concentration in the epithelium and smooth muscle of the oviduct reached its maximum during the follicular phase (pro-oestrus and oestrus). According to these researchers, NO secreted by eNOS during pro-oestrus and oestrus enables the relaxation of the oviduct, which in return facilitates the capture and fertilization of the released ovum. The same researchers reported the lowest marking intensity of eNOS during metestrus and diestrus. They suggested that this situation, which is an indicator of NO production in the oviduct having decreased, facilitates the active transport of fertilized oocytes to the uterus. Rosselli et al. (1994) also supported this view, indicating that NO could be involved in the decrease in contractions in the cow oviduct. Furthermore, Ekerhovd et al. (1997) stated that NO acted as a dilative agent in the human oviduct. In the present study, the expression of eNOS and iNOS having been observed to be weaker during the luteal phase is also in support of this view. On the other hand, Siemieniuch et al. (2009) suggested that NO did not directly cause muscle relaxation. It either acts a modulator of the muscle tone or eliminates the contractile effect of various factors. Ulbrich et al. (2006) ascertained that the decrease in iNOS in the isthmus during the estrual phase leads to the increase of oviduct motility via the activity of circular smooth muscles. In the present study, it was observed that the level of iNOS expression in the muscles of the isthmus was rather high, particularly during the oestrual phase.

In conclusion, in the present study, it was determined that the cow oviduct displayed the activity of all three NOS enzymes. There was a stronger expression of eNOS and iNOS during the estrual phase. Because the iNOS and nNOS immunoreactivity observed particularly in ciliated cells, it was concluded that NO may have a role in the regulation of ciliary activities in the cow oviduct.

Acknowledgements

This study was supported by the Ankara University Scientific Research Project Unit. Project no: 09B33380022008-2010.

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