

Plasma levels of nitric oxide and nitric oxide synthase vis à vis ovarian steroid hormones at and around estrus in cycling cows - a short communication

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ABSTRACT

Blood samples were collected from cycling Sahiwal cows at -2, -1, 0, +1, and +2 days of the estrus cycle and analyzed for plasma levels of Nitric oxide (NO) and Nitric Oxide Synthase (NOS) vis à vis ovarian steroidal hormones viz. estradiol-17 β and progesterone. The results revealed a gradual increase in NO, NOS and Estradiol-17 β levels from day -2 and they peaked at the day of estrus, followed by a steep fall up to day +2. The values of NO, NOS and estradiol-17 β ranged between 7.93 to 18.92 μ M/L, 0.39 to 0.95 U/L and 5.46 to 20.16 pg/mL, respectively. The levels of progesterone ranged from 0.15 to 0.71 ng/mL and showed a significant decline from day -2 to the day of estrus and then remained statistically parallel. In addition, levels of estradiol-17 β exhibited a highly significant ($P < 0.01$) association with levels of NO and NOS. The present study generated data on NO, NOS, estradiol-17 β and progesterone at and around estrus in cows, which is of practical importance for clinical and experimental interpretation.

Key words: cows, nitric oxide, nitric oxide synthase, estradiol 17 β , progesterone, estrus

Introduction

Nitric Oxide (NO) is a highly reactive free radical gas, endogenously synthesized from oxidation of a guanidine nitrogen atom of L-arginine, to yield L-citrulline and NO, catalyzed by Nitric Oxide Synthase (NOS), (MONCADA et al., 1991). NO has diverse

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physiologic functions, including regulation of vascular resistance, participation in cellular injury and signal transduction (MUTLAG et al., 2013). In addition, it also plays a crucial role in ovarian steroidogenesis, follicular development and growth, follicular apoptosis, maturation of oocytes, ovulation, luteolysis and maintenance of pregnancy (KUMAR et al., 2012). SKARZYNSKI et al. (2000) reported that NO also plays a critical role in the optimal formation of the corpus luteum in the early and middle periods of the luteal phase and participates in the regulation of functional luteolysis at the end of the luteal phase. Keeping in mind the important roles of NO in the physiology of the reproductive cycle of cows, the present study was intended to explicate the roles of nitric acid metabolism and ovarian steroidal hormones in the reproductive cycle by estimating the plasma levels of NO, NOS, estradiol-17 β and progesterone at and around estrus in cycling cows.

Materials and methods

The present study included eight normal cyclic Sahiwal cows (aged 3 to 5 years) maintained under standard management conditions throughout the study period, belonging to the Instructional Livestock Farm Complex (ILFC) of the College of Veterinary Science and Animal Husbandry, Mathura (India). The animals were provided with clean drinking water *ad libitum* and the same balanced rations throughout the study period, as per the recommendations (NRC, 2001). The animals were regularly vaccinated against infectious and contagious diseases, as per the farm schedule. Blood sampling was initiated at the expected day -5 of the estrus cycle up to day +5 of estrus, and the day of estrus was detected with the help of a teaser bull and rectal palpation in selected cows. The blood samples of proestrus (days -2 and -1), estrus (day 0), and metestrus (days +1 and +2) of the estrus cycle (BULBUL et al., 2008) were segregated and analyzed for plasma levels of NO, NOS, estradiol-17 β and progesterone. Blood samples (15-20 mL approximately) were aseptically collected in the morning hours through jugular venipuncture from selected animals before feeding, in dry, heparinized screw-capped vials. The samples were brought to the laboratory on ice, centrifuged at 3000 rpm for 15 min to harvest the plasma, and further stored at -20 °C until analyzed for biochemical parameters. Analysis of plasma samples included nitric oxide and nitric oxide synthase by a Enzchrom™ NOS assay kit, estradiol-17 β hormone ELISA kits of Diagnostics Biochem, Canada and progesterone by the liquid phase Radioimmunoassay (RIA) procedure, using progesterone antisera raised at the Department of Veterinary Gynecology and Obstetrics, GADVASU, Ludhiana, as per the standard kit protocols. Statistical significance was determined by ANOVA followed by Tukey's post-hoc multiple comparison test, using SPSS software for Windows (version 16.0). Pearson's correlation coefficient was calculated to investigate the association between variables. The data are presented as the mean \pm SE. A P value >0.05 was considered to be statistically significant.

Results and discussion

The circulating levels of estradiol-17 β , progesterone, NO and NOS (Mean \pm SE) at and around estrus in cycling cows are presented in Table 1. The results revealed a gradual increase in NO, NOS and estradiol-17 β levels in the proestrus period, the zenith on the day of estrus, and a subsequent steep fall in metestrus below the proestrus levels. In addition, NO and NOS exhibited a highly significant ($P < 0.01$) association with estradiol-17 β . A comparable pattern in levels of blood NO was also reported by BULBUL et al. (2008) in cattle and ROSSELLI et al. (1994) and SHARMA et al. (2015) in buffaloes, which substantiates the findings of the present study. However, the plasma levels of NO recorded in this study are a little higher than the concentrations observed by BULBUL et al. (2008) and MUTLAG et al. (2013) in Brown Swiss and Holstein cows, respectively.

The trend of plasma estradiol-17 β levels observed in the estrus cycle of cows in the present study are in agreement with earlier observations in cows (BULBUL et al., 2008; MUTLAG et al., 2013) and buffaloes (MONDAL et al., 2007; MONDAL et al., 2010; SHARMA et al., 2015) which corroborates the findings of the present study.

Table 1. Comparative profiles of nitric oxide, nitric oxide synthase, estradiol-17 β and progesterone at and around estrus in cow

Parameter	Days of cycle (cows)				
	-2	-1	0	+1	+2
Estradiol 17- β (pg/mL)	15.01 \pm 0.67 ^b	17.58 \pm 0.55 ^c	20.16 \pm 0.74 ^d	7.6 \pm 0.63 ^a	5.46 \pm 0.49 ^a
Progesterone (ng/mL)	0.71 \pm 0.25 ^b	0.33 \pm 0.09 ^{ab}	0.15 \pm 0.02 ^a	0.29 \pm 0.05 ^{ab}	0.42 \pm 0.08 ^{ab}
NO (μ mol/L)	14.97 \pm 0.81 ^b	16.97 \pm 0.83 ^{bc}	18.92 \pm 0.89 ^c	9.62 \pm 0.58 ^a	7.93 \pm 0.29 ^a
NOS (U/L)	0.73 \pm 0.04 ^b	0.83 \pm 0.04 ^{bc}	0.95 \pm 0.03 ^c	0.52 \pm 0.04 ^a	0.39 \pm 0.01 ^a

Values with a common superscript letter in the same row are non-significantly different ($P \geq 0.05$).

NO synthesis depends on the action of the NOS enzyme that exists in three iso-forms, neuronal (nNOS), endothelial (eNOS), which are constitutive and inducible (iNOS). NO is synthesized by several ovarian cells, such as theca, stroma, luteal and particularly granulosa cells in bovines (BASINI et al., 1998; BASINI et al., 2000), involving both eNOS and iNOS (ROSSELLI et al., 1998). The gonadotropins (JABLONKA-SHARIFF and OLSON, 1997) as well as estradiol-17 β , influence the synthesis of NO by inducing the expression of both iNOS and eNOS in the ovaries (AL-HIJJI et al., 2001; DIXIT and PARVIZI, 2001).

TAMANINI et al. (2003) and BULBUL et al. (2008) reported that estradiol-17 β induces generation of NO in ovaries, leading to a concurrent rise in estradiol-17 β , as well as in

NO levels from proestrus to estrus, which plays a critical role in follicular development, steroidogenesis, ovulation and luteolysis. Similarly, the present study also exhibited a highly significant ($P < 0.01$) association between NO, NOS and estradiol-17 β levels, similar to these findings. The increase in NOS activity in ovaries, corresponding to the time of the preovulatory surge suggests that NO may assist the process of ovulation by means of its stimulatory effect on prostaglandin production (SALVEMINI, 1997), thus stimulating the inflammatory process at the time of ovulation (DIXIT and PARVIZI (2001).

The elevated concentration of NO during proestrus (just before estrus), followed by the sudden fall observed in the present study indicates that a certain level of NO is required to play a key role in luteolysis, that may facilitate the oxytocin-mediated release of prostaglandin (SKARZYNSKI et al., 2003). Oxytocin is reported to act by enhancing NOS activity (MOTTA and GIMENO, 1997; MOTTA et al., 1997) and NO has been reported to stimulate the synthesis of PGF2 α in humans, (FRIDEN et al., 2000) and bovine (SKARZYNSKI et al., 2000), which in turn increases NOS activity, thus activating a positive feedback mechanism (MOTTA et al., 2001).

In contrast, plasma levels of progesterone showed a gradual decrease during proestrus, the nadir on the day of estrus, and they then remained statistically invariable through the metestrus. SAGAR et al. (2012) reported an increase in progesterone production by the corpus luteum on inhibition of NOS activity by L-NAME in buffaloes. A similar form of negative association was observed between NO and progesterone levels in the present study.

Conclusions

The findings of the present study provide data on the circulating levels of NO, NOS, estradiol-17 β and progesterone at and around estrus in cows, that may be used for clinical and experimental interpretation. A number of autocrine and paracrine mediators and their complex interplay are involved in the regulation of the estrus cycle in cows.

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SAŽETAK

Uzorci krvi prikupljeni su od krava Sahiwal pasmine tijekom -2, -1, 0, +1, i +2 dana estrusnog dijela spolnog ciklusa. Analizirane su razine dušikova oksida (NO) i sintaze dušikova oksida (NOS) u odnosu na steroidne hormone jajnika, posebno estradiol-17 β i progesteron. Rezultati su pokazali postupno povećanje razine NO, NOS i estradiol-17 β hormona počevši od dana -2. Vrhunac razine postignut je na dan estrusa, a zatim je uslijedilo strmo opadanje do dana +2. Vrijednosti promatranih pokazatelja kretale su se u rasponu od 7,93 do 18,92 μ M/L za NO, od 0,39 do 0,95 U/L za NOS i od 5,46 do 20,16 pg/mL za estradiol-17 β . Razine progesterona bile su u rasponu od 0,15 do 0,71 ng/mL, uz značajan pad od dana -2 do dana estrusa nakon kojeg su ostale statistički nepromijenjene. Osim toga, razina estradiola-17 β hormona pokazala je visoko značajnu ($P < 0,01$) povezanost s razinama NO i NOS. Istraživanje je pokazalo podatke o razinama NO, NOS, estradiol-17 β hormona i progesterona tijekom i oko estrusa krava što je od praktičnog značenja za klinička i eksperimentalna tumačenja ovih pokazatelja.

Ključne riječi: krave, dušikov oksid, sintaza dušikova oksida, estradiol-17 β , progesteron, estrus
