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Nitric Oxide as an Early Diagnostic Tool for Intrauterine Diseases in Animal Health

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Abstract

Background: Intrauterine pathologies in animals are a significant cause of reduced reproductive performance and symptomatic infertility, leading to economic losses in the livestock industry. **Objective:** The study aimed to develop a method for the early diagnosis of intrauterine pathologies in animals by assessing nitric oxide (NO) levels using Electron Paramagnetic Resonance (EPR) spectroscopy. **Methodology:** An experimental model was created using dairy *Escherichia coli* culture, which was injected into the uterus of pregnant rabbits under ultrasound guidance. Determination of the level of nitric oxide was accessed in the laboratories of the FSSI "Federal Research Center for Chemical Physics named after. N.N. Semenov" Russian Academy of Sciences. The study involved three groups of rabbits: two groups infected either intrauterine or intraperitoneally, and a control group. Group I (Intrauterine Infection) consisted of two rabbits. A suspension of *Escherichia coli* was injected directly into the uterus through the cervical canal to induce an intrauterine infection. Group II (Intraperitoneal Infection): This group included five rabbits. The *Escherichia coli* suspension was administered intraperitoneally, serving as a comparative model for systemic infection. Group III (Control): The remaining two rabbits served as the control group. These rabbits were not infected and were used to establish baseline measurements for comparison. **Results:** The results demonstrated that the concentration of nitric oxide derivatives, specifically nitrites and N-nitrosamines (RNNO), significantly increased in the blood plasma of infected rabbits compared to the control group. These changes were observed during the early stages of inflammation, indicating the onset of intrauterine pathologies. **Conclusion:** The developed method based on measuring nitric oxide derivatives in blood plasma is effective for the early diagnosis of intrauterine pathologies in animals. [*Bangladesh Journal of Infectious Diseases, June 2024;11(1):52-58*]

Keywords: Nitric oxide; intrauterine pathologies; diagnostics; biological models

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Introduction

Intrauterine pathologies in animals are one of the most serious causes of decreased reproduction and productivity, subsequently causing symptomatic infertility. The economic consequences of symptomatic infertility have led to the development in many countries of the world with development of various national programs and therapeutic strategies to combat genital infections. For this purpose, pharmaceutical companies and enterprises are developing veterinary pharmacological drugs belonging to various therapeutic classes. However, despite the abundance of pharmacological drugs for the treatment and prevention of symptomatic infertility, significant progress has not been achieved[1,2].

Some research[3] described that postpartum endometritis is one of the most dangerous disorders affecting the subsequent reproductive functions of the animal, namely the occurrence of intrauterine infection. This remains a major economic problem for dairy farming worldwide due to large financial losses caused by low conception rates and increased numbers of cows being culled, resulting in lower profits from dairy herds[4,5].

Under large scale commercial dairy farming, there is an increasing trend of cattle with reproductive complications leading to problems of infertility. Predisposing factors for various diseases of the genitals organs in cows includes higher concentration of livestock in a limited area, type of feeding, physical inactivity negatively affecting body metabolism of the cow. This leads to non-compliance with animal hygienic standards and violation of veterinary and sanitary requirements for keeping animals and as a consequence, depression of the neuroendocrine mechanism for regulating reproductive function and an imbalance of the hormonal status of the body, which contributes to the occurrence of peripartum viz., retention of placenta and postpartum complications viz., atony and sub involution of the uterus, endometritis in female farm animals[6].

For a long period, there was no consensus on the spread and development of endometritis in cows with high milk production, especially in intensive farming system. The causes of endometritis were associated with disturbances in feeding and housing animals as well as a decrease in the body's immune system[7,8]. One of the limiting factors in the growth of dairy farming productivity is the widespread prevalence of pathology of the reproductive organs, most often of an inflammatory nature, among breeding stock[9].

A strong correlation between resumption of postpartum cycles and cytological endometritis, as well as low progesterone levels, was reported by Guadagnin & Cardoso[10]. Treatment usually includes hormonal therapy and antibiotics, alone or in combination. However, successful resolution of inflammation largely depends on the diagnostic tools used to help select appropriate therapy for the same. One such diagnostic tool is Cytokines and Acute Phase Proteins (APP). Additionally, clinical endometritis can be diagnosed by the accumulation of mucopurulent to purulent discharges from the vagina in the postpartum period[11].

In the current trend of rearing cattle in a large scale at a commercial level, it has been noticed that the prevalence of cows with reproductive problems especially postpartum endometritis is on a rise, despite the use of comprehensive programs for its prevention, leading to hampered productive and reproductive performance resulting in early culling of the livestock[12]. A large number of antibacterial drugs used in the treatment of endometritis do not meet modern requirements of veterinary medicine for reasons of insufficient therapeutic effectiveness, longer withdrawal periods in milk leading to discarding milk for long periods, the emergence of resistance to pathogenic microorganisms, inhibition of natural neurohumoral mechanisms of local and general defense of the body[13,14].

The development of bovine endometritis involves highly complex signaling processes, including the detection of bacterial components by innate immune cells, as well as the mobilization of neutrophils followed by phagocytosis of invading pathogens. The production of proteins such as haptoglobin, an acidic glycoprotein and ceruloplasmin or serum amyloid A is also stimulated. All of the above regulatory chains have a direct and clear connection mediated by a crucial signaling molecule i.e., Nitrogen monoxide (NO). According to modern concepts, along with endogenous and exogenous hormones and peptides in the body, simple compounds can act as regulatory molecules or messengers such as nitric oxide (NO), carbon monoxide (CO), as well as hydrogen sulfide (H_2S)[15].

Nitrogen monoxide (NO), along with two other gaseous messengers (CO and H_2S), takes part in

the proper functioning of the immune and nervous systems; its level directly determines the tone of all blood vessels and also the course of many pathological processes. One of the most important functions of NO is to activate the heme-containing enzyme, Guanylate Cyclase (sGC). The special physicochemical properties of this molecule determined by the method of its transportation in the form of nitrosyl complexes with heme iron, S-nitrosothiols (RSNO), as well as high and low molecular weight complexes of non-heme iron with thiyl iron ligands (glutathione, cysteine).

The lifetime of nitric oxide is very short and it is difficult to trace it directly in tissues and body fluids. Physical methods have confirmed that a stable metabolite of nitric oxide is the nitrite anion, NO_2-, the measurement of the level of which allows one to analyze and characterize the intensity of biochemical processes occurring in the body. Like any metabolite, nitrite anion is normally recorded at low concentrations viz., 10^{-6} - 10^{-7} mol/l.

Nitric oxide (NO) plays an important role in many physiological processes, including vasculogenesis, angiogenesis, growth, puberty as well as aging and apoptosis. NO plays an important role in ovarian steroid production, ovulation and follicular apoptosis. In other words, increasing the activity of nitric oxide synthase (NOS) leads to an increase in the amount of NO, which triggers the production of prostaglandins and inflammatory cascades that contribute to follicular rupture and atresia. An increase in NO concentration inhibits the synthesis of steroids in luteal and granulosa cells[16].

Since NO is the main paracrine mediator of various biological processes, as well as a key factor in both the reproductive cycle and embryo implantation, super synthesis of NO in the uterus leads to toxicity, or inflammation of epithelial cells and immune rejection. To Develop a method for early diagnosis of intrauterine pathologies in animals, which is based on a method for assessing nitric oxide (NO) levels using electron paramagnetic resonance (EPR) spectroscopy. It will make it possible to detect and diagnose the development of the inflammatory process in the uterus at the early preclinical stages, arriving at an appropriate treatment plan and also to monitor the effectiveness of the treatment.

Methodology

Study Design: The study was conducted to develop a method for early diagnosis of intrauterine pathologies in animals. Experimental work was carried out at the Department of Disease Diagnostics, Therapy, Obstetrics and Animal Reproduction of the Moscow State Academy of Veterinary Medicine and Biotechnology - MBA named after K.I. Scriabin.

Study Procedure: A total of nine pregnant rabbits were selected for the study. After confirming pregnancy through diagnostic procedures, the rabbits were randomly divided into three groups named as group I, group II and group III. Group I (Intrauterine Infection) consisted of two rabbits. A suspension of *Escherichia coli* was injected directly into the uterus through the cervical canal to induce an intrauterine infection. Group II (Intraperitoneal Infection): This group included five rabbits. The *Escherichia coli* suspension was administered intraperitoneally, serving as a comparative model for systemic infection. Group III (Control): The remaining two rabbits served as the control group. These rabbits were not infected and were used to establish baseline measurements for comparison. To induce intrauterine infection in Group I, a suspension of E. coli at a concentration of 1 billion cells per milliliter was prepared. The suspension was carefully injected into the uterus using a classic milk catheter connected to a disposable syringe. Ultrasound guidance was employed to ensure accurate placement of the suspension within the uterus. In Group II, the same *Escherichia coli* suspension was injected intraperitoneally to simulate a different route of infection and allow for comparison of systemic versus localized infection responses.

Specimens Collection Procedure: Determination of the level of nitric oxide was accessed in the laboratories of the FSSI "Federal Research Center for Chemical Physics named after. N.N. Semenov" Russian Academy of Sciences.

Measurement of nitric oxide: To assess the level of nitric oxide, the method of electron paramagnetic resonance spectroscopy was used, which makes it possible to determine radicals in biological samples with fairly simple preparatory procedures. EPR signals are planned to be recorded on a spectrometer from Bruker (Germany) ECS-106. As is known, nitric oxide forms stable complexes with iron in hemoglobin, allowing it to be determined directly in blood samples by EPR. To determine serum nitric oxide we will use a spin trap method based on dithiocarbamate and iron (II). The method of determining NO used in this work is based on the

reaction of formation of nitrosothiol–nitrosocysteine (RSNO) in an acidic medium (pH=3.5) from the nitrite anion NO_2- and cysteine hydrochloride. Then nitrosocysteine, in the presence of iron (2+) and N-methyl-D, L-glucamine dithiocarbamate (MGD), forms a water-soluble paramagnetic mononitrosyl iron complex MNCJ MHD-Fe-NO. Determination of the nitrite anion NO_2- was carried out. plasma proteins weighing more than 30 kD after defrosting were removed by filtration through a Microcon 30 kD filter, Millipore Corporation, USA for 20 minutes at 14500 rpm on a Mini Spinplus centrifuge, Eppendorf. To 50 µl of cysteine with a concentration of 400 mM, 10-120 µl of serum was added after filtration, the pH of the solution was adjusted to 3.5 by adding 0.01 mM HCl. After 5 minutes, 50 µl of 40 mM iron (II) sulfate, 200 µl of 400 mM buffer (Tris or HEPES) and 200 µl of 250 mM MHD were added. Then the pH of the solution is increased to 7.6 with a 0.06% NaOH solution. Under these conditions MNCJ MHD-Fe-NO will be formed.

To construct a calibration curve, a solution of sodium nitrite with a concentration of 480 µM of various volumes (2-40 µl) was added to 50 µl of cysteine hydrochloride with a concentration of 400 mM; the pH of the solution was adjusted to 3.5 by adding 0.01 mM HCl. After 5 minutes, 50 µl of 40 mM iron sulfate, 200 µl of 200 mM (Tris or HEPES), 200 µl of 250 mM MHD were added, and the pH was adjusted to 7.6 with 0.06% NaOH. After 10 min, the EPR spectrum of MHD-Fe-NO MNLC was recorded. The nitrite concentration in the sample was assessed by the method of double integration and comparison of the areas of the EPR signals of the studied and standard samples. As the latter, we used a synthesized complex of MHD with iron (2+) and nitrogen oxide: MNCJ MHD-Fe-NO. The nitrosyl groups of high and low molecular weight RSNOs in serum can be oxidized to nitrite during prolonged sample handling, and this may also contribute to data variability. We believe that the available low molecular weight RSNOs do not contribute significantly to the nitrite measurement. To catalyze their decomposition and the formation of additional nitrite, monovalent copper (Cu +) must be present in the solution or the medium must be alkaline (pH = 10.5). In our studies, the reduction of nitrite and its inclusion in the MNLC MHD -Fe-NO was carried out at pH = 3.5. In the event that the formation of RSNO occurred from nitrite and glutathione or serum cysteine, and the latter can be in low concentrations, then with the addition of an MHD-iron trap, a paramagnetic MHD-Fe-NO MNIC will be formed with the participation of the nitrosyl group of the newly formed RSNO. For the study, blood samples were taken from the ear vein into tubes with heparin and centrifuged for 10 minutes on a centrifuge model CH80-2S “Armed” at a speed of 3000 rpm to sediment red blood cells. Samples were stored in liquid nitrogen (-196°C). To measure nitrosohemoglobin concentration, whole blood (without heparin) was frozen in cylindrical plastic containers of 35 mm long and 4 mm in diameter. EPR signals were recorded on a spectrometer from Bruker (Germany) ECS-106.

Statistical Analysis: Calculations of the areas (S) of EPR signals were performed using the software of a Bruker ECS-106 EPR spectrometer. The developed method allowed us, firstly, to control the influence of serum proteins on the formation reaction of MGD MNICs; and secondly, to take into account the contribution of high molecular weight RSNO and DNIC as sources of NO.

Ethical Clearance: The experimental procedures were approved and conducted in compliance with ethical standards, as specified by the Moscow State Academy of Veterinary Medicine and Biotechnology's ethical review board, ensuring the welfare and humane treatment of the animals involved in the study.

Results

For intrauterine infection, a daily culture of *Escherichia coli* was used at a concentration of 1 billion/ml, which was administered through the cervical canal using a classic milk catheter connected through an adapter to a disposable syringe. All manipulations were carried out under ultrasound control (Figure I).

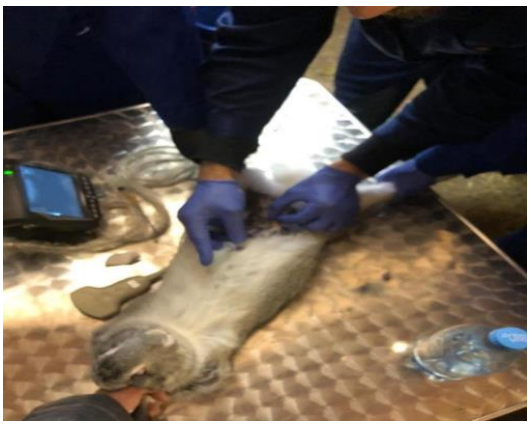

Figure I: Introduction of a daily culture of *Escherichia coli* into the Cervix of a Pregnant Rabbit

To study the nature of pathological changes in pregnant rabbits after infection, we examined the concentration of nitric oxide in the blood serum. Thus, to diagnose an acute inflammatory process, the total concentration of nitrites and RNNO was determined in the rabbit's blood plasma. When its value was over 0.150 μM, the presence of an acute inflammatory process was diagnosed (Table 1).

Table 1: Change in Content (NO2-+RNNO) in the Plasma of Infected Female Rabbits During Recovery

Rabbits No.	Infection method	Content of NO2-+RNNO, μM After Infection	
		5 days	15 days
1	Intrauterine	2.41	0.14
2	Intrauterine	1.99	0.19
3	Intraperitoneal	1.42	0.09
4	Intraperitoneal	0.12	0.11
5	Control	0.11	0.12
6	Control	0.09	0.15

*Source: compiled by authors

Table 1 represents data on the total content of nitrite and RNNO in the blood plasma of control and infected female rabbits obtained at the departments of disease diagnosis, therapy, obstetrics and animal reproduction, immunology and biotechnology.

Discussion

To develop a method for diagnosing fetal infection and monitoring the progress of treatment, we will apply the method of early diagnosis of inflammation according. This method is highly sensitive (up to 40 nM NO2-+ RNNO) and highly specific in the sense that the normal content of NO_2-+RNNO in the blood and in most tissues is less than 50 nM. An increase in the concentration of these compounds in the blood above 150 nM clearly indicates inflammation, regardless of individual characteristics.

But it is not specific to the etiology of inflammation. Therefore, it is necessary to develop a technique that, based on data from a highly sensitive sensor and standard clinical and biochemical criteria, would serve as instructions for making an appropriate diagnosis, suitable method of treatment and monitoring its progress.

As a control, we used conditionally healthy female rabbits kept in the vivarium of the Academy. In the plasma of healthy animals, the content of nitrite and RNNO did not reliably exceed the reference values. In the plasma of rabbits infected intrauterine and intraperitoneal**,** accompanied by the development of an acute inflammatory process, a substance with catalase-inhibiting properties, characteristic of nitrite and RNNO appears. Its total concentration was over 0.15 μM and amounted to 2.41 and 1.99 μM, respectively.

These data suggest that inflammation is indeed associated with the appearance of plasma nitrite and RNNO. By the 15th day after infection, the NO2- + RNNO content decreased to 0.14-0.19 μM, which indicated recovery.

Infection is associated with activation of phagocytes, for detection of which, a method has been developed that allows us to detect in almost 100% of cases. It is based on the fact that activated phagocytes produce superoxide (O2-), which interacts with compounds normally present in the blood plasma - donors (depots) of nitric oxide. They are in plasma and are represented by nitrosyl iron complexes (NICs). As a result of the reaction of reactive oxygen and nitrogen species, peroxynitrite is formed, which decomposes to nitrate, nitrogen nitrite (NO2-) and various non-thiolate nitro compounds (RNO) are also formed.

These final products are captured by the sensor. Normally, their concentration is less than 100 nM. When phagocytes are activated, it increases to several μM. According to our data, an increase in the concentration (NO2-+ RNO) above 150 nM clearly indicates the activation of phagocytes.

The concentration of NO metabolite compounds in blood plasma and cells varies over a wide range. The rate constant for the interaction of superoxide with them, according to a number of researchers, is no less than 10^7 $M^{-1}s^{-1}$. That is, the donor compounds in the blood plasma and cells do not have effective competitors for superoxide.

Activation of phagocytes is an integral part of the development of the inflammatory process. The use of an enzyme sensor for the early diagnosis of mastitis in cattle has been patented. The sensitivity of the sensor exceeded this indicator for the somatic cell count test and mastidine test. An increase in the concentration (NO2-+ RNO) in milk above 0.15 μM clearly indicates the presence of inflammation.

Thus, the appearance of nitrites and RNNO in plasma in concentrations above 150 nM is a sign of the onset of the inflammatory process.

Conclusion

In the course of experimental work, a biological model was created for the diagnosis and therapy of intrauterine pathologies of animals. An original method of intrauterine infection has been developed using a dairy E. coli culture at a concentration of 1 billion /ml, which was injected through the cervical canal using a classic milk catheter connected through an adapter with a disposable syringe. All manipulations were carried out under the guidance of ultrasound. The results of the work allow us to conclude that the level of a nitric oxide derivative, nitrite, in the blood of sick animals varies significantly compared to the control group (healthy animals), which may be due to the development of the inflammatory process and associated oxidative stress reactions. A technique for the early diagnosis of intrauterine pathologies in animals has been developed, which is based on a method for assessing the level of Nitric Oxide (NO) using Electron Paramagnetic Resonance (EPR) spectroscopy. It made it possible to fix the early preclinical stages of the development of the inflammatory process in the uterus. The results of the work allow us to conclude that the level of nitric oxide derivative – nitrite in the blood of diseased animals varies significantly compared to the control group (healthy animals), which may be due to the development of the inflammatory process and associated oxidative stress reactions. Consequently, the developed method, based on determining the concentration of nitrites and N-nitrosamines (RNNO) in plasma, can be used to diagnose intrauterine pathologies and monitor their treatment.

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Conflict of Interest
The authors have no relevant conflicts of interest to declare.

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Contribution to authors:
Artyushina Z, Fedotov S: Conception and design, or design of the research; Serezhenkov V, Sidnev N: the acquisition, analysis, or interpretation of data; conceptualized and designed the overall study; Zherebtsov I, Regan RG: involved in data collection; Artyushina Z: Drafting the manuscript or revising it critically for important intellectual content; Artyushina Z: conducted data analysis; Artyushina Z: drafted the manuscript. All authors reviewed and approved the final manuscript.

Data Availability
Any questions regarding the availability of the study's supporti ng data should be addressed to the corresponding author, who can provide it upon justifiable request.

Ethics Approval and Consent to Participate
The Institutional Review Board granted the study ethical approval. Since this was a prospective study, every study participant provided formal informed consent. Each method followed the appropriate rules and regulations.

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References

1. Belozertseva NS, Fedotov SV, Yakhaev IM. Dependence of the reproductive ability of black-and-white cows on the physiological status. Vet Med. 2019;6:41-4
2. Mussayeva A, Yegorova N, Yerishov M, Dossanova A, Suchshikh V, Namet A, et al. Molecular-biological properties of the attenuated strain of salmonella abortus-equi E-841, used in the creation of a vaccine against abortion of mares. Am J Anim Vet Sci. 2021;16(2):144-50
3. Zubova TV, Pleshkov VA, Smolovskaya OV, Mironov AN, Korobeynikova LN. The use of carotene-containing preparation in cows for the prevention of postpartum complications, Vet World. 2021;14(5):1059-66
4. Tanbayeva G, Barakhov B, Tagayev O, Myrzabekov Z, Ibragimov P, Alpysbayeva G, et al. Effectiveness of antimicrobial preparations for the sanitation of the udder of dairy cows. Int J Vet Sci. 2024;13(5):647-54
5. Omelchuk N. Effect of the Rs-10 radioprotector on protein-steroid interaction in irradiated animals with biphasic adrenocortical response to irradiation. Qubahan Acad J. 2023;3(4):70-6
6. Yakhaev IM, Fedotov SV, Belozertseva NS. Gynecological and mammological medical examination of lactating cows. Vet Med. 2020;6:33-8
7. Shevchenko P, Rychshanova R, Suleimanova K, Bermukhametov Z, Miciński J, Brel-Kisseleva I. Breeding qualities of Aberdeen-Angus cows of different genotypes in Northern Kazakhstan. Am J Anim Vet Sci. 2023;18(3):217-22

8. Dubekova SB, Sarbaev AT, Ydyrys AA, Eserkenov AK, Bastaubaeva SO. Immunological characteristics of winter wheat lines with resistance to rust diseases in Kazakhstan. OnLine J Biol Sci. 2021;21(4):356-65.
9. Kashina E, Yanovskaya G, Fedotkina E, Tesalovsky A, Vetrova E, Shaimerdenova A, et al. Impact of digital farming on sustainable development and planning in agriculture and increasing the competitiveness of the agricultural business. Int J Sustain Dev Plan. 2022;17(8):2413-20
10. Guadagnin AR, Cardoso FC. Association of dry matter intake, milk yield, and days to first ovulation with cytological endometritis in Holstein cows. J Dairy Sci. 2023;106(10):7240-65
11. Toktogulova N, Breidert M, Sultanalieva R, Tuhvatshin R, Kazieva A. Features of metabolic associated fatty liver disease in the Central Asian population in low and high altitudes. Gac Méd Caracas, 2022;130(4):772-80
16. Biofizika. 2020;65:1017-24
12. Petrov AF, Bogdanova OV, Narozhnykh KN, Kamaldinov EV, Shatokhin KS, Gart VV, et al. Clustering of countries based on dairy productivity characteristics of Holstein cattle for breeding material selection. Vet World. 2024;17(5):1108-18
13. Nametov A, Yertleuova B, Orynkhanov K, Semenenko MP, Sidikhov B, Murzabayev K, et al. Evaluation of the antibacterial effect of Artemisia lerchiana compared with various medicines. Braz J Biol. 2023;83:e277641
14. Beishova I, Nametov A, Shamshidin A, Belaya A, Ulyanova T, Kovalchuk A, et al. Effectiveness of the use of genetic markers of meat productivity in the Kazakh White-Headed breed identified using genome-wide association study. OnLine J Biol Sci. 2024;24(4):624-32
15. Serezhenkov VA, Tkachev NA, Artyushina ZS, Kuznetsova MI, Kovach M, Vanin AF. Reduced bioavailability of nitric oxide in horses with colic symptom complex: assessment by EPR spectroscopy.