The use of the electronic nose device for the diagnosis of postpartum metritis in cows

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Abstract. The study aimed to assess the possibilities of applying electronic nose device for early diagnosis of postpartum endometritis in cows using secretions. The experiment included four clinically healthy cows, four cows with endometritis. Samples of secretions were obtained on days 1, 3, 6, 8 after analysis of their odours was carried out with MAG-8 device (Russia). As and measuring array, there were used eight sensors based on piezoelectric crystals frequency of 10.0 MHz and film sorbents on electrodes that respond to compounds of C2-C4 alcohols, linear and cyclic ketones, aldehydes, aliphatic amines, and sulfur-containing organic compounds, acids, ammonia. It was found that during the first three days after calving in cows with postpartum endometritis in comparison with healthy animals, the area of the visual response of the sensors was exceeded by 28.4% the content of alcohols, ketones of linear structure by 5.2-6.0%, aliphatic acids were less by 14.3% and alkyl amines by 9.2%. On 6-8 days after calving, in the clinical manifestation of endometritis, an excess of the “visual imprint” by 6.1 times (p <0.001), a decrease in the content of aliphatic acids by 22.7% (p < 0.05), and an increase in the concentration of sulfur-containing organic compounds - by 4.9%. Therefore, the electronic nose system can distinguish between the composition of the volatile compounds of the cervical postpartum secretions with the normal and pathological course of the postpartum period.

1. Introduction

Postpartum complications in high-yielding dairy cows are widespread and represent an important problem of veterinary obstetrics and are one of the main causes of prolonged infertility. The most common postpartum disease is endometritis, which is of a high percentage and is manifested in 30-70% and more of fresh cows [4, 8, 15, 16]. The economic damage after suffering a disease consists of the shortage of products, the costs for conducting therapeutic measures and premature culling of high-value animals [4, 8, 14, 16].

The main diagnostic methods for acute postpartum endometritis used in practice are clinical-obstetric methods [3, 5], ultrasound methods [2, 5], as well as histological and cytological methods [5]. At rectal palpation, it is possible to assess the size, contents and position of the uterus. However, it is not always possible to obtain information about the character of the uterine contents and the condition of its walls. Over the past decade, ultrasound scanners have been widely used for assessing the state of reproductive organs, diagnosing and predicting complications of the postpartum period, the advantage of which is harmlessness and the possibility of repeated use. Echography can detect changes typical of
Postpartum endometritis, which helps to confirm the clinical diagnosis [2, 5]. Also, an ultrasound examination can determine the size of the uterus, the presence of exudate in its cavity, but it is not always informative. In some cases, it is possible to identify the expanded cavity without visualizing the inflammatory process [2]. The histological and cytological methods for the diagnosis of postpartum endomyometritis in cows are more accurate but are used later. They are expensive, require a certain amount of time, highly qualified specialists and are not technologically advanced in production conditions. Also, biopsy sampling negatively affects the fertility of cows [5]. Therefore, at present, they have not found a wide application in practice for the diagnosis of inflammatory uterine diseases in cows. In most cases, a clinical examination is used for these purposes.

Therefore, the improvement of existing algorithms, the development of new methods for predicting and early diagnosis of acute postpartum endomyometritis in cows is an important vector for solving this problem. There is a need for diagnostic equipment, with the help of which it is possible to make a diagnosis non-invasively, quickly and with high accuracy. Such devices include the electronic nose, which can determine gases and their concentration both in exhaled air and various pathological secrets [1].

In cows, this device was used for determining estrus, mastitis and respiratory diseases. However, the data on sensitivity and specificity have not been described [10, 15, 17, 18]. Therefore, further study of the diagnostic reliability of the electronic nose device for determining metritis is an urgent task.

2. The purpose of the study
The important issue is to study the sensitivity of the electronic nose device developed by the MIP LLC “Sensorika – Novye tehnologii”, as applied to cervical-vaginal discharge samples obtained from fresh cows with a physiological and complicated postpartum period for the diagnosis of acute postpartum metritis in dairy cows. The study aimed to study the equilibrium gas phase of cervical mucus obtained from cows with a physiological and complicated course of the postpartum period.

3. The object of the study
The object of the study was cervical-vaginal mucus obtained from Simmental cows of domestic selection during the early postpartum period with its physiological and pathological course, belonging to one of the farms in the Voronezh region.

4. Materials and methods
The object of the study was cows of Simmental breed of domestic selection belonging to one of the farms of Voronezh region. The animals included in the experiment were subjected to clinical obstetric examination on the 1st-3rd and 6th-8th days after calving under the Methodical Manual for the Prevention of Infertility in Highly Productive Cattle (Voronezh, 2010) [3]. According to the results, they were divided into two groups: with the normal course of the postpartum period (n = 12) and with complicated postpartum metritis (n = 15).

Blood samples were obtained from cows for laboratory tests during the above mentioned periods. Blood content of leukocytes, leukogram, the level of total protein and its fractions, total immunoglobulins, circulating immune complexes were determined following the Methodical recommendations for the diagnosis, therapy and prevention of metabolic disorders in productive animals [6].

Malondialdehyde (MDA) was determined under the Methodical guidelines for the study of free radical oxidation processes and the antioxidant defence system of animals [7].

The parameters of serum bactericidal (SBA) and lysozyme (SLA) activity were determined following the Methodical recommendations for the assessment and correction of nonspecific resistance of animals [9]. At the same time, samples of cervical-vaginal mucus were obtained from animals with the physiological course of the postpartum period (n = 4) and in those with the postpartum endometritis (n = 4).
The obtained cervical-vaginal mucus samples were analyzed on an electronic nose device according to the method of piezoelectric quartz micro-weighing using an array of sensors of the main classes of volatile odour compounds in the equilibrium gas phase above the sample.

The odour was studied at the NIL MIP LLC “Sensorika – Novye tekhnomii” (Scientific Research Laboratory of Materials and their Application “Sensorika-New Technologies”) (SNT) on the laboratory (experimental) odour analyzer “MAG-8” with the electronic nose methodology (manufactured in Russia). As a measuring array, eight sensors based on piezoelectric quartz OAV-type resonators with a base oscillation frequency of 10.0 MHz with different film sorbents on the electrodes were used. Coatings were selected so that volatile biomolecules-markers of both the natural metabolism of living organisms and its abnormal course due to various reasons were easily sorbed. Sorbents are micron-thick polymer films and nanostructured phases: -S8 is the serial number of the sensor in the array. Sorption coatings of sensors: PEGSb - polyethylene glycol sebacinate (sensor labelled S1), TH-100 – Triton X-100 (sensor labelled S2), DTSG18K6 – Dicyclohexane-18-Crown-6 (sensor labelled S3), Tween – Tween- 40 (sensor labelled S4), R6G – Rhodamine 6G (sensor labelled S5), BCG – bromocresol green (sensor labelled S6), MUNT – carboxylated multilayer carbon nanotubes (sensor labelled S7), PEG-2000 – Polyethylene glycol 2000 (sensor labelled S8). Preliminarily, the array was trained in pairs of test compounds of various classes, establishing the characteristics of the sensor array response to these compounds (ketones, alcohols, aldehydes, aliphatic and cyclic amines, acids, ammonia, water).

The qualitative and quantitative characteristics of the output curves of piezo sensors (Figure 1 – a, b) were selected as the criteria for assessing the difference in the odour of the analyzed samples.

![General view of the workplace with an electronic nose “MAG-8” and a piezo sensor](image1.png)

**Figure 1.** General view of the workplace with an electronic nose “MAG-8” and a piezo sensor (a), output curves of an array of 8 piezo sensors (b)

The method of mucus analysis was the following: the samples were thawed and heated at room temperature until equilibrium in a closed sterile tube. An equilibrium gas phase above the mucus was taken with a 5 cm³ syringe and introduced into a closed detection cell, the sensors' responses were recorded in the software for 200 s. Then, we analyzed the data array from all sensors during the measurement. To detect the impurity content of volatile compounds in the equilibrium gas phase above the biological samples, we compared the response values of all selected sensors in the array and the quantitative integrated signal of the electronic nose - the area of the “visual print” of the responses, Sv.im. Hz’s. Substances in the equilibrium gas phase above the samples were identified by the pre-detected properties of the selectivity of sorbents on piezo sensors in the array.

Mathematical and statistical processing of the obtained data was performed using the SPSS version 22 program (IBM Corp, Version 22.0, Armonk, NY, USA, 2013). The Student criterion calculated its arithmetic mean and its error (M ± m) and the significance of the difference (p).
5. Results and discussion

Based on clinical and obstetric studies during the first day after calving, abundant bloody discharges were recorded in cows with subsequent postpartum metritis, and the presence of a mucous plug in most animals was not observed. Also, in 50% of animals of this group, the placenta was separated 10-12 hours after calving. In cows with the physiological course of the postpartum period, the third stage of calving ended in 5.65 hours, the mucous plug in the form of a yellowish weird appeared at the end of the first day after calving. On the 6th-8th days, the cows with postpartum metritis were diagnosed with cervical purulent-catarhal discharges. When the animals were lying, their number significantly increased, and crusts of dried exudate were recorded on the base of the tail. During the rectal examination, it was registered that the uterus was strongly lowered into the abdominal cavity and enlarged, its wall was thickened, and the vibration of the middle uterine arteries was also observed. In animals with a physiological course of the postpartum period, a discharge of cherry-coloured lochia of mucous consistency was registered.

Table 1. Morphological, biochemical and immunobiological blood parameters of cows 1-3 days after calving with a different character of the postpartum period course

| Parameters               | With the physiological course of the postpartum period, n=12 | With postpartum metritis, n=15 |
|--------------------------|-------------------------------------------------------------|--------------------------------|
| Leukocytes, 109/L        | 7.3±0.57                                                    | 6.5±0.51                       |
| Eosinophils, %           | 2.8±0.19                                                    | 5.0 ±0.31***                   |
| Neutrophils, %           |                                                            |                                |
| Stab                     | 2.86±0.24                                                  | 3.4±0.22                       |
| Segmented                | 37.3±3.1                                                   | 33.5±3.2                       |
| Monocytes, %             | 3.7±0.18                                                   | 3.1±0.21                       |
| Lymphocytes, %           | 53.1±4.6                                                   | 58.1±4.2                       |
| Total protein, g/L       | 76.2±5.2                                                   | 69.8±3.0                       |
| Albumins, %              | 54.5±3.8                                                   | 54.2±2.8                       |
| α-globulins, %           | 8.7±0.57                                                   | 8.9±0.3                        |
| β-globulins, %           | 17.5±1.5                                                   | 16.9±0.9                       |
| γ-globulins, %           | 19.3±0.9                                                   | 20.4±1.6                       |
| Total Jg, g/L            | 28.6±1.3                                                   | 23.4±1.3                       |
| CIC, g/L                 | 0.13±0.01                                                  | 0.17±0.01***                   |
| SBA, %                   | 64.6±3.1                                                   | 56.4±1.9                       |
| SLA, µg/ml               | 0.14±0.01                                                  | 0.2±0.01***                    |
| IEI                      | 4.8±0.62                                                   | 6.0±0.51                       |
| MMP c u                  | 0.29±0.02                                                  | 0.46±0.02                      |
| MDA µmol/L               | 2.2±0.12                                                   | 2.7±0.14**                     |

A laboratory blood test showed (Table 1) that cows with postpartum metritis 1-3 days after calving demonstrated a decrease in the organism’s immunological resistance against the background of increased endogenous intoxication and lipid peroxidation, which was confirmed by a decrease in leukocyte concentration by 9.0%, segmented neutrophils – by 9.1%, monocytes – by 16.3%, total protein – by 8.0%, the total immunoglobulins – by 8.0%, serum bactericidal activity – by 12.7%, with an increase in the content of lymphocytes – by 8.7%, stab neutrophils – by 16.1%, eosinophils – on 44.0% (P <0.001), circulating immune complexes – by 23.6% (P <0.01), malondialdehyde – by 18.6% (P <0.01), index of endogenous intoxication – by 20.0%, serum lysozyme activity – by 30.0% (P <0.001). The detected differences in the parameters of the immunobiochemical status of the blood during the first three days after calving indicate the onset of the inflammatory process development in a subclinical form in animals subsequently affected by metritis.
**Table 2.** Morphological, biochemical and immunobiological blood parameters of cows 6-8 days after calving with a different character of the postpartum period course

| Parameters                  | Clinically healthy, n=12 | Postpartum endometritis, n=15 |
|-----------------------------|--------------------------|-------------------------------|
| Leukocytes, 10^9/L          | 7.03±0.37                | 6.8±0.58                      |
| Eosinophils, %              | 2.1±0.21                 | 3.67±0.25***                 |
| Neutrophils, %              |                          |                               |
|    Stab                     | 3.1±0.31                 | 3.0±0.22                      |
|    Segmented                | 47.2±3.64                | 37.0±3.45*                   |
| Monocytes, %                | 2.3±0.2                  | 2.43±0.26                    |
| Lymphocytes, %              | 45.3±2.87                | 53.9±5.2                     |
| Total protein, g/L          | 79.1±4.45                | 72.6±2.87                    |
| Albumins, %                 | 46.3±2.85                | 51.5±2.95                    |
| α-globulins, %              | 8.9±0.47                 | 8.4±0.31                     |
| β-globulins, %              | 19.4±1.06                | 17.9±1.1                     |
| γ-globulins, %              | 25.4±1.83                | 22.2±1.96                    |
| Total Jg, g/L               | 34.3±2.34                | 24.3±1.43                    |
| CIC, g/L                    | 0.17±0.01                | 0.14±0.01                    |
| SBA, %                      | 65.8±2.8                 | 58.5±2.8                     |
| SLA, µg/ml                  | 0.29±0.01                | 0.19±0.01***                 |
| IEI                         | 8.7±0.71                 | 7.6±1.13                     |
| MMP c u                     | 0.39±0.02                | 0.32±0.02                    |
| MDA µmol/L                  | 2.4±0.13                 | 2.43±0.22                    |

Note. *- P<0.05; **- P<0.01; ***- P<0.001

On the 6th-8th days after calving, cows with postpartum metritis also showed a decrease in immunological resistance (Table 2). So, the concentration of segmented neutrophils is lower by 21.7% (P <0.05), the total protein – by 8.3%, β-globulins – by 7.8%, γ-globulins – by 12.6%, serum bactericidal activity – by 11.1%, serum lysozyme activity – by 34.5% (P <0.001), with an increase in the concentration of eosinophils – by 42.8% (P <0.001), which indicates a decrease in total nonspecific resistance allergization of their organisms and the beginning of the clinical manifestation of the pathological process.

Laboratory studies of cervical-vaginal mucus confirmed the results of immunobiochemical studies of cows’ blood by the total area of the complete “visual print” of sensor signals between the groups with physiological (group “norm”) and complicated (group “endometritis”) postpartum period (Table 3).

It has been detected that during the first three days of the postpartum period, the area of the “visual print” of the sensor signals in animals with endometritis is by 28.4% higher than that of cows with the physiological course of the postpartum period. More pronounced differences in the areas of the “visual print” by 6.1 times (P <0.001) were established among the groups on the 6th–8th days after calving.

Thus, during the first three days of the postpartum period, a smaller difference in the composition of volatile bioassay compounds is typical than on the 6th-8th days. A greater number of volatile compounds in the equilibrium gas phase above biological samples of animals from the group “endometritis” on the 6th-8th days after calving indicates the development of a subsequent inflammatory process.

The results of studying the qualitative composition of the volatile odour fraction above the samples and the samples themselves are presented in Tables 4.

It was detected that the shape of the “visual print” of the responses of the sensors in the array revealed differences in the chemical composition of the equilibrium gas phase above the samples within the groups, with particular differences noticeable between the groups. This fact confirms the correct selection of the object of study for additional diagnostic information.
Table 3. The area of the “visual print” of the sensor signals in the equilibrium gas phase above the samples

| Group of animals | Days after calving | 1-3            | 6-8            |
|------------------|--------------------|----------------|----------------|
|                  | The total area of the complete “visual print” | $S_{v.im.0±50, Hz.s}$ |
| Norm             |                    | 790.7±51.2     | 1276.8±94.1    |
| Endometritis     |                    | 1015.1±81.2    | 7769.6±129.6*** |

Note: *- $P<0.05$; **- $P<0.01$; ***- $P<0.001$

The results of changes in the quantitative composition of the odour above the samples according to the relative content of the main classes of volatile compounds, which are configured with an array of sensors, are presented in Table 5.

It was detected that the qualitative composition of the equilibrium gas phase above samples taken from animals 1-3 days after calving, subsequently diagnosed with metritis, significantly differed from cows with a physiological course of the postpartum period. So, in cows with endomyometritis, the content of alcohols, linear and cyclic ketones was higher by 5.2-6.0%, with a decrease in the level of aliphatic acids – by 14.3% and alkylamines – by 9.2% during the early postpartum period.

With the inflammatory process development (6-8 days after calving), similar changes are detected in comparison with the early postpartum period. However, they are more pronounced. So, in cows with postpartum endomyometritis, there was a decrease in acid content by 22.7%, compared with clinically healthy animals, alkylamines – by 8.4%, with an increase in the level of $C_2-C_4$ alcohols, ketones, including cyclic ones – by 6.0%. The manifestation of the disease is reflected in the emergence of a new group of compounds or an increase in their concentration: sulfur-containing organic compounds – by 4.9%, products of abnormal degradation of proteins, as well as ammonia (selective reaction of the sensor with multilayer carbon nanotubes [11-13]).

Table 4. The odour volatile fraction composition above the samples from animals with a different character of the postpartum period course (the 1st-3rd and 6th-8th days)

| Period after calving Group | “Visual print” of maxima [14] | Kinetic “visual print” [14] |
|---------------------------|-------------------------------|-----------------------------|
|                           | Numbers of sensors in the array | Time of responses fixation  |
|                           | “Norm”                        | “Endometritis”              |
|                           | “Norm”                        | “Endometritis”              |
| 1-3 days                  | ![Graph](image1)               | ![Graph](image2)            |
| 6-8 days                  | ![Graph](image3)               | ![Graph](image4)            |
So, at this stage of the study, according to the signals of the MAG-8 electronic nose sensor array, we can speak about qualitative and quantitative changes in the volatile fraction of the uterine-cervical mucus of cows during the development of endomyometritis after calving: a decrease in aliphatic acids, short-chain amines, an increase in alcohol, ketones of high molar mass, the appearance of ammonia, S-containing compounds. The detected differences in the information of an array of chemical sensors in the odour of bioassays correlate with the diagnosis and bias of conventionally determined blood parameters.

Thus, at this stage of the study, using signals from the MAG-8 array of electronic nose sensors, we can speak about qualitative and quantitative changes in the volatile fraction of uterine-cervical mucus obtained 1-3 and 6-8 days after calving from clinically healthy cows and cows with metritis. They have a similar character: the accumulation of alcohols (ethanol, butanol), ketones, including cyclic, a decrease in the proportion of aliphatic acids and alkylamines. However, the manifestation of the inflammatory process in the uterus is characterized by an increase in the concentration of sulfur-containing organic compounds, products of abnormal destruction of proteins, as well as ammonia.

Table 5. The relative content of the components in the samples, \( \omega \% \) of the mass.

| Group of animals | Selectivity of sensors to the compounds [15,16] |
|------------------|-----------------------------------------------|
|                  | S1 – amines | S2 – sulfur-containing | S3 – acids, alcohols | S4 – acids | S5 – acids amines | S6 – amides | S7 – universal | S8 – alcohols, ketones |
| 1-3 days after calving | 10.4±0.61 | 22.6±0.2 | 6.3±0.14 | 2.1±0.12 | 11.5±0.2 | 11.9±0.4 | 10.9±0.3 | 23.3±0.2 |
| Metritis | 9.9±0.19 | 22.4±0.2 | 6.8±0.11 | 1.8±0.11 | 11.8±0.4 | 10.8±0.3 | 11.3±0.1 | 24.5±0.3 |
| 6-8 days after calving | 10.4±0.6 | 22.6±0.5 | 6.4±0.15 | 2.2±0.13 | 11.3±0.3 | 11.0±0.4 | 11.0±0.2 | 23.4±0.4 |
| Metritis | 10.2±0.3 | 23.7±0.3 | 6.2±0.12 | 1.7±0.11 | 11.1±0.4 | 10.9±0.2 | 11.1±0.5 | 24.8±0.3 |

6. Conclusion
The differences were detected in the morphobiochemical and immunological status of cows during the early postpartum period, subsequently with metritis. The detected differences indicate a decrease in the immunological resistance of their organisms against the background of increased endogenous intoxication and lipid peroxidation due to microbial pressure. At the same time, an inflammatory process intensifies in the uterine cavity, proceeding at first in the premorbid stage (1-3 days after calving), and then in a clinically pronounced (6-8 days) form.

The studies on the qualitative and quantitative composition of the equilibrium gas phase above cervical mucus samples of cows with the physiological course of the postpartum period and complicated endomyometritis indicate significant changes in the volatile fraction during the development of endomyometritis. Endomyometritis is characterized by a decrease in the content of aliphatic acids, short-chain amines, an increase in the level of alcohol, ketones of high molecular weight, the appearance of ammonia, sulfur-containing compounds. Therefore, the electronic nose system can distinguish the composition of volatile compounds above bioassays from various groups on the selected array of sensors.

The registered changes in the qualitative and quantitative composition of the equilibrium gas phase in cows with endomyometritis on the 1st-3rd and 6th-8th days of the postpartum period are similar. They can be used as markers for early diagnosis of the inflammatory process development in the uterus.

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