Sensing of the Functional State of Fertility of Cows

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***Abstract*: Sensing of cow functional state of fertility is important for the cattle and diary industries. Usual medical techniques, like the hormone test in urine or milk and the liquid based cytology, are not ideal when farm environment is considered and there is a need to develop improved methods. The method presented in this paper is based on fiber optic capillaries techniques using neural network analysis. We have investigated the vaginal fluids of healthy cows and of those suffering from vaginits and have proved that the method allows establishing the estrous state and distinguishing between healthy and sick animals. We present the experimental setup and analyze various aspects of the sensor design and operation principle with special consideration to the ANN part. The results of the measured signals analysis of ANN training showed a correlation of 0.987 for estrus and 0.986 for the classification of functional state of fertile phase. The proposed sensor introduced automatically precise information of possible vaginitis obtained by establishing the presence of antibodies in the vaginal fluid.**

Keywords: cow estrous detection; cow vaginitis detection; leukocytes detection; fiber optic sensors, fiber optic capillary sensors.

I. INTRODUCTION

The estrous cycle of cows has been investigated with increasing intensity in the recent years [1-8]. Nowadays, the estrous cycles of cows are often resynchronized [9]. Methods of fertile phase of estrus detection are under continuous investigation and improvement [10-18]. Mathematical, methods and methods using fuzzy logic have also been in development [19-20]. Of interest are methods using radiotelemetry [21] and the future may well see mobile radio networks being used for monitoring [22], in which case the energy consumption is an issue [23].

The state of the art of estrus cycle determination was presented in a comparison of four methods for detection of estrus in dairy cows with resynchronized by artificial insemination estrus cycles by Cavalieri et al. [24]. In that study the sensitivity and predictive values of pedometers, radiotelemetric transmitters (HeatWatch) tail-paint and heat-mount detectors were compared. The semi-laboratory method based on measuring the milk progesterone concentration was used as the reference standard for cows being in estrus. Sensitivity was defined as the percentage of estrus cows detected to the number of cows in estrus and the positive predictive value as the number of correct detections divided by the sum of correct and false positive detections. The conclusion was that all four methods gave over 80% of the sensitivity, with the pedometer method scoring lowest at 81.4% and the tail-paint method scoring highest at 91.3%. In terms of positive predictive value, the results were closer and ranged from 87.5% for the pedometer method to 100% for the radiotelemetry method, with the other two methods showing about 92% predictive value [24].

An important problem of cow reproduction is keeping the health of the vaginal duct. These are known methods of veterinary medicine used for that purpose, but they are not automated and when the vaginal changes are visible the illness is usually seriously advanced. Moreover, the bacterial vaginitis can be in its initial and hidden state invisible [25].

Therefore, we set ourselves the following objectives:

- To investigate and develop a sensor which would provide the information of functional state of fertility useable to make decisions on insemination of the cows with a significantly higher sensitivity than the existing field methods,
- To develop a sensor which also could be operated in the farm environment, not only in laboratory environment,
- A developing a method that would yield itself easily to automation, miniaturization and be of low cost,
- To evaluate and further optimize the technology of fiber optic capillary sensors with artificial neural network analysis of data from the point of view of the above main objectives.

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II. SENSOR SET-UP

A. *Principle of sensor work*

In this work we present new developments and advances on intelligent photonic sensors and their applications working on the principle of monitoring optical intensity changes that take place in dynamically forced measurement cycles that were first postulated in [26]. The sensors use fiber optic capillaries in which the phase of the filling liquid changes locally to gas when forced by local heating, while the propagation of light across the capillary is monitored, Fig. 1. Therefore, the sensors examine simultaneously many liquid parameters which are then processed in artificial neural networks [27]. When analyzing biological fluids, in order to achieve good reproducibility and to avoid cross-contamination from sample to sample, it is advantageous to use disposable optrodes. The low cost of capillaries make their disposability practically possible [28].

Before the test the capillary optrode is partially filed with liquid, after which both its ends are closed. Care must be taken that there are no bubbles of gas present after filling the optrode; otherwise the optrode has to be eliminated from the tests [29]. When the capillaries are filled uniformly by the liquid, the initial levels of reflected and scattered signals are measured. As the cow's vaginal fluid in functional state is semitransparent, we expect initially high reflection signal levels and low scatter signal levels. When the bubble appears, the reflected and scatter signal decrease.

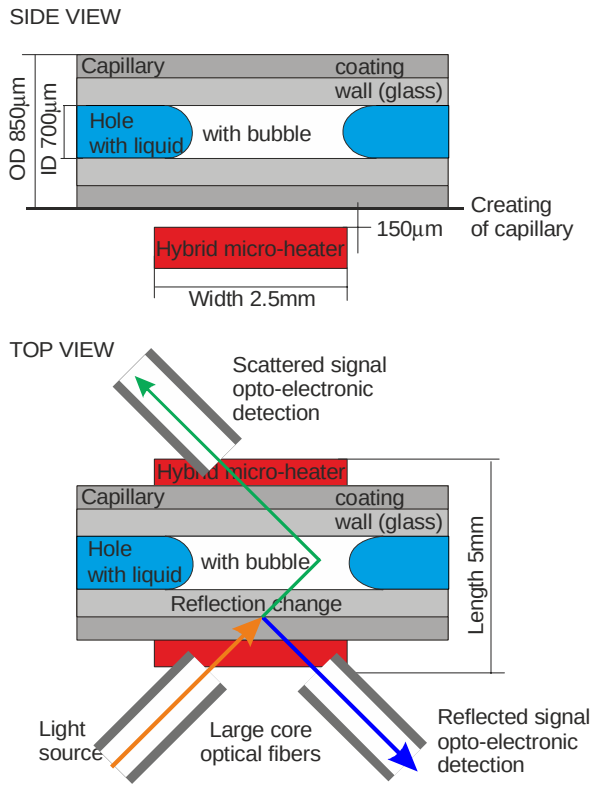

Figure 1. Schematic construction of the capillary head.

When the reflected signal decreases rapidly it gives the impulse to switch off the microheater. Depending on the thermo-dynamical conditions, the gas phase is absorbed in the liquid or remains in the form of a series of small bubbles. Due to structural changes in vaginal fluid induced by heating, the scattered signal can increase.

B. *Sensor construction*

The capillaries used in our experiments were cut into 10 cm long sections from Polymicro Inc. TSP 700 850 fiber. They had both ends closed. In the measuring head we also installed large core optical fibers BFH 37-600 from Thorlabs, the outer diameter of cladding of which is almost the same as the outer diameter of the capillary. In the previous sensor construction for fertility phase examination we used separate bases and replaceable capillary optrodes [30]. In the present work we implemented improvements of the fiber positioning method in the head base, which were key factors for improvement of the repeatability of signal measured from head to head. We also introduced the detection of the scattered signal, Fig 1. As the light source we used the fiber coupled laser source S1FC675 from Thorlabs at a level from 0.01 to 0.2mW. The light source was modulated with a function generator DG2021 from Rigol. The signal was transmitted to the BFH 37-600 fiber by graded-index multimode patch cables with a FC/SMA adapter. The optoelectronic detection unit of our own construction had an SMA fiber input and consisted of an integrated photo-amplifier and a band-pass filter with amplification and RMS detection. The optoelectronic unit was connected to a personal computer by analog input of IOtech personal Daq 3000 16bit/1MHz USB data acquisition system. We fed the heater from the laboratory power supply through an electric switch. The hybrid micro heater was powered with 5W in periods shorter than 30s during which time it showed resistance changes of 0.5% that can be considered as being stable. We also used two LM35DT [31] circuits connected, with the Daq 3000 system, by low pass filters to monitor the temperatures of the measuring head base and of the surrounding ambient. To operate the system we designed a script in DasyLab 10 with 0.1s sampling rate.

The sensor was calibrated by filling the capillary with deionized water and performing a measurement procedure as on Fig. 2. The deionized water started boiling in a time range from 17.1 to 18.5 seconds of the measurement cycle for different disposable optrodes, but the characteristic shape of the signal was considered repeatable. The formation of the gas bubble caused a decrease of the reflected and the scattered signals. The scattered signal in this experiment came from two layers of capillary coating. However, when the bubble was present the scattered signal came mainly from one only layer of capillary coating and that was the reason of the decrease of the signal. After the heater was switched off, the bubble in the water changed from the gas to the liquid phase. There were no changes in the scattered signal after gas phase resorption. We performed experiments when the ambient temperature was in range from 22°C to 24°C and when the head base temperature was not greater than 0.5°C over the surrounding ambient.

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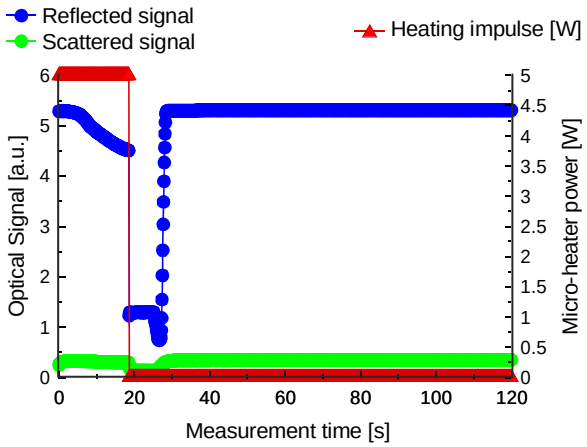

Figure 2. Measurement procedure signals of deionized water.

During the calibration cycle we also registered the temperature distribution in the capillary optrode with a R300 NEC thermo-vision camera and InfReC Analyzer software. The map of temperature just before local boiling of water is presented in Fig. 3. The temperature map at the moment just after the start of boiling is presented in Fig. 4. The positions of the capillary on Fig. 3 and Fig. 4 are rotated by 90 degrees compared with Fig. 1.

The points a, b, c in Figs. 3 and 4 are on the axis of the capillary. The temperature of the capillary was measured in points over the outer edges of the micro-heater. The temperature in point b was determined by the power dissipated in the micro-heater, and exceeded the boiling point of water, putting the steam in the bubble in a superheated condition. Due to good heat conductivity of water the temperature inside the capillary could be considered as almost uniform on the line a-c.

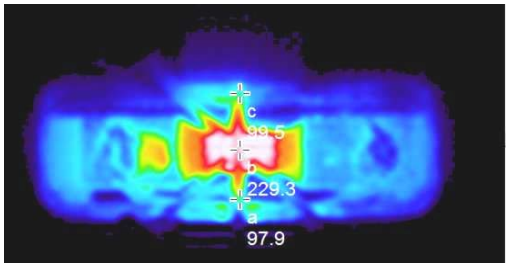

Figure 3. Temperature map just before boiling at 17s of the measurement procedure.

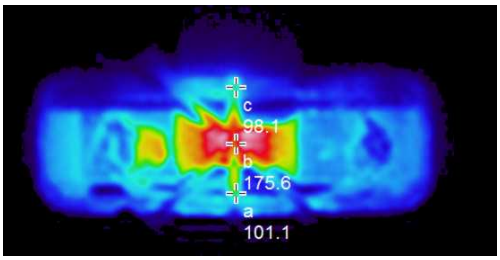

Figure 4. Temperature map just after boiling at 18s of the measurement procedure. The movement of the boiling vapor phase is visible in point a.

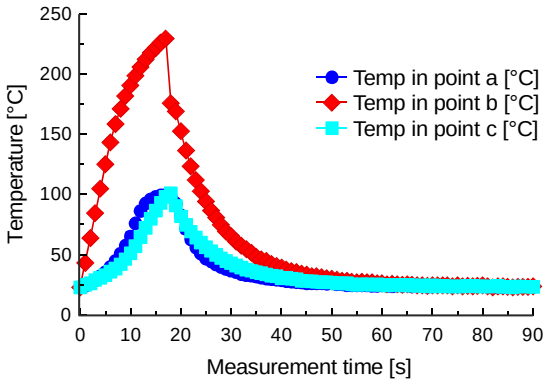

Figure 5. The temperature versus time curve of deionized water filling the capillary measured in the points showed in Fig. 4 and Fig. 5.

The temperature increase in the liquid was considered almost stable in time (see Fig. 5).

III. EXPERIMENT RESULTS

Our experiments were divided in two parts. In the first part we analyzed the optical signals from different components of the vaginal fluids. In the second part we examined the vaginal fluid of cows in the most fertile phase for healthy cows and for cows suffering from vaginitis.

A. Examination of components of vaginal fluid

We used Permeabilization and Blocking Solutions (PBS) from Sigma Aldrich Inc to fabricate the mixture containing antibodies. The purified PBS measurement procedure gave signals very close to those for deionized water. The measurement procedure with PBS antibodies solution is presented in Fig. 6. The gas phase was created in 5.1 second of the measuring procedure, which was three times earlier than for case of deionized water. This phenomenon was repeatable but we found it not easy to interpret. The scattered signal increased significantly from 0.18 a.u. at the initial point of procedure to 0.3 a.u. just before turning the heating off and to average level 1.2 a.u. at the end of procedure. The liquid just above the heater after heating off resorbs the gas phase, the color of this liquid becomes foggy white. This can be explained as antibody protein denaturation. Glycerol is sometimes used as lubricant of cow's vaginal duct. Therefore, we examined next the mixture of e-coli bacteria with glycerol (see Fig. 7). Glycerol and water showed different characteristics of signal reflection, but when the bubble was resorbed, a small increase of the scattered signal from initial 0.76 a.u. to 1.1 a.u. was observed.

The mixtures of antibody and bacteria that we analyzed had similar concentrations of biological components, which combined with the similarity of the amount of energy transferred to the capillary, led us to the conclusion that the analyzed antibody was easier denaturated by temperature than e-coli, and that the scattered signal changes that could be connected with their presence may enable the detection of vaginitis.

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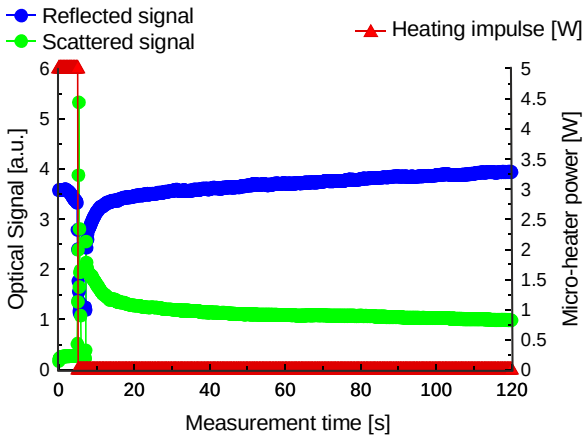

Figure 6. Measurement procedure signals of pbs antibody mixture

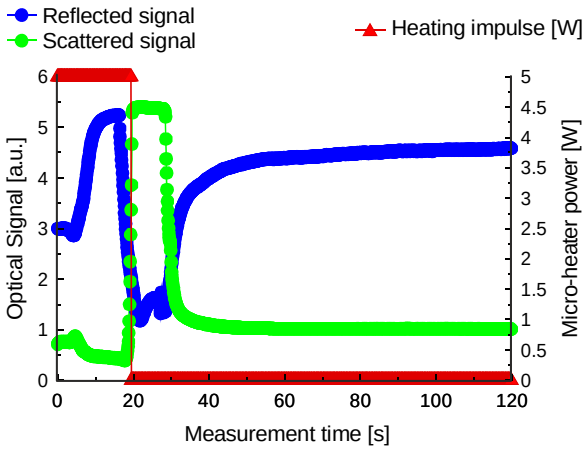

Figure 7. Measurement procedure signals of glicerol e-coli mixture.

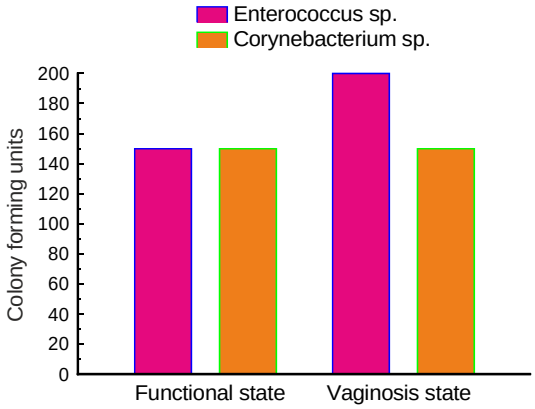

Figure 8. Microbiology classyfication of vaginal fluids.

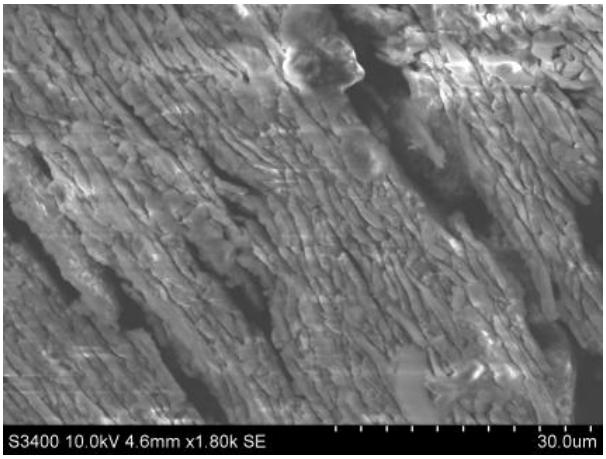

Figure 9. Vaginal fluid arborization test of cow in functional state of fertility, photo made with AFM S3400

B. *Examination of cow's vaginal fluid*

We analyzed vaginal fluids of Holstein-Friesian and Jersey cow breeds that are typical cattle in Poland. The cows were selected randomly from herds counting from 10 to 100 animals. We examined samples of 15 cows classified as healthy. The vaginal fluid was collected in estrus and the post-estrus state. The vaginal fluid was also collected from 2 cows which were in estrus and had vaginitis diagnosed by a veterinary doctor on the farm and confirmed by microbiological analysis, Fig. 8. The difference between healthy and ill state was not great by the count of the bacteria colony forming units. The vaginal fluid arborization test of cow in functional state of fertility is presented on Fig. 9. The observations of filling of the capillaries with various kinds of vaginal fluids are presented in Table 1.

The vaginal fluid of not fertile phase did not enter the capillary without pumping. This was the simplest test for most fertile period of estrus. When the cow had serious problems of its vaginal duct, the vaginal fluid changes were visible and further diagnostic methods were not required, but there were no visual differences between vaginal fluids of cow with vaginitis or functional state, which called for additional diagnostic tests.

TABLE I. OBSERVATION OF FILLING OF THE CAPPILARIES

Liquid type	Capillary action [mm]	Capillary filling
Water	28	capillary action
Vaginal fluid at most fertile phase with ovulation	20	capillary action
Vaginal fluid at most fertile phase without ovulation	0	syringe pumping working well
Vaginal fluid of cow with vaginosis	20	capillary action
Vaginal fluid of cow with ovarian cysts	0	syringe pumping working badly

Measurements of healthy cow's vaginal fluid using our method showed conformity with the results of examinations just discussed, Fig. 10. We observed that the vapor phase creation time was short and was about 5 seconds. The vapor phase existed for 2 seconds. The scatter signal did not exceed after heating the level of 1.5 a.u. but would increase correspondingly with the count of present bacteria. The limits of the increase of the signal are marked as red lines on the part B in Fig. 10 and Fig. 11. The result of the examination of the vaginal fluid of cows with vaginitis is presented in Fig. 11.

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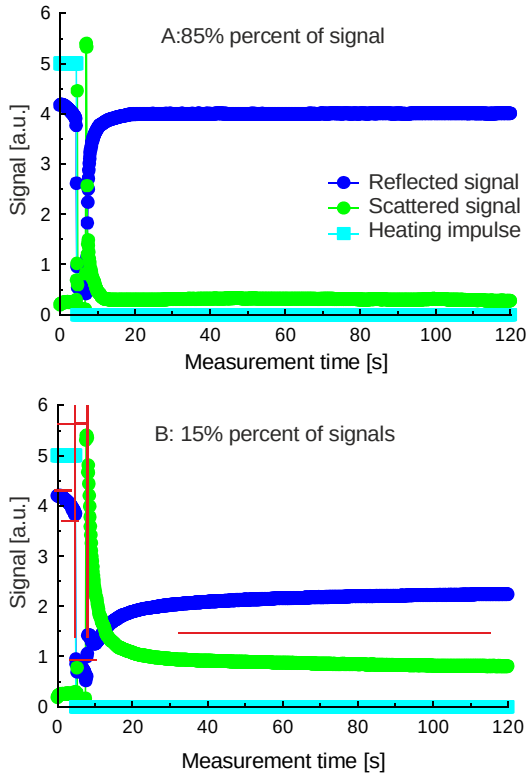

Figure 10. Measurement procedure signals for cows classified as healthy and in estrus state.

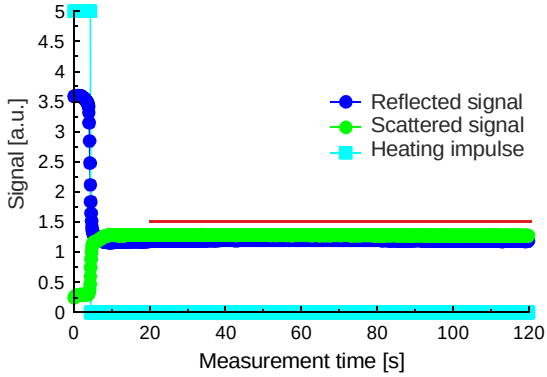

Figure 11. Measurement procedure signal of vaginal fluid of cows with vaginosis.

We observed that the gas phase was either not present or was masked by the denaturation of proteins that would give a significant increase of the scattered signal from the stable level after turning the heating off.

The cow in late estrus state approaches the limits of its readiness for insemination. In our observations on Fig. 12, the signals of vaginal fluid changed compared to Fig. 10. The time of gas phase creation increased significantly to over 10 seconds of the measurement procedure. The generated gas phase accumulated the energy that did not allow the bubble to be resorbed.

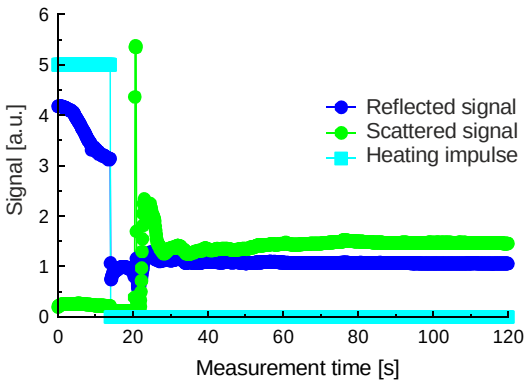

Figure 12. Measurement procedure signals of vaginal fluid of cows after estrus.

IV. Artificial Neural Network For Functional State Of Fertility Classification

The Qnet artificial neural network ANN used for classification of the vaginal fluid samples showed its advantages in sensor data processing. We examined the multilayer perceptron for classification of measured signals. We assumed two output information levels: a) healthy/unhealthy, b) ready for reproduction/ not ready for reproduction. While the cow is in the unhealthy state we assumed that it was not ready for reproduction, but is ready for veterinary treatment. The input information was the levels of signals and timing of the gas phase creation and the duration of the gas phase. The gas phase creation was evidenced by a rapid drop of the reflected signal. The gas phase duration was defined by the difference of time of the maximum level of the scatter signal and the mentioned drop of the reflected signal when the drop of the reflected signal undershot the level of 0.8 a.u. As the levels of the reflected signal we included into the ANN input: the initial level, the minimal level and the level at the measurement end. For the scattered signal we analyzed the: initial level, the maximum level and the last measured level. Therefore our ANN had 8 inputs, 2 outputs and 4 hidden layers in which the perception used the sigmoid activation function and one hidden input with constant -1 value.

Such ANN training gave a 0.986 correlation coefficient in the training set and a 5% RMS error of outputs when cases from monitored cows but not contained in the training set were analyzed. The output responsible for healthy state with a correlation coefficient of 0.999 was more precise than the estrus output where it was 0.987.

We made test of network output for cow that is not included in training set and has first estrus after miscarry. The RMS error of most fertile state was 21%. It is a large figure, but the third estrus state after miscarry is standard consider as fertile and a question arise of mentioned cow healthy state. Therefore, the 5% of output tolerance of fertile state classification seems reasonable.

The QNet software enabled us to analyze the input node contribution of output signals, Tab. 2. The data analysis showed that in our method the functional state of the cow

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examined on base of vaginal fluid was directly and firmly connected with gas phase creation. Taking into account the time of gas phase creation and the time of its lasting, we have a level of 84% of contribution for cow functional state classification. We also could see that the gas creation was also the main information for cow readiness for reproduction at a level of 65% of contribution.

TABLE II. ANN ANALYSIS OF INPUT TO OUTPUT CONTRIBUTION

Input	Input contribution [%]	
	Output for cows ready for reproduction	***Output for cows in functional state***
Initial level of reflected signal	14	1
Time of gas phase creation	35	83
Time of gas phase lasting	30	1
Minimal level of reflected signal	8	3
End level of reflected signal	2	0.5
Initial level of scattered signal	5	0.5
Maximum level of scattered signal	4	0.5
End level of scattered signal	2	0.5

V. CONCLUSION AND FUTURE WORK

We have proved that the fiber optic capillary sensors with artificial network analysis can provide information on cow estrus state with a sensitivity superior to other existing methods and in addition provide information as to the presence of cow vaginitis.

The results of the measured signals analysis of ANN training showed a correlation of 0.987 for estrus and 0.986 for the classification of functional state of fertile phase. The proposed sensor introduced automatically precise information of possible vaginitis which can be of practical usefulness. We showed that the information on vaginitis was obtained mainly by establishing the presence of antibodies in the vaginal fluid. These antibodies are normally transparent in visible light in standard situations. Moreover, the capillary filling capability can act as an indicator of the most fertile state of cow estrus.

Therefore, we conclude that the proposed construction may be in future the base of commercially marketable instruments. For this purpose the sensor construction has to be integrated into a complete instrument and therefore more resistant to use in harsh environment. The examination of greater number of cases could lead to artificial multilayer perceptron network optimization, mainly by precise the range of output tolerances for cows contained and not contained in training set.

ACKNOWLEDGMENT

This work was supported by the European Union structural funds grant MNS-DIAG task 2.

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