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(54) Title: METHOD TO DETERMINE PROGESTERONE IN RAW MILK

(57) Abstract: The invention relates to a method and a system for determining the amount of progesterone in raw milk. According to the invention, a biosensor is contacted with the raw milk whose progesterone concentration is to be determined. The concentration of progesterone in the milk is subsequently determined with the aid of the biosensor.

METHOD TO DETERMINE PROGESTERONE IN RAW MILK

This invention relates to a method and system for determining the amount of progesterone in raw milk.

In dairy farming, it is of importance to know the estrous/heat cycle of a cow. On the basis of this cycle, the farmer can determine at what time the cow is in heat and what the subsequent time of insemination should be.

The time of insemination is difficult to determine. Accordingly, it happens regularly that the artificial insemination occurs too early or too late, so that fertilization does not take place. This is partly due to the fact that the cycle of each individual cow is different. It is therefore desired to use an objective method of determining the time of artificial insemination.

An important parameter for determining the time of insemination is the progesterone concentration in the milk the cow produces. When the time at which the cow can be inseminated approaches, the progesterone concentration in the milk falls below 4 ng/ml. After the insemination the progesterone concentration rises above 20 ng/ml and when a fertilization has taken place, the progesterone concentration will remain above 20 ng/ml. When the progesterone concentration decreases again, a new artificial insemination can take place. As each individual cow has a different cycle, it is desirable to be able to follow the cycle of each cow separately. To more accurately determine the time of insemination, it is not sufficient to know when the progesterone concentration is below or above the lower or upper limit. Especially reliable information about the concentration range between 0 and 7 ng/ml of progesterone is of importance. Most preferably, the farmer wants to make use of a method

for progesterone determination which is automated to a high degree, has a high reliability and has a low cost price per sample.

It is known that the farmer determines the time of insemination on the basis of the condition of the cow or by utilizing field analysis methods for determining the progesterone concentration in raw milk.

The condition of the cow can be determined inter alia by means of visual observation of the animal behavior and/or an activity measurement. For this purpose, the cow has a step counter with which the number of steps can be counted which the animal makes during a defined time interval. This requires the farmer to observe his cows at least two or three times a day for 15 to 30 minutes. In addition, the farmer also looks at the behavior of the cow. When the cow is in heat, the cow regularly mounts other cows and is mounted herself.

In addition to this century-old method, nowadays use is made of newly developed field analysis methods. To be considered are, for instance, dipstick tests, whereby the farmer takes a sample of milk from the cow (this is a manual operation and is not automated) and holds a test strip in the milk. After a minimum incubation period in which the progesterone in the milk reacts with the reagents in the test strip, it can be read what the concentration of progesterone in the milk under test is. In most cases, this reading is done visually, but in some cases the reading may be objectified by utilizing a photometer. The accuracy of the analytic assay is not very high, so that a pronouncement can be made only on whether the concentration is above or below a set limit.

On the basis of the concentration, not only the estrous status can be checked, but also an early gestation diagnosis can be performed. Furthermore, it can be established whether the animal possibly has a defect of the reproductive apparatus.

For the farmer, it would be ideal if the determination of the concentration of progesterone in raw milk could be automated to a large

extent. The farmer then has his hands free for carrying out other activities and, what is more, the farmer is not trained to perform an analytic assay.

In addition, it would be desirable to be able to determine the progesterone concentration with a high accuracy through the entire range between 0 and 40 ng/ml. This gives the farmer more information about the cycle of the individual cow and he can determine the time of insemination better for each cow. This will result in a reduction of the number of failed inseminations per cow, so that the productivity per cow will rise.

The object of the invention is to provide a solution to the above-mentioned disadvantages as well as to meet the wishes on the part of the farmer. In addition, the invention also has good utility for detecting status and/or defects in fertility.

The method according to the invention is accordingly characterized in that a biosensor is contacted with the raw milk, whereby the amount of progesterone in the milk is determined with the aid of the biosensor. In particular, it holds that the biosensor is connected with a computer for the on-line determination of the amount of progesterone in milk. In this way, it is possible to condition the concentration determination of progesterone in raw milk to a high degree.

Preferably, it holds that further the identity of the animal is determined, the amount of progesterone is determined from raw milk which has been produced by the cow a plurality of times spread in time, while the identity of the animal, together with the determined amounts of progesterone and information about the times at which these amounts were determined and/or the milk was produced, are stored in a database. In this way, the course of the concentration of progesterone in raw milk, spread in time, can be determined per animal, be stored and be processed further. On the basis of the time-dependent concentration curve, it can be

accurately determined when the cow is in heat, whether the cow is in-calf, or whether the cow possibly has a defect of its reproductive apparatus. In particular, it holds that the amount of progesterone in the milk is determined during milking. If the cow is milked with the aid of a milking robot plant, the biosensor can, if desired, be placed in the path along which the raw milk flows through the milking robot plant.

The invention also relates to a system comprising a computer and a biosensor connected thereto, which system is arranged for carrying out the method according to the invention.

The invention will presently be further elucidated with reference to the drawing. In the drawing:

Fig. 1 schematically shows a system according to the invention for carrying out a method according to the invention;

Fig. 2 shows the use according to the invention of a first type of biosensor;

Fig. 3 shows the alternative use according to the invention of a second type; and

Fig. 4 shows the curve in time of the concentration of progesterone in raw milk of a cow.

In Fig. 1 reference numeral 1 designates a system for carrying out a method according to the invention. The system comprises a biosensor 2, a computer 4, and a line 6 connecting the biosensor 2 with the computer 4.

For determining the amount of progesterone in the raw milk, the biosensor 2 is contacted with the raw milk 8 of which, in this case, the progesterone concentration is to be determined. The raw milk 8 in this example is contained in a container 10 which has many embodiments, as will be discussed hereinafter.

The raw milk 8 can consist, for instance, of a sample of the raw milk which is produced by the cow when being milked. This sample can then be introduced into the container 10.

The biosensor 2 is of a type known per se and is arranged to determine the concentration of progesterone in the raw milk 8. The biosensor 2 generates on line 6 a signal that represents the measured concentration of progesterone in the raw milk. In this example, this concentration is stored in a database of the computer 4. In this example, via an input 12 of the computer, also the identity of the animal from which the raw milk originates is stored in the database. The identity of the animal can be determined in a manner known per se. To be considered here are, for instance, determining the identity on the basis of a neck responder, an earmark, an implant and/or a bolus, etc. The system can be provided, for instance, with an identification device 14, known per se, which establishes the identity of the animal and feeds it accordingly to the input 12 of the computer 4.

In particular, it holds that in addition to the identity of the animal, the amount of progesterone is determined of raw milk which has been produced by the animal a plurality of times spread in time. The identity of the animal is then stored in the database together with the determined amounts of progesterone as well as information about the times at which the amounts were determined and/or the milk was produced.

In this way, an insight is obtained into the course of the concentration in time, see also Fig. 4, where the horizontal axis plots time in days and the vertical axis plots the concentration in nanograms per milliliter. The time at which the animal is in heat is denoted by day zero and coincides with a dip in the concentration curve in time of the amount of progesterone in the raw milk. Twenty-one days later the animal proves to be in heat again. By presently signalling the time at which the concentration starts to decrease from a value of, for instance, 40 ng/ml to a value of aroun 4 ng/ml, the time of estrus can be accurately determined. When the concentration does not decrease roundabout the twenty-sixth day, the animal is either gestating or the animal possibly

has a defect of its reproductive apparatus. Preferably, it holds that for about two weeks (fourteen days) after a change in the determined amount of progesterone in time has reached a minimum, the progesterone content of the milk of the cow is not determined. As appears from Fig. 4, in general only after the fourteenth day, when the animal is not gestating and/or does not have a defect of its reproductive apparatus, will the concentration decrease again. Only after fourteen days, therefore, is it of interest again to start monitoring the change over time of the amount of progesterone in the milk. After the fourteenth day, the concentration of progesterone in the raw milk of the animal can be determined, for instance, every two or three days.

Preferably, the amount of progesterone is determined during milking with the aid of a milking robot. The biosensor can then be positioned at a suitable position in a path along which the raw milk of the cow flows through the milking robot. To be considered here are, for instance, the milk line and/or the milk claw. In that case, therefore, the milk claw or the milk line constitutes the container 10 in Fig. 1. It is also possible, however, that in the milking robot in an automatic manner a sample is taken of the raw milk which flows along the milk flow path of the milking robot. This sample is then placed in a container 10 separate from the milk flow path. This is useful in particular when a biosensor is used that consists of a plate on which a coating of progesterone bodies is provided, as will be further elucidated hereinafter.

In that case, as shown in Fig. 2, the biosensor 2 can be provided with a plate 16 on which a coating is applied which comprises progesterone bodies 18. Also designated in Fig. 2 is the raw milk 8 which comprises progesterone 20. Further, to the sample of raw milk shown in Fig. 2 an amount of progesterone antibodies 22 has been added. In Fig. 2 the long tail 24 of the antibodies 22 is schematically shown.

If the concentration of antibodies 22 in the raw milk 8 is gradually raised in a manner known per se, the antibodies 22 will primarily bind to the progesterone bodies 20. As soon as more antibodies 22 have been supplied to the raw milk than there are progesterone bodies present in the raw milk, the antibodies 22 will also start to bind to the progesterone bodies 18 of the plate 16. The result is that the mass connected with the plate 16 will rise. Because the antibodies 22 are of weighted design, the weight increase of the plate 16 will be properly detectable.

When in the raw milk 8 at least substantially no progesterone bodies 20 are present and the antibodies 22 are supplied to the raw milk 8, these antibodies 22 will start to bind directly to the plate 16. The plate 16 will therefore be weighted immediately, which can be measured in a manner known per se.

A further advantage of the method according to Fig. 2 is that the regeneration of the biosensor can be carried out fast and simply. Because the antibodies 22 are weighted, they also dissociate readily from the bodies 18 again. When, therefore, the plate 16 is rinsed clean with, for instance, cleaning fluid, the antibodies 22 will easily detach, so that the biosensor can be used again. Because in the method according to Fig. 2 antibodies are added to the raw milk 8, the concentration of progesterone will preferably be determined on the basis of a separated sample of the raw milk.

It is also possible that the plate of the biosensor 2 is provided with a coating with progesterone antibodies 18, as shown in Fig. 3. The progesterone 20 which is present in the milk 8 will then bind to the antibodies 18'. The result is that the plate 16 will be weighted. The weighting of the plate 16, i.e., the extent to which weighting of the plate 16 occurs, is a measure for the concentration of progesterone in the raw milk 8. This weighting can be measured in a manner known per se. After the concentration of progesterone has been determined, the plate 16 can

be rinsed clean in a manner known per se with, for instance, cleaning fluid for regeneration. The progesterone bodies 20 thereby detach and can be used for new detection of progesterone in raw milk 8.

In the method according to Fig. 3, it is theoretically possible that the biosensor 2 as described hereinbefore is included in the milk flow path of a milking robot.

To detect the weighting of the plate 16, the resonance frequency of the plate can be determined in a manner known per se. If the plate is provided with a metal oxide coating, the refractive index at the surface of the plate is found to be dependent on the amount of bodies (Fig. 3) or antibodies (Fig. 2) bound to the coating. This results in measurable changes in the reflection intensity of a laser beam with which the plate is illuminated. In this way too, through detection of the intensity of the reflected laser beam, the concentration can be determined. It is also possible that the amount of progesterone in the milk is determined but that the analysis of the measuring results does not take place until later. The obtained data can also be coupled with other data of the computer 4. It may also be important to consider the time at which the progesterone is detected in relation to other parameters that are dependent on this point in time. Thus, a pronouncement on fertility can be made on the ground of the determined amount of progesterone in combination with other parameters, such as activity and temperature of the animal. Such variants plus other known methods are all understood to fall within the scope of the invention.

CLAIMS

1. A method for determining the amount of progesterone in raw milk of cows, characterized in that a biosensor is contacted with the raw milk, while the amount of progesterone in the milk is determined with the aid of the biosensor.

2. A method according to claim 1, characterized in that the identity of the animal is determined, the amount of progesterone is determined of raw milk which has been respectively produced by the cow a plurality of times spread over time, while the identity of the animal, together with the determined amounts of progesterone and information about the times at which these amounts were determined and/or the milk was produced, are stored in a database.

3. A method according to any one of the preceding claims, characterized in that the amount of progesterone in the milk is determined during milking.

4. A method according to claim 3, characterized in that the cow is milked with the aid of a milking plant/robot.

5. A method according to claim 4, characterized in that the biosensor is included in the milking plant/robot.

6. A method according to any one of the preceding claims, characterized in that the amount of progesterone is determined of a sample of the raw milk of the cow.

7. A method according to any one of the preceding claims, characterized in that at least on the basis of the determined amount or amounts of progesterone it is determined whether a cow is in heat or in-calf or whether the cow has a defect of its reproductive apparatus.

8. A method according to any one of the preceding claims, characterized in that for about two weeks after a course of the

determined amount of progesterone over time has reached a minimum, the progesterone content of the milk of the cow is not determined.

9. A method according to any one of the preceding claims, characterized in that use is made of a biosensor having a surface which is provided with a coating with antibodies of progesterone.

10. A method according to any one of the preceding claims 1-8, characterized in that to the milk antibodies of progesterone are added and that use is made of a biosensor having a surface provided with a coating with progesterone bodies.

11. A method according to claim 10, characterized in that the antibodies are heavier than progesterone.

12. A method according to any one of the preceding claims, characterized in that the determined amount or amounts of progesterone is or are analyzed at a later time than the time at which the sample is taken.

13. A method according to any one of the preceding claims, characterized in that the determined amount or amounts of progesterone is or are processed in combination with other data from a farm computer.

14. A method according to claim 7, characterized in that the determined amount or amounts of progesterone is or are processed in combination with other data, such as the time of determining the amount of progesterone, the activity and temperature of the animal, etc., for making a pronouncement about the fertility of the animal.

15. A method according to any one of the preceding claims, characterized in that the biosensor is connected with a computer for on-line determination of the amount of progesterone in the milk.

16. A system comprising a computer and a biosensor connected therewith, arranged for carrying out the method according to claim 15.

17. Use of a biosensor for determining the amount of progesterone in raw milk of cows, wherein the biosensor is contacted with the milk for determining the amount of progesterone.

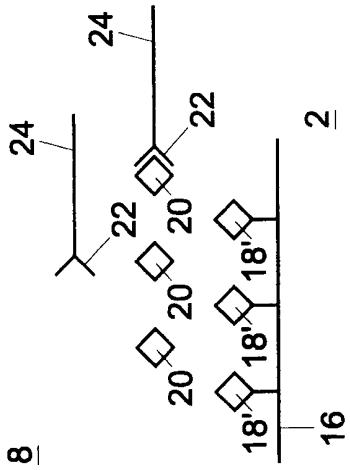

Fig. 2

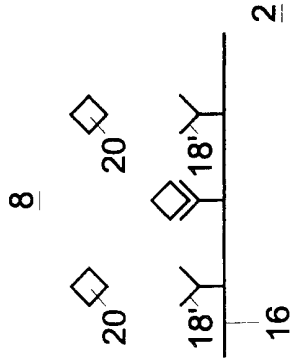

Fig. 3

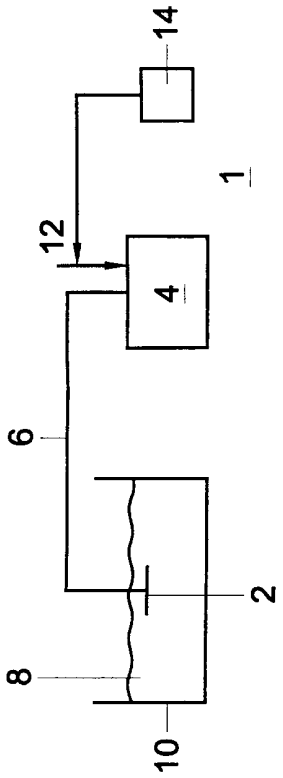

Fig. 1

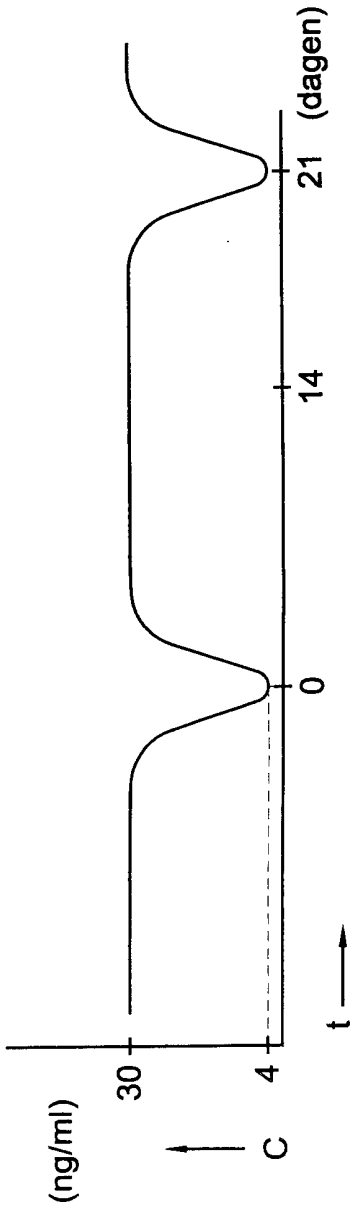

Fig. 4

INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/NL 00/00580

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/74 G01N33/543

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, PAJ, CHEM ABS Data, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	R.M. PEMBERTON ET AL.: "Development of a sensitive, selective electrochemical immunoassay for progesterone in cow's milk based on a disposable screen-printed amperometric biosensor " ELECTROCHIMICA ACTA, vol. 43, no. 23, 1998, pages 3537-3574, XP004130864 Barking UK figure 1 --- -/--	1-17

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
6 October 2000	16/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Van Bohemen, C

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INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/NL 00/00580

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 130, no. 11, 15 March 1999 (1999-03-15) Columbus, Ohio, US; abstract no. 134247, XP002137666 abstract & R. CLAYCOMB ET AL.: "Biosensor for online measurement of bovine" BIOSENSORS AND BIOELECTRONICS , vol. 13, no. 11, 1998, pages 1173-1180, Denver CO USA	1-17

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