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Daily Cow Pregnancy Colloidal Gold Test Strip

【Technical dates and other detailed explanations】

One of the aims of dairy cow feeding management is to improve milk output and shorten the period of non-pregnancy. Detection of pregnancy for dairy cow can offer valuable information producers. For a long time,dairy cow feeders and veterinarians are looking forward to asimple, rapid and effective early pregnancy detection method, which is important for improving feeding management and propagation rate. If the pregnancy isnot promptly detected after mating, it will result in delaying the period of calving and decreasing of propagation rate and milk output. Thus the cost of feeding management will be increased. If the pregnant dairy cow can be detected in early time, the cow can get careful nursing and gestation management, which will increase the pregnancy success rate. Non-pregnant cow can be fertilized early, shorten the period of non-pregnancy and decrease the feeding cost. Thus propagation rate and economical benefit will be increased. Study on the diagnostic skill for early pregnancy of dairy cow is very important to the development of the dairy cow industry.

Progesterone (P4) is a kind of steroid hormone，which was secreted in corpus luteum and placenta. Progesterone concentration change in milk was marked as the specific indication of cow ovary activities. Progesterone plays a most important role in the process of maternal reproductive and maintaining pregnancy.The determination of P4 concentration with radiation immunoassay (RIA) proved that the P4 changes reflect estrus cycle which is consistent with rectal detection.Therefore, the cow reproductive activity can be monitored through testing the P4 concentration.The concentration of P4 has a specific change at the cow different reproductive stages, 1 ~ 5 days after mating ，concentration of progesterone in milk is in low-level,Less than 3ng/ml; along with the increase of days after mating, the progesterone concentration increase gradually; 11 days after mating, progesterone concentration rises rapidly ; 11~ 19 days after mating, progesterone concentration remain at higher level, more than 8 ng/ml;1~ 19 days after mating, the progesterone concentration of pregnant or non-pregnant cow has no obvious difference. 20 days after mating, progesterone concentration in non-pregnant cows milk began to decline to a low level (less than 1 ng/ml), and progesterone concentration in pregnant cows milk continued to maintain at a high level.By the statistical T test，during 21~ 24 days after mating, the progesterone concentration in whole milk of pregnant cow is obvious higher than that of non-pregnant cow（$p<0.01$）.During 0 ~ 29 days after mating, the progesterone concentration in milk of pregnant and non-pregnant cows changes obviously, Through testing of progesterone concentration could make an accurate diagnosis for cow early pregnancy.The test principle of cow pregnancy diagnosis is using double antibody

sandwich colloidal gold test. Progesterone is a hapten small molecular, which could get immunogenicity through connecting with macromolecular protein. The progesterone antigen was synthesized in our company and prepared two different monoclonal antibodies against progesterone. So, the progesterone double antibody sandwich colloidal gold test strips were prepared, that can test progesterone in nanogram level, and the competition method could not get the sensitivity. The test strip is composed of basal plate, nitrocellulose membrane (which is pre-coated with goat anti- progesterone monoclonal antibody on the test band region and goat anti-mouse polyclonal antibody on the control band region),sample absorption layer, and the pad of progesterone monoclonal,antibody labeled with colloidal gold. The two specific monoclonal.

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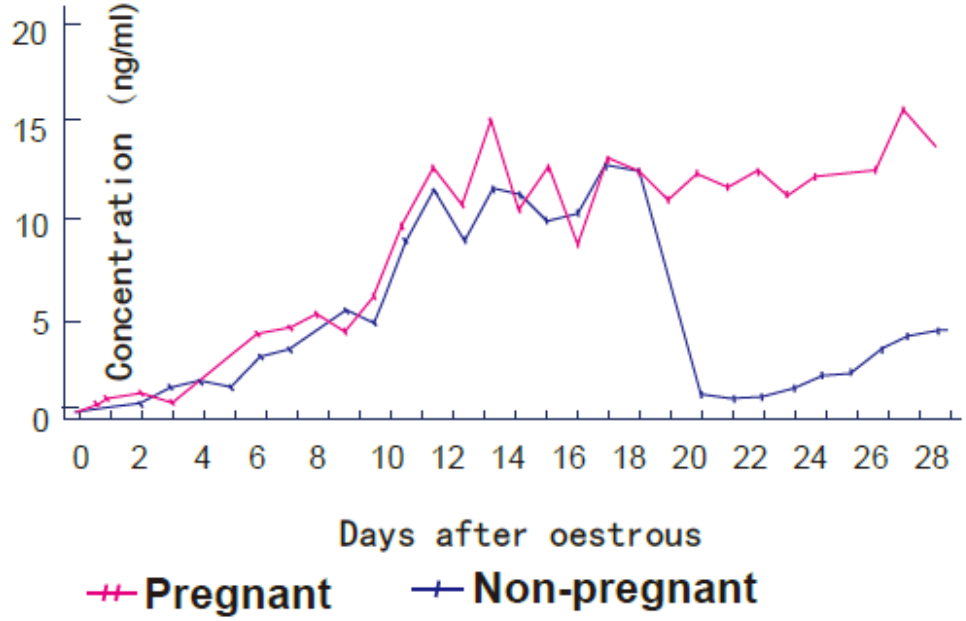
antibodies combine with two different sites of progesterone (P4),according to the color effect of labeled colloidal gold, the concentration of progesterone in whole milk was determined. The detail as follows: The test region of nitrocellulose membrane was pre-coated with anti-progesterone monoclonal antibody Ⅱ and control region was pre-coated with goat anti-mouse polyclonal antibody.When the antigen (such as progesterone)in milk is higher than certain concentration, it would form Ag-Ab-Au complex with anti- progesterone monoclonal antibody Ⅰ , due to capillary chromatography , this complex moves along the membrane, forming two-side sandwich immune complex with anti-progesterone monoclonal antibody Ⅱ which per-coated in nitrocellulose membrane, a color band(T line)will appear in the T area;the surplus gold labled anti-progesterone monoclonal antibody I from goat moves continually, which could combine with goat anti-mouse polyclonal antibody to form a obvious band(C line),so, when test band(T line) and control band(C line)appeared on test strip, that would proved the concentration of progesterone in the milk was higher than 8ng/ml, and the result was positive, which confirmed the cows was pregnant. When the concentration of progesterone in the milk was lower than 4ng/ml, that could not form two-side sandwich immune complex, and there was only C band appeared, which result was negative, that confirmed the cow was not pregnant; It was suspicious when shadow band appeared on test line, the concentration of progesterone in milk was about 4~8ng/ml, the test should be repeated or the clinical examination should be performed at same time.
The test theory is based on variation of P4 in the estrous cycle, to determine whether the early pregnancy of dairy cow by detecting P4 in the end of estrous cycle .The test strip employs colloidal gold immunochromatographic technology for early pregnancy diagnosis of dairy cow, with characteristics of high accuracy, easy to operate, fast result, room temperature storage, easy to carry, safe to mother and baby cow.

The change of progesterone concentration in milk during estrous cycle



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Research and clinical test
The clinical test of 1559 dairy cows showed that 1316 cows confirmed to be pregnant by rectal examination, in which 1274 cows were positive and 32 were considered to be uncertain tested with colloidal gold strips. 22 out of 32 uncertain cows were positive after the second test. The positive predictive rate was 96.8% at first strip test; and 98.5% after the second strip test. 243 cows were negative control.236 out of 243 cows showed negative and 6 being uncertain; 2 out of 6 uncertain cows showed negative after second test. The negative predictive rate was 97.1% at the first test and 97.9% after the second test

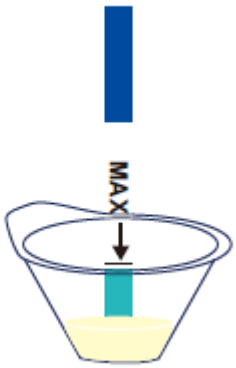
Scope of use
The test can be used to determine the early pregnancy of dairy cow, reexamine dairy cows, and guide the artificial fertilization at right time.

Specificity
Specificity of this test was determined from cross reaction studies with FSH (100μg/mL), E2(10μg/mL), PRL(100μg/mL), LH(25mIU/ml), DHT(10μg/mL), tetraborane(10μg/mL) and H2O. No cross reactivity was observed.

When collecting sample
Collecting the sample (whole milk/serum) at the 22nd-26th days after mating. Pregnant dairy cow can be reexamined at any time. The negative sample is whole milk colleting at o-5th day in estrous cycle.

The pre-treatment methods of the sample
Take whole milk to test its progesterone level directly. In order to get reliable result, give up the first three milking and then collect sample milk around 5-10ml. Mix the sample milk well before testing.

Assay procedure and result analysis
Test strip:



1. Remove the test strip from the foil pouch.
2. Put the "MAX" end into the sample (the surface of the sample should not exceed the MAX line). Due to the function of capillary, the sample dissolves the solid antibody labeled with colloidal gold and moves forward to the other end. When the liquid moves to the 2 / 3 of the result window or to the end, you can take out the strip. One or two red lines will appear in the result window.
3. Observe the result in 5 minutes.

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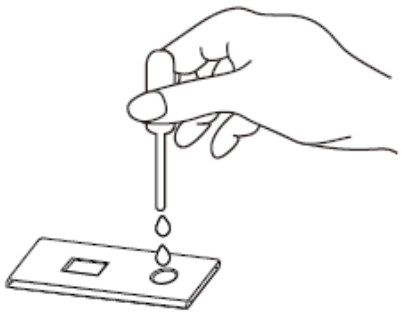
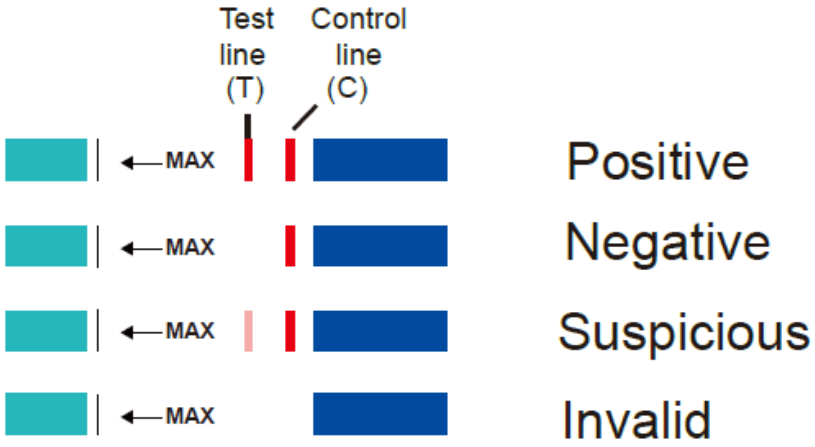
Cassette:



1. Remove the test device and milk dropper from its foil wrapper by tearing along the slice.
2. Using the milk dropper, withdraw the milk sample from the specimen cup and slowly dispense 3～4drops into the circular sample well. The sample liquid moves slowly to the other end due to the capillarity. You will see one or two red lines in the result window.
3. Read results in 5 minutes.

Interpretation of results
Read the result in 5 minutes.
Test strip:



Positive: If both T band and C band are visible, the test result is Positive, which means the cow is pregnant or in middle period of estrous cycle.

Negative: If only C band are visible, the test result is Negative, which means the cow is not pregnant. Suspicious: If the color intensity of the test band (T) is less than that of control band (C), it is necessary to combining with clinical situation or the specimen should be double-checked in next day.

Invalid: When control band does not appear on the membrane, the test is invalid since improper test procedure or deterioration of reagents probably occurred. It is recommended that the test sould be repeated.

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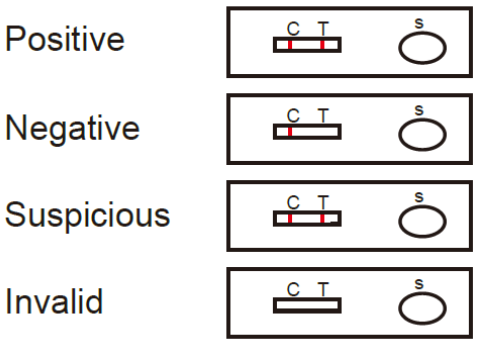

Positive: If both T line and C line are visible, the test result is positive, which indicates the cow is pregnant or in middle period of estrous cycle. Negative: If only C line is visible, the test result is negative, which indicates the cow is not pregnant.
Suspicious: If the color intensity of the test band (T) is less than that of control band (C), it is necessary to combining with clinical situation or the specimen should be double-checked in next day.
Invalid: When control line does not appear on the membrane, the test is invalid due to improper test procedure or deterioration of reagents. It is recommended that the test be repeated.

Sensitivity; Accuracy rate; Precision

The minimum detectable amount: 5-8ng/ml; accuracy rating > 90%; Precision > 90%.

Precaution

(1) Test according to the law of the estrous cycle strictly. Test should be performed at the 22nd to 26th days after mating.
(2) For single use only; do not reuse the test devices.
(3) Don't keep the strip or cassette in the air for a long time after unpacking.
(4) While performing the test, make sure that the surface of sample should not exceed the "MAX" line of strip.
(5)The milk fat percentage influences to the progesterone content. The strips introduced above are suitable to test the milk containing cream ≤ 3.5%; when the cream ≥ 3.5±1%, special design strip provided by the company.
(6) Because of progesterone free is a gradual process in milk, when the test result was negative and which was inconsistent with clinical, reexamination should be performed after 12 hr.(The sample stored in refrigerator at 4℃ in summer).
(7) Do not use test kit after the expiry date.