Development of an ELISA-based kit for the on-farm determination of progesterone in milk

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ABSTRACT: Effective reproductive management is a crucial concern in the dairy industry and missed oestrus and late recognition of unsuccessful insemination can lead to substantial financial loss. Progesterone levels change in a predictable manner with progress of the oestrus cycle and with the onset of pregnancy. We report here the development of a simple ELISA test for the measurement of progesterone levels in bovine milk. The method is based on the use of polyclonal antibodies raised against 11α-hydroxyprogesterone-hemisuccinate and competition for antibody binding sites between free progesterone and horseradish peroxidase-labelled progesterone. Binding is quantified by means of the colour reaction between horseradish peroxidase and 3,3',5,5'-tetramethylbenzidine. The method has proved to be a cost effective, sensitive and easy to perform alternative to currently available methods of oestrus and pregnancy detection. The practical feasibility of the method has been proven under dairy farm conditions.

Keywords: bovine milk; ELISA; oestrus; progesterone; reproduction

Reproductive management is currently a major factor affecting profitability in the dairy industry (Oltenacu et al., 1990; Stott et al., 1999). As dairy herd size has increased, so also has the number of cows per herdsman. This, together with increasing pressure to maximize milk yield whilst at the same time reducing production costs, has negatively affected reproductive performance of herds (Royal et al., 2000; Lucy, 2001). Accurate oestrus detection is crucial for timed and successful artificial insemination and early detection of the success or otherwise of insemination can meaningfully reduce the time delay before repeated insemination if this is necessary. For these reasons it is very important to have a reliable method to accurately detect the occurrence of oestrus and other reproductive states. Several different methods, such as measurement of milk temperature (Maatje and Rossing, 1976), radiotelemetric measurement of vaginal temperature (Kyle et al., 1997) or pedometric technologies (Rorie et al., 2002), have been used in practice for oestrus prediction, but the most widely used, effective and reliable method is determination of the level of progesterone in plasma or milk (Booth et al., 1979; Laing et al., 1979; Friggens and Chagunda, 2005). The concentration of progesterone, secreted by the corpora lutea (CL), changes during the oestrous cycle of the cow. It is minimal on the day of oestrus, but within two days secretion increases as the CL begins to grow. The concentration reaches a maximum on the 10th day after oestrus and remains stable until three to four days before the following oestrus, which occurs on day 21 (Laing and Heap, 1971; Hansel et al., 1973). If insemination is successful, the progesterone concentration remains high, so high levels between day 18 and day 21 can

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indicate pregnancy. These changes in progesterone concentration are detectable in both plasma and milk, although the concentration of progesterone in milk is higher because of the lipophilic character of steroids (Dobson et al., 1975). Typical changes in progesterone production by the CL are also detectable if follicular or luteal cysts occur in the animal, so that the progesterone test can be also used for detection of these reproduction-affecting conditions (Hruska et al., 1983; Nakao et al., 1983; Sprecher et al., 1988; Ruiz et al., 1992).

The use of progesterone concentration as a diagnostic tool has been made possible by the introduction of radioimmunoassay (RIA) techniques (Midgley and Niswender, 1970; Heap et al., 1973; Gowan and Etches, 1979; Holdsworth et al., 1979) and enzyme-linked immunosorbent assay (ELISA) techniques (Dray et al., 1975; Landon et al., 1975; Arnstadt and Cleere, 1981; Sauer et al., 1981). In former Czechoslovakia was developed very effective centralized RIA system of computer-assisted herd management with throughput capacity of more then 1 000 milk samples a day (Hruska and Veznik, 1983; Hruska, 1996). Unfortunately, in addition to being expensive to carry out, RIA techniques require the use of specialized radioisotope facilities and are therefore unsuitable for on-farm use; consequently, ELISA-based techniques have become the most widely used in practice (Nebel, 1988). Here we describe the development of a simple ELISA test for the estimation of progesterone in bovine milk, based on the use of highly specific polyclonal antibodies against 11α-hydroxyprogesterone-hemisuccinate and a conjugate of 11α-hydroxyprogesterone-hemisuccinate with horseradish peroxidase as a labeled hapten. This method, which utilizes the 3,3',5,5'-tetramethylbenzidine (TMB) colour reaction, has proved to be a cost effective, sensitive and easy to perform alternative to methods currently in use to detect oestrus and gravidity. Unlike an earlier investigation using a similar approach (Claycomb et al., 1998) the practical usability, effectiveness and reliability of the method described here has been rigorously tested and confirmed during a field study on a dairy farm.

MATERIAL AND METHODS

Standards. The following steroids were used as standards during the development of the assay procedures: progesterone, aldosterone, androstenedione, cortexolone, corticosterone, cortisone, danazol, dehydroisoandrosterone, β-estradiol, estriol, estrone, etiocholan-3α-ol-17-one, hydrocortisone, norethindrone, prednisolone, Reichstein's substance, testosterone, 11α-hydroxyprogesterone, 14-pregnen-20α-ol-3-one, 16α-hydroxyprogesterone, 17α-hydroxypregnenolone, 17α-hydroxyprogesterone, 19-hydroxyprogesterone, 19-norprogesterone, 4,16-pregnadien-3,20-dione, 4-androsten-3,17-dione, 5α-pregnan-3α,20α-diol, 5α-pregnan-3,20-dione and 5-pregnen-3β-ol-3-one.

All standards were purchased from Sigma (St. Louis, Mo., USA). For long-term storage the steroids were preserved as a 10^{-3}M solution in methanol. For use in ELISA steroid standards were diluted to 10^{-5}M in 4% bovine serum albumin (BSA) in phosphate buffered saline (PBS; 50mM Na_2HPO_4/NaH_2PO_4 buffer, 0.15M NaCl, pH 7.2). For estimation of progesterone concentration in milk samples progesterone standards were used at 20, 10, 5, 2.5, 1.25, 0.625, 0 ng/ml in 4% BSA in PBS.

Chemicals and reagents. 11α-hydroxyprogesterone-hemisuccinate was prepared by Dr. Josef Holik (Isotope Laboratory, Institute of Experimental Botany AS CR, Prague). Its purity was verified by thin layer chromatography (TLC) and nuclear magnetic resonance spectroscopy (NMR). Horseradish-peroxidase (HRP) for enzyme immunoassay (2 500 U/mg), *N,N'*-dicyclohexylcarbodiimide (DCC), *N*-hydroxysuccinimide (NHS), dimethylformamide (DMF), dimethylsulfoxide (DMSO), 1,4-dioxan, tributylamine, isobutylchloroformate, BSA, 3,3',5,5'-tetramethylbenzidine (TMB), thimerosal [2-(ethylmercuriomercapto)benzoic acid sodium salt] and NaN_3 were purchased from Sigma (St. Louis, Mo., USA); ELISA plates were from Nunc A/S (Denmark). All other chemicals were obtained from Lachner (Neratovice, Czech Republic).

Buffers and solutions. The following solutions were used in the ELISA: 50mM carbonate buffer, pH 9.6 was used as a coating buffer. Phosphate buffer saline (PBS, 50mM Na_2HPO_4/NaH_2PO_4 buffer, 0.15M NaCl, pH 7.2) was used for preparation of following solutions: 0.1% TWEEN 20 in PBS used as a wash solution, 4% BSA in PBS for diluting the steroid standards and 0.15% BSA in PBS which served as a reaction solution and after adding of HRP-labeled progesterone as a tracer solution. Substrate diluent solution contained 500 µl 2M CH_3COONa (pH 5.4) and 10 µl of 16% H_2O_2 per 10 ml of MilliQ-water. Substrate solution was prepared by adding

100 µl of 1% TMB solution in DMSO. As a stop solution 2M H_2SO_4 was used.

Immunological reagents. The 11α-hydroxyprogesterone-hemisuccinate was conjugated to BSA using a modification of the mixed anhydride procedure described by Erlanger (1967). The synthesis and use of 11α-hydroxyprogesterone--hemisuccinate for preparation of immunogens and radioligands was discussed in detail by Allen and Redshaw (1978). The hapten was dissolved in DMSO and dioxane and treated with tributylamine and isobutyl chloroformate for 1 h at 4°C (final volume 350 µl, 11α-hydroxyprogesterone-hemisuccinate /tributylamine/isobutyl chloroformate, 1/2/1.5, V/V/V). BSA was dissolved in 50mM phosphate buffer (pH 7.4; 15 mg/ml). The activated hapten solution was carefully added to the BSA and the mixture was gently stirred for 12 h at 4°C. The conjugate was dialyzed against PBS (pH 7.4) for 5 days. 11α-hydroxyprogesterone-hemisuccinate BSA (molar ratio 15:1) was determined using trinitrobenzene sulfonate method (Satake, 1960). The 11α-hydroxyprogesterone-hemisuccinate-horseradish peroxidase conjugate used as labeled antigen in the competitive ELISA was prepared by the carbodiimide method (Gross and Bilk, 1968; O'Sullivan et al., 1979). 11α-hydroxyprogesterone-hemisuccinate (2.46 µmol) was dissolved in 30 µl DMF, mixed with equal amounts of NHS and DCC, and the mixture was stirred for 12 h at room temperature. 50 µl of the activated ester was added dropwise to a solution of horseradish peroxidase (3.4 mg in 300 µl 0.13M $NaHCO_3$). After stirring for 3 h at room temperature, the mixture was dialyzed against PBS (pH 7.4) for 3 days.

Characteristic of antibodies. Polyclonal antibodies against 11α-hydroxyprogesterone-hemisuccinate:BSA were raised in rabbits. The immunization schedule and purification of antibodies was as described previously (Strnad et al., 1997). After the fifth immunization the rabbits were bled and the antisera were precipitated with ammonium sulfate (Harlow and Lane, 1988) and dialyzed for five days. The IgG content in serum was determined by measurement of UV spectra at 280 nm and was calculated 13.53 mg/ml. Purified antibodies were stored frozen at –20°C.

From the antisera obtained, the most promising (M1 and M4) were chosen and tests were carried out on these to determine their immunological characteristics in ELISA. The relative cross-reactivity with steroids structurally related to progesterone was determined as calculated by Abraham (1969). The percent of cross-reaction represents the concentration of cross-reacting compound required to displace 50% of the original hapten bound to the antiserum. For the usage in a practical milk-progesterone assay kit the antibodies which do not react with other sex hormones had to be prepared. Because the hapten was linked to BSA at position 11α, higher cross-reactivity with steroid structures modified on carbon 11 and 19 localized in the area of coupling was predictable (Erlanger et al., 1957; Beale, 1999); however, these steroids should not interfere with progesterone in milk.

For complex comparison we used antibodies Ab 407, also raised against 11α-hydroxyprogesterone-hemisuccinate. These antibodies were prepared in the Veterinary Research Institute, Brno, Czech Republic, where they have been used as reference antibodies for progesterone assays in milk or plasma.

Enzyme-linked immunosorbent assay (ELISA). To determine the characteristics of the prepared antibodies, the following procedure was employed: microtiter plates (Nunc-Maxisorp) were coated with anti-11α-hydroxyprogesterone-hemisuccinate polyclonal antibody (0.55 µg/ml coating buffer) and incubated overnight at 4°C to allow binding to occur. The plates were washed twice with wash buffer before use. After decanting, the wells were filled with 100 µl of reaction solution. If the basic analytical parameters of antibodies were determined, 50 µl of 10^{-5} M progesterone standard in standard diluent was added to the wells A1-H1 and subsequently continually diluted from column 1 to column 11. Whilst if the cross-reactivities of antibodies were determined, progesterone standard was added only to the wells A1 and B1 and to the wells C1-H1 were, also in duplicates, aplicated three different steroid standards. Tracer solution was then added to all wells except the well A12 which served as a blank. The plates were incubated at 4°C for 50 minutes. Unbound conjugate was removed by rinsing the plates three times with wash solution. The plates were then filled with substrate solution and after incubating for 15 min at 25°C the reaction was stopped with 2M H_2SO_4. The final absorbance was measured at 450 nm (Labsystems Multiskan® 314 PLUS, Vantaa, Finland). Sigmoid curves for standards and cross-reacting compounds were linearized by the log-logit transformation:

$$\text{logit } B/B_0 = \ln\{(B/B_0)/(100-B/B_0)\}$$

The resulting data were processed by the ImmunoRustregAnalyser computer program (Laboratory of Growth Regulators, Olomouc, Czech Republic).

For the estimation of progesterone in milk samples, the ELISA procedure described above was slightly modified by changing the pattern of application of the standards. 10 µl of progesterone standards were applied sequentially from well A1 to well G1 at concentrations of 20, 10, 5, 2.5, 1.25, 0.625, 0 ng/ml. Well H1 served as a blank. Milk samples (10 µl) were applied in the next wells. In the assay kit developed for farm use, pre-coated ELISA plates are sealed with self-adhesive plastic film and stored at 4°C before use.

Milk sampling. Samples (approximately 50 ml) were obtained from 34 individual Holstein cows on farms in the Olomouc region of the Czech Republic following hand or machine milking. The samples were preserved with a wide-spectrum preservative (Broad Spectrum Microtabs II: 8 mg Bronopol and 0.3 mg Natamycin per 18 mg tablet; D&F Control Systems, Inc., California, USA).

Validation of the method. Because the ELISA method described here was developed for practical use in the dairy industry, it was necessary to carry out a validation protocol. Linearity of the calibration curve was tested and quantified by the Microcal Origin computer programme (Microcal Software, Inc., Northampton, USA). Variability of the progesterone determination by the ELISA kit was tested on 10 milk samples obtained from dairy farm from Olomouc region, Czech Republic. Six replicates of each sample were tested for progesterone and standard deviations (SD) and coefficients of variation (*CV*) were calculated. The method of standard additions was also used for the validation. Milk sample was spiked with 2.5, 5, 10 and 20 ng/ml progesterone. The total concentration of progesterone in milk sample was then determined to calculate the recovery. The influence of wide-spectrum preservative on the accuracy of progesterone determination was also tested. 10 milk samples were tested for progesteron before and after the addition of preservative. The agreement of the results was confirmed by the paired *t*-test.

Radioimmunoassay (RIA). A Prog-RIA-CT kit (Biosource Europe S.A., Belgium) was used for validation of the ELISA assay. Progesterone concentration was measured in 30 milk samples by the developed ELISA assay and consequently by the commercially available RIA kit. Standards, controls and samples were vortexed and 50 µl of each were transferred to individual sample tubes. 500 µl of ^{125}I-labelled progesterone were dispensed into each tube. After shaking, the tubes were incubated for 3 h at room temperature. The contents of each tube were aspirated, the tubes were washed with 3 ml of wash solution (Tris-HCl) and they were then counted in a γ-counter for 60 seconds (Cobra 5005, Packard Instrument Co., Meriden, Connecticut, USA). Correlation coefficient between the ELISA and the RIA was numbered.

RESULTS AND DISCUSSION

Analytical parameters of ELISA. Antibodies M1 and 407 had lower progesterone detection limits than antibody M4, sensitive linear ranges of measurement and low inter- and intra-assay variabilities (Table 1). In general the degree of sensitivity of Ab M1 to progesterone was similar to antibodies used by other workers to measure progesterone in biological samples (Cleere et al., 1984; Van de Wiel

Table 1. Immunological characteristics of polyclonal anti-progesterone antibodies M1, M4 and 407 determined by ELISA

Characteristics	Antibodies		
	M1	M4	407
Limit of detection (mol/l)	4.4×10^{-9}	1.39×10^{-8}	4.6×10^{-9}
Midrange(B/B0) (mol/l)	5.32×10^{-8}	7.34×10^{-7}	8.16×10^{-8}
Range of measurement (mol/l)	3.6×10^{-7}–4.4×10^{-9}	1.1×10^{-6}–1.4×10^{-8}	1.1×10^{-6}–4.6×10^{-9}
Non-specific binding (%)	1.76	0.53	1.10
Intraassay variability (%)	2.38	3.34	3.78
Interassay variability (%)	3.27	7.60	5.60

and Koops, 1986; Prakash et al., 1988). A standard curve for progesterone with the M1 antibody is shown in Figure 1.

All the antibodies had low cross-reactivity with other related steroids but overall, antibody M1 was superior to the others exhibiting cross-reactivity lower than 1% with most of the steroids tested, including the sex hormones testosterone, androstendione and several estrogens (Table 2). The only steroid for which cross-reactivity exceeded 20% was 5α-pregnan-3,20-dione, the active metabolite of progesterone itself, so it should not affect accuracy of the test. Cross-reactivities of the M1 antibodies were also similar or superior to antibodies used by other workers to determine progesterone (Cleere et al., 1984; Van de Wiel and Koops, 1986; Elder et al., 1987). Consequently, antibody M1 was the one chosen for further development of the ELISA test to estimate progesterone concentration in bovine milk.

Table 2. Cross-reactivity of steroid compounds compared with progesterone for anti-progesterone antibodies M1, M4 and 407

	Cross-reactivity (%)		
	M1	M4	407
Progesterone	100	100	100
β-estradiol	0.02	0.86	0.04
Danasol	< 0.01	0.41	0.02
Norethindrone	0.36	1.48	1.36
Estrone	0.07	0.23	0.07
Hydrocortisone	0.77	0.09	1.52
Estriol	< 0.01	0.1	0.09
Prednisolone	0.43	0.7	0.83
17α-hydroxyprogesterone	< 0.01	< 0.01	0.02
17α-hydroxypregnenolone	< 0.01	0.36	0.51
16α-hydroxyprogesterone	2.93	7.57	30.17
Reichstein's sub	0.42	0.23	1.16
Cortexolone	0.53	0.27	1.31
Dehydroisoandrosterone	0.35	0.93	0.64
Corticosterone	2.84	6.17	13.59
19-hydroxyprogesterone	8.14	6.61	16.71
19-norprogesterone	15.52	37.59	20.44
11α-hydroxyprogesterone	15.42	68.51	7.21
4,16-pregnadien-3,20-dione	0.33	0.05	< 0.01
Etiocholan-3α-ol-17-one	0.36	0.74	0.42
5α-Pregnan-3,20-dione	21.41	21.71	5.75
4-Androsten-3,17-dione	0.46	1.91	1.67
5-Pregnen-3β-ol-20-one	5.51	11.4	3.66
4-Pregnen-20α-ol-3-one	1.15	2.09	6.9
Testosterone	0.07	< 0.01	1.61
Androstendione	0.39	0.41	2.15
Aldosterone	< 0.01	0.21	0.03

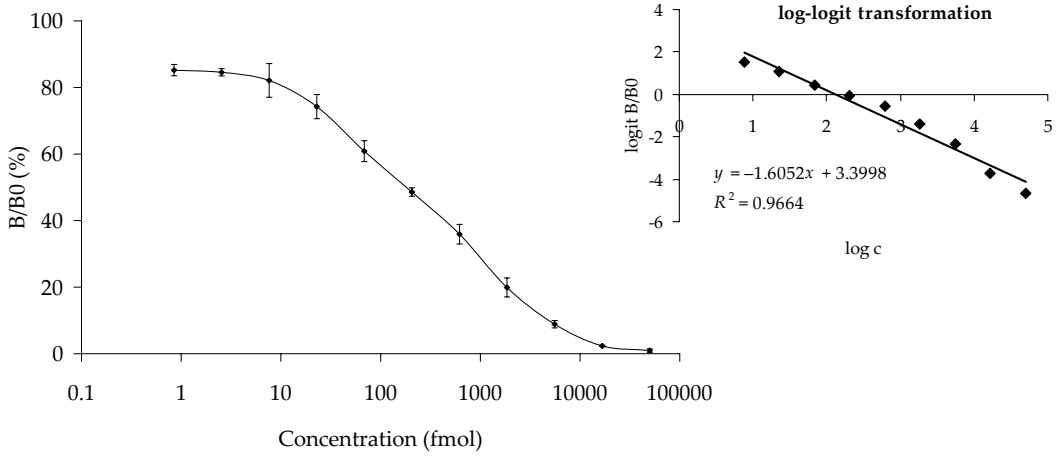

Figure 1. Progesterone standard curve for anti-progesterone antibody M1 and its linearization by logarithmical transformation (vertical bars are ± S.D.; n = 8)

Validation of the method. The validation protocol was carried out with following results. Linearity of the calibration curve was tested. The correlation coefficient of the calibration curve was r = 0.9987, n = 7. The results of progesterone determination variability test are summarized in Table 3. The average SD was ca. 0.5 ng/ml and in no case exceeded 0.8 ng/ml. As a result of this low variability, it was decided that under farm conditions only one measurement would be made on each milk sample at each sampling time. Recovery of standard additions of 2.5, 5, 10 and 20 ng/ml progesterone to milk sample was determined. The percentage recovery ranged from 99% to 119% for the 2.5 ng added. Linear relationships between the added and detected amounts of progesterone are shown in Figure 2. Correlation coefficient was r = 0.9917, n = 8. The influence of wide-spectrum preservative on the accuracy of progesterone determination was also tested. Paired t-test confirmed the agreement of the results with correlation coefficient r = 0.9748, n = 10. The ELISA method was also compared with commercially available RIA kit. Linear regression analysis gave a correlation coefficient r = 0.964 (P < 0.0001; Figure 3). The high degree of correlation between the ELISA and the RIA methods is a general observation noted by other workers (e.g. Cleere et al., 1984; Van de Wiel and Koops, 1986; Elder et al., 1987).

On-farm milk progesterone estimation. The ELISA method described above for the estimation of progesterone concentration in milk was further modified for on-farm use and an ELISA kit was developed. The kit contains progesterone standards, solutions of labelled antigen, and TMB solution, all at 5 × working strength and preserved with thimerosal (Sigma), together with pre-coated strip

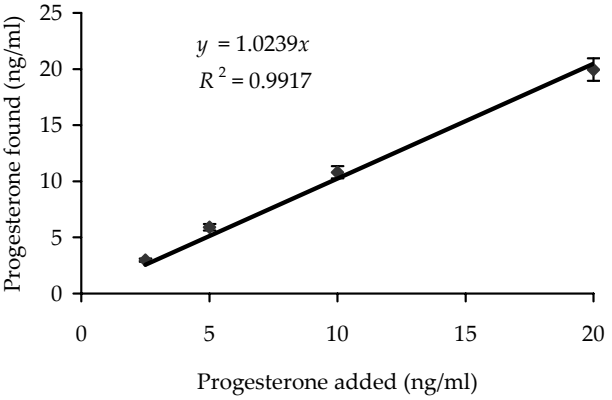

Figure 2. Recovery of defined ammounts of progesterone added to milk sample and measured by developed assay kit (r = 0.9917; n = 8; P < 0.0001; y = 1.0239x)

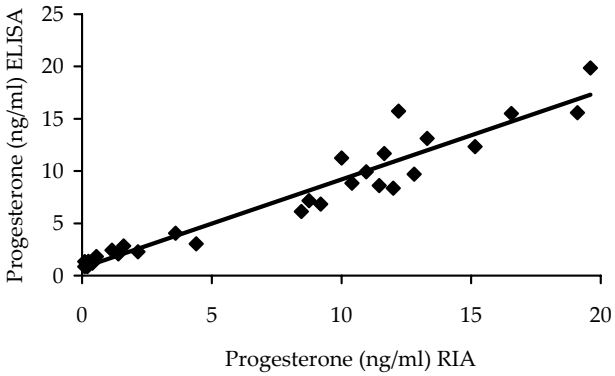

Figure 3. Comparison of progesterone concentrations determined by ELISA and RIA in 30 milk samples (r = 0.964; n = 30; P < 0.0001; y = 0.84339x + 0.7639)

Table 3. Results of variability test of progesterone determination by developed ELISA kit performed on 10 milk samples in six repeats

Sample	Progesterone concentration (ng/ml)						Mean	SD	*CV* (%)
1	7.7	8.1	7.1	7.1	7.6	7.0	7.43	0.3986	5.36
2	11.3	10.8	10.3	11.3	12.1	10.0	10.97	0.6968	6.35
3	1.2	2.0	1.3	1.4	1.1	0.8	1.30	0.3651	28.08
4	7.0	6.0	5.9	6.2	6.0	6.7	6.30	0.4082	6.48
5	7.2	7.4	6.7	7.2	7.3	6.1	6.98	0.4525	6.48
6	4.4	5.1	4.0	4.8	4.4	3.7	4.40	0.4655	10.58
7	7.1	9.4	7.7	8.3	7.3	8.2	7.99	0.7585	9.49
8	2.4	3.3	1.8	1.9	1.0	1.2	1.93	0.7652	39.65
9	3.7	3.6	3.7	4.2	3.7	3.2	3.68	0.2911	7.91
10	1.3	1.8	1.5	1.5	0.9	0.8	1.30	0.3512	27.01

SD = standard deviation. *CV* = coefficient of variation

ELISA plates sealed with self-adhesive plastic film for storage and transport. The stability of all the kit components was tested and found to be in excess of six months. The method is cost effective, sensitive and easy to perform, and the total time needed for measurement does not exceed 60 minutes. The colour response is measured at 450 nm with a manual Microwell Strip Reader EL301 (BioTek Instruments, Inc., Vermont, USA). The method was tested under practical conditions on dairy farm near Olomouc,

Table 4. Progesterone levels (ng/ml) measured by ELISA on Days 18 and 22 after insemination compared with the results of ultrasonic detection of gravidity

Cow	Progesterone Day 18	Progesterone Day 22	Ultra-sound Day 30	Cow	Progesterone Day 18	Progesterone Day 22	Ultra-sound Day 30
1	> 20	10.48	N	18	> 20	> 20	P
2	14.19	> 20	P	19	> 20	19.48	P
3	5.08	4.86	N	20	16.28	2.32	N
4	7.39	2.89	N	21	12.01	> 20	P
5	14.56	3.48	N	22	1.91	5.09	N
6	4.39	4.36	N	23	7.35	3.01	N
7	7.87	4.09	N	24	4.23	3.42	N
8	12.21	> 20	P	25	9.55	15.54	P
9	11.45	3.43	N	26	18.35	11.47	A
10	10.21	7.8	N	27	12.22	> 20	P
11	> 20	> 20	P	28	7.83	0.71	N
12	> 20	3.48	N	29	12.15	> 20	P
13	> 20	> 20	P	30	9.39	2.87	A
14	> 20	> 20	P	31	2.41	1.78	N
15	5.96	7.27	N	32	16.84	10.12	A
16	11.45	6.36	N	33	6.66	1.81	N
17	> 20	> 20	P	34	6.61	4.12	N

P = pregnant, N = non-pregnant, A = early abortion

Czech Republic, where the progesterone concentration in the milk of 34 Holstein cows was measured with the ELISA kit. The first measurement was made 18 days after insemination and was repeated 4 days later in order to detect the onset of pregnancy or failure of insemination.

The first test after 18 days allowed the immediate detection of non-pregnant cows with low progesterone concentrations (< 8 ng progesterone/ml milk 18 days post insemination). This enabled these animals to be re-inseminated just one oestrus cycle after the first insemination. Following the second measurement of milk progesterone concentration (22 days post insemination), cows in which progesterone concentrations remained high were classified as pregnant, and those animals in which progesterone concentration had significantly decreased since the first test were classified as non-pregnant. To test the validity of the ELISA results, the cows were scanned with an ultra sound camera 30 days after insemination and the results were compared (see Table 4). The first ELISA measurement had revealed 12 animals with progesterone concentrations lower than 8 ng/ml milk. Their progesterone remained low at the second measurement and ultra sound scans confirmed all of them to be barren. Of the remaining 22 cows, the 12 animals with stable high progesterone concentration were classified as pregnant; three cases of early abortion were observed; in six animals progesterone concentration decreased below 8 ng/ml on day 22, so they were classified as non-pregnant. The last animal was predicted not to be pregnant although its progesterone concentration remained higher than 10 ng/ml at the second measurement, because the decrease between first and second measurements was greater then 10 ng/ml. A possible explanation for this discrepancy was that a suspected form of embryonic death had occurred or that the animal was not inseminated in oestrus so the oestrus cycle was in the stage of maximum progesterone production by the time of the first progesterone measurement. As shown in Table 4, subsequent ultra-sound scans confirmed all the predictions made from the ELISA measurement of progesterone concentration using the on-farm kit.

CONCLUSIONS

Reproductive management and its effectiveness is currently one of the most difficult problems being solved by dairy and livestock producers. Early detection of the succes or failure of artificial insemination could result in significant shortening of the time delay before eventual reinsemination and thus in indispensable financial savings. Although ultra sound testing is a reliable and currently the most widely used method for the recognition of pregnancy, it can be effectively used only from 28 days post insemination, when it is already too late to re-inseminate in the same cycle. In contrast, the ELISA test presented here can be applied in time to re-inseminate in the current oestrus cycle. The developed ELISA kit combined with a simple manual ELISA strip reader has proven to be a competent diagnostic tool usable in on-farm conditions. It has been successfully used in practice for pregnancy detection, and it is suggested that the test could be used to aid complex diagnosis of animals with poor conception rates and for oestrus detection.

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