DETERMINATION OF FAECAL GESTAGENS IN SOWS BY COMMERCIAL PROGESTERONE EIA KIT

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Abstract

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The aim of the study was to test the applicability of commercial progesterone enzyme immunoassay (EIA) kit for faecal gestagen determination in sows. Faecal and blood samples were collected every second day from oestrus until d 16, and every day from d 16 until d 21 of oestrous cycle or pregnancy. Extraction of gestagens from faeces was performed by methanol. Faecal gestagen and plasma progesterone concentrations were measured by Ovucheck 96 Well Plasma/Serum Progesterone EIA kit. Changes in faecal gestagen (progesterone and its metabolites) concentration during the oestrous cycle or pregnancy significantly correlated with plasma progesterone levels. Faecal gestagen concentrations on days 20 and 21 of oestrous cycle were 2148 ± 474 and 1961 ± 65 nmol/kg. In the same period of pregnancy faecal gestagen concentrations were 7624 ± 8 and 7468 ± 106 nmol/kg, respectively. In pregnant animals, the faecal gestagen concentrations were significantly higher ($P< 0.01$) than in non-pregnant ones. The results indicate that measurement of faecal gestagens in sows by using commercial EIA kit is suitable for progesterone status detection in healthy animals, but is not appropriate in animals suffering from diarrhoea.

Gestagens, progesterone, diarrhoea, oestrous cycle, pregnancy, swine

After completing their function in the organism steroids are deactivated by glucuronate and sulphate conjugation in the liver and excreted in urine by the kidneys or via bile released into the gut (Taylor 1971). However under the influence of intestinal bacteria the steroids in the gut are deconjugated, partially reabsorbed and partially excreted by faeces. In pigs evident enterohepatic circulation of steroids has been described (Ruoff and Dziuk 1994; Symonds et al. 1994). After 14C progesterone administration in pigs, 34 % of the radioactivity is excreted by faeces and 66 % by the urine (Palme et al. 1996). The main progesterone faecal metabolites in sows are: 20-oxo-pregnanes (50.1 %), 20-b-hydroxy pregnanes (24.2 %), 20-a-hydroxy pregnanes (18.9 %) and unidentified metabolites (7.5 %). Only about 1 % of the administered progesterone is excreted in unmetabolised form (Palme et al. 1997).

Determination of steroid hormones in faeces has been described in many species. Detection of pregnancy by measuring oestrogens in faeces was demonstrated in ewes after day 88 (Busch and Bamberg 1990), in mares after day 120 (Youngblood and Williams 1987; Palme et al. 1989) and in sows between day 25 and 30 (Szenci et al. 1993). Significant positive correlation between plasma and faecal gestagens was observed in cows (Schwarzenberger et al. 1990) and in cycling sows (Hulten et al. 1995). The measurement of faecal steroid hormones is sometimes the only suitable method for the

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detection of pregnancy or oestrous cycle in zoo (Safar-Hermann et al. 1987; Wasser et al. 1988; Schwarzenberger et al. 1996) and free ranging animals (Wasser et al. 1991).

The measurement of faecal steroids in sows has at least two advantages. The collection of faecal samples is easier than venepuncture and the animals are not subjected to stress.

Ovucheck 96 Well Plasma/Serum Progesterone EIA kit is a commercial product used for oestrus detection and assessment of pregnancy status or luteal function in cows, sows, goats and it was also validated for horses, sheep and dogs (Eckersall and Harvey 1987).

The aim of the study was to:

1. test the applicability of the commercial progesterone EIA kit for the measurement of feacal gestagens (progesterone and its metabolites) in sows,
2. follow the oestrous cycle dynamics,
3. compare the faceal and blood gestagen concentrations.

Materials and Methods

Animals

The investigation was performed on 17 mature hybrid sows (*Sus scrofa*) aged from 14 months to 4.5 years, kept in individual pens. Two animals were followed through the 21-day oestrous cycle and 15 animals from insemination to the 21st day of pregnancy. The animals were fed twice daily approximately 1 kg of commercial diet mixture containing: barley 35 %, maize 24.46 %, wheat meal 12 %, soya bean meal 7.48 %, fish meal 6.88 %, alfalfa 6.50 %, dried beet pulp 6 %, mollases 0.05 %, mineral-vitamin addition 1.62 % and water *ad libitum*.

Sampling

From the beginning of oestrus to the 16th day of oestrous cycle or pregnancy the faecal and blood samples were taken every second day and daily in the period from the 16th to 21st day. Until analysed, faecal and plasma samples were kept frozen at -20 oC. Diarrhoea was permanently observed in two animals and temporary in two others.

Faecal gestagen analysis

Extraction of faecal gestagens was performed by methanol as described by Möstl and coworkers (Möstl et al. 1993). Extracts were diluted in proportion 1:20 with buffer solution (pH 7.4) containing: Na_2HPO_4 12.21 g, $NaH_2PO_4 \times 2H_2O$ 2.18 g, gelatine 1.00 g, NaN_3 0.50 g, in 1 L of distilled water.

Measurement of gestagens (i.e. progesterone and its metabolites) in diluted faecal extracts was done by following the original instructions for progesterone determination in serum or plasma as described in the Ovucheck 96 Well Plasma/Serum Progesterone EIA kit, using diluted extracts instead of serum or plasma. The cross-reactivity from steroides other than progesterone is less than 1% except for: 11 a-hydroxy-progesterone (66.0 %), 5-Pregnan-3b-ol-20-one (16.0 %), 5b-pregnan-3, 20-dione (4.5 %), 5a-Pregnan-3, 20-dione (3.3 %) and Deoxy-corticosterone acetate (3.0 %). The range of the assay was from 382 to 7632 nmol/kg. The absorbance was measured by EIA reader (MCC/340 Labsistem Multiskan).

The intra-assay coefficient of variation (CV) was 3.59 % (n = 5) and 12.56 % (n = 5) for the concentration of 5950 and 1329 nmol/kg, respectively; the interassay CV was 7.03 % (n = 5) and 14.65 % (n = 5) at the concentration 5969 and 1097 nmol/kg, respectively.

Plasma progesterone analysis

Plasma progesterone analysis was performed by following the original instructions of the kit. The range of the assay was from 1.6 to 31.8 nmol/L.

Measurement of the water content in faeces

Water content was measured in the faeces of the animals with permanent diarrhoea, and in faecal samples from two healthy animals. Approximately 2 g of fresh samples were dried at +105 °C (drying apparatus STE 17 Chirana) until constant weight. The percentage of water was calculated from the difference between fresh and dry weight.

Statistical evaluation

Results were considered significant at the level $P < 0.05$. The data of gestagen concentration and water content in faeces were analysed by Student's t-test, plasma and faecal gestagen concentrations compared by correlation coefficient (r). Statistical package MS Excel version 5.0, statistical function STDEVP was used. Faecal values higher than 7632 nmol/kg were considered as 7632 nmol/kg and plasma values higher than 31.8 nmol/L were considered as 31.8 nmol/L. Results of faecal gestagen concentrations from 4 pregnant animals suffering from diarrhoea were excluded from statistical analysis.

Results

Mean faecal gestagen and plasma progesterone concentrations in sows during the oestrous cycle, during the first three weeks of pregnancy and in animals with diarrhoea are presented in Tables 1, 2, 3, 4, and 5.

Low faecal gestagen concentrations were observed from the beginning till the 6th day of oestrous cycle. From the 8th till the 17th day concentrations were high, thereafter they started to decrease gradually and were low again on the 20th and 21st day of oestrous cycle. In pregnant sows faecal gestagen concentrations gradually increased from the insemination till the14th day and remained high from that day on. Similar pattern was also found in plasma progesterone concentrations. Faecal gestagen concentration in pregnant animals on 20th and 21st day was significantly higher ($P< 0.01$) than in nonpregnant animals at the same period. Significant ($P< 0.01$) positive correlation was found comparing faecal gestagens and plasma progesterone concentrations during the oestrous cycle ($r = 0.74$) and during the first three weeks of pregnancy ($r = 0.97$).

Table 1
Faecal gestagen concentrations (nmol/kg) in sows during the oestrous cycle

	Day of oestrous cycle													
	0	2	4	6	8	10	12	14	16	17	18	19	20	21
Mean	1303	1126	2059	2400	6766	7632	7632	7632	7632	7037	5819	4611	2148	1961
SD	227	156	67	229	591	0	0	0	0	595	1813	1930	668	91
SE	161	111	47	162	419	0	0	0	0	422	1286	1369	474	65

Table 2
Plasma progesterone concentrations (nmol/L) in sows during the oestrous cycle

	Day of oestrous cycle													
	0	2	4	6	8	10	12	14	16	17	18	19	20	21
Mean	5.2	8.7	13.2	24.2	31.0	30.5	31.8	31.8	28.8	15.7	10.0	9.4	9.3	17.8
SD	2.8	0.5	1.1	1.9	0.8	1.3	0	0	3.0	2.1	0.2	0.2	0.7	2.4
SE	2.0	0.3	0.8	1.3	0.6	0.9	0	0	2.1	1.5	0.1	0.1	0.5	1.7

Table 3
Faecal gestagen concentrations(nmol/kg) in sows during the first three weeks of pregnancy

	Day of pregnancy													
	0	2	4	6	8	10	12	14	16	17	18	19	20	21
Mean	1365	1376	1886	3145	5764	5793	6473	7369	7602	7499	7595	7591	7624	7468
SD	856	529	700	873	1819	2075	1882	831	94	421	116	129	26	352
SE	258	159	211	263	548	625	567	250	28	127	35	39	8	106

Table 4
Plasma progesterone concentrations (nmol/L) in sows during the first three weeks of pregnancy

	Day of pregnancy													
	0	2	4	6	8	10	12	14	16	17	18	19	20	21
Mean	3.5	5.2	9.7	14.3	21.0	26.2	27.0	27.5	28.1	30.7	31.5	30.7	31.0	31.2
SD	1.5	2.4	2.5	2.8	3.8	6.3	8.0	7.4	6.6	2.6	0.9	3.5	2.4	1.8
SE	0.4	0.7	0.7	0.8	1.2	1.9	2.4	2.2	2.0	0.8	0.3	1.1	0.7	0.5

In faeces of two animals with diarrhoea mean water content was 73.27 % (SD 4.56; SE 1.22) and 72.74 % (SD 1.98; SE 0.53) respectively. Values were significantly higher ($P< 0.01$) than in two healthy animals where water content was 65.26% (SD 4.56; SE 1.22) and 65.93% (SD 3.08; SE 0.82).

Table 5
Faecal gestagen concentration (nmol/kg) in pregnant animals with diarrhoea on several days after oestrus

	Day of pregnancy													
animal	0	2	4	6	8	10	12	14	16	17	18	19	20	21
1	*3018*	*843*	*636*	*2922*	*7632*	*6176*	*3441*	*3428*	*7632*	*7632*	*5654*	*3962*	*7632*	*7632*
2	*388*	*464*	*1113*	*1259*	*1832*	*1189*	*2210*	*989*	*1953*	*2499*	*3046*	*1313*	*1968*	*1781*
3	652	480	1196	1396	4678	7339	2748	3717	*6541*	*5959*	*3543*	*3638*	*5625*	*3288*
4	576	986	1301	1755	*4252*	*5059*	*3571*	6525	7632	7632	6449	*5473*	*4846*	*4153*

Table 6
Plasma progesterone concentration (nmol/L) in pregnant animals with diarrhoea on several days after oestrus

	Day of pregnancy													
animal	0	2	4	6	8	10	12	14	16	17	18	19	20	21
1	*2.3*	*5.7*	*9.8*	*12.0*	*14.4*	*27.5*	*25.4*	*30.4*	*31.8*	*31.8*	*31.8*	*28.4*	*31.8*	*31.8*
2	*3.6*	*2.8*	*16.1*	*23.6*	*26.6*	*29.2*	*29.2*	*31.8*	*26.4*	*29.0*	*31.8*	*31.8*	*31.8*	*31.8*
3	1.6	7.6	16.6	20.3	20.0	25.4	25.4	26.4	*29.3*	*31.8*	*29.8*	*31.8*	*28.4*	*30.6*
4	4.7	2.4	4.8	15.8	*23.1*	*24.0*	*23.8*	31.8	31.8	31.8	31.8	*31.8*	*31.8*	*31.8*

Legend:
1, 2; sows with permanent diarrhoea
3, 4; sows with temporary diarrhoea
italics; days of sampling when diarrhoea was observed

Discussion

Changes in faecal gestagen (progesterone and its metabolites) concentration during the oestrous cycle and early pregnancy show the pattern typical for progesterone concentration changes in blood (Wang et al. 1985; Choi et al.1986). Significant positive correlation between faecal gestagen and plasma progesterone values indicates that the measurement of progesterone and its metabolites in faeces using Ovucheck 96 Well Plasma/Serum Progesterone EIA kit gives an accurate insight into the progesterone status in sows. Significant difference in faecal gestagen concentration between pregnant and non-pregnant animals on 20th and 21st day shows that this is a suitable method for pregnancy detection.

In animals suffering from diarrhoea faecal gestagen levels were low and the changes in gestagen concentrations were different from those in normal animals. No data could be found to explain the influence of diarrhoea on faecal gestagen content. During the digestive disorders water and electrolyte secretion is stimulated, reabsorption aggravated and the gut content diluted. Because the comparison of gestagen concentration in dry and fresh matter is not significantly different, dilution is probably not the only reason for low gestagen values. Massive peristaltic waves transport the gut content with the gestagens in the bile faster than in normal digestion. This could be another reason for lower gestagen concentration in gut content.

Conclusion could be made that in animals with diarrhoea or other digestive disorders the measurement of faecal progesterone and its metabolites is not appropriate method for reproductive status determination in sows.

The study indicates that measurement of faecal gestagens in sows by using Ovucheck 96 Well Plasma/Serum Progesterone EIA kit is suitable for progesterone status detection in healthy animals, but is inappropriate in animals with diarrhoea.

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