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PREGNANCY DIAGNOSIS IN COWS AND HEIFERS BY DETERMINATION OF OESTRADIOL-17α IN FAECES

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SUMMARY

The concentration of unconjugated oestradiol-17α was measured in faeces of 21 non-pregnant animals and of 39 cows and heifers between the 10th and 25th week of gestation. After the 14th week of gestation all pregnant animals secreted significantly more oestradiol-17α in the faeces than non-pregnant animals. This is a possible method therefore for the confirmation of pregnancy not only in cows but also in heifers.

INTRODUCTION

The measurement of substances produced by the conceptus can be used for the detection of pregnancy. In cows the concentration of oestrogens in the peripheral blood increases as pregnancy progresses (Robertson & King, 1979). Oestrogens are excreted in urine, milk (Monk, Erb & Mollett, 1975) and faeces (Velle, 1975). Heap & Hamon (1979) and Hamon *et al.* (1981) reported the diagnosis of pregnancy in cows based on the detection of oestrone sulphate in milk.

In order to establish a method for pregnancy diagnosis in cows *and* heifers, the present study was designed to measure oestrogen concentrations in the faeces of pregnant and non-pregnant animals.

MATERIALS AND METHODS

Animals and sampling

In a herd of Simenthal and Brown Swiss cows and heifers pregnancy was confirmed by rectal palpation, and faeces samples were collected in small plastic bags from 21 non-pregnant animals at various stages of the oestrous cycle and from 39 pregnant cows and heifers (between 10 and 25 weeks of gestation). The samples were transported to the laboratory at ambient temperature and stored at −20°C until assayed.

Reagents and apparatus

The antisera for total oestrogens and for oestradiol-17ß were kindly donated by Dr B. Cook, The Royal Infirmary, Glasgow. The antiserum for oestradiol-17α was purchased from Bioanalysis Ltd, Cardiff. The cross-reactions of this antiserum (No. 1007) have been described by Dobson & Dean (1974): oestradiol-17α 100%, oestradiol-17ß 0·4%, oestrone 0·2%, other steriods <0·1%. The final dilution of the oestrogen and oestradiol-17ß antisera was 1 : 30000; that of oestradiol-17α 1 : 1000.

Unlabelled steriods and organic solvents for extraction were obtained from Merck (Darmstadt, West Germany) and radioactive oestrone and oestradiol-17ß from New England Nuclear (Dreieich, West Germany).

[^{3}H]-oestradiol-17α was prepared by the method of Hoffmann (1977): about 10 ml heparinized blood were collected from a non-pregnant cow, centrifuged for 15 min at 1500 *g* and the erythrocytes washed three times with 0·9% NaCl solution. The erythrocytes were transferred to a test-tube with 50 µCi [^{3}H]-oestrone dissolved in 10 ml tissue culture medium and incubated overnight at room temperature on a mechanical shaker. The erythrocytes were removed by centrifugation and the medium was extracted twice with 10 ml diethyl ether. [^{3}H]-oestrone and [^{3}H]-oestradiol-17α were separated by sephadex LH-20 column chromatography (20 × 0·5 cm; dichlormethane-methanol: 98 + 2).

Assay

A 0·5-g sample of faeces was extracted with 0·5 ml chloroform and 1·5 ml 2 N NaOH in a test-tube for 30 min and centrifuged at 1500 *g* for 15 min. 0·5 ml of the supernatant were transferred into another tube and extracted with 5 ml of a mixture of petroleum ether (40 to 60°C b.p.) and diethylether (9 + 1) for 30 min. The aqueous phase was then frozen, and the organic layer decanted and dried down by a stream of nitrogen at 60°C in a water bath.

In the faeces samples from 10 cows and heifers the oestrone and oestradiol fractions were separated by column chromatography (20 × 0·5 cm columns of Sephadex LH-20; eluent: dichlormethane-methanol: 98 + 2), the solvents evaporated and the extracts redissolved in 1·2 ml gelatine-phosphate-buffer. The concentration of the oestrone fraction was determined using the antiserum which cross-reacts with several oestrogens. In the oestradiol fraction the concentrations of oestradiol-17α and -17ß were measured using the specific antisera.

All three oestrogen radioimmunoassays were performed as described by Möstl *et al.* (1981) for oestrone. As there were no significant differences in oestradiol-17α concentrations with and without column chromatography, the concentration in the samples was determined by radioimmunoassay without chromatography. [^{3}H]-oestradiol-17ß was used for monitoring procedural losses. The accuracy of the assay was determined after adding known amounts of oestradiol-17α to faeces samples from non-pregnant animals.

RESULTS

The overall recovery of [^{3}H]-oestradiol-17ß added was 50 ± 5% (n = 10) and all values were corrected for their individual procedural losses. Reagent blank values were about 12 pg/tube. The coefficient of variation within the assay was 6·9% (n = 8) in a high level

pool (42·1 ng/g) and 17·4% in a low level pool (4·03 ng/g). The inter-assay coefficient of variation was 12·3%. The linear regression analysis $y = ax + b$ (y = ng found, x = ng added) from data measured after addition of increasing concentrations gave the following results: $a = 0{\cdot}92$, $b = 35{\cdot}8$ and $r = 0{\cdot}992$.

In order to evaluate which oestrogen was present in the faeces of cows and heifers all three oestrogens were determined in 10 samples; the results demonstrated that oestradiol-17α was the predominant oestrogen (Table I).

Table I
Concentration of oestrone, oestradiol–17β and oestradiol–17α (ng/g) in faeces of cows and heifers

Animal no.	*Oestrone*	*Oestradiol–17β*	*Oestradiol–17α*
1	3·1	2·1	27·3
2	2·0	4·4	17·3
3	1·1	1·4	5·7
4	3·6	9·3	23·7
5	3·6	6·2	23·3
6	0·5	0·8	14·2
7	2·7	7·2	50·1
8	1·2	1·2	6·1
9	3·5	3·8	32·3
10	0·2	0·3	2·2

On the basis of these results the specific measurement of oestradiol-17α in faeces was tested for its use in pregnancy diagnosis. The mean concentration of oestradiol-17α of non-pregnant animals was $9{\cdot}86 \pm 3{\cdot}86$ ng/g. Oestradiol concentrations in faeces of cows between the 10th and 13th week of pregnancy were not different from those of non-pregnant animals. After this time the oestradiol-17α concentration in the faeces rose and from the 14th week of gestation onwards it was higher than 22 ng/g. This value was greater than the mean of the oestradiol-17α concentration plus three standard deviations ($\bar{x} + 3$ SD) in the faeces of non-pregnant animals (Fig. 1).

DISCUSSION

The milk progesterone test detects cows which are non-pregnant on day 24 (Booth, Davies & Holdsworth, 1979), but about 20% of those cows with high progesterone levels on day 24 may be confirmed later as non-pregnant because of early embryonic mortality (Ayalon, 1978). Alternatively the determination of substances produced by the conceptus can be used to confirm pregnancy. Evans & Wagner (1981) showed that a 115-day-old

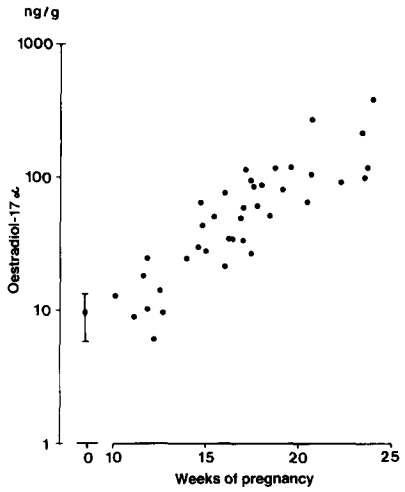

Fig. 1. Concentration of oestradiol-17α (ng/g) in faeces of non-pregnant ($\bar{x} \pm$ SD; n= 21) and pregnant (10 to 25 weeks; n= 39) cows and heifers.

bovine placenta was able to produce oestrogens *in vitro* using labelled androstenedione as precursor. Robertson & King (1979) reported, that plasma oestrogen concentrations increased at about 10 weeks of pregnancy.

Oestradiol-17α, a weak oestrogen, is the major urinary metabolite in cows (Velle, 1975) and the present results showed that it is also excreted in faeces. The concentration of oestradiol-17α in faeces was 10 times higher than that of either oestrone or oestradiol-17ß. Möstl *et al.* (1983) showed that pregnancy diagnosis in cattle can be performed by measuring oestrogens in faeces using an antibody cross-reacting with oestrone (100%), oestradiol-17ß (110%) and oestradiol-17α (30%). The specific determination of oestradiol-17α however gives four times higher oestrogen values, and therefore increases the sensitivity of the method.

From the 14th week of gestation onwards the oestradiol-17α concentration in faeces was found to be significantly higher than in non-pregnant animals. This is in agreement with the pattern of oestrone sulphate concentration in milk (Heap & Hamon, 1979). Compared to the measurement of oestrone sulphate in milk, the determination of oestrogens in faeces offers the advantage that it can be done not only in cows but also in heifers.

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