

Pregnancy Diagnosis in Mares by Determination of Oestradiol-17- β Hormone in Faeces

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Abstract: The aim of the present study was the confirmation of pregnancy diagnosis in mares by the determination of oestradiol-17 β in faeces. The research was carried out on 30 cyclic mares. Faeces samples were collected through pregnancy at monthly intervals. The faeces samples of pregnant and non-pregnant mares contained a mean oestradiol-17 β concentration of 9.39 ± 2.63 and 7.70 ± 2.00 ng/g, respectively in the 4th month of gestation. From the 5th month of pregnancy the oestradiol-17 β concentration in pregnant mares was significantly higher than in non-pregnant mares. Oestradiol-17 β levels were estimated to be 71.20 ± 8.00 and 4.60 ± 2.20 ng/g in pregnant and non-pregnant mares in the 5th month of pregnancy, respectively.

However, the confirmation of pregnancy with the enzyme – immunoassay technique was possible after the 5th month of gestation; faeces samples collected at short intervals would be necessary to obtain a reliable pregnancy diagnosis.

Key Words: Pregnancy diagnosis, oestradiol-17 β , faeces, mare

Kısrak Dışkısında Östradiol 17- β Hormonu Tayini ile Gebeliğin Teşhisi

Özet: Bu çalışmada kısrak dışkısında östradiol-17 β hormonu EIA tekniği ile ölçülerek gebeliğin teşhis edilmesi amaçlandı. Çalışma 30 kısrak üzerinde gerçekleştirildi. Gebelik boyunca bir ay aralıklarla dışkı örnekleri toplandı. Gebeliğin 4. ayında ortalama östradiol 17- β konsantrasyonları gebelerde 9.39 ± 2.63 , gebe olmayanlarda 7.70 ± 2.00 ng/g ölçüldü. Gebeliğin 5. ayından itibaren östradiol-17 β miktarı gebe kısraklarda gebe olmayanlardan belirgin derecede yüksekti, 5. ayda gebelerde 71.20 ± 8.00 gebe olmayanlarda ise 4.60 ± 2.20 ng/g bulundu.

EIA metodu ile kısraklarda 5. aydan itibaren gebeliğin teşhis edilmesi mümkün olmasına karşın, gebelerdeki östradiol-17 β miktarının gebe olmayanlara göre ne zaman farklılaşmaya başladığının belirlenmesi için dışkı örneklerinin daha kısa aralıklarla toplanmasının uygun olacağı kanısına varıldı.

Anahtar Sözcükler: Gebelik teşhisi, östradiol-17 β , dışkı, kısrak

Introduction

Oestrogens can be used as an indicator for pregnancy diagnosis in animal species in which the foeto-placental unit is the source of large quantities of oestrogens. In most studies on faecal oestrogen determinations, unconjugated oestrone and/or oestradiol-17 β or oestradiol-17 α levels were measured using specific oestrogen antibodies or antibodies against total unconjugated oestrogens. Steroid concentrations in faeces exhibit a similar pattern to those in plasma, but have a lag time which, depending upon the species, can be from 12 h to more than 2 days (1). Faecal oestrogen evaluations for pregnancy determination were used in

domesticated hoof stock (1-5). Consecutively, faecal oestrogen analysis was applied to non-domesticated ungulate and primate species and successful pregnancy diagnosis was reported in the red buffalo, yak, Grevy's zebra and Nubian ibex (6), as well as in the muskox (7), the gorilla, orangutan, Mhorr gazelle, Przewalski's mare and Malaysian tapir (8,9), and in the yellow baboon (10) and sable antelope (11). Oestrone and oestradiol-17 β are the main oestrogens in mare faeces, while in cattle oestradiol-17 α predominates (5).

The aim of the present study was the confirmation of pregnancy diagnosis in mares by the determination of oestradiol-17 β levels in faeces.

Materials and Methods

The study was carried out on 30 cyclic mares. The oestrus of mares was detected and they were mated from the beginning of day 2 of oestrus and every other day until the end of oestrus. Faeces samples were collected throughout the pregnancy at monthly intervals. Faecal oestradiol-17 β was determined by enzyme-immunoassay (EIA) technique.

Extraction: To 0.5 g faeces 1.5 ml KOH (1 mol/l) and 0.5 ml of a mixture of chloroform + n-hexane (6/4) were added. After shaking (30 min) and centrifugation (1500 x g; 15 min) 10 μ l were transferred to another vial and diluted (1/50) with assay buffer containing 0.1% Tween (5).

Enzyme-immunoassay: Oestradiol-17 β in faeces was determined using the EIA technique described by Meyer and Hoffmann (12). Anti-17 β oestradiol (E-2885) and oestradiol-peroxidase (E-8883) were purchased from Sigma. EIA was performed on microtitre plates which were coated with goat anti-rabbit IgG (1 μ g per well).

Statistical analysis: Comparisons between pregnant and non-pregnant groups were made using Student's t test. All data are presented as the mean \pm sd.

Results

Out of 30 mares only 24 produced foals. The results obtained from the oestradiol-17 β determination in faeces samples between months 1 and 12 are shown in the Table. Oestradiol-17 β concentrations in the faeces of pregnant mares between months 1 and 4 of pregnancy were no different from those of non-pregnant mares. After this time, the oestradiol-17 β concentrations in the faeces of pregnant mares increased and reached 71 ng/g in the 5th month of pregnancy, while it was 4 ng/g in non-pregnant mares. It remained at high levels until the end of pregnancy. The oestradiol-17 β concentrations in faeces collected after parturition decreased (5.00 \pm 1.40).

Discussion

Möstl et al. (13) have shown that faecal oestrogen content in non-pregnant mares averaged 4.1 ng/g. For 65 mares in the 15th – 48th week of pregnancy, the faecal oestrogen level ranged from 20.8 to 400 ng/g;

Table. Levels of oestradiol-17 β in faeces of pregnant and non-pregnant mares.

Month	Pregnant (n = 24)	Non-pregnant (n = 6)
1	3.27 \pm 0.57	2.61 \pm 0.58
2	4.45 \pm 0.94	3.92 \pm 1.65
3	5.14 \pm 1.78	5.10 \pm 1.33
4	9.40 \pm 2.63	7.70 \pm 2.00
5	71.20 \pm 8.00	4.60 \pm 2.20
6	82.64 \pm 6.70	6.00 \pm 1.54
7	69.90 \pm 5.83	4.75 \pm 2.22
8	77.65 \pm 4.89	4.83 \pm 1.47
9	75.50 \pm 9.45	7.18 \pm 2.79
10	62.30 \pm 2.20	7.17 \pm 3.34
11	68.30 \pm 5.00	5.00 \pm 1.55
12	5.00 \pm 1.40	4.17 \pm 1.00

Oestradiol-17 β (ng/g), n = Number of animals.

throughout this period it was significantly higher than in non-pregnant mares.

Faecal oestrogen determination in mares after 120 days of pregnancy has been shown to be a reliable indicator of pregnancy in mares by Bamberg et al. (14) and faecal oestrogen concentrations in pregnant mares were higher than in non-pregnant mares. In another trial, Bamberg et al. (2) reported that the oestrogen concentration in faeces of non-pregnant mares was 3.7 \pm 2.2 ng/g between day 90 and 150; from the 17th week of pregnancy onwards the oestrogen concentration was significantly higher than in non-pregnant mares and reached values of 166 \pm 15 ng/g after the 20th week of gestation.

In our study, although the oestradiol-17 β concentration of pregnant mares started to increase in the 4th month of gestation, it was not significantly higher than that of non-pregnant mares ($p > 0.05$). However, from the 5th month of pregnancy, the oestradiol-17 β concentration in the faeces of pregnant mares was significantly higher than that of non-pregnant mares ($p < 0.05$).

In conclusion, the confirmation of pregnancy with the EIA method was only possible after the 5th month of gestation, and faeces samples collected at weekly intervals would be necessary to obtain reliable pregnancy diagnosis. This technique combines the advantages of sample collection without restraint or invasiveness. Therefore, this pregnancy diagnosis can also be performed for zoo animals when blood collection causes problems.

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