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Suitability of serum oestrone sulphate measurement to verify pregnancy in red deer

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ABSTRACT

The mean ± s.e.m. concentration of oestrone sulphate (OS) in serum from 31 non-pregnant red deer was 2.0±0.2 ng/ml. The value 3 standard deviations above this mean was 6.2 ng/ml which represents the highest serum OS concentration likely to be found in non-pregnant deer. Only 2 out of 40 serum samples obtained from 15 deer between 118 and 182 days pregnant had an OS concentration >6.2 ng/ml, the overall mean ± s.e.m. value for the 40 samples being 2.4±0.3 ng/ml. On days 195 and 209 of gestation, 5 of 10 deer and 7 of 9 deer respectively had serum OS concentrations above 6.2 ng/ml; the mean ± s.e.m. concentrations on days 195 and 209 being 7.2±1.3 ng/ml and 14.9±3.4 ng/ml respectively. These results show that mean serum OS concentrations rise markedly in red deer during late gestation, and that by day 209 serum OS concentrations have risen above 6.2 ng/ml in most hinds. Measuring serum OS concentrations in the last month before expected calving may offer an alternative to ultrasonography for verifying pregnancy status in red deer.

Keywords: Oestrone sulphate; deer; serum; pregnancy; enzymeimmunoassay.

INTRODUCTION

The association with pregnancy of elevated concentrations of oestrone sulphate (OS) in serum, milk or faeces is well documented for several species including cows (Henderson *et al*., 1994), goats (McArthur and Geary, 1986), horses (Henderson *et al*., 1997) and pigs (Moenter *et al*., 1992). Measurement of OS provides an accurate means of determining pregnancy status in these species. In deer there is only limited information concerning the association of OS or other oestrogens with pregnancy. A study by Barrell and Bos (1989) in red deer indicated that in general the presence of measurable OS levels in serum of hinds was associated with pregnancy. In fallow deer (*Dama dama*) mean serum OS concentrations were significantly higher in pregnant hinds at 49 days of gestation than in non-pregnant females (Wilker *et al*., 1993). In white-tailed deer (*Odocoileus virginianus*), mean plasma concentrations of oestrone and oestradiol increased 2- to 4-fold in the last month of gestation (Harder and Woolf, 1976).

The purpose of the present study was to determine if OS concentrations were elevated in serum during late pregnancy in red deer which have a gestation period of approximately 233 days. If so, this might offer an alternative to ultrasonography for verifying pregnancy status close to the expected time of calving.

MATERIALS AND METHODS

This study involved 31 non-pregnant, mature red deer hinds, and 15 pregnant red deer hinds in which pregnancy was established by embryo transfer and then confirmed by ultrasonography and subsequently from calving records. The deer were farmed under normal husbandry conditions. A single blood sample (10 to 20 ml) was obtained from each non-pregnant hind by jugular venepuncture. Four jugular blood samples were obtained at fortnightly intervals from the pregnant deer (except for 1 hind which was only bled 3 times) when the animals were between 118 and 209 days pregnant. Serum was prepared from each of the blood samples and stored frozen until assayed for OS.

Oestrone sulphate was measured in serum samples using an antibody-coated enzymeimmunoassay which has been described previously for assaying OS in cows' milk (Henderson *et al*., 1994) and horse faeces (Henderson *et al*., 1997). Briefly, the assay was performed as follows. Wells of microtitre plates (Nunc, Maxisorp C12) were coated with a monoclonal antibody to OS sulphate by overnight incubation. Following blocking with a 0.5% gelatin buffer solution and washing, the antibody-coated wells received a 10 µl aliquot of deer serum, or an aliquot of OS standard, plus an aliquot of oestrone glucuronide-horseradish peroxidase conjugate in a total volume of 0.2 ml. The wells were then incubated overnight at 4°C. After emptying and washing the wells, 0.1 ml of o-phenylenediamine substrate-chromogen solution was added and colour allowed to develop for 20 to 30 minutes before the reaction was stopped with 2M H_2SO_4 (0.05 ml/well) and colour intensity read at 490 nm. The concentration of OS sulphate in the assayed deer serum samples was calculated by interpolation from a standard curve. The working range of the standard curve was from 5 to 200 pg/well. The intra- and inter-assay coefficients of variation of the assay were <11%. Dose-response lines of the OS sulphate standards and dilutions of deer serum ran parallel.

RESULTS

The mean ± s.e.m. concentration of OS in serum obtained from the 31 non-pregnant hinds was 2.0±0.2 ng/ml, and 23 of the 31 deer had a serum OS concentration of ≤2 ng/ml. Less than 1% of non-pregnant deer are likely to have a serum OS concentration greater than the value 3 standard deviations above the mean non-pregnant value. From the present data, this value was calculated to be 6.2 ng/ml.

FIGURE 1: Scatter diagram of the oestrone sulphate concentrations in 59 serum samples obtained from 15 deer between 118 and 209 days of pregnancy. The horizontal line indicates 6.2 ng/ml which is the value 3 standard deviations above the mean serum oestrone sulphate concentration of 2.0 ng/ml found in non-pregnant deer.

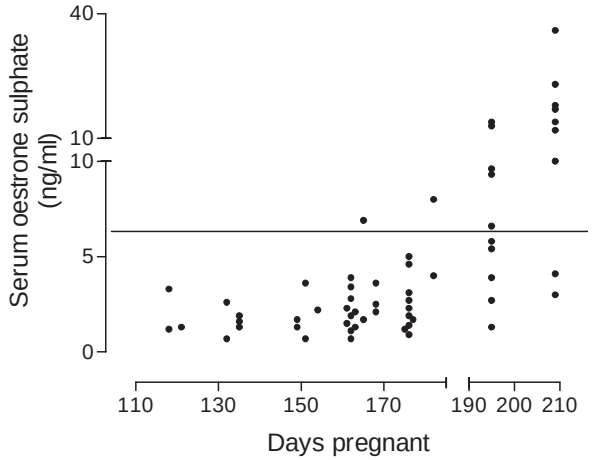

TABLE 1: Frequency table showing the number of deer with serum oestrone sulphate concentrations of <2, 2 to 6.2 and >6.2 ng/ml on different days of pregnancy.

Serum oestrone sulphate conc. (ng/ml)	Days pregnant 118 to 182	195	209
<2	8	1	0
2 to 6.2	9	4	2
>6.2	2	5	7

Figure 1 shows a scatter diagram of the OS concentrations in the 59 serum samples obtained from the 15 pregnant deer between 118 and 209 days of gestation. The data is also shown as a frequency table of deer numbers in Table 1. Between 118 and 182 days of pregnancy there were only 2 serum samples from 2 deer with an OS concentration >6.2 ng/ml. These were obtained on days 165 and 182 and had OS concentrations of 6.9 and 8.0 ng/ml respectively. Twenty of the 40 serum samples obtained between days 118 and 182 had an OS concentration <2 ng/ml. Five of the 10 deer sampled on day 195, and 7 of the 9 deer sampled on day 209 had a serum OS concentration >6.2 ng/ml (Table 1). The mean ± s.e.m. serum OS concentration on days 195 and 209 were 7.2±1.3 ng/ml (n=10 deer) and 14.9±3.4 ng/ml (n=9 deer) respectively. The earliest blood sample obtained from the 9 deer sampled on day 209 was obtained on day 162. The mean ± s.e.m. serum OS concentration on day 162 for these 9 deer was 2.2±0.4 ng/ml, which was significantly lower ($P<0.01$, paired t-test) than the mean ± s.e.m. value of 14.9±3.4 ng/ml found on day 209. The mean ± s.e.m. value of 2.2±0.4 ng/ml found on day 162 did not differ significantly ($P>0.05$, unpaired t-test) from the mean value of 2.0±0.2 ng/ml found for the 31 non-pregnant deer.

DISCUSSION

The results of this study show that in red deer the mean concentrations of OS in serum rise substantially during the final month of gestation. This is consistent with the results of Harder and Woolf (1976) for white-tailed deer (*Odocoileus virginianus*) where it was found that plasma levels of oestrone and oestradiol increased 2 to 4 fold during the last month of pregnancy. In this study, 5/10 and 7/9 deer on days 195 and 209 of pregnancy respectively had serum OS concentrations >6.2 ng/ml which is the highest concentration likely to be found in non-pregnant deer. Serum OS concentrations may continue to rise as parturition approaches. Thus, it is possible that by blood sampling closer to the expected day of calving (day 233) the serum OS concentration in all deer could exceed 6.2 ng/ml. By day 209 of pregnancy none of the deer had a serum OS concentration below 2 ng/ml. Measurement of serum OS concentrations in the last month of gestation may thus provide an alternative to ultrasonography for verifying pregnancy status. Those deer returning a serum OS concentration >6.2 ng/ml can be considered pregnant while those with values <2 ng/ml can be considered non-pregnant. Values between 2 and 6 ng/ml are probably best regarded as returning an inconclusive diagnosis. Re-measuring serum OS concentrations in these deer closer to the expected date of calving, to determine if the OS levels are rising, may allow a conclusive diagnosis to be made. The ability to distinguish deer which are pregnant, and within a month of calving, from deer which are not pregnant or are late calving deer may aid management decisions.

There would be considerable practical potential in being able to use OS measurements to verify pregnancy status accurately earlier than the last month of gestation. Unfortunately, this was not found to be a realistic possibility. Only 2 serum samples from 2 of the deer between 118 and 182 days pregnant had OS concentrations >6.2 ng/ml. The remainder had OS concentrations indistinguishable from those of non-pregnant deer. Blood samples were not obtained earlier than 118 days of pregnancy. Thus, it was not possible to examine the proposal of Barrell and Bos (1989) that measuring OS concentrations in blood samples obtained between 50 and 90 days post-conception may be of value in diagnosing pregnancy status. However, ultrasonography is commonly used to diagnose pregnancy in deer early in gestation. Real-time ultrasound scanning is well accepted as a straightforward and accurate way of pregnancy diagnosing deer (Wilson and Bingham, 1990). The high degree of accuracy and the immediacy of the diagnosis gives this technique considerable advantage over endocrine analysis. Nevertheless, there is some farmer concern that the use of a rectal probe for ultrasound analysis in late pregnancy may be detrimental. Although there is little hard

evidence to support this contention, the use of OS measurement to verify pregnancy status close to the expected time of calving may be one way of addressing such concern. However, ultrasonography will likely remain the most practical means of determining pregnancy status in deer.

ACKNOWLEDGEMENT

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