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Levels of Oestrone Sulphate during Pregnancy in Different Breeds of Cows and its Possible Association with Retained Foetal Membranes

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Abdo, G. A., O. M. Njuguna, G. Fredriksson and A. Madej: Levels of oestrone sulphate during pregnancy in different breeds of cows and its possible association with retained foetal membranes. Acta vet. scand. 1991, 32, 183–188. – The levels of oestrone sulphate in plasma of pregnant cows was followed from 30 days of pregnancy until parturition. The Swedish Jersey Breed (SJB) showed significantly lower levels of oestrone sulphate between 101 and 200 days of pregnancy than either the Swedish Red and White (SRB) or the Swedish Lowland Breed (SLB). No significant difference was noted between SRB and SLB. On days 141–160 of gestation the oestrone sulphate values were still below the 10 nmol/l level for the SJB while they were above this level for the SRB and the SLB, and the difference was significant. In the SJB, levels above 10 nmol/l were reached on days 161–180 of gestation.

In the second part of this study the levels of oestrone sulphate were measured around parturition in SRB cows. At parturition, the levels of oestrone sulphate rose to peak values of 79.9 ± 5.2 nmol/l and then decreased to 6.6 ± 0.5 nmol/l on the day after calving. In one cow peak values of 66.0 nmol/l were reached 2 days prior to parturition, and subsequently dropped to 7.0 nmol/l at parturition. This cow had retained foetal membranes. A possible relationship between low oestrone sulphate levels prior to parturition and retained foetal membranes is discussed.

breed difference; parturition, peripartal period.

Introduction

Of the 2 major oestrogens produced by the foetal placental unit, oestrone and oestradiol-17β, oestrone in its conjugated form, namely oestrone sulphate, is the major oestrogen in maternal circulation (*Thatcher et al.* 1982, *Gaiani et al.* 1982). Measurement of oestrone sulphate is therefore the method of choice in evaluating placental production of oestrogen. While some reports indicate that measurable levels of oestrone sulphate in bovine plasma appear around day 80 of gestation (*Gaiani et al.* 1982), others indicate day 100 (*Warsfold et al.* 1989). It is generally agreed by all workers that the level starts to rise after day 100 of gestation (*Gaiani et al.* 1982, *Warsfold et al.* 1989).

The level of oestrone sulphate in bovine plasma of pregnant cows was shown to be influenced by the sire (*Thatcher et al.* 1982). Holstein heifers inseminated to Angus bulls had lower prepartum concentrations of oestrone sulphate than heifers inseminated to either Holstein or Brahman bulls. To our knowledge, no study has been performed to evaluate breed differences of oestrone sulphate levels. The first objective in this study therefore was to follow plasma oestrone

sulphate levels throughout pregnancy especially considering different breeds.
The second objective was to determine peripartum oestrone sulphate levels and especially relate these to peripartum conditions including retained foetal membranes.

Materials and methods

Animals

The research was carried out on 23 pregnant dairy cows (7 Swedish Jersey Breed (SJB), 12 Swedish Red and White Breed (SRB) and 4 Swedish Lowland Breed (SLB)) covering the gestation period from day 30 of pregnancy until parturition, and an additional 14 SRB cows around parturition. The 23 animals belonged to the experimental herds of the Departments of Animal Breeding and Genetics, and the additional 14 to Animal Nutrition and Management, Swedish University of Agricultural Sciences, Uppsala, Sweden. The study was undertaken from April to November 1989 and all possible pregnant cows were used during this time. This explains the low number of SLB cows. The cows were tied in stalls during the time of the study. Daily outdoor exercise in a paddock was allowed when weather conditions permitted. The cows were fed according to Swedish standards and milked twice daily. All cows were healthy and in good condition during the time of the study.

Blood sampling

In the first experiment, jugular vein blood samples were withdrawn into heparinized vacutainer tubes (Becton-Dickinson, USA) every 14 days starting from day 30 of pregnancy from 23 pregnant dairy cows. The blood was centrifuged immediately and plasma was removed and stored at – 20°C in plastic tubes until hormone analysis.
In the second experiment, blood plasma was collected daily from 14 SRB cows, 7 days prior to the expected date of parturition until 7 days postpartum. The calving dates were calculated from breeding records as well as from clinical signs of approaching parturition. Cows with retained foetal membranes were recorded.

Hormone analysis

Oestrone sulphate concentrations were determined by radioimmunoassay (*Kindahl et al.* 1982, *Mohamed et al.* 1987) using antiserum against oestrone-3-glucuronide-BSA raised in rabbits. Antiserum (K-8860), cross-reacted with oestrone and oestradiol-17β but not with progesterone and cortisol. The final dilution of antiserum was 1:11520. The sensitivity of the assay was 2.5 nmol/l. The intra-assay coefficient of variation for oestrone sulphate concentration in the range of 2 to 100.0 nmol/l was below 15 per cent. The inter-assay coefficient of variation was 14.8 per cent (mean value 53.6 nmol/l).

Statistical analysis

The changes in levels of oestrone sulphate during periods of 20 days interval were evaluated by analysis of variance using the software program Statgraphics STCS, (Inc. Rockville, MD, USA). The Sheffe multiple range test was used to compare means of oestrone sulphate levels in that period. Values in the results are given as mean ± standard error.

Results

Pregnancy

Swedish Jersey Breed. Plasma oestrone sulphate (Fig. 1 & Table 1) was first detectable from day 90 of pregnancy (0.3 ± 0.3 nmol/l). Then there was a gradual but slow increase. The values reached 21.2 ± 5.2 nmol/l on days 201–220 when a sharp increase began up to 48.0 ± 4.4 nmol/l by days 261–280 of pregnancy. Oestrone sul-

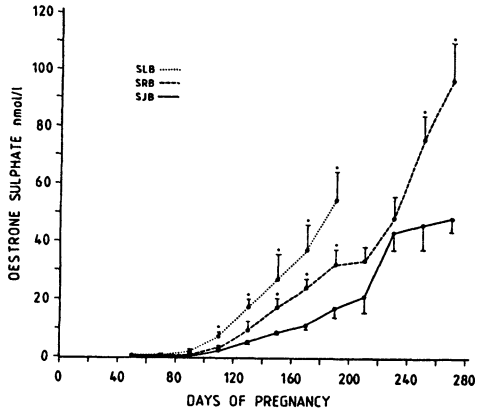

Figure 1. Oestrone sulphate concentration in plasma of SJB, SRB, and SLB during pregnancy. * indicates statistical difference in the values of SRB and SLB compared to SJB ($p < 0.05$).

phate values passed 5.0 nmol/l in the interval 121–140 days and 10 nmol/l between 161–180 days.

Swedish Red and White Breed. The plasma level of oestrone sulphate in SRB (Fig. 1 & Table 1) was low, 0.3 ± 0.1 to 0.8 ± 0.3 until day 100 of pregnancy. Then it increased progressively to 48.2 ± 7.5 nmol/l in the interval 221–240 days and rose sharply to reach a concentration of 96.8 ± 12.8 nmol/l by days 261–280. The level of oestrone sulphate was above 5 nmol/l at 121–140 days of pregnancy but still below 10 nmol/l. By the interval 141–160 days it was above 10 nmol/l.

Swedish Lowland Breed. Plasma oestrone sulphate (Fig. 1 & Table 1) was low, 0.6 ± 0.2 to 2 ± 0.6 nmol/l, from day 41 to 100 of pregnancy and then there was a gradual and continuous increase reaching 54.6 ± 9.8 nmol/l in the interval 181–200 days. The level of oestrone sulphate passed 5 nmol/l limit during the period of 101–120 days and was above the 10 nmol/l limit between 121–140 days.

Table 1. Plasma levels of oestrone sulphate, days of pregnancy in cows of different breeds by nmol/l.

Days of pregnancy	SJB		SRB		SLB	
	Value	n	Value	n	Value	n
41–60	0	2	0.3 ± 0.1	3	0.6 ± 0.2	4
61–80	0	2	0.1 ± 0.1	4	0.5 ± 0.1	7
81–100	0.3 ± 0.3	4	0.8 ± 0.3	5	2.0 ± 0.6	5
101–120	2.5 ± 0.7	3	3.2 ± 0.6	4	7.3 ± 1.1*	5
121–140	5.2 ± 0.6	9	9.4 ± 2.9	7	17.2 ± 2.6*	5
141–160	8.8 ± 0.6	6	17.2 ± 2.7*	6	26.9 ± 8.8*	5
161–180	11.3 ± 1.4	9	24.0 ± 2.8*	7	37.1 ± 8.9*	5
181–200	17.6 ± 3.1	7	32.3 ± 4.9*	8	54.6 ± 9.8*	5
201–220	21.2 ± 5.3	5	33.3 ± 5.3	7	–	
221–240	43.7 ± 6.1	7	48.2 ± 7.2	11	–	
241–260	46.5 ± 8.3	5	75.7 ± 8.2*	10	–	
261–280	48.0 ± 4.4	5	96.8 ± 12.8*	11	–	

* Statistical significant difference in the levels of oestrone sulphate between SRB/SLB, and SJB at $p < 0.05$.
– No observations.

The level of oestrone sulphate in blood plasma of SJB was lower than that in SRB and SLB in different periods of pregnancy (Table 1). This difference of oestrone sulphate levels between either SRB or SLB and SJB was found statistically significant ($p < 0.05$). No significant difference was noted between SRB and SLB.

Parturition

Only 1 of 14 SRB cows had retained foetal membrane (RFM) more than 12 h after calving. Dystocia was not observed in any of the cows. The prepartum concentrations of oestrone sulphate in blood plasma of cows with normal drop of foetal membranes fluctuated between 77.3 ± 6.3 and 79.9 ± 5.2 nmol/l during the 7 days prior to parturition and then decreased abruptly to 6.6 ± 0.5 nmol/l by day 1 after calving. The levels then continued to decrease and at 7 days after calving 1.1 ± 0.2 nmol/l was monitored (Fig. 2).

On the other hand in the cow with RFM, oestrone sulphate levels were lower than in normal cows before calving and started to decrease 2 days before calving from a peak value of 66.0 nmol/l to 12.8 nmol/l 1 day before calving and then to 7.0 nmol/l on the day of calving. It continued to decrease and 1.2 nmol/l was measured 7 days postpartum (Fig. 2).

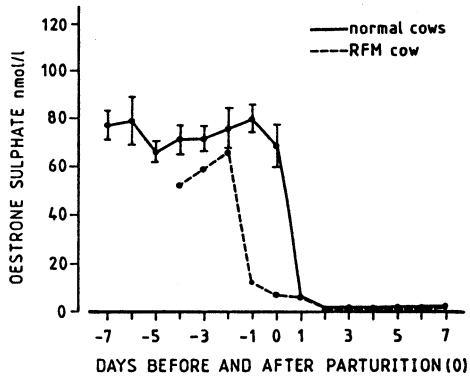

Figure 2. Oestrone sulphate concentration in blood plasma of SRB cows around parturition.
——— cow with normal drop of foetal membranes (n = 13) (x ± SE).
- - - - cow with retained foetal membranes (RFM).

Discussion

The levels of oestrone sulphate in maternal plasma remained relatively low in all the 3 breeds before day 100 of gestation, which is in agreement with other workers (*Gaiani et al.* 1982, *Warsfold et al.* 1989). This may reflect low production of oestrone by the conceptus, or a low ratio of sulphokinase to sulphatase activity with the oestrone leaving the conceptus in the unconjugated state being rapidly excreted as oestrone glucuronide (*Robertson & King* 1979).

It is also possible that the foetal production of oestrone is too low to be detected in the peripheral circulation of maternal plasma. The SJB showed significantly lower levels of oestrone sulphate between 101 and 200 days of gestation than either the SRB or the SLB. On days 141–160 the oestrone sulphate levels for the SJB were still below the 10 nmol/l level unlike those of either the SRB or the SLB. This difference was found to be significant. However, no significant difference was observed during the whole interval from day 101 to day 200 of pregnancy between the SRB and the SLB. This may be due to a low number of animals in the SLB group and the large variation of the values in that group. These breed differences in timing of start of detectable levels of oestrone sulphate as well as actual levels of the hormone are of importance when used for positive pregnancy diagnosis.

Mohamed et al. (1987) observed a lower value of plasma oestrone sulphate in a Jersey (7.8 nmol/l) compared to the Friesians or

Ayrshire included in their study. The variation in oestrone sulphate levels between breeds may be explained by the difference in foetal sizes. The weight of the foetus at birth and oestrone sulphate levels have been shown to have a positive correlation (*Thatcher et al.* 1982). The SJB have lower sizes and weights than the SRB which in turn are lower than the SLB. It would also be of interest to find out whether this is reflected in the sum of the size of the placentomes as a larger foetus would have a larger placental support. A sharp increase in oestrone sulphate values was observed in the interval from day 241 to day 280 of pregnancy. This is in agreement with findings by other workers (*Thatcher et al.* 1980). This is also the time of rapid increase of the weight and size of the foetus. Due to our method of sampling, we cannot report on the values of oestrone sulphate around parturition in the first experiment.

The low level of oestrone sulphate observed in the cow which had retained foetal membranes probably reflects disturbances in the foetal placental unit. The amount of oestrogens for the maturation process of the placentomes will be insufficient. General uterine contractibility would also be impaired with insufficient levels of oestrogens. An association between retained foetal membranes and high pre-partal cortisol level was reported by *Peter & Bosu* 1987. An association also probably exists between low prepartal oestrone concentration in maternal plasma and retained foetal membranes. This has also been reported recently in an experimental study by *Grunert et al.* 1989. However, as only 1 cow had retained foetal membranes, this may not be significant and more work needs to be done.

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Sammanfattning

Östronsulfatnivån under dräktigheten hos olika koraser samt dess eventuella samband med kvarbliven efterbörd.

Östronsulfatnivåerna i plasma följdes hos 23 kor av 3 olika raser från 30 dagars dräktighet till slutet av dräktigheten. Svenska jerseykor uppvisade signifikant lägre nivåer av östronsulfat mellan dag 141–200 och dag 241–280 jämfört med svensk röd och vit boskap och mellan dag 101–200 jämfört med svenska låglandskor. I intervallet 141–160 av dräktigheten var östronsulfatnivåerna fortfarande under 10 nmol/l för jerseykorna medan de hade passerat denna nivå för de andra raserna. Denna skillnad var signifikant. Jerseykorna uppnådde denna nivå i intervallet 161–180 dagars dräktighet.

I denna studies andra del mättes östronsulfatnivåerna vid tiden runt kalvningen hos svenska röda och vita kor. Vid kalvningen uppnåddes maximala koncentrationer av hormonet på 79,9 ± 5,2 nmol/l för att minska till 6,6 ± 0,5 nmol/l en dag efter kalvningen. Hos en ko nåddes maximala värden på 66,0 nmol/l 2 dagar innan kalvningen och sjönk sedan till 7,0 nmol/l på kalvningsdagen. Denna ko hade kvarbliven efterbörd. Sambandet mellan låga östronsulfatnivåer omedelbart innan kalvningen och kvarbliven efterbörd diskuteras.

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