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# Faecal concentration of cortisol metabolites in prepartum ewes

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**Summary**

This study was performed to determine the endocrinological starting point of parturition in ewes and to study correlations between the viability of offspring and the concentrations of faecal cortisol metabolites in the dam using 11-oxoetiocholanolone EIA (measuring 11,17-dioxoandrostanes). The levels of faecal cortisol metabolites of 10 ewes positive for ovine pulmonary adenocarcinoma (OPA) were compared against 10 negative ewes during the last week of gestation and 12 h after delivery. The mean concentration in the OPA group was higher (500-600 ng/g) than that observed in the control group (150-200 ng/g). The mean cortisol level showed a significant ($p$<0.05) increase during the last two days of pregnancy in all subjects (OPA and controls). The increase in faecal cortisol metabolites was not significant ($p$>0.05) in three animals (2 OPA and 1 control); their offspring showed low viability and died soon after delivery. These results underline that the mean baseline faecal cortisol metabolite concentration in ewes does not affect physiological pregnancy while, to ensure viability of lambs, it is essential that the cortisol level increases significantly (90%), regardless of its baseline level.

**Keywords**

Cortisol, Faecal metabolites, Parturition, Neonate, Sheep, Viability.

## Introduction

Parturition signals the end of the gestation period and thus the time when the foetus becomes independent of the mother and is able to survive in the outside world. It is initiated by the transmission of numerous biochemical signals from the foetus to the mother (4). These are emitted only when the foetus has reached complete maturity of its pulmonary, circulatory and digestive glandular systems and overall neurohormonal growth. Pulmonary maturity is characterised by an alteration of the biochemical ratio of lecithins and sphingomyelins. The rise in lecithins impedes the collapse of the pulmonary alveoli, thus ensuring that the neonate is able to breathe autonomously. This biochemical modification is controlled by the serum cortisol level, whether endogenous or administered ad hoc by the mother as needed. The action of foetal cortisol and hypothalamus-pituitary-adrenal integrity are therefore essential to trigger the parturition mechanism (2, 5).

The observation that adrenal gland and foetal brain abnormalities prolong gestation has been confirmed by numerous experiments in which hypophysectomy or foetal adrenalectomy delay parturition (4), while the administration of glucocorticoids causes premature onset of parturition (6). During the final stages of pregnancy, increased growth of the foetal

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adrenal cortex and a higher glucocorticoid synthesis are observed. In the last week of gestation, there is a considerable increase in plasma cortisol in the foetus which stimulates the endometrium and placenta to produce prostaglandin F2$\alpha$ (PGF2$\alpha$) (3). In general, the increase in foetal corticosteroids during the last month of gestation also appears to be responsible for the activation of particular enzyme systems in the cotyledons which increase the ability of the placenta to convert steroids (C21) into androgens (C19) and oestrogens necessary to trigger the birth mechanism. Labour can progress normally only when there is harmonious interaction between the foetus, the expulsive forces and the preparation of the birth canal: all this is possible only in the presence of the right hormonal balance. When the normal succession of endocrinal, biochemical, receptor and functional events undergoes any modification, the risk of a more complex labour increases (1). Given the capacity of foetal cortisol to cross the placental barrier (13), it was considered opportune to measure the variations of this hormone in the dam, starting from the foetal matrix (10). The glucocorticoids in the blood flow are metabolised in the liver through conjugation with glucuronic acid and sulphates and excreted as catabolites in the urine and faeces, passing through the bile ducts in the intestinal lumen where they undergo further transformation by the intestinal flora (7, 11). Studies conducted with glucocorticoids labelled with radioactive isotopes have revealed excretion times and modes for these substances (7). In ewes, although mainly excreted in the urine, steroidal hormone metabolites can also be measured in the faeces approximately 12 h after the release of the hormone into the circulation (9, 11).

A total of 21 cortisol metabolites were found in faeces; the most significant were steroids $C_{21}O_4$ and $C_{19}O_3$ with a molecular weight of from 302-308 to 350 Da, mainly in the non-conjugated form (7).

This study had the following objectives: identify the moment that parturition is triggered endocrinologically by measuring faecal cortisol metabolites (11,17-dioxoandrostanes) in the dam, apply the technique proposed by Palme *et al.* (9) and investigate any correlation between their concentration and neonatal viability.

## Materials and methods

The survey was conducted on 20 half-breed ewes held in the farm belonging to the *Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise* 'G. Caporale' in the province of Teramo. Of these, 10 were positive for ovine pulmonary adenocarcinoma (OPA) (group A), and the remaining 10 healthy ewes constituted the control group (group B). Group A ewes were found positive for Jaagsiekte sheep retrovirus (JSRV), detected with the semi-nested long terminal repeat (LTR) polymerase chain reaction (PCR) method (sn-LTR-PCR) (8). All animals were stabled in a shelter with an outside paddock, automatic water troughs and self-harnessing devices. During the trial, all animals received a diet of first-cut hay and concentrated feed. Ewes were synchronised by the application for 12 days of intravaginal pregnenolone sponges and administration of pregnant mare serum gonadotropin (PMSG) after removal of the sponges and 48 h before introduction of the rams.

Faecal material was taken between 8.00 and 10.00 am daily from each animal directly from the rectal ampulla for 15 days before parturition. A further sample was taken 12 h after delivery, corresponding to the delay between the peak in blood cortisol at parturition and the faecal excretion of metabolites. To identify the time of parturition, ewes were observed every 6 h for the entire trial period. All lambs underwent Apgar assessment for viability and efficiency of their principal bodily functions. This test was conducted twice, 1 and 5 min after birth. Parameters considered were heart and breathing rate, muscle tone, grimace response and mucous membrane colour, each of which was assigned a number on a 0-2 scale. The Apgar score is obtained from the sum of these five vital parameters and had a minimum value of 0 and a maximum value of 10. A score

of 7-10 is considered indicative of an animal in good condition, 4-7 indicates a moderately depressed animal and scores below 4 reveal a strongly depressed animal.

Animals were kept in accordance with the standards set for animal testing by the Italian Legislative Decree 116/92, with the approval of the Ministry of Health.

Faecal samples were placed in suitable containers immediately and identified with a progressive number and then frozen at –20°C until the extraction procedure, so as to avoid any deterioration caused by their bacterial content. At the end of the collection period, the samples were prepared, selecting only those from the final week of gestation and those taken 12 h after delivery. After thawing, homogenisation and weighing, 5 ml of an 80% methanol in water solution (4 ml methanol, 1 ml water) was added to a representative 0.5 g aliquot of the sample for the extraction of faecal metabolites. After hand vortex mixing for 1-2 min and multishaker mixing for 45 min at 250 rpm, the samples were centrifuged for 15 min at 2 500 rpm as described by Palme and Mostl (10). Then, 1 ml of supernatant was taken from each sample and transferred into vials sealed with a rubber cap and metal ferrule. The samples were despatched in polystyrol containers used for the transport of refrigerated materials by express courier to the 'Ludwig Boltzmann' Biochemical Laboratory at the Vienna Veterinary Faculty of Medicine. Stability was checked and they were then subjected to the 11-oxoetiocholanolone immunoenzymatic test (EIA) according to the technique validated and reported by Palme and Mostl (10).

Data were then subjected to statistical analysis using the Friedman test. Differences were considered significant for $p \leq 0.05$.

## Results

The mean values ± standard error of the mean (SEM) of the faecal cortisol metabolites found in the two experimental groups are presented in Figure 1. The results reveal that the mean level for the OPA-positive group (500-600 ng/g) is significantly ($p<0.01$) higher than that of the control subjects (150-200 ng/mg). There was no significant difference in metabolite concentration from day 7 to day 2 before parturition in either group. From day –2 onwards, an increase in cortisol metabolites was observed in both groups, with a statistically significant difference ($p<0.05$) compared with the values observed 12 h after delivery. This increase is in line with the 'physiological course' of the hormone concentration examined, the blood peak of which is observed at parturition. This increase was faster in the OPA-positive group (group A) than in the control animals (group B).

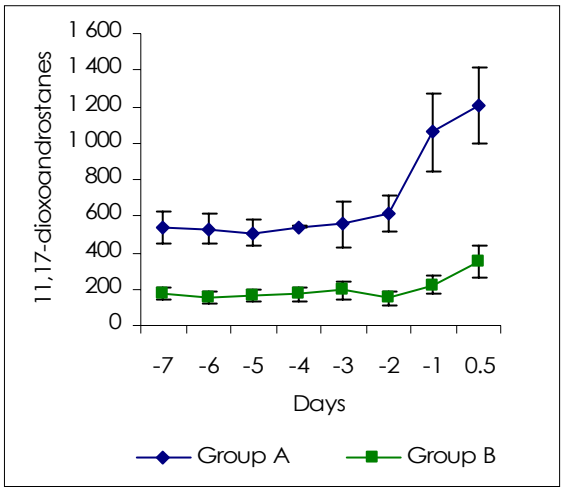


Figure 1
Mean values ± standard error of the mean of metabolite concentrations (11,17-dioxoandrostanes) in groups A and B

As can be seen in Figure 2, only 3 animals: 2 from the OPA group (ewes 1 and 2) and one from the control group (ewe 3), showed no increase in cortisol levels at parturition. The lambs born by these three animals showed poor viability, with an Apgar score below 4, and died immediately after birth.

## Discussion

The faecal cortisol metabolite measurement technique proposed by Palme and Mostl (10) was found suitable for this trial. The data obtained mirrored the events related to the triggering of parturition.

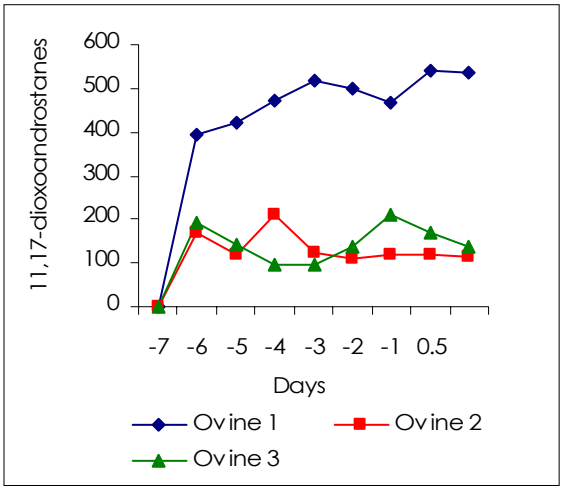


Figure 2
Faecal cortisol metabolite (11,17-dioxoandrostanes) values in ewes 1 and 2 (group A) and ewe 3 (group B)

The use of faeces offers a number of benefits: they are easy to collect and repeated samples can be taken without disturbing the animal, and thus without affecting its endocrinal condition (7, 10). This also enables monitoring of both short-term changes, linked to particular events and the long-term profile during longitudinal studies, such as hormone changes in the run-up to parturition and maturation of the foetal pituitary adrenal axis. Faecal cortisol metabolite concentration is related to a time interval, not to the sampling time only (12).

This study demonstrates that the mean baseline faecal cortisol metabolite level in the ewe does not affect the normal progress of pregnancy and that an increase of cortisol over the baseline is essential for normal parturition and good neonate viability. Given that the faecal cortisol peak is 12 h after the blood peak and that the increase in metabolites begins 2 days before parturition, it can be confirmed that in ewes, the preparation for parturition begins 60 h prior to delivery (with a maximum interval of –6 h, equal to the time between the last observation and delivery). The faecal cortisol metabolite concentration could therefore be used as a predictive value for the endocrinological triggering of parturition to ensure the wellbeing of the mother and neonate.

From our data, it can be postulated that there is a correlation between the concentration of faecal metabolites found in the mother and the viability of the neonate; in the three cases in which the lambs died immediately after birth (two in group A and one in group B), no raised cortisol level in the mother was found from day –2. The low number of dead lambs did not enable any statistical demonstration of this correlation and consequently further studies on a larger number of animals are necessary.

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