The morphofunctional state of pregnant sheep in the last stages of gestation is normal and with symptoms of eclampsia

Rinat N. Bulatov¹, and Vladimir S. Avdeenko²,*

¹Volgograd State Agrarian University, Volgograd, Russia
²St. Petersburg State University of Veterinary Medicine, St. Petersburg, Russia

Abstract. Sheep in the last stages of pregnancy showed signs of eclampsia, such as proteinuria, hypertension, swelling of the abdominal wall, and coma. Based on the diagnoses, two groups of 20 sheep with eclampsia were created: typical and atypical signs, in each, and one group of clinically healthy animals. From each group, 5 animals were slaughtered. In sheep suffering from eclampsia, a decrease in the total mass of the placenta and its cotyledons, as well as an increase in the length of the umbilical cord, was also found. Standard histological and histochemical studies were performed on the obtained placental samples. As a result, it was found that the average thickness of the integumentary epithelium of the caruncles in ewes with a typical form of eclampsia was 14.3 ± 1.7 μm, and in sheep with an atypical form - 15.5 ± 1.9 μm, compared with 27.9 ± 1.9 μm in the physiological course of gestation. This leads to a general decrease in the thickness of the layers of connective tissue separating the crypt and villi of the cotyledons. In addition, the cytoplasm of cells exhibits dystrophic modifications along with a decrease in the ability to store glycogen. Dystrophic changes are present in the cytoplasm of cells, which contributes to a decrease in glycogen storage.

1 Introduction

The problem of eclampsia in sheep is relevant in veterinary medicine, biology and medicine. According to recent studies [1, 2], eclampsia in pregnant sheep in the last stages of gestation develops as a result of disruption of the uteroplacental and fetal placental blood flow and changes in the coagulation properties of the blood, leading to placental insufficiency. The analysis of studies [3, 4] on the cause-and-effect relationships of this disease indicates the polyhedral nature of the occurrence and course of eclampsia in small ruminant cattle in the last stages of gestation, during lambing and in the first days after birth. The obtained genetic data [5] indicate the incompatibility of fetal and maternal antigens and insufficient immunological changes that are necessary for the normal intrauterine development of the fetus/fetuses and their placenta. According to data [6], the offspring of sheep that suffered eclampsia in the last stages of gestation are characterized by a low viability index. This circumstance emphasizes the need to study the role of morphofunctional changes in the placenta in ewes in establishing the pathogenesis of eclampsia.

* Corresponding author: avdeenko0106@mail.ru

© The Authors, published by EDP Sciences. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/).
Purpose of the study is to study the morphofunctional state of the placenta of sheep with eclampsia.

2 Research methodology

The study was conducted at sheep breeding enterprises in the Volgograd region of the Russian Federation from 2016 to 2024. A flock (700 young sheep) of the Volgograd breed took part in the experiment. An obstetric examination was carried out. Based on the diagnoses, two groups of sheep with eclampsia were created: with typical and atypical signs, containing 20 animals each and one group of clinically healthy animals. From each group, 5 animals were slaughtered. We obtained placental tissue samples, which were placed in a 10% neutral formaldehyde solution and kept in it at a temperature of 4°C. Tissue samples were subjected to a rigorous procedure involving gradual dehydration in a series of alcohol solutions, followed by a thorough clearing process using chloroform, all performed with precision on a Shendon Cytadel 2000 histoprocessor. The samples were then embedded in high-quality Histomix paraffin media sourced from Biovitrum, ensuring optimal preservation and structural integrity during all subsequent processing steps. The state-of-the-art MICROM HM 340E rotary microtome was used to create precise and consistent 5-micron sections with expert precision. These carefully processed sections were then carefully mounted on special slides, paving the way for further detailed analysis and research. A sophisticated CHIC method was used to identify neutral glycosaminoglycans (GAGs) using an improved McManus modification to provide insight into the structural components of the samples studied. In addition, staining of cell nuclei with Mayer's hematoxylin provided a vital contrast for highlighting cellular organization and morphology in tissue sections. To further investigate the complex molecular composition, Alcian blue staining was performed at a specific pH of 3.0 using the Steedman exact method to accurately determine the presence of acidic glycosaminoglycans (GAGs). This meticulous approach provided a comprehensive and nuanced analysis of the biochemical composition of the tissue sample, shedding light on the complex molecular intricacies embedded in the tissue microenvironment. Through these careful processing steps and analytical techniques, a greater understanding of the molecular and structural composition of the tissue was achieved, paving the way for further research and discoveries in the field of histology and tissue analysis. Microspecimens were studied using an AxioScope.A1 microscope (ZEISS) and recorded with a high-resolution digital camera AxioCamMRc5. The resulting images were processed with the ZENpro 2012 program (ZEISS). RNA quantification was carried out according to the method outlined by Schmidt and Tannhäuser, and additional measurements were made using dual-wavelength UV spectrophotometry. The activity of the G-6 phase was assessed using the Swenson method with modifications, including certain conditions (including glucose-6-phosphate substrate at a concentration of 50 μM and 0.1 ml of a suspension of microsomal fraction per sample) in a 0.25 m sucrose solution in a Tris-HCl buffer medium pH 7.4. Enzyme activity was quantified in micromoles of inorganic phosphate (pH) for each 1 mg of specialized microsomal protein.

Statistical analysis of the data was carried out using the standard “Statistics” program adapted to Microsoft Excel 2000 SPSS 10.0.5 for Windows 10.

3 Research results

The 29.2% in the atypical form and 24.5% in the typical form of the total number of cases. In sheep with results of the study showed that the incidence of eclampsia in sheep in the last stages of gestation was eclampsia, a decrease in the size of cotyledons (fetal part of thickness and volume) was observed.

From the data in Table 1 it follows that the difference in the weight of caruncles with fetal membranes was 0.768 kg in sick ewes, compared with clinically healthy ewes (p<0.05).
Table 1. Organometric indicators of the placenta and the amount of amniotic fluid in sheep in normal conditions and when pregnancy is complicated by eclampsia

<table>
<thead>
<tr>
<th>Expected date before lambing days</th>
<th>Diagnosis</th>
<th>Weight of placenta on the day of extraction, kg</th>
<th>Dimensions of the umbilical cord, cm</th>
<th>Amount of fetal fluid, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>thirty</td>
<td>Atypical eclampsia</td>
<td>1.432±0.02</td>
<td>18.93±0.23*</td>
<td>832.5±2.05*</td>
</tr>
<tr>
<td></td>
<td>Typical eclampsia</td>
<td>1.379±0.04*</td>
<td>19.68±0.17**</td>
<td>798.6±5.15*</td>
</tr>
<tr>
<td></td>
<td>Healthy animals</td>
<td>1.482±0.02</td>
<td>17.33±0.14</td>
<td>932.5±2.15</td>
</tr>
<tr>
<td>15</td>
<td>Atypical eclampsia</td>
<td>1.732±0.02**</td>
<td>21.33±0.13*</td>
<td>937.5±2.75*</td>
</tr>
<tr>
<td></td>
<td>Typical eclampsia</td>
<td>1.699±0.06**</td>
<td>23.8±0.19*</td>
<td>918.7±3.56*</td>
</tr>
<tr>
<td></td>
<td>Healthy animals</td>
<td>1.982±0.02</td>
<td>17.93±0.21</td>
<td>1097.5±2.14</td>
</tr>
<tr>
<td>5</td>
<td>Atypical eclampsia</td>
<td>1.792±0.12*</td>
<td>23.23±0.13*</td>
<td>987.5±1.15*</td>
</tr>
<tr>
<td></td>
<td>Typical eclampsia</td>
<td>1.697±0.21*</td>
<td>24.52±0.32*</td>
<td>952.2±2.05*</td>
</tr>
<tr>
<td></td>
<td>Healthy animals</td>
<td>2.062±0.12</td>
<td>21.23±0.13</td>
<td>1197.5±1.32</td>
</tr>
</tbody>
</table>

Note: *p<0.05; **p<0.02; *** p<0.001, hereinafter

Before lambing, the weight of the placenta in sick sheep was 1.732±0.03 kg, and in healthy sheep it was 1.982±0.02 kg (p<0.01). 5 days before the expected lambing, the difference in the weight of the placenta increased by an average of 270 g (p<0.05). One can note a noticeable delay in placental mass in relation to fetal growth, a decrease in the functionally active area of the chorion and a decrease in the capacity of the interstitial space. The length of the umbilical cord in sheep with eclampsia was significantly longer, ranging from 2.6 to 3.4 cm. A similar thing was observed with regard to the amount of collected amniotic fluid, the volume of which was smaller - from 100 to 200 ml. With eclampsia, the average size was 17.3x14.6 cm, with a thickness in the central sections of 2.42 cm, and in the marginal sections - 1.06 cm. In 2/3 of the sheep, infarctions and foci of calcification were found in the placenta, which were most often small in size and were located mainly on the periphery.

With complications of pregnancy in sheep, we observed changes in the morphometric parameters of the fetal part of the placenta, which are presented in Table 2.

Table 2. Morphometric indicators of the fetal part of the placenta of ewes.

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Complication of pregnancy</th>
<th>Healthy ewes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Typical eclampsia</td>
<td>Atypical eclampsia</td>
</tr>
<tr>
<td>Number of placentomas, pcs.</td>
<td>61.02 ±1.32*</td>
<td>77.04 ±1.43*</td>
</tr>
<tr>
<td>Cotyledon area, cm2</td>
<td>3.73 ±0.83**</td>
<td>5.13 ±0.32</td>
</tr>
<tr>
<td>Fetal-placental ratio</td>
<td>23.02</td>
<td>18.62</td>
</tr>
</tbody>
</table>

Analysis of the data in Table 2 shows that in animals suffering from eclampsia, there was a decrease in the area of the fetal part of the placenta (53.73±0.83 cm2) with a significance of p<0.01. As a result of the experiment, it was found that the average number (placentomas) of caruncles and cotyledons in the typical form of eclampsia was reduced by an average of 18.94 pieces (p<0.05), and in the atypical form by 9.45 pieces (p<0.05), in comparison with the physiological course of pregnancy, where the average number of caruncles was 83.83±1.23 pieces. A significant distance
between cotyledons was also observed (5.38) and differed from healthy ewes (83.83±1.23 and 77.04±0.32 cm²; 3.73±0.63 cm and 4.36±0.62 cm, respectively). In 53.63% of cases, small foci of calcification were found in the tissues of the placenta, often located along the periphery, with an increased number of small terminal villi tightly surrounding the stem villi, next to the chorionic plate.

The placental-fetal coefficient was 0.155±0.001 in sheep with eclampsia, and in healthy sheep it was 0.122±0.003. Micro-morphometric studies showed that the average thickness of the integumentary epithelium of the caruncles in ewes with a typical form of eclampsia was 14.3±1.7 µm (p<0.05), and in sheep with an atypical form - 15.5±1.9 µm (p<0.05), in comparison with physiological indicators of the course of gestation - 27.9±1.9 µm. The composition and specific surface area of villous stroma, villous vessels, interstitial space, avascular villi, and fibrinoid are constantly increasing. Changes in the structure of the fetal membranes are manifested by diffuse fusion of the connective tissue components of the amnion and chorion with noticeable hydration. The cumulative thickness of the connective tissue layer between the amnion and chorion decreases to 30-40 µm (from 50-80 µm in normal pregnancy), indicating a decrease in fiber folding and representing a form of tissue aging. Cotyledons were formed from villi emanating from the chorioallantoic membrane, the stromal part of which, in the case of eclampsia, consisted of a collagen framework. In more than half of the pregnant sheep with eclampsia, unformed chorionic villi were found in certain areas of the placenta, where Kashchenko-Hoffbauer cells were present in the edematous stroma.

The stromal part of the allantoic membrane and the membrane of the allantoic chorion is represented by a dense network of collagen fibers of various diameters, with fibroblasts, as shown in Figure 1. The largest number of syncytial nodes was noted in the placenta of ewes suffering from a typical form of eclampsia, amounting to 28.9%. This figure decreases significantly to 10.2% in ewes with an atypical form of eclampsia. In ewes with a typical form of eclampsia, a higher number of microthrombi was observed - 2.8 p/z compared to 0.8 p/z in clinically healthy animals. The area of microinfarctions and necrosis in the placenta of ewes with eclampsia was significantly higher and amounted to 1.5 times. The capillary basement membrane had uneven thickness, showing areas lacking structure. Inside the endothelial cells, a light composition containing single pinocytic vesicles is observed. The presence of mitochondria is limited and the matrix is swollen and pale, while the caruncle crypts are minimally visible. Chromatin appears rough, forming clumps along the inner surface of the nuclear envelope. The vascular walls of the villi have thickening, sclerosis and signs of hyalinosis, as well as cases of fibrinoid necrosis in certain areas. The lumens of the vessels narrow, close and are localized by thrombotic masses.

![Fig. 1](image1.png)  ![Fig. 2](image2.png)

**Fig. 1.** Longitudinal section of a sheep placentome: 1) normal - stained with hematoxylin-eosin. Total increase 100; 2) for eclampsia - trichrome staining according to Masson Total increase 100.

When studying endothelial cells, it was found that the vessels in the central part of the supporting and intermediate villi have limited luminal spaces. The terminal villi had numerous narrow capillaries located mainly in the stromal core. The absence of syncytial capillary membranes was noted, since the capillaries adjacent to the epithelium were shielded by a layer of mesenchymal cells. In addition,
narrow openings were noticeable in the supporting villi of the second and third order, indicating the onset of obliterating angiopathy. The syncytiotrophoblast appears reduced in thickness, accompanied by numerous exposed villi and nonfunctional syncytial formations. In the cytoplasm of the decidual tissue, the stroma of the middle stem villi and near the blood vessels, small glycogen granules were noted, visually presented in Figure 2.

![Fig. 2. Cross section of a sheep lacentome](image)

1) normal (Combined Alcian blue staining and McManus PIC reaction. Co-staining with Mayer’s hematoxylin. Total magnification 200). 2) for eclampsia (Staining with hematoxylin-osin). Total increase: 100.

Membranes beneath the endothelial and epithelial layers show thickening, deformation, fragmentation in localized areas and exhibit a highly positive PIC response. Significant amounts of neutral and depolymerized mucopolysaccharides were found in the hardened stroma of the villi and the walls of blood vessels. Eclampsia is characterized by an increased level of glycoproteins, which is primarily associated with a significant accumulation of fibrin, as well as a decrease in glycogen content in these structures. In the syncytiun there is a decrease in the content of RNA, carboxyl groups and, to a lesser extent, sulphydryl groups. A heterogeneous distribution of ribose-type nucleic acids is observed in the syncytial layer. The presented data, as shown in Figure 3, indicate that G-6-Phase activity during physiological pregnancy is $0.31 \pm 0.07 \mu\text{mol RC per 1 mg}$ of microsomal protein. At the same time, there is a significant decrease in G-6-Phase activity in the tissues of the ovine placentome during eclampsia ($0.15 \mu\text{mol RC per 1 mg}$ of microsomal protein), which is apparently due to changes in the structure of microsomal membranes.

![Uncomplicated Pregnancy vs Complicated Pregnancy Eclampsia](chart)
Moreover, in 75% of cases, all RNA is concentrated precisely in the microsomal fraction, while in mitochondria only in 18% of cases, while in the nuclear fraction only in 7% of cases.

4 Conclusion

The results of morphological studies showed that the surface of the uterine part of the placenta has a cellular structure. In the typical form of eclampsia, the number of caruncles averaged 47.34±1.12 (p<0.05), and in the atypical form - 56.78±2.15, in comparison with the physiological course of pregnancy, where the average was 76.23 ±1.43. The average thickness of the integumentary epithelium of the caruncles in ewes with a typical form of eclampsia was 14.3±1.7 microns, and with an atypical form - 15.5±1.9 microns, in comparison with the physiological course of gestation, where the thickness was 27.9±1.9 microns. In sheep suffering from eclampsia, there was a decrease in the total mass of the placenta, the size of its cotyledons (the fetal part of the thickness and volume), as well as an increase in the length of the umbilical cord. In the tissues of the cotyledons, small foci of calcification were identified, an increased number of small villi, which were densely grouped around the stem villi, and unformed chorionic villi were found in certain areas of the placenta in more than half of the ewes with pregnancy complicated by eclampsia. Changes in the structure of the placenta in ewes in the last stages of gestation with complications of pregnancy eclampsia have distinctive features characterized by the formation of a chorioallantoic organization with limited development of the villi of the cotyledons of the fetal membranes and certain areas of the uterine caruncles, which apparently is an adaptive response to the increased need of the fetus for nutrients and energy to complete intrauterine development.

Reference