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Volumetric proportions of the goat placenta structural components throughout gestation*

Proporção volumétrica dos componentes estruturais da placenta caprina ao longo da gestação

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SUMMARY

The goal of this research was to determine the volumetric proportions of the structural components of the goat placenta throughout gestation. We used nine goats of nondefined breed, separated into groups A, B and C, with 90, 120 and 150 days of gestation, respectively. From each animal fragments of three placentomes were obtained, one removed from the base, another from the middle part and the other from the end of the gestational uterine horn. The fragments were fixed in Bouin solution and histologically processed according to the standard techniques and stained with hematoxilin-eosin. Microscopically, a 25-point circular grid (Zeiss KPL ocular 6.3x with 25 points) was randomly used across the placentome to determine the volumetric proportion of the maternal and fetal connective tissue, syncytium, trophoblastic epithelium and binucleate cells. An increase of volumetric proportion of the syncytium was found at the end of gestation and also a decrease of volumetric proportion of fetal connective tissue in the same period.

UNITERMS: Placenta; Morphology; Goat.

INTRODUCTION

The caprine placenta, characterized by the development of restricted areas of interdigitation between fetal and maternal tissues known as placentomes, is classified as cotiledonary[3].

Histologically the placentome is composed of the caruncular (maternal) tissue with its cripts filled with ramified projections of the chorion known as chorionic villi[2]. In the placentome, the maternal blood circulation is separated from the fetal circulation by six tissue layers: maternal vascular endothelium, maternal connective tissue, syncytium, thophoblastic epithelium, fetal mesenchyma and fetal vascular endothelium, resulting that the caprine placenta is also classified as epitheliochorial[3]. Wooding[18] has proposed that the ruminant placenta be classified as synepheliocorial due to the occurrence of migration of fetal cells through maternal-fetal junction and fusion of these cells with the maternal epithelial cells.

The maternal epithelium of the caruncular cripts persists throughout gestation as a syncytium, which is the result of binucleate corionic cells fusion[18,19]. The trophoblastic epithelium is composed by columnar epithelial intermixed with binucleate chorionic cells, wich migrate through maternal-fetal interface and fuse with the syncytial cells[18]. The binucleate cells correspond to 15 to 20 per cent of the epithelial trophoblastic cells and approximately 1/7 of these cells are seen migrating to the caruncular maternal tissue[19].

Although the ruminant placentome has been a subject for studies by many authors[3,8,18], and Stegeman[15] had studied some morfometric parameters and their relation to fetal development in sheep, there is no histometric evaluation of the main histological components of the caprine placentome, and no information about changes in its structural components throughout gestation. Moreover, the maturation process of the caprine placenta, including morphological changes at the end of gestation, is not well-known. In other ruminant species, mainly in bovine, it is known that many structural changes occur in the placentome at the end of gestation and these changes are related to the normal delivery of the placenta, including decrease in the number of binucleate cells[16] and in the number of epithelial cells in the caruncular cripts[1,11].

Stegeman[15] showed that the average ratio between maternal and fetal tissues in the ovine placentome ranges from 0.4432 to 0.7293, and that the fetal connective tissue decreases, even with the increase in the degree of branching in the corionic villi throughout gestation. Also, the degree of vascularization of the corionic villi increases after the 10th week of gestation.

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The goal of this study was to quantify, by morphometric method, the main structural components of the caprine placentome, from different regions of the uterus, and to evaluate its structural changes throughout gestation.

MATERIAL AND METHOD

The study was carried out in the Veterinary College of Federal University of Minas Gerais. Nine adult goats of unspecified breed were used, and the animals were submitted to controlled mating and underwent gestation diagnosis by x-ray 80 days after mating. The animals were confined and given water *ad libitum* and fed hay, grass and a standard concentrated ration.

The goats were separated into groups A, B and C, based on 90, 120 and 150 days of gestation, respectively. Each group was composed of three goats. Three placentomes were obtained from each animal of all groups and fragments were taken by sagital section of the placentomes for histological purposes. The first placentome was removed from the base, the second from the middle part and the third from the end of the gestational uterine horn. All of the goats had twins. The animals from groups A and B were euthanized, while those from Group C were submitted to caesarean section to obtain the placentomes. The fragments taken were fixed in Bouin solution for 24 hours, processed histologically according to the usual parafin inclusion techniques, and five micrometer sections were cut perpendicular to endometrial surface and stained with hematoxilin-eosin[10].

A light microscope with 250x magnification, with a 25-point ocular (Zeiss KPL ocular 6.3x with 25 points) was used to evaluate the slides. Only one slide of each placentome was used. Microscopically, a 25-point square array grid was superimposed across 40 microscopic fields randomly selected, corresponding to 1,000 points per slide. The number of times in which the points were superimposed on a determined structure was counted to establish volumetric proportion of this component. Volumetric proportion was calculated as percentage according to the following formula[6]:

$$\text{Volumetric proportion} = \frac{\text{points per parameter}}{\text{total points counted}} \times 100$$

The results were taken as percentage corresponding to volumetric proportion of maternal connective tissue (including cells, fibers and blood vessels), syncytium, fetal connective tissue (including cells, fibers and blood vessels), trophoblastic epithelium and binucleate cells. The number of microscopic fields to be analysed in each slide was determined in order to obtain a confidence interval of 95% with ± 10% of variation of mean volumetric proportion of a tissue component with a proportion of about 0.30, using the formula[14]:

$L = 2\,[p(1-p)/n]^{0.5}$;

where: p = proportion;

n = number of points;

L = limit (confidence interval at 95% of probability).

When ocular points fell on technical artifacts or on retraction areas between maternal and fetal tissues, they were considered only for the evaluation of the histotechnical quality of the slides, but not for the computation of volumetric proportions.

The *split-plot* experimental design was used and after angular transformation, the data were submitted to analysis of variance[14].

RESULTS

The histological picture of the placentomes at 90, 120 and 150 days of gestation is illustrated in the Fig. 1 to 3. The maternal tissue as a whole showed a tendency to an increase in its volumetric proportion while the fetal tissue showed a tendency to a decrease after the 120th day of gestation, so the ratio between the maternal and fetal tissue also showed a tendency to increase, which was significant only for the placentomes of the middle part of the uterine horn at 150 days of gestation ($p<0.05$). The ratio between maternal and fetal tissues (the average number of points counted in the maternal tissue divided by the average number of points counted in the fetal tissue) ranged from 0.8841 at 90 days of gestation to 1,1246 at 150 days of gestation.

The volumetric proportion of maternal connective tissue did not show any significant difference between groups and uterine regions (Tab. 1). The volumetric proportion of the syncytium was similar for all uterine regions at 90 days of gestation, but at 120th day, for the syncytium, it was greater at the end of the gestational uterine horn than in its base. At the end of the gestation (150 days) there was no significant difference in the volumetric proportion of the syncytium between placentomes from different regions of the gestational uterine horn, and all of them showed an increase in volumetric proportion of the syncytium at 150 days of gestation (Tab. 2).

The volumetric proportion of the fetal connective tissue of placentomes from different regions of the gestational uterine horn was different only at 90 days of gestation, when this proportion at the middle part was greater than that at the end of gestational uterine horn. The volumetric proportion of the fetal connective tissue also showed a decrease for all uterine positions at 150 days of gestation (Tab. 3).

The volumetric proportion of the trophoblastic epithelium was greater only at the end of the gestational uterine horn than in its middle part (Tab. 4).

The volumetric proportion of binucleate cells was greater for the middle part than at the base of gestational uterine horn, and there was no significant difference between groups for the volumetric proportion of the binucleate cells (Tab. 5).

Table 1
Volumetric proportion of maternal connective tissue in caprine placentomes located at the base, middle part and end of gestational uterine horn at 90, 120 and 150 days of gestation.

Stage of gestation	Volumetric proportion (%) in different regions of the uterus		
	Base	Middle part	End
90 days	40.85 ± 1.79	37.19 ± 1.80	37.97 ± 3.85
120 days	42.44 ± 1.82	40.94 ± 1.04	40.72 ± 1.25
150 days	38.91 ± 4.74	40.67 ± 6.21	37.77 ± 2.66

There is no significant difference ($p>0.05$).

Table 2

Volumetric proportion of syncytium in caprine placentomes located at the base, middle part and end of gestational uterine horn, at 90, 120 and 150 days of gestation.

Stage of gestation	Volumetric proportion (%) in different regions of the uterus		
	Base	Middle part	End
90 days	9.33 ± 0.82^{a*}	9.70 ± 0.57^{a*}	9.18 ± 0.44^{a*}
120 days	8.55 ± 0.45^{a*}	9.86 ± 1.00^{ab*}	10.34 ± 1.04^{b*†}
150 days	10.69 ± 2.87^{a†}	11.99 ± 1.86^{a†}	11.58 ± 1.67^{a†}

^{ab} Different letters in the same row indicate significant difference ($p<0.05$);
*† Different symbols in the same column indicate significant difference ($p<0.05$).

Table 3

Volumetric proportion of fetal connective tissue in caprine placentomes located at the base, middle part and end of gestational uterine horn at 90, 120 and 150 days of gestation.

Stage of gestation	Volumetric proportion (%) in different regions of the uterus		
	Base	Middle part	End
90 days	12.96 ± 4.25$^{a\ b*}$	16.62 ± 2.85^{a*}	12.66 ± 2.84^{b*}
120 days	12.29 ± 1.95^{a*†}	12.02 ± 1.09^{a*}	10.99 ± 0.77^{a*}
150 days	7.09 ± 3.14^{a†}	6.45 ± 2.57^{a†}	6.01 ± 2.36^{a†}

^{ab} Different letters in the same row indicate significant difference ($p<0.05$);
*† Different symbols in the same column indicate significant difference ($p<0.05$).

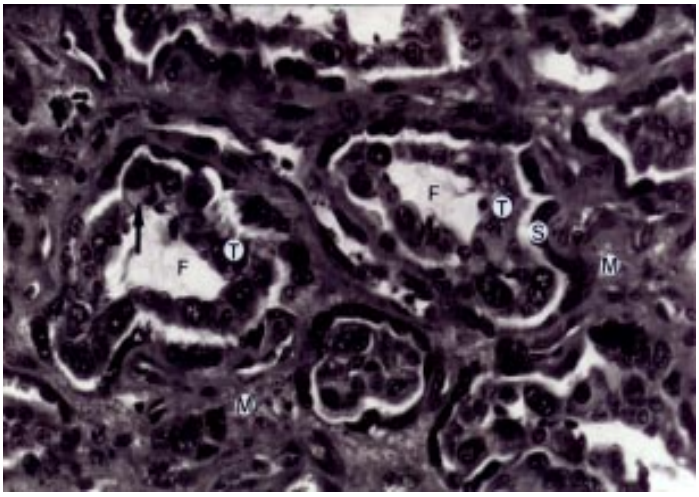

Figure 1

Histological aspect of goat placentomes at 90 days of gestation: maternal connective tissue (M), syncytium (S), fetal connective tissue (F), trophoblastic epithelium (T) and binucleate cells (arrow).

Table 4

Volumetric proportion of trophoblastic epithelium in caprine placentomes located at the base, middle part and end of gestational uterine horn at 90, 120 and 150 days of gestation.

Stage of gestation	Volumetric proportion (%) in different regions of the uterus		
	Base	Middle part	End
90 days	34.86 ± 3.57^{ab}	34.07 ± 4.34^{a}	38.31 ± 7.20^{b}
120 days	35.32 ± 1.52^{a}	35.86 ± 2.68^{a}	36.58 ± 0.97^{a}
150 days	41.62 ± 0.86^{a}	39.47 ± 1.73^{a}	41.42 ± 2.38^{a}

^{ab} Different letters in the same row indicate significant difference ($p<0.05$);
There is no significant difference between stages of gestation ($p>0.05$).

Table 5

Volumetric proportion of binucleate cells in caprine placentomes located at the base, middle part and end of gestational uterine horn at 90, 120 and 150 days of gestation.

Stage of gestation	Volumetric proportion (%) in different regions of the uterus		
	Base	Middle part	End
90 days	1.27 ± 0.49^{a}	2.37 ± 1.13^{b}	1.83 ± 0.80^{ab}
120 days	1.37 ± 0.70^{a}	1.27 ± 0.43^{a}	1.34 ± 0.34^{a}
150 days	1.65 ± 0.09^{a}	1.39 ± 0.33^{a}	1.18 ± 0.06^{a}

^{ab} Different letters in the same row indicate significant difference ($p<0.05$);
There is no significant difference between stages of gestation ($p>0.05$).

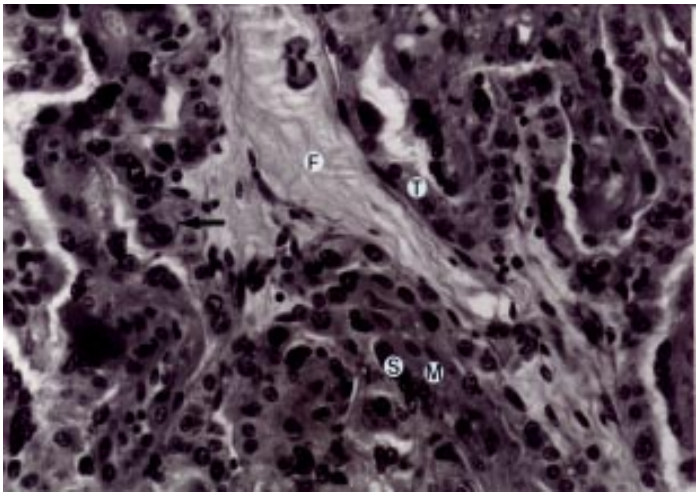

Figure 2

Histological aspect of goat placentomes at 120 days of gestation: maternal connective tissue (M), syncytium (S), fetal connective tissue (F), trophoblastic epithelium (T) and binucleate cells (arrow).

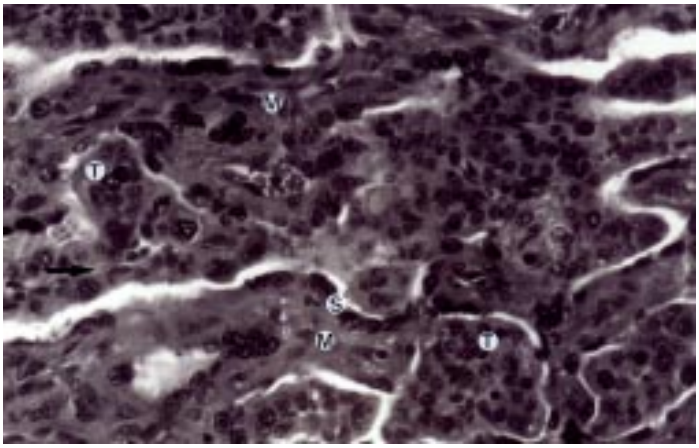

Figure 3
Histological aspect of goat placentomes at 150 days of gestation: maternal connective tissue (M), syncytium (S), fetal connective tissue (arrow) and trophoblastic epithelium (T).

DISCUSSION

The ratio between maternal and fetal tissue observed in this study was different from the one reported by Stegeman[15] in the ovine placenta. Apparently, the ratio of maternal tissue in the ovine placentome is lower than the one in the caprine placentome, nevertheless in the ovine there is also a tendency to an increase in the ratio of maternal tissue at the end of gestation[15]. Likewise it occurs in the caprine placenta according to our results.

The maternal connective tissue was stable during gestation and did not show any change in its volumetric proportion for the different regions of the uterus. It is important to note that this *stability* occurs after the 90th day of gestation. If the study had been made in earlier periods of gestation, before the 90th day, it would possibly show different results due to the morphological changes that occur in the beginning of the placentation process[3,9].

The fetal connective tissue, the trophoblastic epithelium and the binucleate cells showed some variation in their volumetric proportions for different regions of the uterus at the 90th day of gestation, with interaction between stage of gestation and uterine region at the 90th day of gestation. Probably this difference of volumetric proportion in the fetal components of placentome from different regions of the uterus is related to the difference of maturity between the placentomes of different regions. It was found that some placentomes develop earlier than others[8] and are bigger during the whole gestation, with a tendency to a higher development of the bovine placentomes located in the middle part of uterine gestational horn[1]. Yet, the variation observed in the volumetric proportion of the syncytium in different regions in the uterus at 120 days of gestation could not be explained based on the available information discussed above.

The syncytium showed an increase in volumetric proportion at the 150th day of gestation, possibly due to the migration of binucleate cells, which migrate continuously during gestation from the trophoblastic epithelium through the maternal-fetal junction to the syncytium which lines the maternal connective tissue[18]. In bovine there is no formation of syncytium and the number of epithelial cells of the caruncular cripts decreases at the end of gestation, what is important to the normal delivery of the placenta after fetal expulsion[7,11].

According to Wooding[19] the percentage of binucleate cells in the trophoblastic epithelium tends to decrease at the end of gestation in caprine. In the present study no significant difference was found for the volumetric proportion of those cells at different stages of gestation. It is important to consider that the method of morphometric analysis used in the present study is not the best one to evaluate the population of binucleate cells, because that corresponds to a low percentage of the whole tissue so the confidence interval of the percentage is very wide. Thus, a reliable evaluation of this component would demand the counting of a higher number of points than that counted in this study. In bovines the binucleate cells decrease in number at the end of gestation and this process is important to the normal delivery of the placenta[16], but in caprine the role of these cells in maturation and delivery of placenta is yet unknown.

The fetal connective tissue showed a significant decrease in volumetric proportion at 150th day of gestation in all uterine regions studied. Wimsatt[17] in his histological description of ovine placentome reported that the fetal mesenquima initially increases its mass and at the end of gestation becomes thinner. Although this observation was made long ago, the quantification of this tissue has not been done until the present time and this is the first quantitative determination of decrease in the fetal connective tissue in caprine at the end of gestation. Sharpe *et al.*[12] evaluated the volumetric proportion of maternal and fetal collagen in bovine placentomes and demonstrated that there is a raise in the volumetric proportion of fetal collagen between 90th to 270th days of gestation and decrease of volumetric proportion of maternal collagen during the same period. Probably the volumetric proportion of collagen has a high and positive correlation with the volumetric proportion of the connective tissue, since the collagen is its main extra cellular component. Yet, Sharpe *et al.*[12] did not find any difference in the volumetric proportion of collagen in cows with normal delivery and with placental retention. However, there are evidences that the predominant type of collagen in the placentome of cows with placental retention is the type III[13] and that the collagenase is important in the delivery process of the fetal portion of the bovine placenta[4,5]. In goats, the mechanisms responsible for the decrease of volumetric proportion of connective tissue and possibly for the collagen are unknown; however, we can speculate that this structural change has some importance in placental maturation and delivery in this species. Stegeman[15] observed that the decrease in the mesenquimal mass in the ovine corionic villi was associated with the branching of the villi, which occurs steeply from 9th to 16th week of gestation. He also reported that there is an increase in the degree of vascularization of the villi throughout gestation, partly caused by the disappearance of the thin connective tissue.

Based on our findings and on the available information we can suggest that the structural changes that occur in the caprine placenta are different from those found in the bovine.

In conclusion, there is an increase in the volumetric proportion of the syncytium in the caprine placentome at the end of gestation and a decrease in it for the fetal connective tissue in the same period, and probably this decrease is related to the delivery of the placenta in this species.

RESUMO

O objetivo deste estudo foi determinar as proporções volumétricas dos componentes estruturais da placenta caprina ao longo da gestação. Foram utilizadas nove cabras adultas sem raça definida, separadas em grupos A, B e C, com 90, 120 e 150 dias de gestação, respectivamente. De cada animal foram colhidos fragmentos de três placentomas, sendo um localizado na base do corno uterino, outro no terço médio e o último da extremidade. Os fragmentos foram fixados em solução de Bouin, processados segundo as técnicas rotineiras de inclusão em parafina e corados pela hematoxilina e eosina. Microscopicamente, uma ocular integradora (Ocular Zeiss KPL 6,3x com 25 pontos) foi aleatoriamente sobreposta sobre a secção do placentoma para a determinação da proporção volumétrica do tecido conjuntivo materno e fetal, do sincício, do epitélio trofoblástico e das células binucleadas. Foi observado aumento na proporção volumétrica do sincício ao final da gestação e diminuição do tecido conjuntivo fetal no mesmo período.

UNITERMOS: Placenta; Morfologia; Caprinos.

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