

# Morphometric analysis of collagen in placentomes of dairy cows with normal delivery and with placental retention

Análise morfométrica do colágeno no placentoma de vacas leiteiras com liberação normal e com retenção de placentas

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## SUMMARY

The goal of this study was to quantify, by morphometric method, the collagen of maternal and fetal portions of dairy cows placentomes with normal delivery and with placental retention. Twelve cows were used and a placentome was obtained from each one soon after fetal expulsion. Eight cows showed normal placental delivery (Group 1) and four cows showed placental retention (Group 2). From each placentome a fragment was obtained, histologically processed and stained by Masson's Trichromic. Volumetric proportions of maternal and fetal collagen were determined with a 25 point ocular (Zeiss KPL ocular 10x with 25 points). There was no significant difference between groups ( $p > 0.05$ ) in volumetric proportion of maternal collagen ( $34.57 \pm 5.47$  and  $32.04 \pm 5.82$ , respectively) and in volumetric proportion of fetal collagen ( $9.85 \pm 2.04$  and  $10.73 \pm 2.47$ , respectively).

UNITERMS: Placental retention; Collagen; Puerperium; Placenta; Bovine

## INTRODUCTION

Placental retention is one of the most frequent puerperal disorders in dairy cows, and its etiology and pathogenesis is complex and not completely known<sup>4</sup>.

Some evidences have appeared recently on the action of the collagenase enzyme in the process of separation of maternal and fetal tissues in the bovine placentome at parturition<sup>1,2</sup>. However, no difference was demonstrated in the amount of collagen in the placentome of cows with normal delivery and in those with placental retention<sup>6</sup>, but Sharpe *et al.*<sup>7</sup> (1990), observed that collagen type III predominates in the placentome of cows with placental retention.

The evidences in the literature led to the goal of this study, that was to quantify, by morphometric method, the collagen of maternal and fetal portions of dairy cows placentomes with normal delivery and with placental retention.

## MATERIAL AND METHODS

This work was carried out in the Veterinary College of the Federal University of Minas Gerais and the samples were obtained at the São José Farm, located in Tapiratiba (São Paulo). Twelve cross-breed dairy cows with predominance of Holstein breed features were used.

After fetal expulsion, the time spent by each animal for release of the fetal membranes was determined, placental retention

being considered when the placenta remained attached for more than eight hours after fetal expulsion. Immediately after it, cows were submitted to a surgical procedure that made use of an ovariectomy via vagina for the obtention of a placentome, located in the caudal region of the uterus. Eight cows delivered the placenta in normal time and constituted Group 1, whereas four cows presented placental retention and constituted Group 2.

A fragment from each removed placentome was obtained by sagittal section. The fragments measured approximately 0,5 cm width and were fixed in Bouin solution for 24 hours. Then, they were histologically processed according to the usual paraffin inclusion techniques, and the slides were stained by Masson's Trichromic<sup>5</sup>.

A light microscope with 400x magnification, with a 25-point ocular (Zeiss KPL ocular 10x with 25 points) was used. Microscopically, a 25-point square array grid was repetitively superimposed across 80 microscopic fields randomly selected, corresponding to 2000 points per slide. By Masson's Trichromic the collagen is stained blue and other tissues components are stained red. Thus, all points superimposed on blue stained tissue components were counted, in order to estimate the volumetric proportion of maternal or fetal collagen according to its location. Volumetric proportion was calculated as percent using the following formula<sup>3</sup>:

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

The number of microscopic fields to be analysed in each slide was determined, in order to obtain a confidence interval of 95% with  $\pm 10\%$  of variation of mean volumetric proportion of a tissue component with a proportion about 0.15, using the following formula<sup>8</sup>:

$$L = 2 \sqrt{\frac{p(1-p)}{n}}$$

where: p = proportion  
n = number of points  
L = limit (confidence interval at 95% of probability)

When ocular points coincided with technic artifacts or with the retraction areas between maternal and fetal tissues, they were computed to validate histotechnic quality of the slide. For the computation of volumetric proportions, those ocular points were disregarded.

After angular transformation the data were submitted to analysis of variance, and the means were compared by Student's t-test<sup>8</sup>.

## RESULTS

The volumetric proportion of fetal collagen was  $9.85 \pm 2.04$  and  $10.73 \pm 2.47\%$  for groups 1 and 2, respectively. Maternal collagen presented the following volumetric proportions:  $34.57 \pm 5.47$  and  $32.04 \pm 5.82\%$  for groups 1 and 2, respectively. Volumetric proportion of collagen in fetal tissue, as much as in maternal tissue, did not show any significant difference ( $p > 0.05$ ) between groups (Tab. 1 and Fig. 1).

TABLE 1

Volumetric proportions of maternal and fetal collagen in placentomes of dairy cows (Group 1 - n=8: normal delivery and Group 2 - n=4: placental retention). Tapiratiba - SP. March and April, 1995.

	Group 1	Group 2
Maternal collagen (%) <sup>*</sup>	$34.57 \pm 5.47$	$32.04 \pm 5.82$
Fetal collagen (%) <sup>*</sup>	$9.85 \pm 2.04$	$10.73 \pm 2.47$

<sup>\*</sup> There is no significant difference between groups ( $p > 0.05$ )

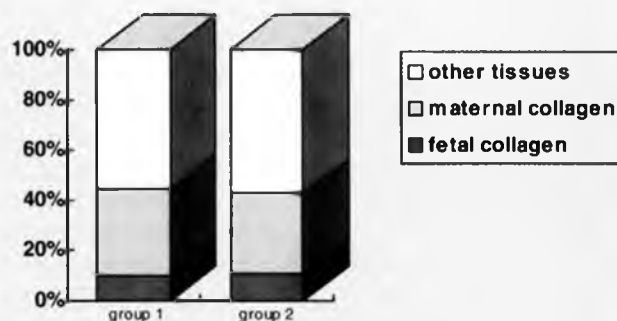


FIGURE 1

Volumetric proportions of maternal and fetal collagen in the placentome of dairy cows (Group 1 - n=8: normal delivery and Group 2 - n=4: placental retention). Tapiratiba - SP. March and April, 1995).

## DISCUSSION

Our results are similar to those obtained by Sharpe *et al.*<sup>6</sup> (1989), who did a morphometric analysis of maternal and fetal collagen in the placentome throughout gestation and during post-partum period of cows with normal delivery and with induced placental retention.

Sharpe *et al.*<sup>6</sup> (1989) did some chemical analyses (colorimetric assay for hydroxyproline and protein) to validate the morphometric results. Results of the chemical analysis were compatible with those of morphometric analysis that is, both of them showed similar tendencies. Those authors obtained a volumetric proportion of fetal collagen in placentomes collected 2 hours after fetal expulsion, which varied from 0.18 to 0.21, superior than the volumetric proportion obtained in this study. Also, the volumetric proportion of maternal collagen at the same time was 0.45 and it was also superior to the volumetric proportion of maternal collagen obtained in the present study. The divergence with our results can be explained by the use of a 100x magnification by Sharpe *et al.*<sup>6</sup> (1989), in contrast with a 400x magnification in this study, that gives better accuracy to identify the structures.

The results of this study indicate that there are no changes in the amount of collagen in the placentome in placental retention. However, it was demonstrated that in of placental retention the placentome shows a greater proportion of collagen type III<sup>7</sup>, which implies the action of collagenase in the placental separation process<sup>1</sup> and also justifies the experimental therapeutic results obtained with administration of collagenase through fetal blood vessels of umbilicus cord<sup>2</sup>.

## CONCLUSION

In conclusion, based on the conditions in wich this study was carried out, there are no differences in volumetric proportion of collagen in fetal and maternal tissues of cows with normal delivery and with placental retention.

## RESUMO

O objetivo deste estudo foi quantificar, através de método morfométrico, o colágeno da porção fetal e da porção materna do placentoma de vacas leiteiras com liberação normal e com retenção de placenta. Foram utilizadas 12 vacas, das quais foi colhido um placentoma, imediatamente após a expulsão fetal, sendo que oito apresentaram liberação normal da placenta e constituíram o Grupo 1 e quatro apresentaram retenção e constituíram o Grupo 2. De cada placentoma foi obtido um fragmento que foi processado histologicamente. As lâminas foram coradas pelo Tricrômico de Masson. As proporções volumétricas do colágeno materno e do colágeno fetal foram determinadas com o auxílio de uma ocular integradora (Ocular integradora Zeiss KPL 10x com 25 pontos). Não houve diferença significativa entre grupos 1 e 2 ( $p > 0,05$ ), tanto na proporção volumétrica do colágeno materno ( $34,57 \pm 5,47$  e  $32,04 \pm 5,82$ , respectivamente) quanto na proporção volumétrica do colágeno fetal ( $9,85 \pm 2,04$  e  $10,73 \pm 2,47$ , respectivamente).

UNITERMOS: Retenção de placenta; Colágeno; Puerpério; Placenta; Bovinos

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