From the Departments of Pathology, University of California, San Diego and the San Diego Zoo

6541

THE PLACENTA OF THE RHINOCEROTIDAE

By K. Benirschke and Linda J. Lowenstine

Introduction

It is rarely possible to examine the placenta of rhinoceroses except following the birth of offspring in Zoological Gardens. It is therefore not surprising that the only specimens described so far have come from the Basel Zoo through the efforts of Ernst Lang. Only the Indian rhinoceros' placenta has been fully studied, yet it would be of considerable interest to know more about the structure of the placenta of all five species of these animals. For one thing, the fact that this mammalian family is composed of four genera is astounding, and one may thus wonder whether this taxonomic oddity is reflected in significant placental differences. Further, if ever one may wish to practice embryo transfer for artificial rearing of a severely endangered form, the function of the placenta is critical and must be better understood. This knowledge must of course extend beyond the analysis of anatomical features, but they are a first beginning. This paper describes the placenta of three species of rhinoceros; it cannot be anticipated presently that Javan and Sumatran rhinoceros placentas will become available soon. The former is not in captivity and the latter animals have not bred thus far in zoos that hold them.

The placentas of the other four species of rhinoceros, other than that of the Indian rhinoceros, have heretofore not been fully described. It is further important to recall that MOSSMAN (1987) correctly stated in his extensive monograph on comparative placentation, that the rhinoceros placenta has been described only from delivered term pregnancies. None has yet been studied in utero, attached to the endometrium. It is important that the in situ placenta of rhinoceroses be observed so as to achieve answers to some critical questions posed in this paper. Mossman described the placenta of the Indian rhinoceros (*Rhinoceros unicomis*), the only studied organ at the time of his study, to be an "omphaloplacenta, i.e. a disc of bilaminar omphalopleure", and viewed it as being similar to that of the tapir. Previous authors quoted by Mossman had considered the organ to be intermediate between that of the horse and cattle.

LUDWIG (1962) was the first to describe the microscopic features of an African rhinoceros (*Diceros bicornis L.*) and considered it to be a "placenta villosa diffusa". It was composed of broad-based villous tufts with leaf-shaped branches. LUDWIG and MÜLLER (1965) studied the afterbirth of an Indian rhinoceros (*Rhinoceros unicornis L.*) from the Basel Zoo histochemically. Because of those areas that were found to be free of villi (the a-villous "streets"), they described this organ to be a "Placenta villosa diffusa incompleta". Perhaps the previous specimen of which Ludwig had been able to obtain only small samples had similar villus-free areas but they had not been submitted for study. Two different types of villi were described for the first time: "leaf-like villi" and "folded villi". The epithelium of the former was cuboidal to flat, and the chorionic plate was covered by a more cylindrical epithelium. Conversely, the folded villi were covered by tall cylindrical trophoblast. A variety of histochemical reactions were possible on this unusually well-preserved tissue. These authors also compared their findings with those in cattle placentas and found great similarities. They also identified occasional binucleate trophoblastic cells and sought similarities to the diplokaryocytes of cattle placentas.

In their description of the fine structure of the placenta of an Indian rhinoceros, LUDWIG and VILLIGER (1965) found that the epithelium of the leaf-like villi is identical to that of equidae. An exception were the "diplokaryoctes" which had many more mitochondria and endoplasmic reticulum. Many transport vesicles were found in the trophoblastic epithelium.

DOLINAR et al. (1965) observed the freshly delivered placenta of another Indian rhinoceros and were also able to obtain very fresh tissue for histology. It came from a term pregnancy with a 68 kg male infant. The placenta weighed 4,960 g, had an amnionic cavity of approximately 78 liter content, a 57.8 cm umbilical cord and resembled the organ described for tapir and black rhinoceros. The villus-free "streets" were well depicted as were the fibrous strands that connect allantois to chorion. These thin fibers also attach to and perhaps anchor the large muscular vessels that course within the space between allantois and chorion. Diplokaryocytes were

again noted and the tall epithelium of the villus-free chorion was likened to the focal scars of horse placentas that are believed to overlie the uterine glands and may absorb "uterine milk". Amnionic pearls or hippomanes were not identified.
Details concerning the weights and some reproductive and gestational features of the Indian rhinoceros can be found in LANG's paper (1967). He described the average weight of six neonates to be 65.6 kg, and the average gestational length as being 477 days.

Macroscopic Observations

At the Zoological Parks of San Diego and San Pasqual we have been able to collect the placentas from the following species:

Indian rhinoceros (*Rhinoceros unicornis L.*)	No. 16
Black rhinoceros (*Diceros bicornis L.*)	No. 6
Southern white rhinoceros (*Ceratotherium s. simum L.*)	No. 6

The available weights of neonates and placentas of these animals and those obtained from the literature are summarized in Table 1. The placenta of rhinoceroses is very thin and large, and it conforms roughly to the bicornuate uterus of the animal. The overall length reaches 230 cm in an Indian rhinoceros, with maximal circumference of up to 120 cm. One horn of the bicornuate placenta (Figs. 1,2) is considerably larger and contains the fetus than the other horn which is largely filled with membranes and fluid. The umbilical cord inserts nearly at the junction of the two sides, also near the site of rupture during delivery, the cervical outpouching of the sac. The fetus is contained within the amnionic sac whose membrane is intimately fused with the allantoic membrane (Fig.1). The reflected allantois, in turn, is fused with the chorionic membrane on whose outside reside the trophoblastic epithelium and villi. The entire thickness of the placenta is never greater than 3 mm, and often it is thinner. This is especially true in the larger streets ("Strassen") of avillous membranes which were described especially well by DOLINAR et al. (1965). When we witnessed the delivery of an Indian rhinoceros at night, the dam lay on her side and the amnionic sac containing the fetus slid out slowly and without great maternal effort. The cord ruptured spontaneously; thereafter the dam stood up, exhibited Flehmen, and then turned to the amnion-enclosed fetus and proceeded to lick off the sac, thus freeing the neonate from its enclosure. It is for this reason that the amnionic sac often comes separately and that the placenta is delivered at a later time without amnion. It also explains the incomplete presence of umbilical cords in submitted specimens.

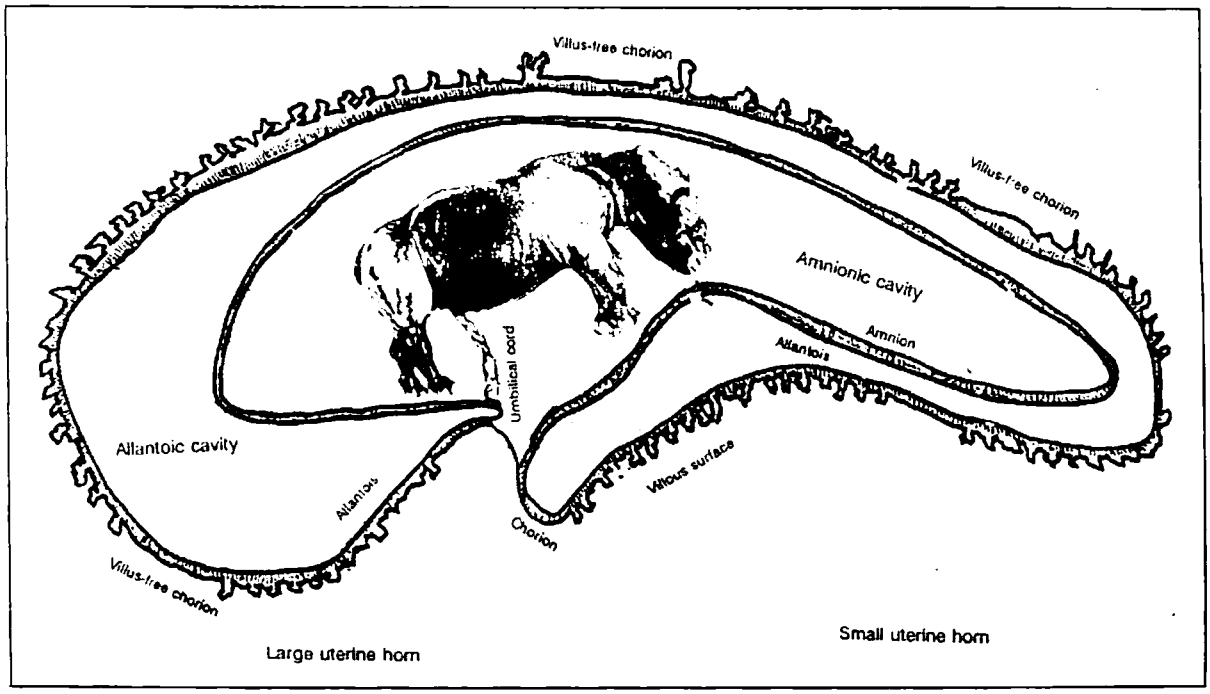

Fig. 1: Diagrammatic representation of a fetal rhinoceros in its placenta. Note the very minimal cord twist of this short cord of an actual specimen.

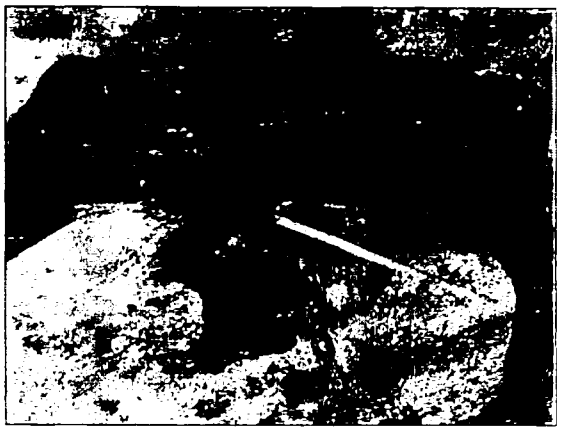

Fig. 2: Placenta of a Southern White Rhinoceros (7,600 g). The lesser uterine horn is to the right, the cervical portion is central and below. The remains of umbilical cord and shredded amnion are in the center. The overall length of this placenta was 192 cm; its largest width was 100 cm.

The umbilical cord severs spontaneously during the birth of rhinoceroses, most often near its fetal end. The length of most umbilical cords of the specimens described was not available to us, most of it remained on the neonate. It was also not measured as it was not made available and seemed of little importance. Only DOLINAR et al. (1965) gave an accurate measurement for the entire length of one umbilical cord of an Indian rhinoceros at term. It was 57.8 cm long. Aside from the three or four large allantoic vessels and the numerous tiny allantoic vessels, the umbilical cord contains a huge allantoic duct that connect dome of bladder with the allantoic cavity. In an attempt to assess aspects of placental efficacy, KLOOSTERMAN and HUIDEKOPER (1954) have calculated a "placental coefficient", the ratio of placental to fetal weight. They found for the human development that this ratio declined from 2 at 8 weeks gestation to 0.13-0.19 at term (40 weeks). The importance of this ratio is difficult to validate exactly and that is true even more so in these rhinoceros specimens. It lies at 0.089 in the Indian rhinoceros, at 0.115 in the Black rhinoceros, and in the White rhinoceros it is at 0.075. Because of some frequently contaminating dirt and because of the excessively large quantity of amnion present in these animals, this placental/fetal ratio is probably meaningless for our specimens. In other species, a fetal to cotyledonary weight ratio has been used to infer some functional capacity of the placenta, but even this has given rise to much inconclusive discussion (DIXON and ROBERTSON, 1969). Such figures that have been arrived at are probably of little use. Nevertheless, in order to provide as much information as possible, the weights available to us for fetus and placentas are listed in Table 1. They may be useful in future comparative studies.

In several species the amnion and/or allantois possesses "pustules", projections on the surface of the membranes or the umbilical cord. They may be very pronounced and were seen in only few of the rhinoceros placentas. One such case is illustrated in Figure 3, the allantoic surface of an Indian rhinoceros. There were about 20 such 0.5-2 cm, slightly pedunculated nodules located towards the end of the smaller horn. Their brown surfaces were smooth and the content was soft. Microscopically, they are epithelium-lined that has keratin production. Perhaps when these nodules are degenerating they produce part of the hippomanes. We were not impressed by a marked difference in the villous structures described (leaf-like or folded), either in their macroscopic or microscopic details.

Fig. 3: Allantoic surface of an Indian Rhinoceros placenta (3,600 g). Numerous round "pustules" are irregularly distributed near the end of the lesser horn.

Microscopic Observations

The placenta of rhinoceroses is an epithelial-chorial organ without invasion of the maternal tissues by the trophoblast. Nevertheless, in endometria available to us from post partum uteri it is evident that some endometrial hemorrhage must occur after delivery, since hemosiderin-laden macrophages are abundant in the endometrial stroma. We found no significant histological differences among the microscopic features of the three species of rhinoceros examined here. Villus-free areas of chorion (Figs. 1,4) were found in all our specimens. These are membranous areas that DOLINAR et al. (1965) speculated lie over endometrial glands and that may serve to absorb uterine milk. They likened this feature to the anatomy of the horse placenta, as depicted by AMOROSO (1952). In situ observations have not yet been made and will be of interest in the future. This is especially so as these areas in the horse placenta are much smaller and less abundant than in the rhinoceroses studied.

The simply branched villi (Fig. 4) contain few elements other than the fetal vessels, but there was occasional yellow-green pigment within the villous connective tissue whose origin is uncertain. Macrophages, similar to the Hofbauer cells of primate placentas, were not identified. The trophoblastic cover of the villi is usually tall columnar, with basal nuclei and prominent brush border (microvilli) toward the endometrium.

Fig. 4: Microscopic appearance of the placenta of a Southern White Rhinoceros. The allantoic sac (above) is loosely connected to the chorionic membrane© that carries the fetal vessels to the main stem villi and its branches (center). The chorionic membrane has villus-free areas ("streets") (S) lined by a tall columnar epithelium. (H&E x 100).

Occasional binucleate cells ("diplokaryocytes") are found but they were not especially prominent in any species. Therefore, we hesitate to reiterate the analogy to bovine placentas made in previous studies, especially so because only so few of these cells were found. Moreover, the villi of rhinoceros placentas are fairly uniformly distributed, excepting the villus-free streets, and they have no similarity to the cotyledonary structures of bovidae. We found no trophoblastic mitoses. In many areas the cylindrical epithelium becomes more cuboidal without there being any obvious special localization of these changes. There were a few remarkably enlarged vacuoles in some areas of the placenta of an Indian rhinoceros (Fig. 5). They extended into the superficial villous stroma and originated in the trophoblast of the placenta. Many vacuoles had an eosinophilic proteinaceous content and projected slightly on the epithelial surface and were found primarily in the non-villous chorionic "streets". The nature of these large vacuoles is uncertain, even though transport vesicles have been described electronmicroscopically (LUDWIG and VILLIGER, 1965). The vacuoles that we observed in this case were much larger than the usual transport vacuoles of trophoblast, and they were mostly confined to the superficial villous connective tissue but apparently originated from the trophoblastic cytoplasm. In no case were

the villous capillaries protruding into the trophoblastic cover, such as is described for human syncytiocapillary membranes. The vacuoles do not represent such a vascular adaptations as they never contained blood.

Fig. 5: Unusual area of villous trophoblast in an Indian Rhinoceros placenta. Large, empty vacuoles are present in trophoblast and subtrophoblastic villous connective tissue. They are largely empty and may represent unusual transport vesicles (H&E x 256).

Amnion and allantois are densely adherent in the thin sac that encloses the fetus (Fig. 6). The amnionic epithelium is flat and that of the allantois mostly cuboidal but it is also usually not very well preserved. An abundance of capillaries and larger vessels course in the allantoic connective tissue, none are present in the amnion, or they are stray from the allantois. Allantois and chorion are more loosely connected and, especially when pulled apart, they leave characteristic thin connective tissue strands (Fig. 7). The area in between these two sheets is also the position of the very muscular fetal placental surface vessels that carry blood from cord to the villous branches and that run in the chorionic membrane.

Fig. 6: Amnionic sac of an Indian Rhinoceros. The amnion with well-preserved epithelium is densely fused to the allantoic membrane that carries the allantoic vessels. The allantoic epithelium is usually poorly preserved (H&E x 160)

Fig. 7: Chorio-allantoic tissue of an Indian Rhinoceros placenta with large fetal blood vessel coursing within the chorionic membrane. The allantoic epithelium (A) is better preserved, and the attachment with numerous thin, elastic connective tissue bands (CTB) between allantois and chorion, including its vessels, is clearly evident. Villi are below. (H&E x 100).

Contrary to earlier observations, keratinized squamous metaplasia was found on the cord of an Indian rhinoceros, and typical squamous keratin "pearls" were observed in amnion, on the cord surface, and in allantoic areas of the placenta of a black rhinoceros (Fig. 8). They are the apparent equivalents of amnionic "pustules" described for so many animals (RAMSEY, 1975; BENIRSCHKE and CALLE, 1994). In contrast to the findings of earlier studies, we found several animals to possess large hippomanes, mostly in the narrow horn, and weighing up to 180 and 168 g, and measuring 14 x 6 cm (Table 1). They were olive-green or brownish and had an abundance of polarizing crystals within the mostly acellular debris. The crystals are presumably derived from urinary products. The centers of these protrusions were soft and they either attached to the allantoic surface or lay free.

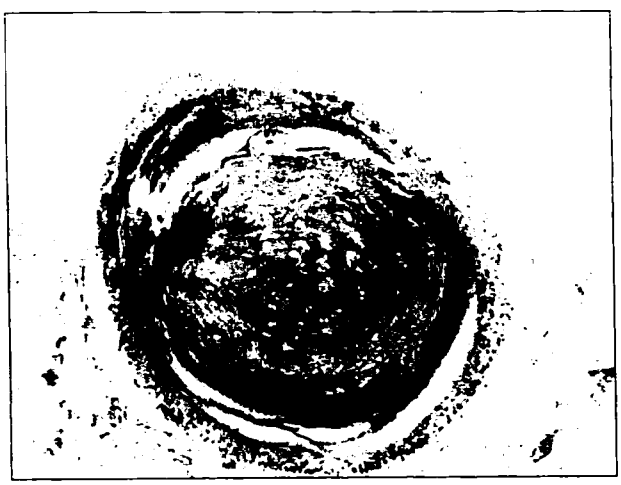

Fig. 8: Keratinized epithelial "pearl" or pustule within allantois of a black rhinoceros placenta. The allantoic epithelium is degenerated, but the fibrous strands to the chorion are readily seen at lower right (H&E x 100).

Table 1: Placentas of Rhinocerotidae: Weights of fetus and placentas in three species of rhinoceros.

Species	Fetal weight	Placental weight	Special Findings	Literature
Indian rhinoceros	55 kg	3,600 g	Amnion nodules	
	66.8 ♂	?	Hippomanes 168g	
	?	5,300 g		
	?	6,300 g		
	?	14,000 g	Hippomanes, amnion nodules	
	68 kg ♂	4,960 g	No hippomanes	DOLINAR et al. (1965)
	65 kg ♀	8,925 g	Term stillborn	
	68 kg ♂	(717 g amnion)	Term stillborn	
	76 kg ♂	3,680 g		
	10.82 kg ♂	2,875 g	Very premature	
	? ♂	1,211 g	Neonatal death	
	47 kg ♂	6,200 g		
	64.6 kg ♀	6,300 g		
	71.4 kg ♂	6,114 g	Amnion plaques	
	63.6 kg ♂	5,600 g		
	70.5 kg ♀	4,300 g		
Black rhinoceros	?	3,525 g	Keratin pearls	
	28.5 kg ♂	4,300 g	Stillborn	
	30.9 kg ♂	4,200 g		
	?	3,700 g		
	41 kg ♂	4,025 g		
	40 kg ♂	3,650 g	20 cm cord, many hippomanes	
White rhinoceros	?	4,200 g		
	?	6,800 g	Hippomanes	
	?	7,600 g		
	?	20,000 g		
	66.8 kg ♂	5,015 g		
	?	4,930 g		

Conclusions

The large rhinoceros placenta consists of a thin membrane of allantochorion with rather diffusely scattered villi Occasional villus-free regions are prominent findings of all species and are referred to as "streets". No significant differences were found in the structure of the placenta from these three species of rhinoceros examined, irrespective of their generic differences in classification. Before one can conclude that the placentas are sufficiently similar that inter-specific (-generic) transfer of embryos may be possible, more detailed biological studies are needed. For instance, no information on placental hormone production is yet available. While the rhinoceros fetal gonads resemble those of horse fetuses, it would be premature to deduce that rhinoceros placentas have similar endocrine activities to stimulate fetal tissues.

Summary

The placenta of the Rhinocerotidae

We describe the placentas of three rhinoceros species. In this review we studied sixteen placentas of the Indian rhinoceros, six specimens of the black rhinoceros, and six placentas of the Southern white rhinoceros. Generally speaking, these placentas are very similar although their size differs markedly. The rhinoceros placenta is similar in structure to that of horses, representing a diffuse epithelio-chorial type with large areas of villus-free regions, so-called "streets". These are covered by a simple columnar epithelium, as are the villi which have but few branches. In several cases hippomanes were found, not previously described in the few specimens examined heretofore. In addition, some placentas exhibited epithelial sprouts, "pustules" with keratinization. The possibility of inter-species embryo transfer among rhinoceros species is briefly discussed.

Zusammenfassung

Die Plazenta der Rhinocerotidae

Es werden die Nachgeburten von drei Nashornarten beschrieben. Es konnten sechzehn Plazenten vom Indischen Panzernashorn, sechs vom Spitzmaulnashorn, und sechs vom Breitmaulnashorn untersucht werden. Im allgemeinen sind die Plazenten dieser drei Nashornarten übereinstimmend, wenn auch in der Grösse unterschiedlich. Es handelt sich um eine dem Pferd ähnliche Nachgeburt des diffusen epithelio-chorialen Typus, mit Strassen von zottenfreien Arealen, und von einem einfachen Epithel überzogenen, wenig verzweigten Zotten. In verschiedenen Fällen fanden wir Hippomanes, was von den wenigen vorher beschriebenen Plazenten nicht bekannt war. Außerdem gab es in einigen Nachgeburten Epithelknospen, die als "pustules" bei anderen Tierarten verschiedentlich beschrieben wurden. Die Möglichkeit einer Interspecies-Embryo-übertragung wird diskutiert.

Resumé

Le placenta des rhinocérotidés

Les auteurs décrivent les placentas de trois espèces de rhinocérotidés. Ont été examinés seize placentas du rhinocéros unicorne des Indes, six placentas du rhinocéros noir et 6 du rhinocéros blanc. En général, les placentas des trois espèces de rhinocéros sont identiques quoique leur grandeur diffère. Il s'agit, en l'occurrence, d'un placenta semblable à celui du cheval représentant un type épitélio-chorial diffus avec des soi-disant rues sans régions de villis couvertes d'un simple épithélium, et il en est de même pour les villis qui n'ont que peu de branches. Dans plusieurs cas, nous avons trouvé des hippomanes, fait qui n'a pas été décrit auparavant en rapport avec les placentas. En plus de cela, nous avons déterminé dans quelques placentas des gemmations épithéliales décrites parfois comme "pustules" chez d'autres espèces animales. Les auteurs soumettent à la discussion le problème de la possibilité d'un transfert embryonal entre les différentes espèces de rhinocéros.

References

AMOROSO, E.C. (1952):
Placentation. In, Marshall's Physiology of Reproduction. A.S. Parkes, ed., 3rd ed., Vol. II. London: Longmans, Green.

BENIRSCHKE, K. and P.P. CALLE (1994):
The placenta of the Beluga whale (Delphinapterus leuca). Verh. ber. Erkrg. Zootiere 36:309-314.

DIXON, H.G. and W.B. ROBERTSON (1969):
The growth of the conceptus and its blood supply. In, Foetus and Placenta. Klopper, A. and E. Diczfalusy, eds. Oxford and Edinburgh: Blackwell. pp. 1-32.

DOLINAR, Z.J., LUDWIG, K.S. and E. MÜLLER (1965):
Ein weiterer Beitrag zur Kenntnis der Placenten der Ordnung Perissodactyla: Zwei Geburtsplacenten des Indischen Panzernashorn (Rhinoceros unicornis L.). Acta anat. 61:331-354.

KLOOSTERMAN, G.J. and B.L. HUIDEKOPER (1954):
The significance of the placenta in obstetrical mortality. A study of 2.000 births. Gynaecologia 138:529-550.

LANG, E.M. (1967):

Einige biologische Daten vom Panzernashorn (Rhinoceros unicornis). Rev. Suisse Zool. Genéve 74:603-607.LUDWIG, K.S. (1962): Zur Kenntnis der Geburtsplacenten der Ordnung Perissodactyla. Acta anat. 49:154-167.

LUDWIG, K.S. and E. MÜLLER (1965):

Zur Histochemie der Plazenta des Panzernashorns (Rhinoceros unicornis L.). Verhandl. Anat. Gesellschaft, Vienna. Anat. Anz. Suppl. 115:155-159.

LUDWIG, K.S. and W. VILLIGER (1965):

Zur Ultrastruktur der Blattzottenepithelien in der Plazenta des Indischen Panzernashorns (Rhinoceros unicornis L.). Acta anat. 62:593-605.

MOSSMAN, H.W. (1987):

Vertebrate Fetal Membranes. Houndmills, Great Britain: The MacMillan Press.

RAMSEY, E.M. (1975):

The Placenta of Laboratory Animals and Man. New York: Holt, Rinehart and Winston.

Adress of authors:

Kurt B e n i r s c h k e, M.D.
University of California, San Diego Medical Center
200 West Arbor Drive
San Diego, CA 92103-8321 (USA)