

**Morphology of peri-partal placentomes and post-partal foetal membranes in African buffalo (*Syncerus caffer*) and comparative aspects with cattle (*Bos taurus*)**

By

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## Declaration

I, Susanne Schmidt, do hereby declare that the research presented in this dissertation, was conceived and executed by myself, and apart from the normal guidance from my supervisors, I have received no assistance.

Neither the substance, nor any part of this dissertation has been submitted in the past, or is to be submitted for a degree at this university or any other university.

This dissertation is presented in partial fulfilment for the requirements for the MSc in production Animal Studies.

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Signed .....  
Susanne Schmidt

Date .....

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**LIST OF ABBREVIATIONS**

BL	Basal lamina	PAGPs	Pregnancy associated glycoproteins
BM	Basement membrane	PBS	Phosphate buffered saline
BNC	Binucleate cell	PL	Placental lactogens
CAP	Capillary	PSPs	Pregnancy specific proteins
CEC	Crypt epithelial cell	PV	Primary villus
CE	Crypt epithelium	rER	Rough endoplasmic reticulum
CP	Chorionic plate	SEM	Scanning electron microscopy
CRL	Crown rump length	sER	Smooth endoplasmic reticulum
CW	Crypt wall	SV	Secondary villus
DLB	Double laminar body	TB	Toluidine blue
ER	Endoplasmic reticulum	TE	Trophoblast epithelium
ERY	Erythrocyte	TEC	Trophoblast epithelial cells
GA	Golgi apparatus	TEM	Transmission electron microscopy
H&E	Haematoxylin and Eosin	TV	Tertiary villus
LM	Light microscopy		
MIVI	Microvilli		
MNC	Multi nucleate cell		
MP	Maternal plate		
MVJ	Microvillar junction		
MYA	Million years ago		
NU	Nucleus		
p.c.	Post conception		

**SUMMARY**

**Morphology of peri-partal placentomes and post partal foetal membranes in African buffalo (*Syncerus caffer*) and comparative aspects with cattle (*Bos taurus*)**

By

Susanne Schmidt

**Supervisor:** Prof. D. Gerber

**Co-Supervisors:** Prof. J. T. Soley, Prof. T. Aire and Prof. A. Boos

**Department:** Production Animal Studies

**Degree:** MSc

The aim of this study was to describe the histo-morphology of the full-term placenta of African buffalo and to compare placental morphology between African buffalo and cattle.

African buffalo, water buffalo and cattle differ, besides numerous external features, in various reproductive parameters such as gestational length. Interest in reproduction in African buffalo, including the application of assisted reproductive technologies, has gained momentum in recent years, with the aim of finding an efficient way of a) increasing genetic diversity and b) producing “disease free” offspring of this wild ruminant species. In contrast to the many studies on placentation in domesticated bovids (cattle and water buffalo), the placenta of African buffalo has been almost completely neglected. A polycotyledonary, synepitheliochorial placenta, characterized by the development of numerous placentomes is generally described for members of the bovid family. Cattle placentomes are stalked, mushroom shaped and represent sites of anchorage and close contact between mother and foetus via interdigitation of foetal cotyledonary villi within corresponding caruncular crypts.

Placentomes from 3 peri-partal, and foetal membranes from 7 post-partal African buffalo cows were collected and placentomal and cotyledonary samples prepared for light microscopy (LM), scanning- and transmission Electron Microscopy (SEM, TEM). The morphology and villous-crypt architecture of buffalo placentomes as well as the histology and ultrastructure of their structural components were described. Complete foetal membranes were macroscopically examined and cotyledonary villi were studied by SEM. A comparison with cattle placentomes and foetal membranes was performed directly or via comparison with relevant descriptions available in the literature.

Comparison between buffalo and cattle placentae revealed that distribution pattern and placentome size were similar in both genera but that buffalo displayed considerably more placentomes than cattle. Buffalo placentomes were, in contrast to those of cattle, non-stalked. Differences in foetal villosity included long, slender and moderately branched villi in buffalo compared to broad, conical and complexly branched villi in cattle. Comparable cell types were involved in the synepitheliochorial interhaemal placental barrier in both genera but histological evidence for the process of placental maturation seems to be less pronounced in the buffalo than in the cattle placenta.

The simpler villi in the non-stalked placentomes of the African buffalo form less complex fetomaternal interdigitations, which is interpreted as providing a somewhat less efficient nutrient supply to the developing foetus. This might partly explain the longer gestation period in buffalo compared to cattle. The placenta of water buffalo also contains non-stalked placentomes, thus resembling the African buffalo placenta in this respect, indicating a closer phylogenetic relationship between the two buffalo genera than between buffalo and cattle.

Results of this first study of placentomes and foetal membranes of African buffalo fills large gaps existing in ruminant placentation and may provide the basis for further research in buffalo reproduction. The similarity in placental morphology between the African buffalo and water buffalo may enhance future trials of intergeneric embryo transfer between the two buffalo genera.

OPSOMMING

**Morfologie van peri-partale plasentome en post-partale geboortevliese in die Afrika buffel (*Syncerus caffer*) en vergelykende aspekte met beeste (*Bos taurus*)**

Deur

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**Departement:** Produksiedierstudies

**Graad:** MSc

Die doel van die studie was om die histomorfologie van die volterm plasenta van die Afrika buffel te beskryf en om plasentale morfologie van die Afrika buffel te vergelyk met die van beeste.

Buiten verskeie eksterne gelaatstrekke verskil Afrika buffels, waterbuffels en beeste in verskeie reprodusiewe maatstawwe soos onder andere draagtydperk. Belangstelling in voortplanting by Afrika buffels, ingesluit die toepassing van kunsmatige voortplantingstegnieke, het in die afgelope jare toegeneem, met die doel om 'n doeltreffende metode te vind om a) genetiese diversiteit te vermeerder en b) "siektevrue" afstammeling van hierdie wilde herkouerspesie te produseer. In teenstelling met verskeie studies van die plasentasie van gedomestikeerde beesspesies (beeste en waterbuffels), is studies van die plasenta van die Afrika buffel karig. Plasentasie van die familie *Bovidae* word algemeen beskryf as plasentasie van die polikotiledonêre, sinepiteliochoriale tipe, gekenmerk deur die ontwikkeling van vele plasentome. Beesplasentome het 'n stingel, is sampioenvormig en verteenwoordig areas van verankering en nabye kontak tussen moeder en fetus via interdigitasie van fetale kotiledonêre villi met die korresponderende karunkulêre krupte.

Plasentome van 3 peri-partale en geboortevliese van 7 post-partale Afrika buffels is versamel en die plasentomale en kotiledonêre monsters voorberei vir ligmikroskopie (LM), skanderings- en transmissie elektronmikroskopie (SEM, TEM). Die morfologie en villus-kript argitektuur van buffel plasentome sowel as die histologie en ultrastruktuur van hul strukturele komponente, is beskryf. Volledige geboortevliese is makroskopies ondersoek en kotiledonêre villi is per SEM bestudeer. 'n Direkte vergelyking is getrek met beesplasentome en –geboortevliese of via die relevante beskikbare literatuur.

Vergelyking van buffel en bees plasentas het onthul dat die verspreidingspatroon en plasentoomgrootte soortgelyk was in beide genera maar buffels het beduidend meer plasentome vertoon as beeste. Buffel plasentome het in teenstelling met die van beeste, geen stingels nie. Verskille in fetale villusagtigheid het ingesluit lang, slank en matig vertakte villi in buffels vergeleke met breë, koniese en komplekse vertakking in beeste. Vergelykbare seltipes was betrokke in die sinepiteliochoriale interhemale plasentale skans in beide genera maar histologiese bewyse vir die plasentale maturasieproses blyk minder uitgesproke te wees in buffel- as in die beesplasenta.

Die meer eenvoudige villi in die stingellose plasentome van die Afrika buffel vorm minder komplekse fetomaternale interdigitasies, wat vertolk word om ietwat minder doeltreffende voeding te voorsien aan die ontwikkelende fetus. Hierdie verskynsel mag 'n gedeeltelike verklaring wees vir die langer draagtydperk van buffels. Die plasenta van waterbuffels bevat ook stingellose plasentome, en is dus soortgelyk aan Afrika buffel plasentatie in hierdie opsig. Hierdie ooreenkoms toon 'n nadere filogenetiese verwantskap tussen die twee buffel genera as tussen beeste en buffels.

Hierdie eerste studie van die plasentome en geboortevliese van die Afrika buffel vul groot gebreke aan in bestaande kennis van plasentasie by herkouters en kan die basis vorm van toekomstige navorsing in voortplanting by buffels. Die eendersheid van plasentale morfologie van die water en Afrika buffel kan toekomstige eksperimente aangaande intergeneriese embryo-oordrag tussen die twee buffel genera ondersteun.

## 1 Introduction

The African buffalo (*Syncerus caffer*, *S. caffer*) is an important member of South Africa's Big Five and plays an essential role in the South African wildlife industry.

An increasing demand for genetic diversity and for "disease-free" buffalo has led to growing interest in buffalo reproduction including the application of assisted reproductive technologies (ART).

African buffalo, water buffalo (*Bubalus bubalis*, *B. bubalis*) and cattle (*Bos taurus*, *B. taurus*) represent different genera within the large family of bovidae. Similar in outer appearance, differences in reproductive physiology between these three bovids are reflected most obviously by the differing mean gestation lengths, being 343 days in *S. caffer*, 315 days in *B. bubalis* and 280 days in *B. taurus*. This is noteworthy, considering the comparably similar weight of dams and calves at birth, the latter weighing an approximate average of 38 – 40 kg in all three genera.

Applying intergeneric embryo transfer as one of the ART between *S. caffer* (donor) and *B. taurus* (recipient), abortion of all buffalo conceptuses occurred after some two months of gestation. As morphological features of reproductive organs of cattle and buffalo are similar it was attempted to determine whether differences in placentation could be responsible for these abortions. The present study was therefore designed to study gross morphology, cyto-morphology and ultrastructure of the full term African buffalo placenta and to compare these features with the corresponding tissues in cattle.

## 2 Literature review

### 2.1 Phylogenetic relationships in the family Bovidae

The order Artiodactyla that comprises the even-toed ungulates represents the most dramatic and spectacular evolutionary radiation of large mammals in the world. Approximately 275 extant species have been described and a rich fossil record for this group exists, revealing tremendous evolutionary diversity throughout historical times. The subfamily Bovinae (family Bovidae) contains some of the most economically important and well-studied taxa in the order Artiodactyla. Typically, extant bovine taxa have been divided into three tribes: the Bovini, containing cattle (genus *Bos*) and their wild relatives including bison (*Bison*), Asian water buffalo and anoa (*Bubalus*), and the African buffalo (*Syncerus*); the Boselaphini, containing the Asian nilgai (*Boselaphus*) and four-horned antelope (*Tetracerus*); and the Tragelaphini, containing the African bushbuck, kudu (*Tragelaphus*), and eland (*Taurotragus*) (Simpson 1945). Data of various phylogenetic studies suggest a synonymisation of the genera *Bos* (2n=60) and *Bison* (2n=60) according to anatomical (Groves 1981) and genetic (Modi *et al.*

1996; Ritz *et al.* 2000) criteria as well as the ability of cattle and bison to produce intergeneric hybrids (Modi *et al.* 1996). Most authors recognise the two Buffalo genera, *Bubalus* ( $2n=48/50$  in River and Swamp buffalo, respectively) and *Syncerus* ( $2n=52/54$  see 1.2), to be more closely related to each other than either is to the genus *Bos* (Groves 1981; Janecek *et al.* 1996; Hassanin & Douzery 1999; Gallagher *et al.* 1999; Buntjer *et al.* 2002). Phylogenetic analyses using microsatellites (Ritz *et al.* 2000) demonstrate a substantial divergence among species. *Bos taurus* and *Bos indicus* were estimated to have diverged 0.31-0.82 million years ago (MYA), *Bos* and *Bison*: 0.46-1.23 MYA, *Bos* and *Bubalus*: 1.85-4.93 MYA and *Bos* and *Syncerus*: 0.98-2.16 MYA. The latter indicates an almost half divergence between *Bos* and *Syncerus* when compared to *Bos* and *Bubalus*. However, Ritz *et al.* (2000) doubt the closer relationship indicated between *Bos* and *Syncerus* than that between *Bos* and *Bubalus*, and mention the power of microsatellites for phylogenetic interference to decrease as evolutionary divergence increases. Craniological studies by Groves (1981) suggest that the bovini last shared a common ancestor about 2-4 MYA whereas the divergence time between *Bubalus* and *Syncerus* is assumed to be 5.1-7.3 million years derived from mitochondrial DNA sequence data (Van Hooft *et al.* 2002).

## 2.2 Subspecies of the African buffalo

Three subspecies of the African buffalo (*Syncerus caffer*) have been identified: Cape (Savannah) buffalo on the savannahs of eastern and southern Africa (*Syncerus caffer caffer*,  $2n=52$ ), the Forest buffalo in the rain forests of western and central Africa (*Syncerus caffer nanus*,  $2n=54$ ) and the West African buffalo from the Sahel-Sudan savannahs (*Syncerus caffer brachyceros*,  $2n=?$ ) (Van Hooft *et al.* 2002).

The Forest buffalo is the smallest ("dwarf" = *nanus*) of the three and has longer, red-brown hair, whereas the Savannah buffalo is larger, darker and less hairy. Positioned between these subspecies are the red, less hairy buffalo of west African origin. Hybridisation occurs occasionally between the Savannah and the Forest buffalo (Benirschke 2005).

## 2.3 Reproductive characteristics of female African buffalo, water buffalo and cattle

Differences in reproductive physiology between the three bovini (domestic cow, water buffalo and African buffalo) are reflected most vividly by their differing mean gestation lengths, being 343 days in *Syncerus* (Knechtel 1993), 315 days in *Bubalus* and 280 days in *Bos* (Hafez & Hafez 2000). This is a remarkable fact considering the comparatively similar calf birth weight of an approximate average of 38 - 40 kg in all three genera (Noakes 1986; Benirschke 2005; Hufana-Duran *et al.* 2004). Female reproductive characteristics of the three bovini are presented in Table 2.1.



**Table 2.1. Reproductive characteristics of female cattle (*Bos*), water buffalo (*Bubalus*) and African buffalo (*Syncerus*).**

	<b><i>Bos</i><sup>1</sup></b>	<b><i>Bubalus</i><sup>1</sup></b>	<b><i>Syncerus</i><sup>2</sup></b>	<b>References</b>
	mean (range)	mean (range)	mean (range)	
<b>Sexual season</b>	polyestrous	polyestrous	polyestrous	<sup>1</sup> Hafez & Hafez (2000) <sup>2</sup> Knechtel (1993)
<b>Estrous cycle</b>				
<b>Length (d)</b>	21 (14-29)	21 (18-21)	18.4 (18-22)	<sup>1</sup> Hafez & Hafez (2000) <sup>2</sup> Knechtel (1993) 23
<b>Estrus (h)</b>	18 (12-30)	21 (17-24)	24	<sup>2</sup> Pienaar (1969) <sup>1</sup> Hafez & Hafez (2000) <sup>2</sup> Knechtel (1993)
<b>Gestation length (d)</b>	280 (278-293)	315 (305-330)	343 340	<sup>1</sup> Hafez & Hafez (2000) <sup>2</sup> Knechtel (1993) <sup>1</sup> Hafez & Hafez (2000) <sup>2</sup> Vidler <i>et al.</i> (1963)
<b>Age of first calving (mo)</b>	30 (24-36)	42 (36-56)	66	<sup>1</sup> Hafez & Hafez (2000) <sup>2</sup> Grimsdell (1973)
<b>Post partum intervals (d)</b>				
<b>Uterine involution</b>	45 (32-50)	35 (16-60)	< 21	<sup>1</sup> Hafez & Hafez (2000) <sup>2</sup> Gerber, pers.comm.
<b>First ovulation</b>	30 (10-110)	75 (35-180)	< 21	<sup>1</sup> Hafez & Hafez (2000) <sup>2</sup> Gerber, pers.comm.
<b>Calving interval (mo)</b>	13 (12-14)	18 (15-21)	12-24.6	<sup>1</sup> Hafez & Hafez (2000) <sup>2</sup> Bertschinger (1996) <sup>2</sup> personal observation

Although African buffalo calve all year round, wherever they occur, the majority of animals conceive during the rainy season and especially towards the end of the rainy season, which generally coincides with peak grazing conditions of their habitat (Grimsdell 1973).

## 2.4 The placenta in mammals

A principal feature of mammalian embryonic development is the formation of a placenta, which serves as an organ of nutrient supply, waste-product elimination, insulation and source of hormones and enzymes. Placentation has been defined as “development of membranes that are apposed to or fused with the uterine mucosa for the purpose of physiological exchange” (Mossman 1987). An extraordinary variability of placental structures has developed throughout mammalian species with each ensuring optimal conditions for the developing offspring.

The mammalian placenta is classified according to various morphological features:

- Placental shape (diffuse, cotyledonary, zonary, discoidal)
- Placental feto-maternal interdigitation (villous, folded, lamellar, labyrinthine)
- Layering of interhaemal membrane (epitheliochorial, synepitheliochorial, endotheliochorial, haemochorial)
- Placental separation at birth (deciduate, non-deciduate)
- Maternofoetal bloodflow interrelationship (concurrent, multivillous, crosscurrent, countercurrent)

According to the above classification, the bovine placenta is variably classified as:

- 1) Cotyledonary (Andresen 1927)
- 2) Villous (Mossman 1987)
- 3) Non-deciduate (semideciduate (Dyce *et al.* 1987))
- 4) Epitheliochorial of synepitheliochorial subtype (Wooding 1992)
- 5) Cross- and countercurrent (Ebert 1993; Leiser *et al.* 1997a)

Ad 1)

Caruncles are permanent endometrial elevations in the ruminant uterus. They are formed early in the foetal uterus and persist throughout life unless destroyed pathologically. During pregnancy, localized foetal villous proliferations, the cotyledons, develop on the chorionic membrane. Cotyledons, together with the uterine caruncles, form functional units, the placentomes. The term placentome is appropriately used to designate the composite structure of both the foetal cotyledon and the maternal caruncle. In the cotyledonary-type placenta, placentomes of varying numbers and sizes are formed and constitute transitional and localized structures where an intimate feto-maternal contact is established (Hafez 1954).

Ad 2)

A placenta is classified as “villous” if “the villi and their branches are free from one another, i.e., never or only occasionally interconnected by fusions of their trophoblast”(Mossman 1987).

Ad 3)

In a non-deciduate placenta, the foetal membranes detach at birth from the uterine mucosa without causing mucosal destruction (Nickel *et al.* 1992). Little loss of maternal tissue during parturition with subsequent repair is described as semideciduate (Dyce *et al.* 1987).

Ad 4)

The epitheliochorial interhaemal barrier consists of 3 foetal (capillary endothelium, connective tissue, trophoblast epithelium) and 3 maternal (crypt epithelium, maternal connective tissue, capillary endothelium) components. The non-invasive chorion (trophoblast) is attached to an intact uterine epithelium throughout gestation. The term “synepitheliochorial“ indicates the migration and fusion of trophoblast-originating binucleated cells with uterine epithelial cells (Wooding 1992).

Ad 5)

In the crosscurrent system, foetal and maternal capillaries are arranged to cross one another. Efficacy of passive diffusion is better than in the multivillous form but less so in the countercurrent form (Ebert 1993). In the countercurrent system, foetal and maternal capillaries are arranged in parallel to one another but have an opposite blood flow. This is the most effective vascular arrangement for passive diffusion (Leiser & Kaufmann 1994).

#### **2.4.1 The cotyledonary placenta**

The cotyledonary placenta is divided into two subtypes (Mossman 1987):

- Oligocotyledonary, with only 5 – 8 placentomes, characteristic for the deer (Mossman 1987)
- Polycotyledonary, with about 50-175 placentomes, characteristic for bovines (Mossman 1987)

In the bovine polycotyledonary placenta, placentomes are formed from day 30 of gestation onwards (Melton *et al.* 1951; Greenstein *et al.* 1958). They are arranged in a relatively orderly manner and vary remarkably in size and shape during the course of pregnancy. Foetal cotyledonary villi display a complex interdigitation with complementary caruncular crypts within each placentome (Leiser & Kaufmann 1994). This feto-maternal interdigitation is responsible for a substantial enlargement of the contact area between mother and foetus, reaching approximately 130 m<sup>2</sup> (Rüsse 1991). The

chorioallantois that exists between placentomes is apposed to the endometrium in flat to gentle folds and is referred to as either the “interplacentomal“, “intercotyledonary” or “smooth” chorioallantois. The extremities of the chorioallantois that occupy the very tip of each uterine horn usually undergo degeneration and coagulative necrosis. These “necrotic placental tips” are very common and their size varies with some approaching 3-5 cm in length (Schlafer *et al.* 2000). Accessory absorptive and phagocytic structures within the bovine placenta include areolae (a chorionic fold forming over a glandular opening), arcade systems (chorion situated between the entrance of two caruncular crypts) and marginal folds (chorionic folds at the transition between placentome and interplacentomal areas) (Mossman 1987).

## **2.4.2 Ruminant placentomes**

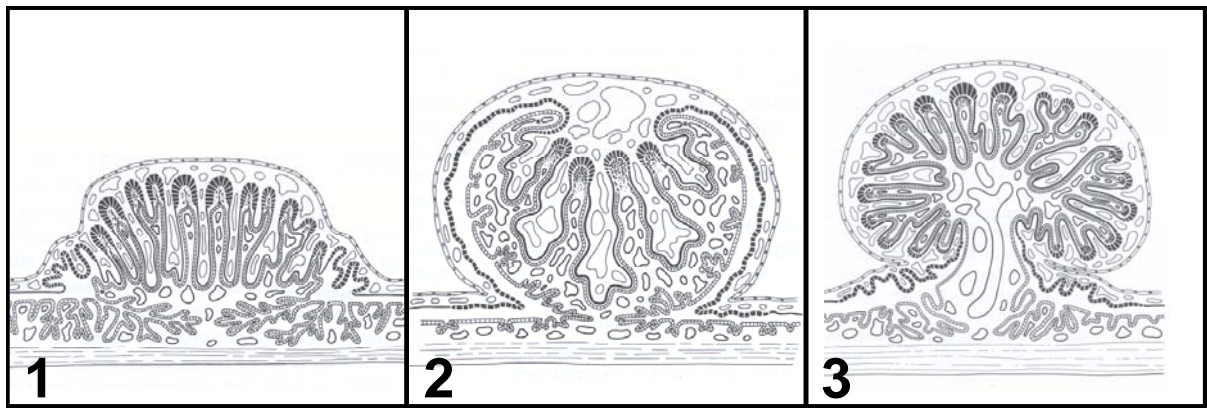
Placentomal morphology has been studied in many domestic and wild ruminant species including the domestic cow (Andresen 1927; Björkman 1954; Laven & Peters 2001), water buffalo (Abdel-Raouf & Bawadi 1966; Yap 1974; Hafez 1954; Abd-Elnaeim *et al.* 2003), sheep and goat (Andresen 1927; Mossman 1987; Hradecky *et al.* 1988a), deer (Strahl 1911; Andresen 1927) and in a large number of antelope species e.g. Tragelaphinae, Hippotraginae, Cephalophinae, Taurotraginae etc. (Strahl 1911; Andresen 1927; Hradecky *et al.* 1987a; Hradecky *et al.* 1988a; Benirschke 2005). The above-mentioned papers illustrate the great variety of placentome shapes occurring within the order Artiodactyla.

### **2.4.2.1 Placentome shape**

Placentome shape changes during placental development. Therefore it is important to investigate placentomes obtained during the last trimester of pregnancy in order to ascertain their definitive shape. However, intergradations between placentome types appear, even in late gestation when differences become most obvious (Mossman 1987). Changes in placentome shape, especially during early developmental stages at the beginning of pregnancy, might explain the slightly confusing classification of placentome types prevalent in literature.

Three types of placentomes are usually recognised (Figure 1.1) (Andresen 1927; Mossman 1987):

- 1) Flat
- 2) Concave
- 3) Convex
  - a) Pedunculated (mushroom-shaped)
  - b) Non-pedunculated



**Figure 2.1. Placentome types in Artiodactyla. The flat (1) placentome is typical for certain deer species. The concave (2) placentome is found in sheep and goat. The convex (3) placentome is typical for most bovid species. Note the development of a distinct caruncular stalk (pendunculated) in the third placentome type (from Mossman, 1987).**

Ad 1)

The term “flat“ is apparently based on different criteria among authors (Andresen 1927; Mossman 1987; Laven & Peters 2001), which might be misleading in some cases. The “flat“ type described by Mossman (1987) lacks a caruncular stalk but displays a convex shape (see Figure 2.1). The elk (*Cervus*) is a typical example of this group (Mossman 1987). Andresen (1927) describes flat placentomes as structures “which hardly rise over the uterine surface” and mentions, in accordance with Benirschke (2005), the dik-dik (*Madoqua*) as well as certain deer species, as typical examples. Therefore, the flat placentome defined by Mossman (1987) rather equals the non-pedunculated, convex form described by Andresen (1927) (see below). Another definition for “flat” is used by Laven and Peters (2001), who illustrate stalked placentomes which, “when placed on a level surface, rested mainly on caruncular rather than cotyledonary tissue“ after the placentomal tissue proper was removed from the caruncular stalk.

Ad 2

The concave placentome is characteristic of the *Caprini*, e.g. in members of domestic and wild sheep and goats (*Capra hircus*, *Ovis aries*, *Capra ibex ibex* etc) and is easily recognized (Mossman 1987).

Ad 3

Convex placentomes are further divided into pedunculated and non-pedunculated placentomes (Andresen 1927).

Pedunculated placentomes, those placentomes which demonstrate a narrowness at their base (caruncular stalk), occur in some members of the *Cervidae* (e.g. red brocket deer (*Macama*)), reindeer (*Rangifer*), roe deer (*Capreolus*) and in *Bos* species (e.g. domestic cow) (Benirschke 2005).

Non-pedunculated placentomes, which lack a caruncular stalk, resemble an ovoid loaf of bread lying sessile on the uterine wall (Andresen 1927). This type of placentome is found in the blackbuck (*Cervicapra*), red deer (*Cervus*), and partly in the domestic cow and the roe deer (*Capreolus*). The occurrence of both pedunculated and non-pedunculated placentomes in *Bos* and *Capreolus* species is explained by the fact that at a certain stage of gestation, formerly non-pedunculated placentomes develop a caruncular stalk and become secondary pedunculated, thus mushroom shaped (Andresen 1927). Furthermore, Mossman (1987) states that all three placentome types appear flat in early developmental stages as the stalk only appears later during gestation. When peripheral placentomal tissue grows as gestation proceeds, the underlying endometrial tissue is narrowed and forms the caruncular stalk. An unusual situation is described in the Uganda kob (*Andenota kob thomasi*), where early pedunculated placentomes become sessile, thus non-pedunculated later during gestation (Hradecky *et al.* 1988a).

To allow assigning a placentome to a specific type, it is important to investigate placentomes cut in transverse versus longitudinal section, and medium-sized, circular placentomes are recommended to be ideal for examination (Andresen 1927; Hradecky *et al.* 1988a).

In Water buffalo, pedunculated and non-pedunculated placentomes are described. Hafez (1954), who investigated placentomes in early and mid-pregnancy, makes no mention of the formation or lack of a caruncular stalk. Abdel-Raouf *et al.* (1966) report the occurrence of entirely non-pedunculated placentomes in the water buffalo placenta, whereas Yap (1974) and Abd-Elnaeim (2003), in more recent studies, describe the occurrence of a short, broad stalk, which becomes inconspicuous during late developmental stages (Ram and Chandra 1984).

#### **2.4.2.2 Placentome components**

Each placentome consists of two components, the maternal caruncle and the foetal cotyledon. The maternal tissue constituting the caruncular stalk, if present, forms the placentomal basal plate and the caruncular septae. The basal plate (maternal plate) varies from convex to flat depending on the shape of the placentome. The cotyledon forms the more or less convex (or concave in sheep and goat) chorionic plate (foetal plate), thus covering the maternal caruncle like a cap. (As maternal and chorionic plate both arch “upwards” within a single placentome when viewed laterally, both tissues

will be described as “convex” in this study.) The chorionic plate projects numerous villous trees towards the maternal tissue. The orientation of villous trees relative to the uterine surface also depends on placentomal shape (Mossman 1987; Leiser *et al.* 1997a). Flat placentomes are composed of parallel, vertically orientated and only slightly branched foetal villi within corresponding caruncular crypts (Mossman 1987). Convex placentomes display conical villous trees, each consisting of a large base narrowing towards the tip much like a Christmas tree (Mossman 1987; Leiser *et al.* 1997a).

#### **2.4.2.3 Number of placentomes (cotyledons) in *Bos*, *Bubalus* and *Syncerus***

The number of caruncles present in the non-pregnant cattle uterus (75 – 120 (Rüsse 1991)) does not correlate exactly with the number of placentomes, which develop during gestation. So-called “spare” caruncles, which are not covered by a cotyledon, are commonly reported to occur in the pregnant bovine uterus (Laven & Peters 2001). An “extreme” of the latter situation is described by Laven and Peters (2001) who reported no placentome development in the non-pregnant horn in 10 of 47 bovine uteri examined in their study. The reason and cause of this phenomenon are unclear. According to Mossman (1987), a polycotyledonary type placenta containing 50 – 175 placentomes is characteristic of bovids. Laven and Peters (2001), who investigated bovine placentae at various gestational stages, did not find a significant change in the total number of placentomes during gestation. However, a significant difference in the number of placentomes is reported between the pregnant and the non-pregnant uterine horn, the former usually containing more placentomes. Mean placentome numbers counted at a gestational age of over 251 days (Laven & Peters 2001) and mean numbers of cotyledons counted on expelled foetal membranes (Bertolini *et al.* 2002) of domestic cows were 48.9 (range 40 – 58) and 69 in the pregnant horn and 22.8 (range 0 – 51) and 33 in the non-pregnant horn, respectively. The latter author reports the range of cotyledonary numbers in the whole uterus to be 52 – 153.

Total numbers of *Bubalus* placentomes (or cotyledons of expelled foetal membranes) and corresponding numbers for the pregnant / non-pregnant horn (if available) were 117.8 and 62.7 / 54.7 (Roy and Luktuke, 1962 cited by Ranjhan & Pathak 1993), 124.0 and 53.6 / 45.4 (Bhosrekar and Sharma, 1972 cited by Ranjhan & Pathak 1993), 108.0 (Sharma and Gupta, 1978 cited by Ranjhan & Pathak 1993), 92.0 and 62 / 30 (Abd-Elnaeim *et al.* 2003) and 126.0 and 68 / 55 (Abdel-Raouf & Bawadi 1966). Contrary to the situation in cattle, Abdel-Raouf and Bawadi (1966) and Hafez (1954) mention an increase in placentome numbers during the course of gestation in water buffalo. Placentome numbers rise from 31.4 (pregnant horn) and 10.6 (non-pregnant horn) in early stages to 89.0 (pregnant horn) and 57.5 (non-pregnant horn) in late stages of pregnancy (Hafez 1954). In the latter study, placentome numbers reached a maximum at a mean crown-rump-length (CRL) of 19 cm, and not at the end of pregnancy.



In the African buffalo, three expelled foetal membranes have been investigated by Benirschke (2005). To date this is the only available source of information on placental features at term in this genus. Reflecting 34, 52 and 54 cotyledons per afterbirth respectively, the numbers are far lower when compared to corresponding numbers in cattle and water buffalo.

#### **2.4.2.4 Distribution of placentomes (cotyledons) in *Bos*, *Bubalus* and *Syncerus***

Placentomes in cattle are arranged in four rows that run lengthwise along both uterine horns (Andresen 1927; Schlafer *et al.* 2000). Two rows are found parallel to the border of insertion of the mesometrium (ventral) and the other two parallel to the antimesometrial border (dorsal) (Andresen 1927). Placentomes from the dorsal and ventral rows are arranged in an alternating manner (Andresen 1927). Irregularities of placentome distribution along some parts of the uterus are also reported (Andresen 1927) but most likely represent accessory placentation as described by Björkman (1954).

Placentome distribution in water buffalo is similar to cattle (Hafez 1954; Abdel-Raouf & Bawadi 1966; Ranjhan & Pathak 1993; Abd-Elnaeim *et al.* 2003). Hafez (1954) observed 2-6 rows of placentomes in the uterine body and 2 rows at the extremities of the horns. Abdel-Raouf *et al.* (1966), who separated the uterine horns into cranial, middle and caudal parts, described 2, 4 and 4 rows in each part, respectively. According to the same authors, the uterine body may contain four rows of irregularly arranged placentomes.

Reports on placentome / cotyledonary distribution in the African buffalo are lacking. Images of expelled foetal membranes published by Benirschke (2005) do not allow a certain conclusion in this matter.

#### **2.4.2.5 Villous and crypt formation in bovine placentomes**

The question has been raised (Andresen 1927) regarding which tissue, foetal or maternal, might be responsible for formation and shape of placentomes. Björkman (1954) and Hradecky *et al.* (1987a) share the opinion that crypts were preformed and subsequently invaded by trophoblast villi whereas Melton *et al.* (1951) and Wild (1964) suggested that crypts develop in response to villous proliferation. A simultaneous villous-crypt formation is also suggested (King *et al.* 1979; Hradecky *et al.* 1987a). Björkman (1954) described crypt formation in the cow as “chorio-passive”, a term indicating that “maternal elements of the placentome are found before the chorionic villi come into close contact with them”. Hradecky *et al.* (1987a) implied that crypt and villi formation might have similar regulatory mechanisms but do not necessarily depend on each other.



#### 2.4.2.6 Villous and crypt classification

The nomenclature of the various components of villous trees is based on their constituting microvasculature, which has been well described in various genera, e.g. *Bos* (Ebert 1993; Leiser *et al.* 1997a; Pfarrer *et al.* 2001), *Bubalus* (Abd-Elnaeim *et al.* 2003), and human (Kaufmann *et al.* 1979; Castellucci *et al.* 1990). Chorionic villi and caruncular crypts are classified according to their vascular supply as follows: Vessels in the core of maternal primary (stem) septa and foetal primary (stem) villi are stem arteries and stem veins. The areas surrounded by primary septa are therefore termed primary (stem) crypts. Arteries and veins ramify into arterioles and venules, which constitute branches of second order, thus secondary (intermediate) septa and villi. Arterioles and venules supply blood to/from the capillary bed in tertiary structures, hence tertiary (terminal) septa and villi. Enclosed crypts are therefore classified as secondary and tertiary crypts, respectively (Leiser *et al.* 1997a; Pfarrer *et al.* 2001).

#### 2.4.2.7 Villous-crypt architecture in *Bos*, *Bubalus* and *Syncerus*

Vascular architecture of foetal villous trees and caruncular crypts has been studied in cattle and water buffalo using corrosion cast techniques in combination with light microscopic observations (Ebert 1993; Leiser *et al.* 1997a; Pfarrer *et al.* 2001; Abd-Elnaeim *et al.* 2003). The basic principle of this technique is that vessels follow every contour of the tissue surface, thus demonstrating a three-dimensional image of foetal villi and maternal crypts. Foetal villi consist of the vascular villous tree, surrounding stromal components and covering trophoblast. The corresponding maternal crypts consist of the vascular framework, maternal stroma and maternal crypt epithelium.

In cattle and water buffalo foetal villous trees ramify up to the third order and interlock with corresponding caruncular crypts (Leiser *et al.* 1997a; Abd-Elnaeim *et al.* 2003). In the early bovine placenta, villous trees resemble a “Thuja tree” in shape (Ebert 1993), with a wide base at the chorionic plate and a narrow tip ending along the maternal plate. At midgestation, foetal villi are characteristically described as “Christmas-tree-like“ (Leiser *et al.* 1997a), with a similar appearance to that mentioned above. Chorionic villous trees radiate in maternal direction, eventually ramifying to form several distal divisions (treetops). Intermediate villi project at an acute angle along the entire length of the stem villus. Intermediate villi are variable in their ramification and length. They are simpler in form during early pregnancy and at the treetops but complex during advanced gestation and at the base of the villous trees. During later gestational stages (second half), the conical, Christmas-tree-like villous trees become more slender and tall with secondary villi mainly ramifying at right angles from the stem. The angle of terminal villi relative to the main axis of intermediate villi is generally described as being 45 degrees but varies from no angle, e.g. at the treetop to obtuse angles.

Ramifications of terminal villi sometimes result in the formation of fan-like structures (Leiser *et al.* 1997a) which occur over the entire villus with the exception of the treetop (Ebert 1993). Two different forms of terminal villi were demonstrated in the study done by Ebert (1993) in cattle, a “sling-type“ and a “basket-type“. The sling type ends in a thin tip whereas the basket type displays a roundish form. Fewer terminal “baskets“ occur than terminal “slings“, especially during late gestation. Depending on placentomal size villous trees at 7 months of gestation reach a length of 15 mm (Ebert 1993).

In water buffalo placentomes, according to Abd-Elnaeim *et al.* (2003), conical or cylindrical shaped villous trees run, in contrast to those in cattle, parallel to one another in a feto-maternal direction. From the sixth month of pregnancy most villous trees are well separated from each other at the chorionic plate. Contrary to the situation in cattle, a smooth and a rough villous type is described to exist throughout gestation in water buffalo, the rough type dominating towards the end of gestation. The smooth villous type projects few or no intermediate and terminal branches whereas the rough type projects distinct branches of secondary and tertiary orders. The angles of ramification of villi of higher orders are similar to those described in cattle.

Crypt walls correspond intimately with the borders of the villous trees and will therefore not be additionally described.

In general, the intricate interdigitation of cotyledonary villous trees and caruncular crypts assigns the ruminant placenta to a rather complex type (Leiser *et al.* 1998). However, Abd-Elnaeim *et al.* (2003) report that a firmer materno-foetal connection exists in water buffalo than in cattle placentomes. This is believed to be promoted by the longer gestation period in water buffalo, which allows for a more elaborated interdigitation between villi and crypts in water buffalo.

SEM observations of African buffalo cotyledons (villous trees) include, to date, early gestational stages only, (Schmidt *et al.* 2005) and the blood vessel corrosion cast technique was not performed. At a foetal CRL of 17 cm, cotyledons comprised long and slender stem villi, which resembled a slender “Tuscany-cypress“ (Schmidt *et al.* 2005) rather than a “Thuja-tree“ as described in cattle at similar foetal CRL (Ebert 1993). Secondary branching was restricted to small, lateral budding along the primary villus whereas ramification up to the third order has not yet been observed. Smooth areas lacking any secondary structures are also described (Schmidt *et al.* 2005).

Similar techniques to those employed for the *Syncerus* placenta (without corrosion casts) have been used to describe foetal villousity in e.g. the horse (Mc Donald *et al.* 2000), the rhesus monkey (King &

Mais 1982) and the human placenta (Kaufmann *et al.* 1979). As far as could be ascertained, Sobiray (1992) shows the only SEM images of *Bos*-foetal villi prepared by a similar technique.

#### **2.4.2.8 Placentomal histology**

##### 2.4.2.8.1 Trophoblast epithelium (TE)

Chorionic villi consist of a vascularized mesenchyme provided with a simple layer of trophoblast epithelial cells, which are arranged in an irregular manner. Cells rest on a distinct, undulating basement membrane, which is frequently indented by foetal capillary loops, so-called “intraepithelial capillaries”. The trophoblast epithelium consists of typically uni-nucleate trophoblast epithelial cells (TEC) and binucleate cells (BNC, giant cells), scattered at random throughout the TEC (Björkman 1954; 1969).

Trophoblast epithelial cells have a large, round to ovoid nucleus which is situated basally or centrally within the cell. The karyoplasm contains one or more nucleoli. The cells display a light cytoplasm within the apical region in which numerous mitochondria with cristae occur. Numerous electron-dense vesicles, granules and membrane bound vacuoles are found within that part of the cell adjacent to the maternal crypt epithelium. Sparse profiles of rough and smooth endoplasmic reticulum (rER, sER) as well as scanty free ribosomes are scattered throughout the cytoplasm. The Golgi apparatus (GA) is poorly developed. Neighbouring trophoblast epithelial cells are connected via labyrinth-like interdigitation of their lateral membranes as well as by desmosomes and apical tight junctions. The apical cell membrane projects numerous microvilli which interdigitate intimately with microvilli of the crypt epithelial cells, thus forming the microvillar junction (MVJ) (Björkman 1954; 1969; Björkman & Bloom 1957; Hager 1983; Schoon 1989). From a functional point of view, TEC therefore demonstrate the typical picture of a highly resorptive epithelium (numerous microvilli, apical vesicles in combination with many mitochondria). TEC are capable of active substance uptake, even against a concentration gradient, as well as the transport of these substances through the cell and their release towards underlying cells and capillary lumina. However, para- or intercellular transport of substances seems to be impeded by intercellular occlusions such as tight junction, thus possibly indicating a minor role for this transport route. Intact apical microvilli, the lack of apical cytoplasmic protrusions and poorly developed GA and ER cisternae further indicate a lack of synthesis capacity and secretory activity of TEC (Hager 1983).

Any trophoblastic epithelial cell can give rise to a BNC. This is achieved via division of a TEC, which produces two cells, one of which has no apical tight junctions. The nucleus of this cell divides mitotically without subsequent cytokinesis, thereby forming a young BNC (Wimsatt 1951; Wooding

1982a). Young BNC are found as early as 17 days *post conception* (p.c.) within trophoblast epithelium (Wimsatt 1951). BNC rapidly lose contact with the basement membrane and migrate towards the MVJ. No tight or gap junctions between BNC and TEC have been described and the maturation process is completed with only rare and tiny desmosomal-like contacts with neighbouring cells being observed (Wooding 1982a). BNC grow in size and rapidly develop an extensive system of swollen rER cisternae and a very large GA. The most characteristic feature of a BNC is the increasing number of cytoplasmic granules, accounting for over 50% of cytoplasmic volume in a mature BNC (Wooding 1992). These granules are membrane bound, round to ovoid vesicles of different densities, each containing microvesicles (Wooding 1982b; Byatt *et al.* 1986; Wooding & Flint 1994; Wooding *et al.* 1996). The surface of BNC lack microvilli during all stages of development (Björkman 1968; Leiser 1975; Wathes & Wooding 1980). Mature, fully granulated cells reach a considerable size of 30-50  $\mu\text{m}$  (Duello *et al.* 1986; Wooding 1992) and can contain one or more lipid droplets and/or crystalline inclusions (Wimsatt 1951; Wooding 1982a). A unique structural element in BNC is the double laminar body (DLB) (double lamellar body/apparatus), which is present in all ruminant species so far examined (Björkman & Bloom 1957; Wooding & Flint 1994; Wooding *et al.* 1997). It is a small area of double membranes immediately continuous with the ER cisternae, is usually located close to the GA and branches in a characteristic manner.

Approximately 15-20% of all cells within the trophoblast epithelium are BNC and their numbers remain fairly constant throughout gestation (Wooding 1982b; Duello *et al.* 1986). Numerous studies have revealed evidence for BNC migration through the MVJ and subsequent fusion with an uterine epithelial cell (crypt epithelial cell, CEC). The resulting hybrid or so-called “cryptal giant cell“, initially contains three nuclei but grows due to further migration and fusion of additional BNC, producing multinucleate cells (MNC) (Wooding 1982b). Binucleate cells and tri- or multinucleate cells within the uterine epithelium are described as early as day 18 p.c. (Wathes & Wooding 1980). In most trinucleate cells, two nuclei have a similar density while the other differs, which is seen as an indicator of cell fusion (Wooding & Beckers 1987). During the remainder of pregnancy, migrating BNC will fuse with a single crypt epithelial cell to form a transient trinucleate cell, which dies after its granules have been released and is then phagocytosed by the chorionic epithelium (Wooding & Wathes 1980). Occurring signs of cellular degeneration (vacuolation and fragmentation of nuclei and cytoplasm) in both foetal and maternal bi- or trinucleate cells underlines the hypothesis of cell death after granule delivery (Duello *et al.* 1986). However, the occurrence of transient trinucleate cells during the entire pregnancy does not explain the existence of large, multinucleate cells within the uterine epithelium during all stages of pregnancy as described by Björkman (1954, 1969).

Granule transfer and the exocytotic release of their contents close to the maternal circulation seems to be the primary function of BNC migration in cattle. Granule content includes the two main groups of placental proteins produced in ruminant BNC: placental lactogens (PL) and pregnancy-specific (PSPs) and/or pregnancy -associated glycoproteins (PAGPs) (Beckers *et al.* 1998). Compared to sheep and goat, modification of the uterine epithelium is only transitional in cattle and the trinucleate cells subsequently produced in the maternal epithelium do not appear to play a barrier- or structural role (Wooding 1992). Fusion of ruminant BNC with maternal tissue might be a successful way to allow foetal access to maternal circulation while avoiding an attack by maternal lymphocytes. Delivery of BNC granular content might be critical in developing a successful metabolite and/or immunological dialogue between mother and foetus (Wooding 1992).

#### 2.4.2.8.2 Crypt epithelium (CE)

The trophoblast epithelium is in intimate contact with the epithelium lining caruncular crypts, the maternal crypt epithelium. This epithelium is composed of simple, cuboidal or columnar epithelial cells (crypt epithelial cells, CEC) which rest on a distinct basement membrane that is less undulating than its foetal counterpart. Lateral cell membranes lack invaginations or interdigitations between neighbouring cells. Desmosomes as well as apical tight junctions are described (Björkman & Bloom 1957; Björkman 1969; King *et al.* 1979; Hager 1983). Scanning electron microscopic observations demonstrate narrow apical cell surfaces covered by microvilli which appear to continue onto adjacent cells resulting in indistinguishable cell borders. Parallel microfilaments anchor the microvilli within the apical cytoplasm (Schoon 1989). Round to ovoid nuclei are situated towards the base of CEC within electron lucent cytoplasm and the nuclei contain one or more nucleoli. Cytoplasmic organelles are sparse and include mitochondria with cristae (mainly apically situated), rER, sER and free ribosomes as well as a sparsely developed GA. Occasional “membrane whorls”, consisting of concentric lamellar ER-tubuli reflect a high synthetic capacity. Lipid droplets, vacuoles and microvesicles in apical and basal cytoplasm represent cellular inclusions (Björkman & Bloom 1957; Björkman 1969; King *et al.* 1979; Hager 1983; Schoon 1989). The development of a second type of CEC, the maternal tri- or multinucleate cell (MNC) has been mentioned above. MNC differ from BNC in so far as they are generally smaller in size (but larger than CEC) and possess microvilli on their apical cell border which participate in the formation of the MVJ. MNC are scattered among the CEC from early implantation onwards and they are reported to occupy up to 70% of the uterine epithelium. MNC contain two or more nuclei, scattered long profiles of rER, small round to ovoid mitochondria (predominately located in the cell apex) and occasional large lipid droplets (Wathes & Wooding 1980).

### 2.4.3 Placental maturation at end of gestation and placental release

During the last weeks of pregnancy morphological changes in the maternal epithelium (see below) shorten the distance between foetal and maternal blood supply. This is an important mechanism whereby the placenta meets the growing demands of the foetus and it is referred to as “placental maturation” (Woicke *et al.* 1986). Moreover, placental maturation is necessary to allow for an uncomplicated release of foetal membranes in cattle post partum.

Throughout gestation, degeneration of maternal CEC, and, to a lesser extent of TEC, is detectable. This degeneration reflects a normal tissue turnover and also indicates the contribution of maternal epithelium to embryotrophe (Björkman 1954; Björkman 1969; King *et al.* 1979, 1981; Hoffmann & Schuler 2002, Boos *et al.* 2003a). Morphological changes occurring during placental maturation reach their maximum about 5 days prior to parturition (Woicke *et al.* 1986). At the LM level, all authors describe a reduction of CEC numbers and of cell height (mean 8.45  $\mu\text{m}$  (Willms 1986) and 8,2  $\mu\text{m}$  (Tolhuysen 1990)) in mature placentomes of cattle with normal foetal membrane delivery (Björkman 1954; Björkman & Sollen 1960; Holm *et al.* 1964; Willms 1986; Woicke *et al.* 1986; Schoon 1989; Sobiray 1992). A partial (Björkman & Sollen 1960; Holm *et al.* 1964; Woicke *et al.* 1986) or complete (Schoon 1989) loss of maternal epithelium is also reported, leading to the formation of a syndesmochorial-type placenta (Schulz & Merkt 1956; Schoon 1989) around parturition. Sobiray (1992) and Willms (1986) relate this phenomenon to the location of the maternal epithelium within the placentome. In superficial parts of placentomes, a significantly lower epithelium or a complete lack of epithelium occurs more frequently than in deep secondary and tertiary crypt walls and closer to the placentome stalk.

Other parameters typical for mature placentomes are increased amounts of connective tissue within crypt walls (Schoon 1989; Tolhuysen 1990) and occurrence of intense oedema (Woicke *et al.* 1986; Schoon 1989). The increase in connective tissue is accompanied by a decrease in number of fibrocytes per area of tissue (Willms 1986) and an increase in septum and villous thickness (Björkman 1954; Woicke *et al.* 1986). Disagreement exists regarding the involvement of leukocytes with maturation and separation process of foetal membranes. Woicke *et al.* (1986) and Schoon *et al.* (1989) report an increase in leukocyte stasis and tissue infiltration as well as a thrombocyte aggregation in placentomes of animals with physiologic foetal membrane release, whereas Sobiray (1992) and Tolhuysen (1990) do not confirm these findings. Difference in investigation procedures (location within the placentome from where the samples were taken) might be responsible for the above mentioned discrepancies (Tolhuysen 1990). Gunnik (cited by Davies *et al.* 2004) suggests that an inflammatory response is involved in normal foetal membrane separation. It has been demonstrated that placentomes from cattle



with normal foetal membrane separation contain a chemotactic factor for leucocytes and that this factor was lacking in placentomes from cattle with retained placentas. Furthermore, leucocytes from cows with retained placentas were in a less activated state than those from cows with normal foetal membrane release (Kimura *et al.* cited Davies *et al.* 2004). Authors of a recent publication (Davies *et al.* 2004) therefore suggest that maternal immunological recognition of foetal MHC class 1 proteins (Major histocompatibility complex) expressed by trophoblast cells triggers an immune/inflammatory response that contributes to placental separations during parturition in cattle (Davies *et al.* 2004).

At both the light microscopy and ultrastructural level, degeneration of CEC is commonly described during the last five days of gestation (Woicke *et al.* 1986; Schoon 1989; Sobiray 1992). Signs of degeneration are nuclear pycnosis, cell vacuolisation and occurrence of myelin figures (Woicke *et al.* 1986). Boos *et al.* (2003a) report an increase in number of apoptotic bodies in maternal and foetal epithelial cells in domestic cows during the last trimester of pregnancy. The trophoblast epithelium displays signs of degeneration such as cell fragmentation, myelin figures, residual bodies and lipid droplets within the cytoplasm as well as indistinguishable lateral cell borders (Schoon 1989). Disintegration of microvilli and the appearance of small feto-maternal gaps are described to occur in a discontinuous fashion and from the periphery to more central regions of the placentome. In addition, Sobiray (1992) reports that most TEC display abnormal features such as a light cytoplasm and electron lucent nuclei. Whereas some authors demonstrate a disintegration of feto-maternal contact zones (Schoon 1989; Sobiray 1992), Björkman and Sollen (1960) describe an intensely stained MVJ with closely apposed cells in mature pre-partum and post-partum placentomes. The MVJ represents interdigitating microvilli and forms the natural site of contact and thus separation between the trophoblast and crypt epithelium.

A large peri-partal decrease in number of BNC is observed when foetal membranes are subsequently released normally and it has been suggested that declining BNC numbers are related to placental separation rather than to parturition in cattle (Gross *et al.* 1991). The rare BNC contain typical granules but demonstrate signs of degeneration such as disintegrating organelles, myelin figures, dense bodies and lipid droplets within the cytoplasm (Schoon 1989). Placentomes from different locations within the uterus do not differ regarding changes during maturation (Willms 1986).

#### **2.4.4 Placenta foetalis**

##### **2.4.4.1 Basic functions of the foetal membranes**

Foetal membranes enclose two fluid filled cavities, the amniotic and allantoic cavities, surrounding the embryo. The embryo, floating in amniotic fluid is therefore insulated against external insults and is protected against dehydration.

Foetal membranes also constitute the organs of nutrition and substance exchange for the growing foetus as they provide feto-maternal contact until parturition. The allantoic cavity receives and stores foetal urine. A mechanical anchorage between foetus and mother is achieved via multiple vascularized chorionic villi within maternal caruncular crypts (Wild 1964).

##### **2.4.4.2 Expulsion of the placenta foetalis (afterbirth)**

During expulsion of the foetus, uterine contractions lead to changes in intrauterine pressure which result in intermittent anaemia and hyperaemia of foetal chorionic villi. This processes causes loosening between the chorion and the uterine epithelium leading to a mechanical displacement, which first occurs in the proximity of the caruncular stalk.

Tearing of the umbilical cord following expulsion of the foetus and the resulting blood loss from the umbilical vessels causes a decrease in villous size due to a local anaemia within villous capillaries. Furthermore, the release of the placenta depends upon changes in molecular forces maintaining microvillar interdigitation (Wooding & Flint 1994). Post-partial uterine contractions and uterine involution finalise the detachment process of the foetal placenta (Schulz & Merkt 1956), which is physiologically released within 12 hours post partum. Membranes are generally passed with the allantoic surface (smooth and shiny) of the allantochorion outermost (Arthur *et al.* 1996).

##### **2.4.4.3 Structural features of the afterbirth following normal delivery**

Björkman and Sollen (1960) reported that chorionic villi from afterbirths, taken immediately after normal delivery, demonstrate similar morphology to those before expulsion of foetal membranes. Villi are to a large extent provided with an intact trophoblast epithelium, an observation which is in accordance with descriptions by Willms (1986). However, some villi, especially those of higher order, display signs of mechanical or necrotic damage of TEC (Björkman & Sollen 1960). Areas of complete loss of TEC are reported and demonstrated by Sobiray (1992).



Amniotic epithelial proliferations, also termed amniotic plaques, epithelioms or epithelial islands are physiologically normal flat, white to yellow structures present on the inner aspect of the amnion (Schlusche 1983).

### **3 Materials and Methods**

#### **3.1 Buffalo breeding facilities, quarantine facilities, animals and animal handling**

Placentome samples and afterbirths were collected from adult African buffalo cows housed at a buffalo-breeding centre in Limpopo province, South Africa. Breeding facilities are located on a 185 ha farm, housing 65 female and 7 male adult buffalo, 33 buffalo calves (newborn to approximately 7 months of age) and 21 Jersey cows (November 2004). Buffalo calves and Jersey cows are kept together in a quarantine area. To prevent direct contact between calves, Jerseys and adult buffalo, a double electric fence and a 2 m wide bush and grass free area, the latter to prevent infestation with ticks, enclose the quarantine area.

The breeding facility for adult buffalo is situated approximately 30 m from the quarantine area. Most adult buffalo roam free on the farm throughout the year but tend to stay in vicinity of bomas where they are fed with lucerne twice a day during the dry season. Reproductively active, non-pregnant buffalo cows are kept in small groups of 4-5 females together with one sexually mature buffalo bull in a 500 m<sup>2</sup> boma. Animals are observed 24 hours a day for 365 days a year. After mating has been observed in each cow, all animals are released and are allowed to roam free on the farm for the following 9 to 10 months. Cows showing obvious signs of estrous after being released are regarded as unsuccessfully mated and are brought back for another breeding attempt.

To ensure close observation of cows during their last 2 months of gestation, late pregnant cows are again kept in small groups (4-5 animals) in bomas and observed 24 hours a day. Parturition is closely monitored and as soon as the calf has been born, the cow is immobilised with 5-8 mg M99® (etorphine hydrochloride) and 100-150 mg Stresnil®. Approximately 6-8 minutes later, the cow goes down and is immediately stabilized in sternal recumbency, blindfolded and the calf removed. Following sample collection (see below), the cow is injected intramuscularly with 20 ml Clamoxyl® (amoxicillin trihydrate/potassium clavulanate) to prevent any bacterial infection and with 4 ml Fentocin® (oxytocin synthetic) to ensure uterine contraction after sample taking. Finally, the antidote M5050® (diprenorphine) is given intravenously using a dosage of approximately 2-3 times the dosage of M99®.

#### **3.2 Sample collection from peri partal (n=3) buffalo cows**

As soon as an immobilized cow was in sternal position, the uterus was examined per vagina. All reachable placentomes were carefully palpated and examined for possible stalk formation. One easily reachable, medium sized, round to ovoid (ca 3 x 4 cm) placentome was chosen for collection per cow.

Several attempts at blunt extirpation by twisting off a placentome proved unsuccessful due to the broad connection between the placentome and the uterine wall. Therefore, a sling of fetotomy wire, passed through a modified 20 ml syringe, was used to remove each placentome.

### **3.2.1 Collection, fixation and tissue processing of sampled placentomes for light microscopy (LM) and transmission electron microscopy (TEM)**

Immediately after removal of a placentome, two 4 mm thick slices were cut from its centre. For light microscopy (LM), specimens were immediately fixed in 10% buffered formalin for a minimum of 24 hours. An automatic tissue processor was used for dehydration, clearing and wax impregnation of tissue samples. After processing, samples were embedded in Paraplast®, 4µm sections were cut and stained with Haematoxylin and Eosin (H&E).

For transmission electron microscopy (TEM), small 2 mm<sup>3</sup> blocks were cut from the centre of each fresh placentome and immersed for a minimum of 24 hours in 2.5% glutaraldehyde in Millonig's phosphate buffer. The samples were washed in Millonig's phosphate buffer and post-fixed in similarly buffered 1% osmium tetroxide for 1 to 2 hours. Samples were dehydrated in a graded series of ethanol (50%, 70%, 80%, 90%, 96%, 100% x 15 minutes / step) and the ethanol replaced with propylene oxide (100% x 2 – 15 minutes / step). Blocks were routinely infiltrated with Epoxy resin (Embed 812) overnight, embedded in flat moulds and allowed to dry overnight at 60°C. Semi-thin sections (0.5 µm) were cut and stained with toluidine blue (TB). Suitable regions of interest were chosen and ultra-thin sections (0.1µm) were cut and contrasted with uranyl acetate and lead citrate.

### **3.3 Collection, fixation and tissue processing of samples from expelled foetal membranes for scanning electron microscopy (SEM) and Stereo microscopy (n=5)**

Seven foetal membranes were collected immediately after their natural expulsion, 2-4 hours after the birth of a healthy calf. Samples for microscopic observations were only collected from five fresh afterbirths as the remaining two had to be deep-frozen for later examination and were excluded for SEM purposes. Two medium sized (4 x 5 cm) cotyledons per afterbirth were chosen from the centre of the pregnant horn. Tissue samples of approximately 1 square centimetre were cut from the centre of each cotyledon and rinsed thoroughly with Millonig's phosphate buffer. Some samples were pinned on a wax plate via the chorionic plate and the entire sample was immersed up-side-down in 2.5% glutaraldehyde in Millonig's phosphate buffer for a minimum of 24 hours. In other samples, single

villous trees were separated at their origin at the chorionic plate and processed separately in order to obtain a three-dimensional view of complete, single villous trees.

Following fixation, both types of samples were rinsed in Millonig's phosphate buffer, dehydrated in a graded ethanol series using the same concentrations as for TEM but for longer periods (20 minutes / step). When applicable, samples were removed from the wax plate and critical point dried in a SPI critical point drying apparatus using liquid CO<sub>2</sub>. The dried samples were mounted on aluminium stubs with carbon adhesive tape and coated with palladium in a Polaron E 5100 sputter coater.

### **3.3.1 Data collection from expelled foetal membranes (n=7)**

Following sample collection for SEM purposes, or following a slow and careful defrosting process (n=2), each afterbirth was rinsed thoroughly with water. Foetal membranes were cut open along the mesometrial attachment and spread flat on the ground. The pregnant and non-pregnant horn were separated and assessed separately. During foetal membrane release most cotyledons were turned inside out when compared to the situation before placental detachment, i.e. the chorionic villi were now exposed on the surface. Cotyledons where the chorionic surface was not exposed had to be turned inside out (Figure 5.1) to enable the evaluation of cotyledonary shape and size.

Distribution, number, size (length and width in cm) and shape of all cotyledons were recorded and images taken using a digital camera. The number of cotyledons in the pregnant and non-pregnant horn was compared with a students t-test at a significance level of  $p < 0.05$ . The program NCSS, version 2001 was used.

### **3.4 Placentome samples from cattle for LM (n=7)**

H&E-stained slides of cattle placentomes were used for a direct micro-morphological comparison between placentomes of buffalo and cattle. Slides were available from an earlier study by Boos *et al.* (2003a) and originated from cows slaughtered during the last month of gestation as well as from cows immediately post partum. For details see Boos *et al.* (2003a).

### **3.5 Collection and tissue sampling from cattle afterbirths for scanning electron microscopy (SEM) (n=4)**

Holstein-Friesian cows used for this study were kept at the Department of Farm Animals, Clinic of Reproduction of the Vetsuisse Faculty in Zurich, Switzerland. Sample collection from fresh afterbirths and their processing for SEM was done in exactly the same way as described for buffalo. Foetal

membranes were examined for possible pathological lesions but morphological recordings were not performed due to the large amount of information available in literature.

### **3.6 Microscopic equipment and computer software**

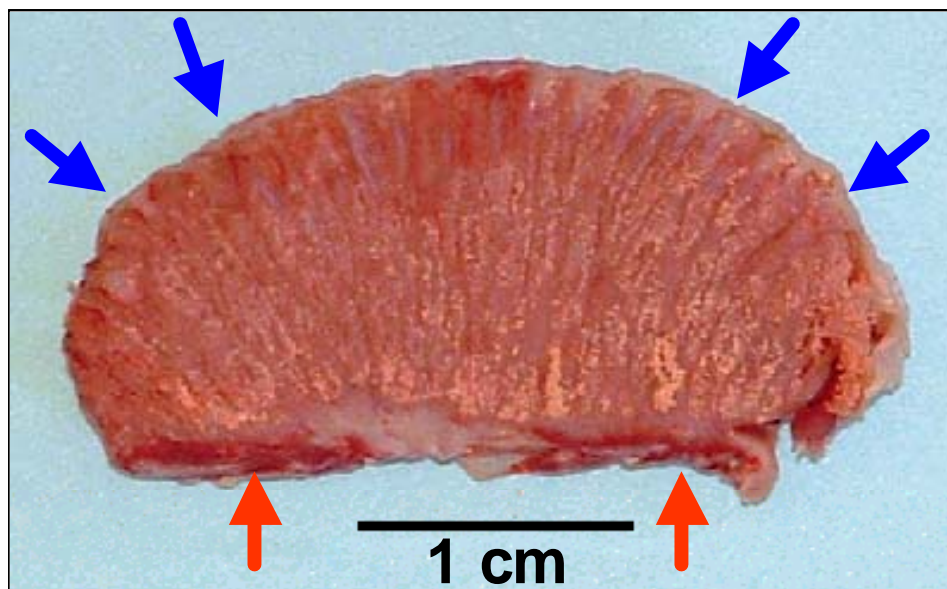
Light microscopic observations were carried out using a standard Olympus-Light Microscope. A Colorview 12® camera and the AnalySIS Prp® (version 3.2; build 757, Soft Imaging System, Münster, Germany) program were used for measurements and photo documentation. A “Philips CM 10” Transmission Electron Microscope operated at 80kV was used for ultrastructural observations. A “Philips XL 20” Scanning Electron Microscope operated at 7kV was employed for examination of SEM samples.

## 4 Results

### 4.1 Placentomes of African buffalo

#### 4.1.1 Macroscopic morphology

Placentomes were ovoid and measured approximately 4 cm in length and 2.5-3 cm in width. The cut edge at the placentome base, thus the area connecting the placentome proper to the underlying endometrium, took up most of the basal placentome surface. Placentomes were dome-shaped in cross-section, displaying a rather flat, broad base (maternal plate, MP) and a convex periphery, covered by the convex chorionic plate (CP) (Figure 4.1). One of the three collected placentomes demonstrated a minor narrowing at its base, resulting in a slight inward bending of the placentome periphery.



**Figure 4.1. Macroscopic photograph of a buffalo placentome. The dome-shaped placentome rests on a straight maternal plate (red arrows). The chorionic plate (blue arrows) covers the convex placentome like a cap and projects villous trees into corresponding caruncular crypts.**

#### 4.1.2 Morphology and villous-crypt architecture

Throughout each placentome, most chorionic villi were found within corresponding caruncular crypts, which represented the exact “negative” impression of each chorionic villus. Generally, primary (stem) villi emerged separately and mainly vertically from the chorionic plate and were arranged in parallel formation (Figure 4.2). A slightly oblique course of villous trees in relation to the maternal plate was found at the placentome periphery only. All distal villous tips ended in an approximate straight line at the straight maternal plate (Figure 4.2). Each villous tree was relatively broad along much of its length prior to narrowing slightly towards the tip. Crypt walls entirely surrounded each villous tree, thus creating “compartments” within the placentome (Figure 4.3).



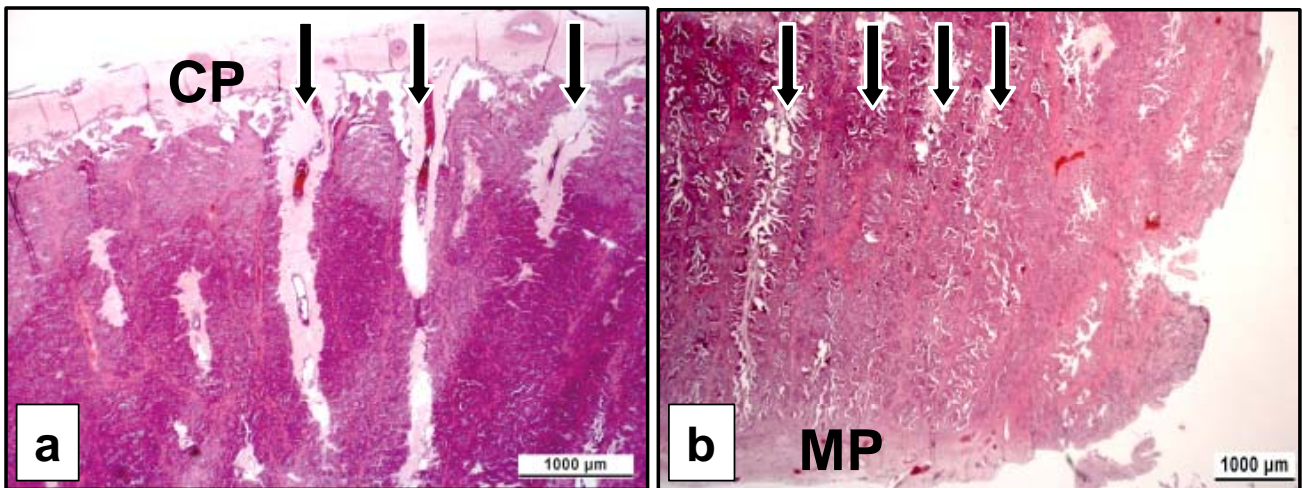


Figure 4.2. Light micrographs of buffalo placentomes. [a] placentome periphery: Chorionic villi (arrows) project separately from the chorionic plate (CP) and are arranged in parallel throughout the placentome. [b] placentome base: Chorionic villi (arrows) end in a straight line along the maternal plate (MP). (H&E)

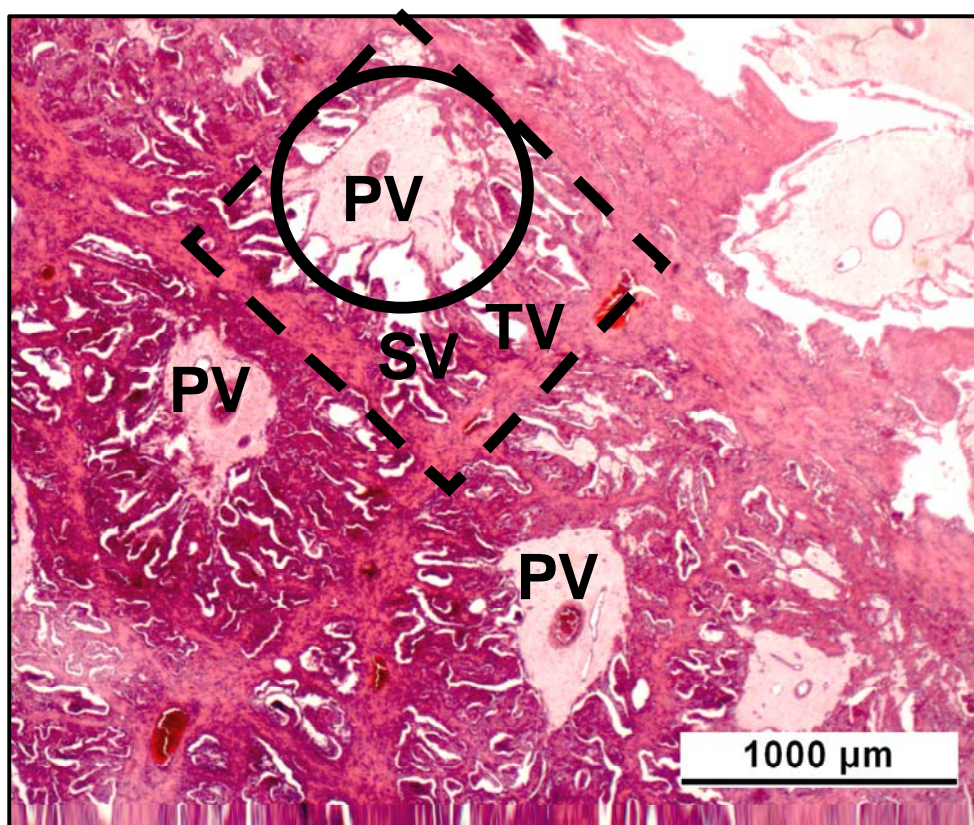
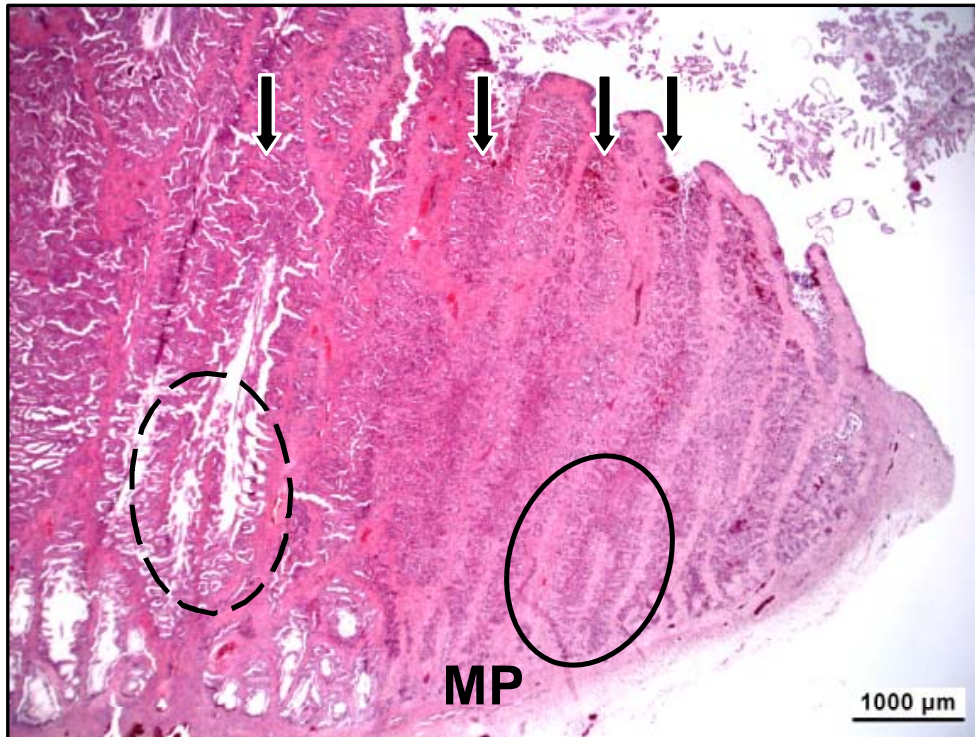


Figure 4.3. Light micrograph of a buffalo placentome in horizontal cross-section. Each primary villus (PV) with its attendant branches (secondary and tertiary villi: SV, TV) is entirely surrounded by the crypt walls (broken square), thus demonstrating compartments (main crypts) within the placentome. The primary villus has a thick villous core and occupies a large area (circle) within the available crypt space when compared to the area occupied by branches of higher order. Villous cores of secondary and tertiary villi (SV, TV) are inconspicuous due to their small diameter (H&E).

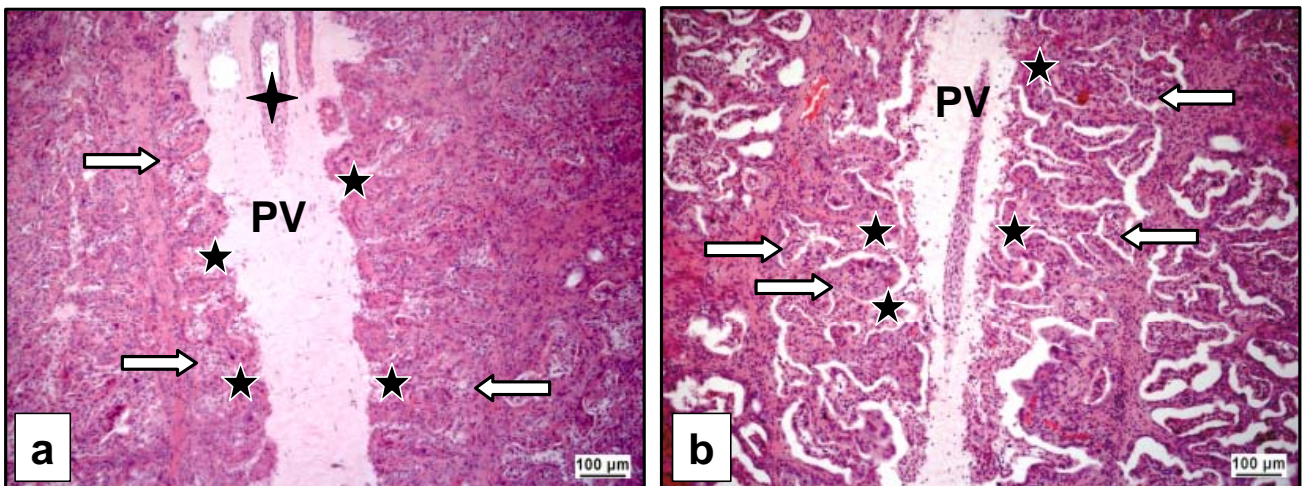
#### 4.1.3 Chorionic villous branching pattern

Some stem villi divided at an acute angle into two slightly more slender stem villi running in fetomaternal direction (Figure 4.4). Throughout the entire length of each primary villus, finger-like secondary villi branched off at approximately right angles. Secondary villi were rather short and frequently branched along their length and at their tips into tertiary structures, the terminal villi. Ramification angles of tertiary structures varied greatly (Figure 4.3 and Figure 4.5)



**Figure 4.4.** Light micrograph of a buffalo placentome. Chorionic villi (arrows) are arranged in parallel and frequently branch in an acute angle into further stem villi running in a fetomaternal direction. Villous branching occurs at any level throughout the placentome. Branching at the placentome centre (broken circle) and base (maternal plate, MP) are illustrated (continuous circle). (H&E)





**Figure 4.5. Light micrographs of single villous trees within corresponding caruncular crypts. The primary villi (PV) are broad, contain large, centrally located blood vessels (cross) and project secondary villi (stars) at approximately right angles. Tertiary villi (arrows) emerge from secondary structures in an irregular manner. Trophoblast and crypt epithelium are apposed to each other in [a], whereas this contact was lost in [b]. The gap occurring between foetal and maternal tissue in [b] is an artefact facilitating recognition of villous structures. (H&E)**

#### 4.1.4 Histology of caruncular tissue (LM and TEM)

##### LM

Caruncular tissue was composed of variably sized caruncular crypts lined by a simple epithelium and supported by a cell-rich connective tissue.

In most H&E-stained sections, intimate contact between trophoblast (TE) and crypt epithelium (CE) was lost in the majority of secondary and tertiary structures and an artificial gap of varying thickness occurred between the two epithelia (Figure 4.5.b). This facilitated the clear differentiation between villi of higher orders (Figure 4.5.b). Generally, crypt walls throughout the placentome were lined by a continuous epithelium. Crypt epithelial cells (CEC) ranged in shape from squamous to low cuboidal, containing an elongated or round nucleus, respectively (Figure 4.6). Epithelial cell height ranged from 6 µm to 15 µm with an average height of approximately 12 µm. The rather pale nuclei of cuboidal cells displayed one or more distinct nucleoli. Lateral cell borders could not be distinguished. A moderate number of epithelial cells contained two, and some cells three, round, well-separated nuclei. Intermingled between cuboidal cells were single cells with dense, pycnotic and sometimes-irregular nuclei. In sections containing crypts devoid of chorionic villi the existence of a largely continuous epithelium could be easily demonstrated (Figure 4.7). Peripheral capillaries frequently indented the epithelial basement membrane, resulting in a somewhat uneven crypt profile.

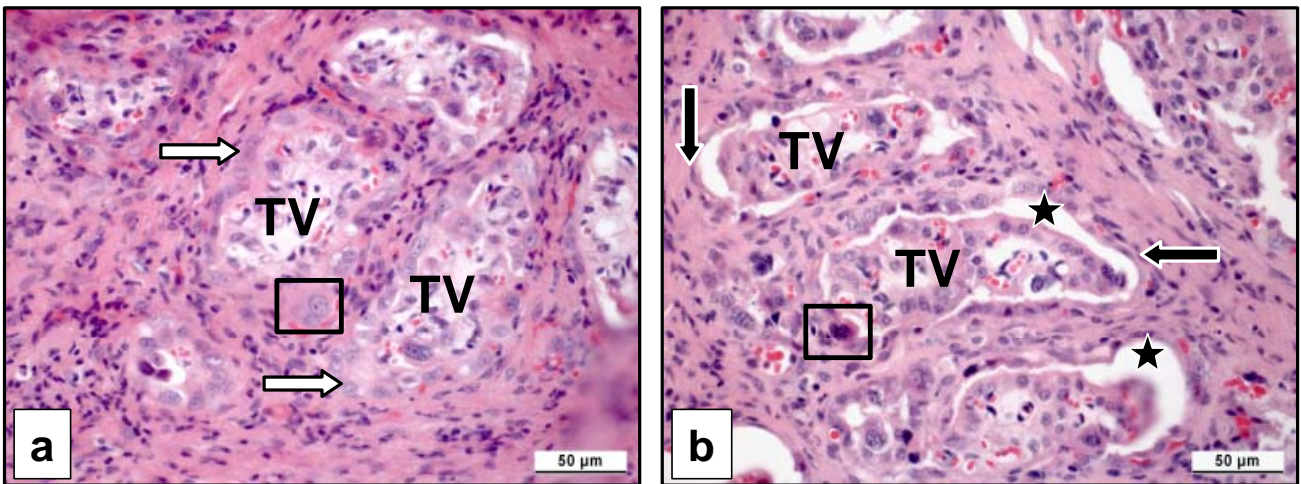


Figure 4.6. Light micrographs of tertiary villi (TV) within caruncular crypts of a buffalo placentome. Tertiary crypts are lined by a continuous epithelium of cuboidal cells with a round nucleus ([a]: white arrows) or by squamous cells with an elongated nucleus ([b]: black arrows). Multinucleate cells within the maternal epithelium are marked by a rectangle ([a] and [b]). Feto-maternal contact was artificially lost in [b] and a gap (stars) occurs between the foetal and maternal epithelium. (H&E)

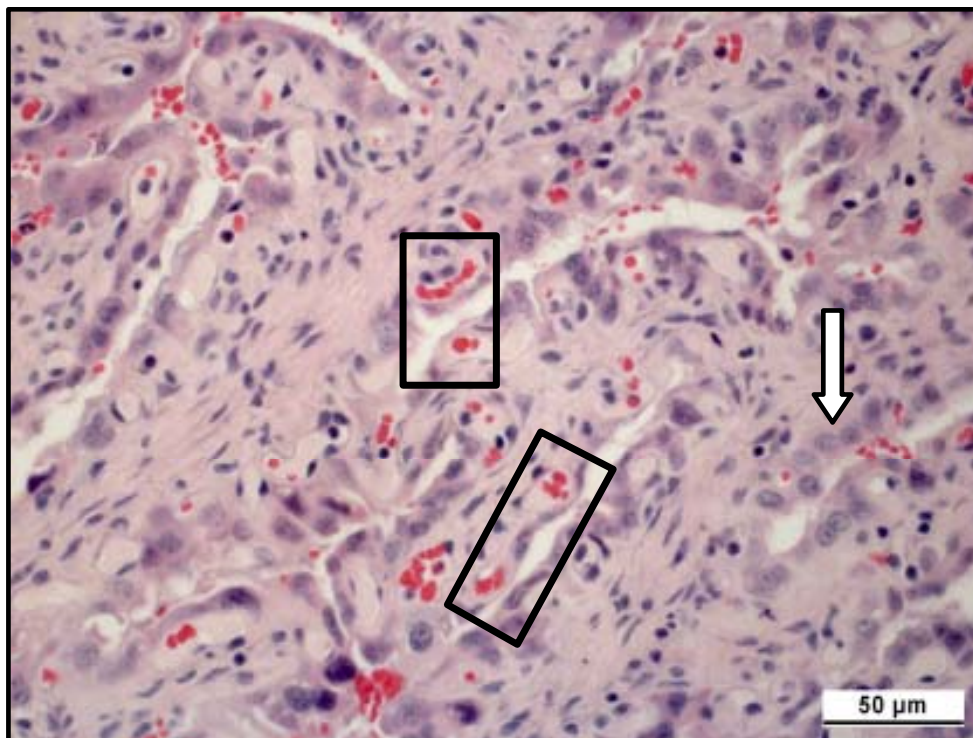
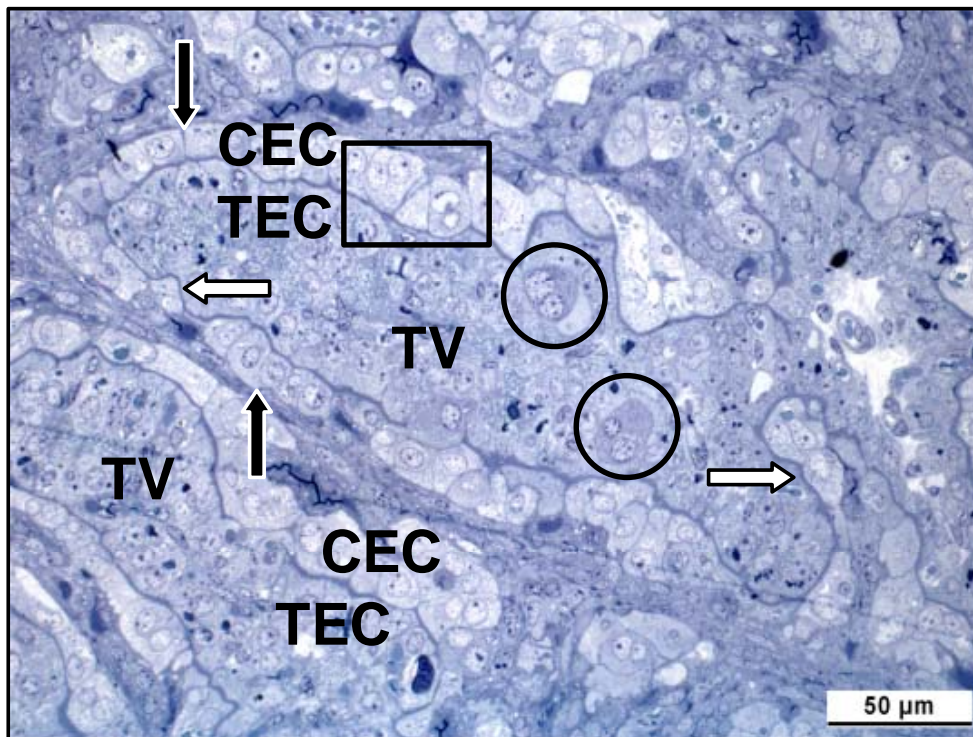


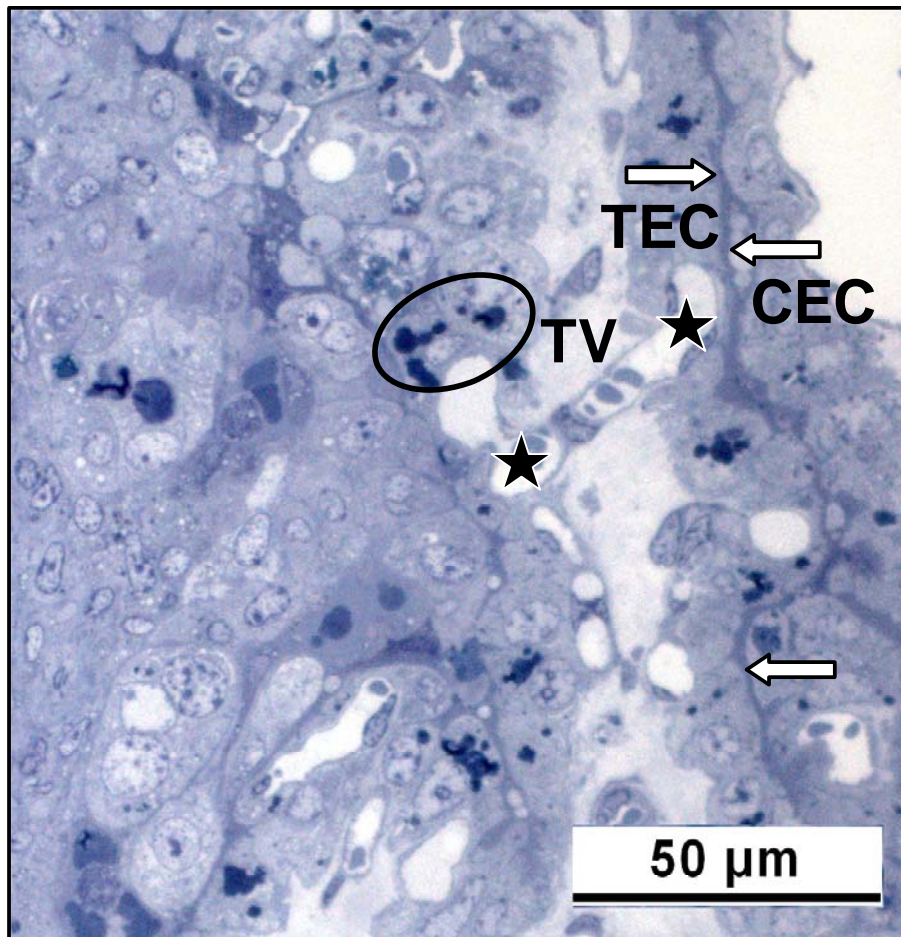
Figure 4.7. Light micrograph of empty caruncular crypts of a buffalo placentome. Crypt walls are lined by a largely continuous epithelium of cuboidal cells (arrow). Areas where subepithelial capillaries indent the maternal basement membrane are enclosed in rectangles. The latter areas are also partially lined by a squamous epithelium. (H&E)



Toluidine blue-stained (TB-stained), semi-thin sections from specimens collected for TEM demonstrated a completely different pattern when compared to most H&E-stained slides. As a rule, the TE and CE were closely apposed to each other via a distinct and intensely stained line representing the microvillar junction (MVJ) (Figure 4.8 and Figure 4.9). Distinctly visible lateral cell membranes between crypt epithelial cells clearly revealed the simple nature of the epithelium. Crypt epithelial cells generally appeared as rather large, cuboidal, pale cells with one, or sometimes more nuclei (maximum of three). Crypt epithelial cell height varied between 12 - 18.7  $\mu\text{m}$  with an average of 15  $\mu\text{m}$ . Nuclei were round to ovoid in shape and were located in the basal or central cell compartment. The light-staining cytoplasm and rather large cell size accounted for a sometimes swollen appearance of CEC. Areas of flattened or even missing epithelial cells were very rare when compared to observations on H&E-stained sections (Figure 4.8 and Figure 4.9). Subepithelial capillaries indenting the basement membrane seemed to occur intraepithelially and were termed “intraepithelial capillaries.

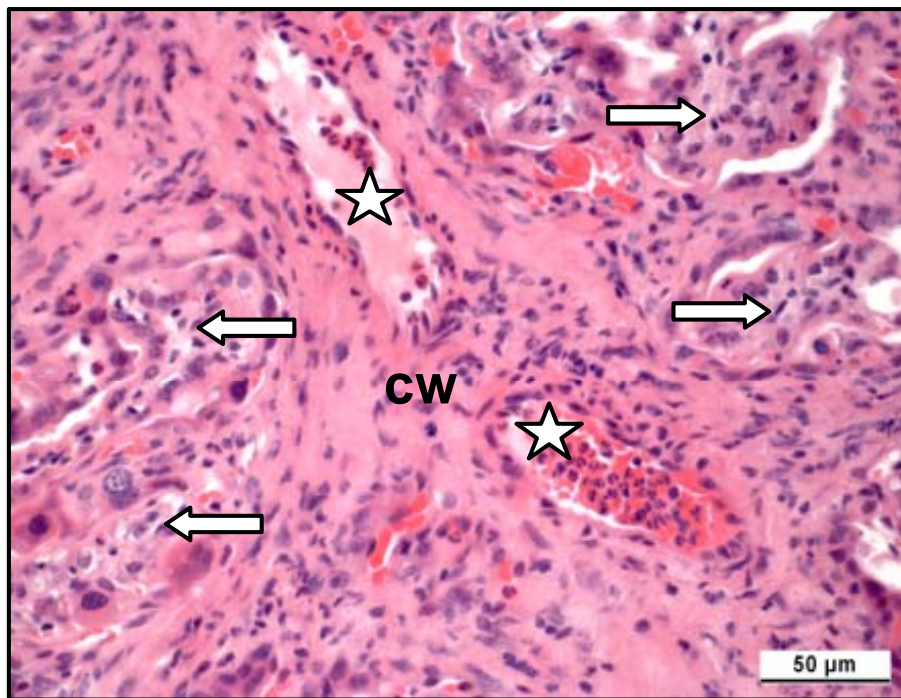


**Figure 4.8. Light micrograph of tertiary villi (TV) within caruncular crypts of a buffalo placentome. Crypt epithelial cells (CEC) stain less intensely compared to trophoblast epithelial cells (TEC). CEC often contain more than one nucleus (rectangle) and display distinct lateral cell borders (black arrows) Microvillar junctions (blue line, marked by white arrows) are clearly identifiable. Foetal binucleate cells (circles) stain darker than the surrounding uninucleate TEC. The fetomaternal contact area is undisturbed (semi-thin section, TB-stained).**



**Figure 4.9.** Light micrograph of tertiary villi (TV) within caruncular crypts of a buffalo placentome. The crypt and trophoblast epithelial cells (CEC, TEC) are closely attached via the microvillar junction (blue line marked by white arrows). A foetal capillary (stars) indents the trophoblast epithelium on both sides of the villus. Note the close proximity of the capillary wall to the MVJ, effectively forming an “intraepithelial” capillary. Dark-staining, irregular intracytoplasmic structures within the trophoblast epithelial cells are highlighted in the circle (semi-thin section, TB-stained).

The epithelial lining of the crypt walls (primary, secondary and tertiary structures) was supported by a cell-rich connective tissue. Fibrocytes contained dense, irregularly shaped nuclei. The intercellular matrix was dark staining compared to that of foetal connective tissue. Large blood vessels emanating from the maternal plate were observed deep within the walls of primary crypts. Smaller vessels (arterioles and venules) were found within the walls of secondary crypts whereas numerous capillary sections were seen close to secondary and tertiary septal surfaces. Most large blood vessels were filled with erythrocytes only but sporadically contained rather high numbers of mature granulocytes, often adherent to the vessel's wall (Figure 4.10).

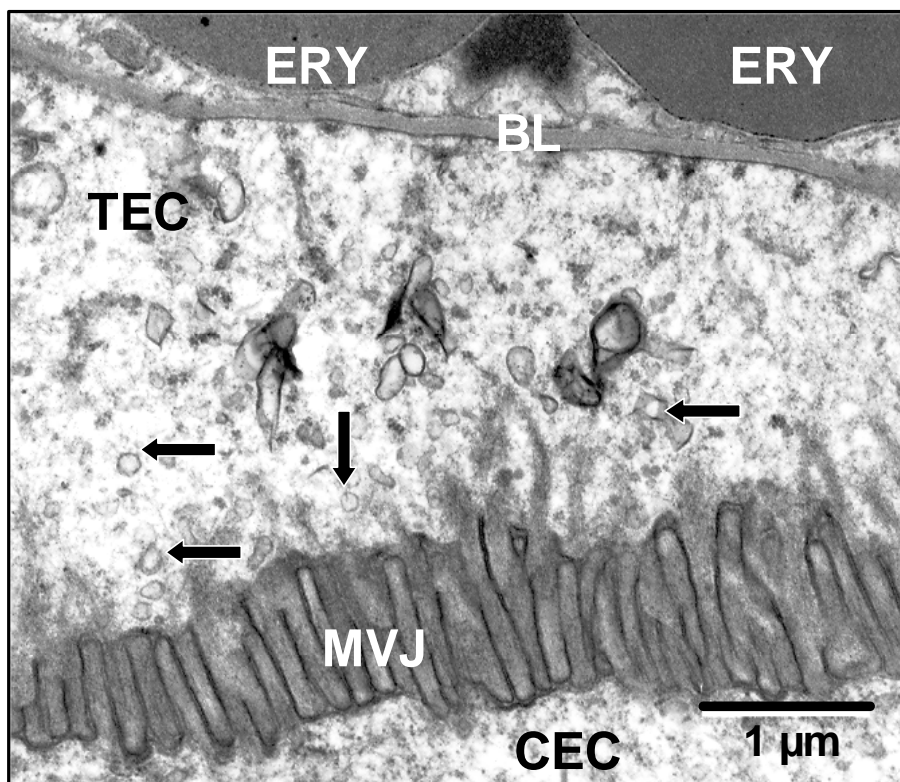


**Figure 4.10. Light micrograph of a primary crypt wall (CW) demonstrating longitudinal sections of large blood vessels (stars) containing erythrocytes and granulocytes. Arrows demonstrate tertiary foetal villi. (H&E)**



## TEM

Transmission electron microscopy revealed that the MVJ between the TEC and the CEC was represented by the intimate interdigitation of foetal and maternal microvilli (Figure 4.11). Crypt epithelial cells were very large and sometimes reached the size of up to three “corresponding” TEC. Crypt epithelial cells and TEC each rested on a distinct basal lamina (BL), which often appeared scalloped when indented by “intraepithelial” maternal/foetal capillaries (Figure 4.12). The basal lamina of the capillary and the TEC appeared to have fused as only one structure was visible (Figure 4.12). Lateral cell membranes were straight and demonstrated low numbers of indistinct desmosomes. Crypt epithelial cells had a swollen appearance, the cytoplasm was pale and contained only a few organelles. Nuclei were round and their numbers varied between one and three per cell. The cytoplasm of MNC did not differ from that of uninucleate cells. Round to ovoid mitochondria, often swollen, occurred scattered throughout the cytoplasm. Their cristae were hardly recognizable. A few profiles of small and often swollen rough endoplasmic reticulum cisternae could be observed. Multivesicular bodies and lipid droplets occurred occasionally. Small membrane whorls (myelin figures) were found in the apical part of some cells or between adjacent CEC (Figure 4.13).



**Figure 4.11. TEM micrograph demonstrating interdigitation between a trophoblast and crypt epithelial cell (TEC, CEC) of a buffalo placentome. The microvillar junction (MVJ) is formed by interdigitating microvilli on the luminal surface of both cell types. Vesicles (arrows) of various sizes occur within the apical cytoplasm of the trophoblast epithelial cell. Two erythrocytes (ERY) are visible within a foetal capillary (“intraepithelial” capillary), indenting the foetal basal lamina and closely approaching the microvillar junction. The basal laminae (BL) of the capillary and the TEC appear fused into a single structure.**

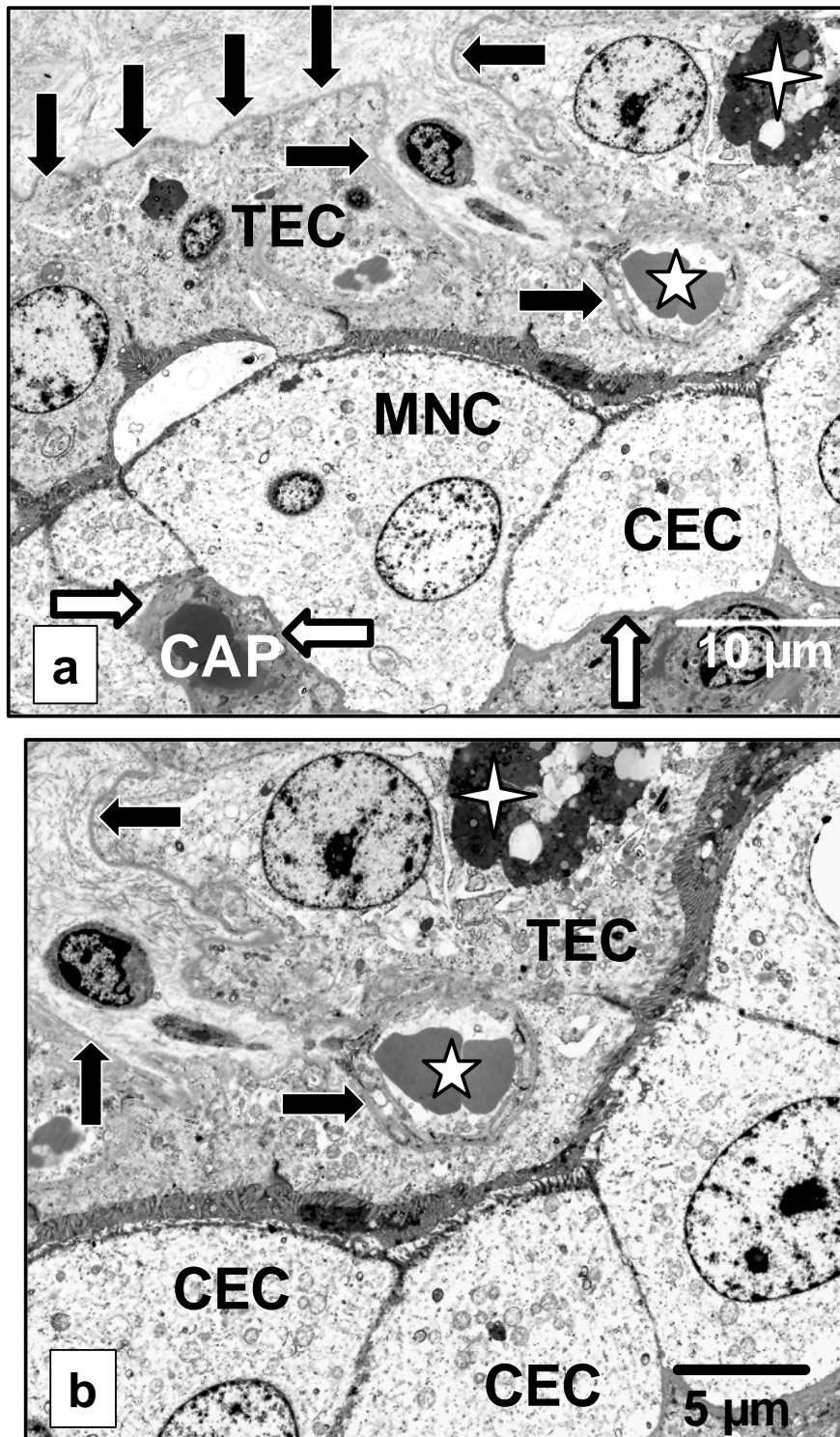
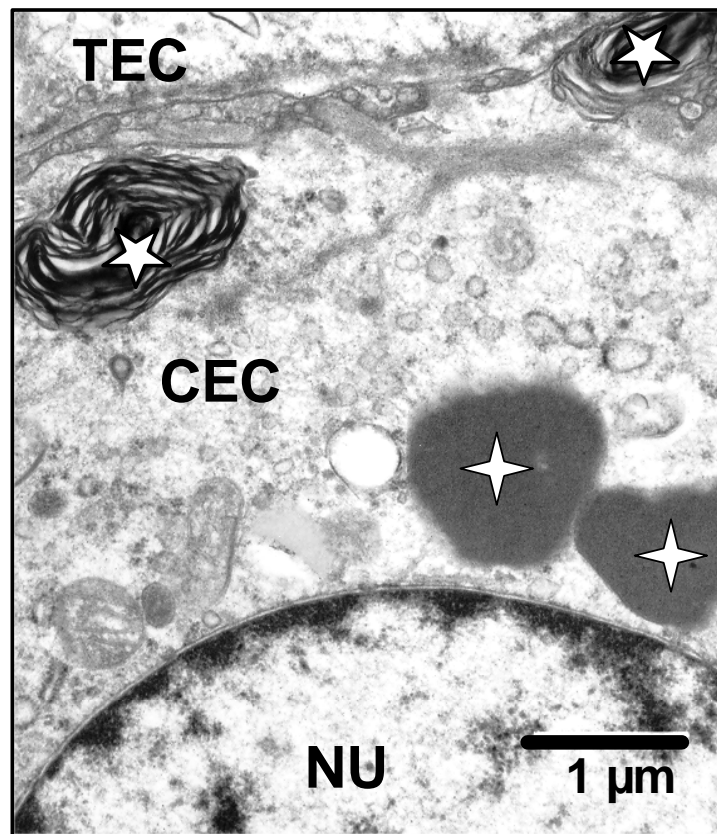


Figure 4.12. TEM micrographs of trophoblast and crypt epithelial cells of a buffalo placentome connected via a MVJ. Uni- and multinucleate crypt epithelial cells (CEC and MNC) are large and pale and have straight lateral cell membranes. [a] A maternal capillary (CAP) indents the maternal basal lamina (white arrows) and appears intraepithelial. Trophoblast epithelial cells (TEC) generally appear darker when compared to CEC ([a] and [b]). A foetal capillary (star) indents the foetal basal lamina (black arrows) and the capillary lumen comes as close as 3 µm towards the MVJ, thus reducing the distance between foetal and maternal capillaries remarkably. A large lipofuscin granule (cross) occurs within one trophoblast epithelial cell.





**Figure 4.13. TEM micrograph of parts of a crypt and trophoblast epithelial cell (CEC, TEC) of a buffalo placentome. The microvillar junction is not visible in this micrograph. Two lipid droplets can be seen (crosses) close to the crypt epithelial cell nucleus (NU). Stars indicate membrane whorls (myelin figures) within the cytoplasm.**

#### **4.1.5 Histology of cotyledonary tissue (LM and TEM)**

##### **LM**

A pale, cell-poor connective tissue constituted the core of each primary villus, which contained longitudinal profiles of large blood vessels in its centre (Figure 4.5). Very few fibrocytes were encountered and when present contained dense and elongated or pale and ovoid nuclei. Numerous sections of smaller blood vessels and capillaries were found within more cell-rich connective tissue of secondary and tertiary villi. However, the core of these villi of higher order sometimes appeared inconspicuous due to the very small villous diameter (Figure 4.3).

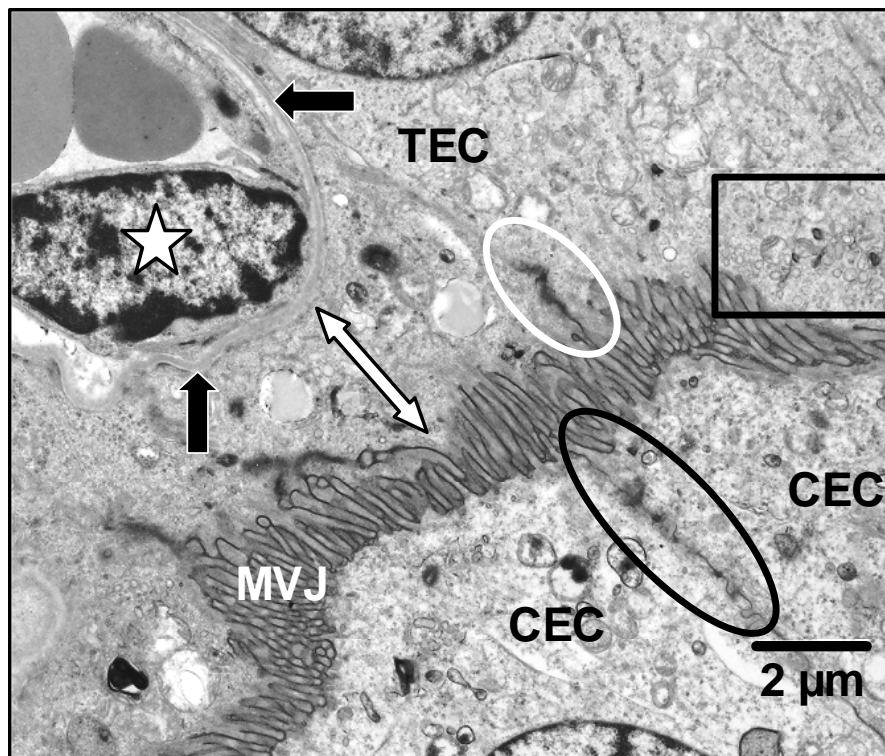
In H&E-stained sections, bizarre shapes of secondary and tertiary villi often made it difficult to give an accurate description of the irregular trophoblast epithelium. The epithelium comprised two cell types, uninucleate trophoblast epithelial cells (TEC) and binucleate cells (BNC). Trophoblast epithelial cells displayed a great variety of sizes and shapes whereas BNC were generally larger and ovoid in shape. Nuclei and cytoplasm of BNC were more intensely stained than the TEC, in both H&E (Figure 4.6) and TB-stained (Figure 4.8) sections. Few BNC were identified and occurred at various levels within



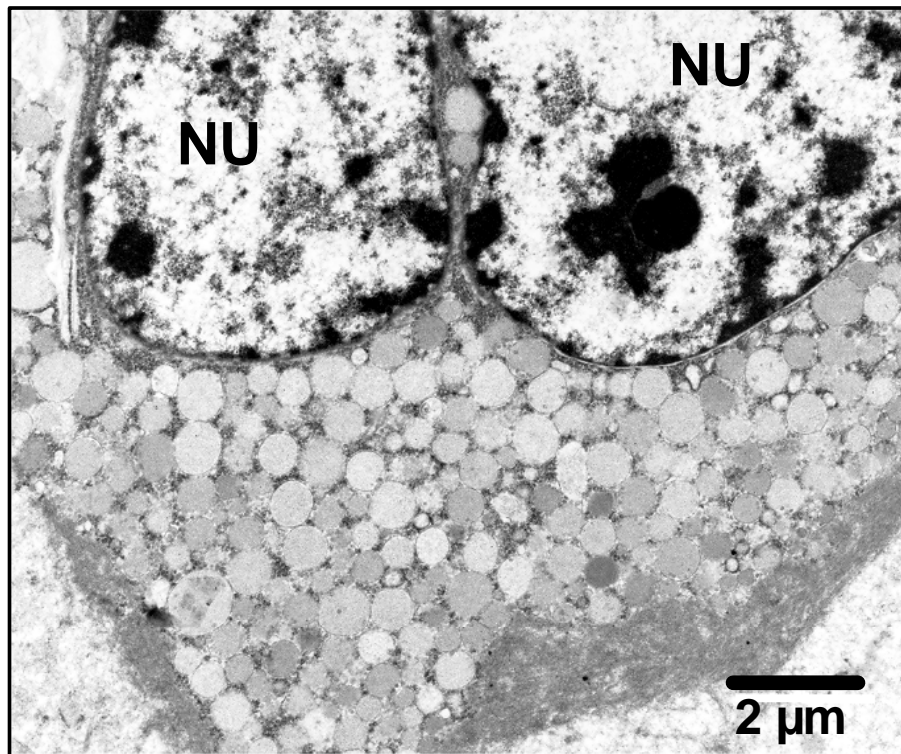
the trophoblast epithelium. A clearly visible basal lamina was lacking, which, together with the varying forms and shapes of TEC, made it difficult to assign the TE to a specific epithelial type. Numerous capillaries within the connective tissue were observed to indent the basal lamina and adopted an “intraepithelial” position, displacing the TEC in the process (Figure 4.9).

## **TEM**

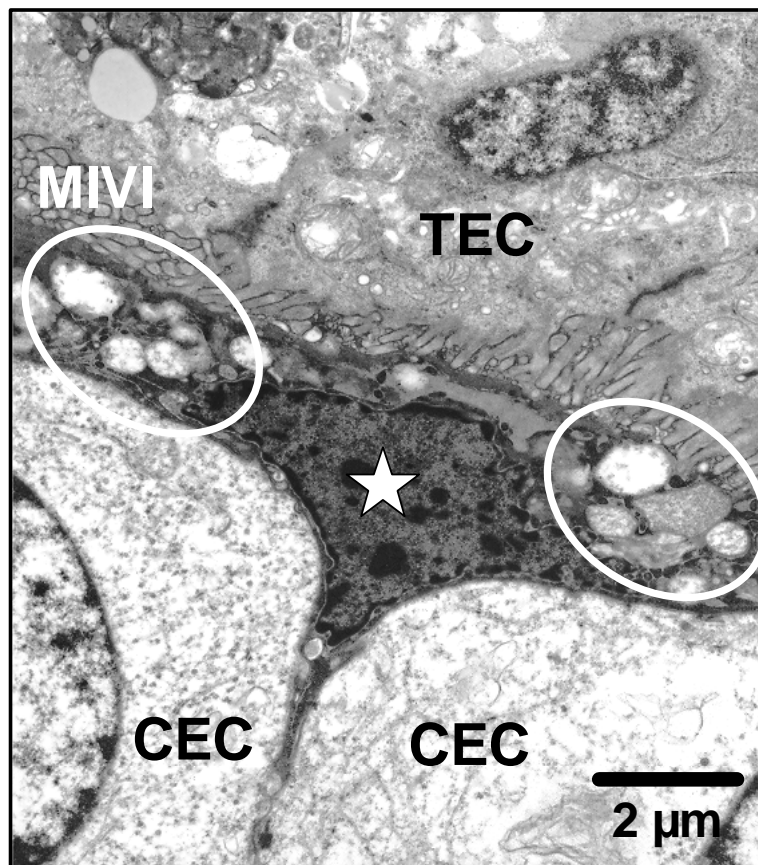
Transmission electron microscopy of the TE confirmed the great variety of TEC shapes. The basal lamina was now clearly identifiable and was often indented by capillaries, which approached as close as 2-4  $\mu\text{m}$  to the MVJ (Figure 4.12). Trophoblast epithelial cell cytoplasm was generally more electron dense when compared to CEC (Figure 4.14). The content of TEC cytoplasm differed from that in CEC in that large, irregular, dark structures occurred within numerous cells. These structures contained lipid droplets and resembled lipofuscin granules (Figure 4.12). Myelin figures were also present. In the sub-microvillous cytoplasm, numerous small vesicles could be observed (Figure 4.14). Binucleate cells were easily recognizable by their large size and dark cytoplasm. Most BNC contained moderate amounts of round to ovoid granules at either the sub- or supranuclear region. Granules were of grainy appearance and contained small numbers of microvesicles (Figure 4.15). Similar looking granules were occasionally observed within small intercellular spaces between CEC and TEC, where the MVJ appeared slightly loosened. Furthermore, pycnotic nuclei or nuclear/cytoplasmic remnants occurred intercellularly between maternal and foetal epithelium in some areas (Figure 4.16).



**Figure 4.14.** TEM micrograph of trophoblast and crypt epithelial cells connected via an intact microvillar junction (MVJ) in a buffalo placentome. Note the darker appearance of trophoblast epithelial cell (TEC) cytoplasm when compared to crypt epithelial cells (CEC). A capillary (endothelial cell nucleus, star) indents the trophoblast basal lamina (black arrows) and approaches the microvillar junction to as close as approximately 3-4  $\mu\text{m}$  (white double arrow). The basal laminae of both capillary endothelium and TEC appear fused. There are small vesicles (rectangle) within the sub-microvillous cytoplasm of trophoblast epithelial cells. Note the junctional complexes between adjacent trophoblast epithelial cells (white circle) and between crypt epithelial cells (black circle).



**Figure 4.15.** TEM micrograph of a trophoblast binucleate cell (BNC) of a buffalo placentome. Two well-separated nuclei (NU) are clearly visible within the cell. Numerous cytoplasmic granules fill the supranuclear cell compartment. The granules have a grainy appearance and occur in various sizes. Many granules contain microvesicles, the latter being too small to be recognized at this magnification.



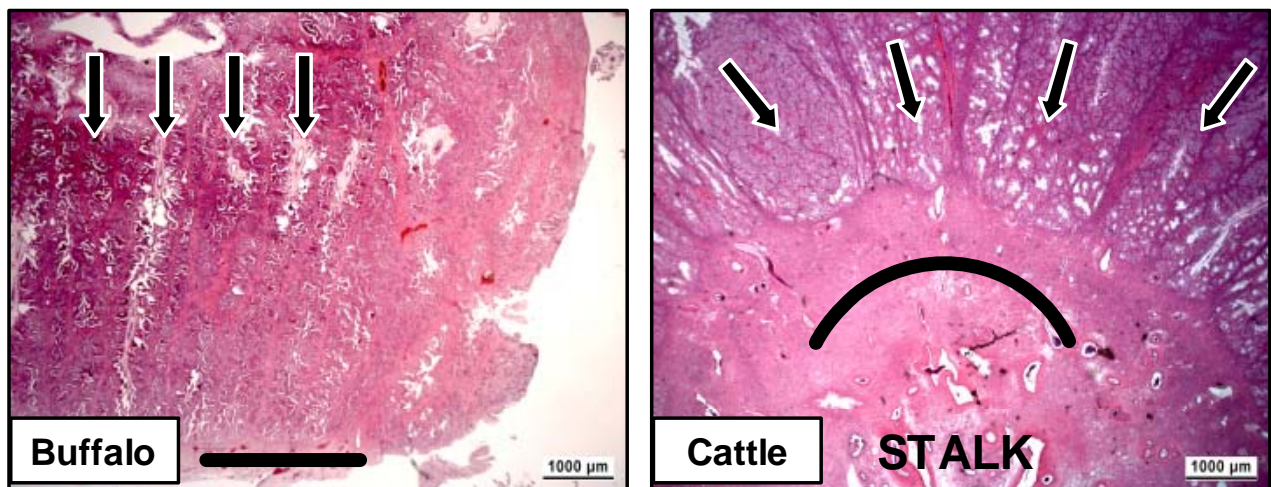
**Figure 4.16. TEM micrograph of adjacent trophoblast and crypt epithelial cells (TEC, CEC) of a buffalo placentome. Note the darker cytoplasm of trophoblast epithelial cells when compared to the crypt epithelial cells. Microvilli (MIVI) are visible on the surface of the former but the microvillar junction appears disrupted possibly due to the plane of section. A pycnotic nucleus (star) and ovoid structures resembling degenerated granules or swollen mitochondria (circles) are found in the intercellular space between trophoblast and crypt epithelium.**



## 4.2 Placentomes of cattle (peri partum) – comparative aspects

### 4.2.1 Microscopic morphology and villous-crypt architecture

The macroscopic appearance of cattle placentomes used in the present study, thus their outer shape and size were unknown, as only H&E-stained placentomal cross sections were available for observation. All cattle placentomes examined displayed a convex maternal plate, thus indicating the presence of a caruncular stalk (Figure 4.17). At the placentomal surface, each stem villus radiated separately and at an approximately right angle from the chorionic plate towards the convex maternal plate. Villous trees in the placentomal centre were therefore vertically orientated whereas those at the placentomal periphery demonstrated a horizontal or even upward direction when related to the interplacentomal endometrial surface. Villous tips thus ended in a curved line along the maternal plate. Villous bases were broad at their origin and decreased in diameter towards their tips. The narrowing of the primary villi in combination with the presence of long secondary villi emanating from the villous base, and the branching of increasingly shorter secondary villi towards the primary villous tip resulted in a rather triangular/conical shape of the entire villous tree (Figure 4.18). Secondary villi were generally longer when compared to corresponding branches in the buffalo, and were slender and well equipped with distinct, pointed tertiary branches (Figure 4.19).



**Figure 4.17.** Light micrographs of parts of a buffalo and cattle placentome. The maternal plate (black bar) appears straight in the buffalo and convex in cattle. The latter is caused by caruncular stalk formation, which was apparent in all cattle samples examined. Chorionic villi (black arrows) are arranged in parallel in the buffalo but radiate from various directions in cattle and end in a straight (buffalo) or convex (cattle) line at the maternal plate. (H&E)

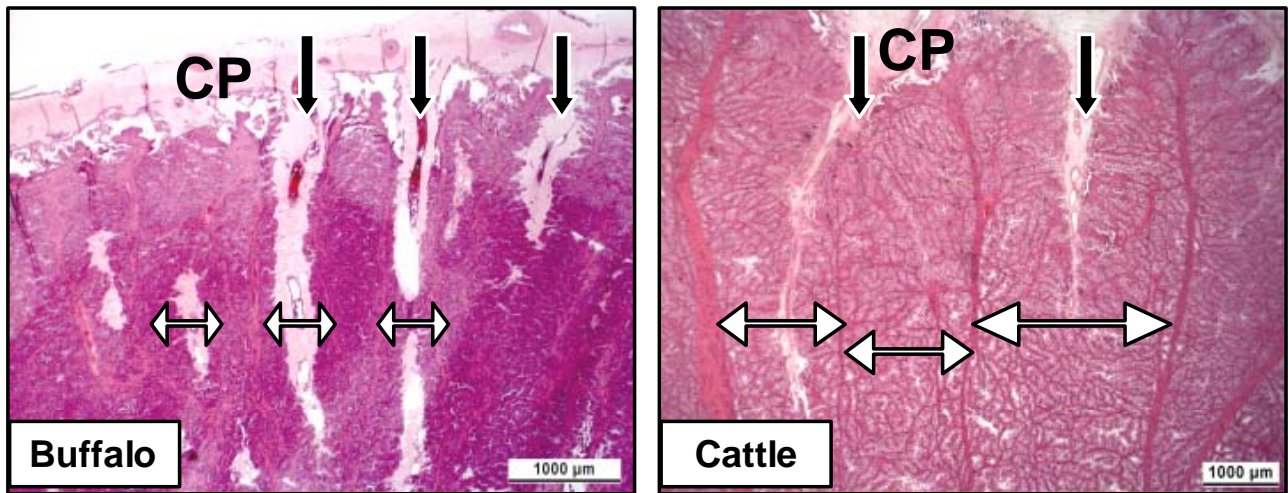


Figure 4.18. Light micrographs of villous trees within caruncular crypts of buffalo and cattle placentomes. The width of villous trees (white double arrows) varies greatly between buffalo and cattle. Note the large diameter of buffalo stem villi (black arrows) projecting rather short villi of higher order, which results in a comparably narrower villous tree. A slender villous stem (black arrows) projecting long and complexly branched secondary and tertiary villi are responsible for the wide villous diameter in cattle. (H&E)

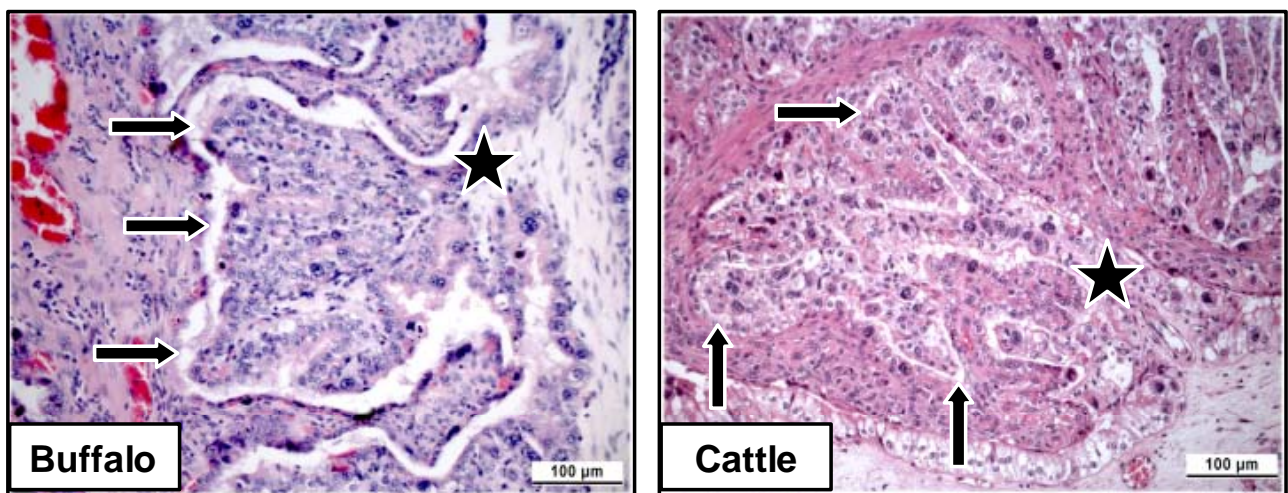
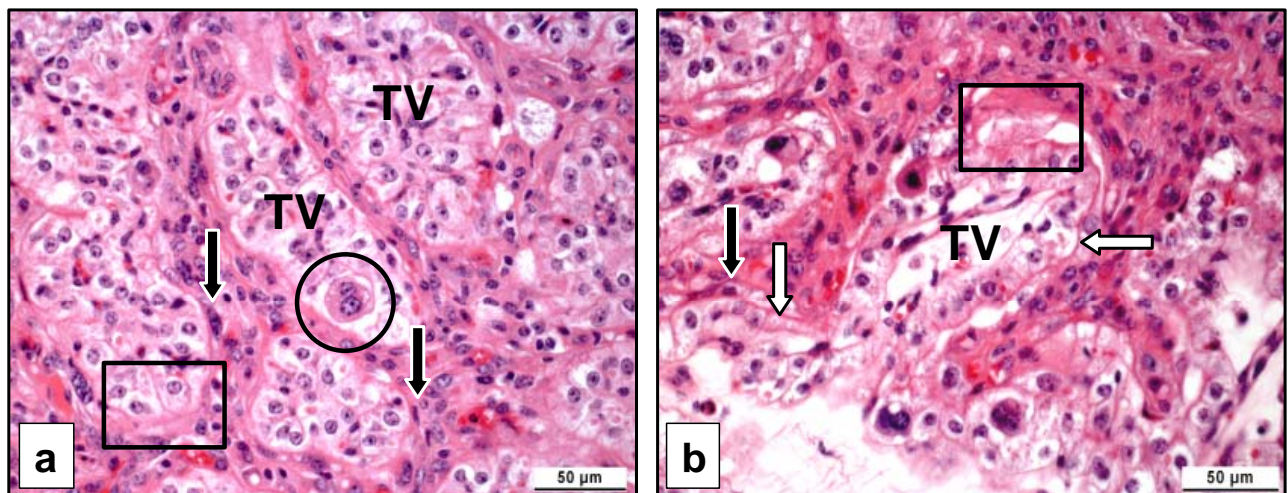


Figure 4.19. Light micrographs of chorionic villi of higher order within corresponding caruncular crypts of buffalo and cattle placentomes. Secondary (star) and tertiary (arrows) villi are broader and simpler in the buffalo when compared to the long and slender structures in cattle. Note the apparent disappearance of secondary and tertiary villous cores (connective tissue) in buffalo and cattle samples, creating the impression that higher order villi are entirely composed of trophoblast epithelium. (H&E)

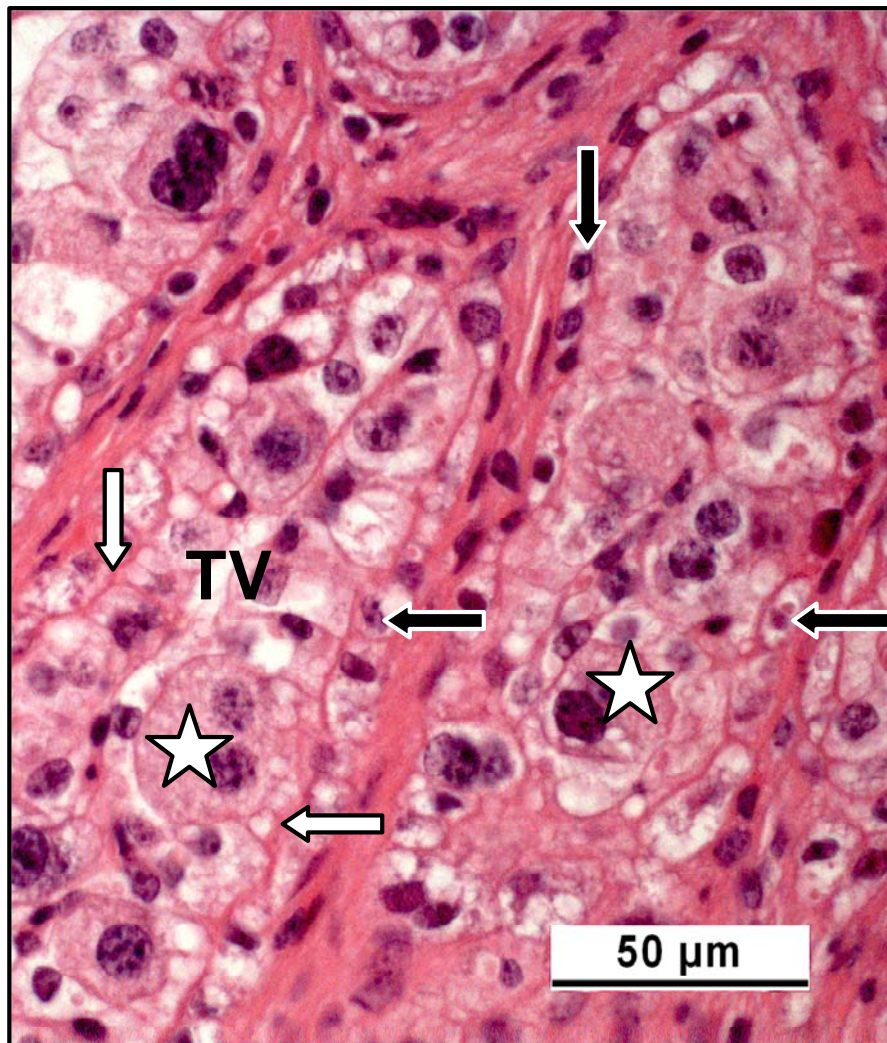


#### 4.2.2 Histology of caruncular and cotyledonary tissue (LM)

Tissues constituting the cores of the villi and crypts were similar to those described in the buffalo. Granulocytes were encountered in moderate numbers in some of the larger blood vessels. In H&E-stained specimens, the contact between foetal and maternal epithelium (when present) was still intact over most parts of the placentome. The appearance of the crypt epithelium was manifold and the recognition of CEC was very difficult in many areas. Whereas some crypts were lined by a low cuboidal or squamous epithelium, a complete lack of epithelium was frequently apparent (Figure 4.20). Crypt epithelial cell height (randomly measured in areas where the epithelium was clearly identifiable) ranged from 8.2 – 15.3  $\mu\text{m}$  (average 11.2  $\mu\text{m}$ ). Many of the nuclei of CEC were pycnotic or surrounded by a light halo. A consistent feature was the rather pale, vacuolated and mostly degenerated cytoplasm of CEC. It was uncommon to see more than one nucleus per CEC. The MVJ was clearly visible as a continuous, eosinophilic line between the TE and CE. Two cell types, the TEC and the BNC constituted the TE (Figure 4.21). Both cell types displayed the typical features described in the buffalo.



**Figure 4.20. Light micrographs of tertiary villi within caruncular crypts of cattle placentomes. The trophoblast epithelium is in close contact with the crypt epithelium (when present) throughout all [a] or most [b] of the tertiary villi (TV). The microvillar junction is visible as a distinct, red line (white arrows in [b]). Crypt epithelial cells are squamous (black arrows) or apparently entirely lacking over short distances (rectangles). A binucleate cell is marked (circle) within the trophoblast epithelium in a region where the maternal epithelium is again inconspicuous. (H&E)**



**Figure 4.21.** Light micrograph of tertiary villi (TV) within caruncular crypts of a cattle placentome. A cuboidal crypt epithelium can be identified over large parts of the tertiary crypt walls. However, crypt cell nuclei appear pycnotic and are often surrounded by a light halo (black arrows), thus giving the CEC a vacuolated appearance. A distinct MVJ is visible (white arrows). BNC (stars) are present within the trophoblast epithelium. (H&E)



### 4.3 Macroscopic description of the buffalo afterbirth

#### 4.3.1 General information

All foetal membranes (n=7) were delivered naturally between 2 to 3.5 hours after birth of a healthy buffalo calf. One afterbirth was so badly torn that only some areas of the pregnant horn were available for investigation. Data from this specimen were therefore not included in the presented graphs but selected cotyledons are demonstrated in Figure 3.26. In two of the six complete foetal membranes, an exact separation between the pregnant and the non-pregnant horn could not be performed and corresponding data are therefore lacking.

To closely examine the cotyledons on the foetal membranes of the buffalo and the domestic cow it was sometimes necessary to turn single cotyledons inside out in order to place them flat on the underlying surface. During this procedure, differences in outer shape between buffalo and cattle cotyledons were encountered. While buffalo cotyledons could be turned inside out without any difficulties, certain force was necessary to achieve the same effect on the cotyledons of cattle (see Figure 5.1 under Discussion).

#### 4.3.2 Number and size of cotyledons on buffalo foetal membranes

Total numbers of cotyledons as well as the number per horn are given in Table 4.1. Note the great variation in total numbers of cotyledons from 89 to double that number. Two foetal membranes contained slightly more cotyledons in the pregnant compared to the non-pregnant horn (animals 2 and 6) whereas the opposite was true in the two remaining membranes (animals 1 and 3). On average, the pregnant horn contained 50.3 % of all cotyledons.

<b>Animal</b>	<b>Cotyl total</b>	<b>Cotyl ph</b>	<b>Cotyl np</b>	<b>Cotyl</b>
<b>No</b>	<b>No</b>	<b>No</b>	<b>No</b>	<b>ph / np %</b>
<b>1</b>	<b>89</b>	<b>42</b>	<b>47</b>	<b>47.2 / 52.8</b>
<b>2</b>	<b>109</b>	<b>59</b>	<b>50</b>	<b>54.1 / 45.9</b>
<b>3</b>	<b>115</b>	<b>57</b>	<b>58</b>	<b>49.5 / 50.5</b>
<b>4</b>	<b>156</b>			
<b>5</b>	<b>168</b>			
<b>6</b>	<b>178</b>	<b>90</b>	<b>88</b>	<b>50.5 / 49.5</b>
<b>Average</b>	<b>136</b>	<b>62</b>	<b>61</b>	<b>50.3 / 49.7</b>

**Table 4.1. Total number of cotyledons (Cotyl total), number of cotyledons on the pregnant horn (Cotyl ph), number of cotyledons on the non-pregnant horn (Cotyl np) and the ratio of the number of cotyledons between the ph and the np expressed as a percentage (cotyl ph / np %).**

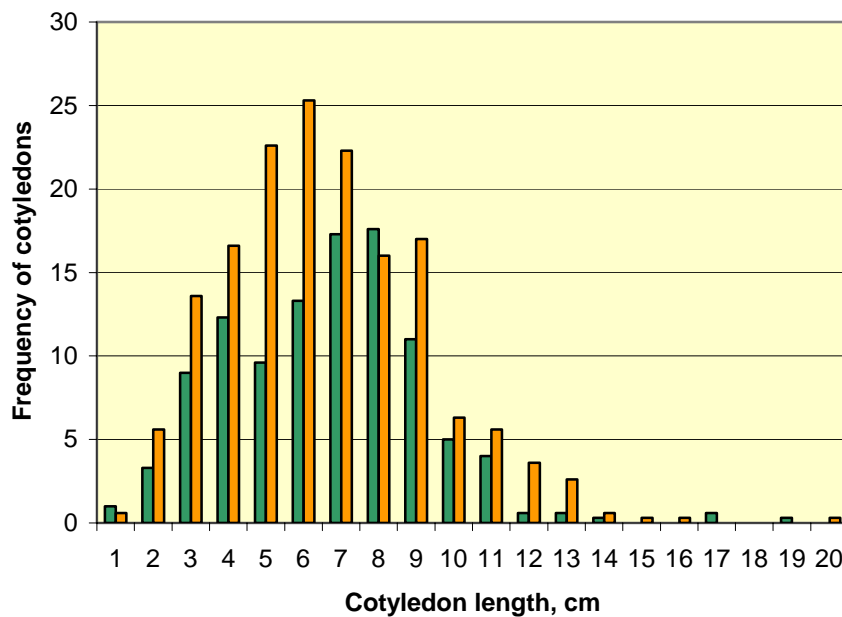
The sizes of cotyledons (length and width) are given in Table 4.2. Although displaying a rather consistent average length and width within “total cotyledons”, a distinct difference of 1.4 - 2.3 cm in cotyledonary length and 0.8 - 1.9 cm in cotyledonary width is demonstrated between cotyledons in the pregnant and those in the non-pregnant horns, respectively.

<b>Animal</b>	<b>Cotyl total</b>	<b>Cotyl ph</b>	<b>Cotyl np</b>
<b>No</b>	<b>Average size in cm</b>		
<b>1</b>	<b>6.8 x 4.5</b>	<b>7.5 x 5.5</b>	<b>6.1 x 3.6</b>
<b>2</b>	<b>7.2 x 3.9</b>	<b>8.1 x 4.8</b>	<b>6.3 x 3.1</b>
<b>3</b>	<b>6.5 x 3.8</b>	<b>7.0 x 4.2</b>	<b>6.0 x 3.4</b>
<b>4</b>	<b>6.4 x 3.7</b>		
<b>5</b>	<b>6.7 x 4.3</b>		
<b>6</b>	<b>6.7 x 3.8</b>	<b>7.9 x 4.5</b>	<b>5.6 x 3.2</b>
<b>Average</b>	<b>6.7 x 4.0</b>	<b>7.6 x 4.7</b>	<b>6.0 x 3.3</b>

**Table 4.2. Average size (length/width) of cotyledons in both horns (Cotyl total), in the pregnant (Cotyl ph) and in the non-pregnant horn (Cotyl np).**

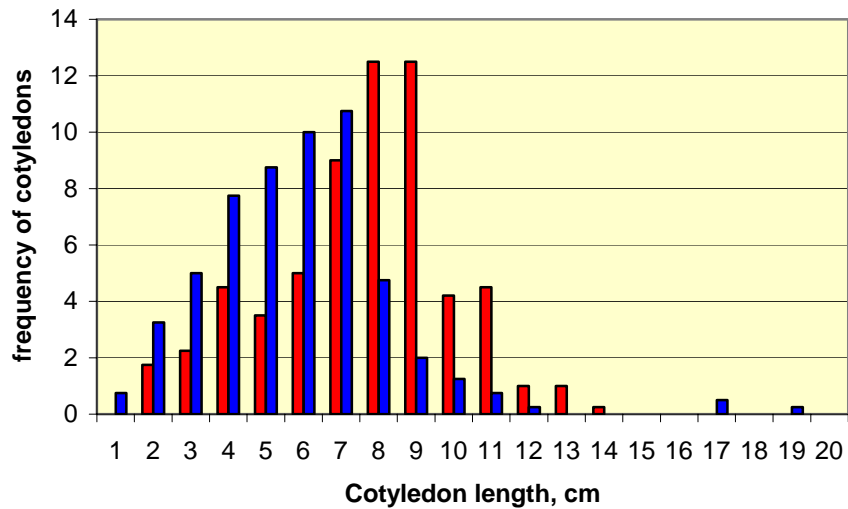
**4.3.3 Distribution of different cotyledonary lengths in relation to total number of cotyledons in buffalo foetal membranes**

In order to compare cotyledonary lengths in relation to the frequency of their occurrence, animals 1, 2 and 3 were grouped to represent foetal membranes with an average total number of 104.3 cotyledons, and animals 4, 5 and 6 were grouped to represent foetal membranes with an average total number of 167.3 cotyledons. Frequency of cotyledons in relation to their length is illustrated in Graph 4.1. In foetal membranes with an average of 104.3 cotyledons, the most frequently occurring cotyledons measured between 8.0 and 8.9 cm in length whereas those on foetal membranes with an average of 167.3 cotyledons, measure between 6.0 and 6.9 cm in length.



**Graph 4.1. Distribution of cotyledons according to their average length. Animals 1, 2 and 3 are grouped representing foetal membranes with an average total number of 104.3 cotyledons (green). Animals 4, 5 and 6 are grouped representing foetal membranes with an average total number of 167.3 cotyledons (orange).**

Distribution of cotyledons between the pregnant and the non-pregnant horn in relation to their length is demonstrated in Graph 4.2.



**Graph 4.2. Distribution of cotyledons between the pregnant (red) and the non-pregnant (blue) horn in relation to their average length.**

Cotyledons longer than 8 cm occurred more frequently in the pregnant horn than the non-pregnant horn whereas the opposite was true for cotyledons smaller than 8 cm (Graph 3.2). However, the largest cotyledon (length=19.5 cm) was found in the non-pregnant horn. In general, cotyledonary size was largest in the centre and decreased towards the tip of each horn.

#### 4.3.4 Arrangement and shapes of cotyledons in buffalo foetal membranes

In all afterbirths examined, cotyledons occurred throughout the entire harvested foetal membranes, that is, they were encountered in areas of the membrane corresponding to the base, centre and tip of each horn. A clear recognition of the part corresponding to the corpus uteri was generally impossible and cotyledons found in this region were therefore included as part of the base of the horn. Necrotic tips of the membranes were never observed. Along the centre of both uterine horns, the majority of cotyledons were arranged in an orderly manner in the form of two mesometrial and two antimesometrial rows (Figure 4.22, Figure 4.23 and Figure 4.24). Fewer rows of cotyledons as well as a random distribution of cotyledons occurred at the base and towards the tips of some horns (Figure 4.24).

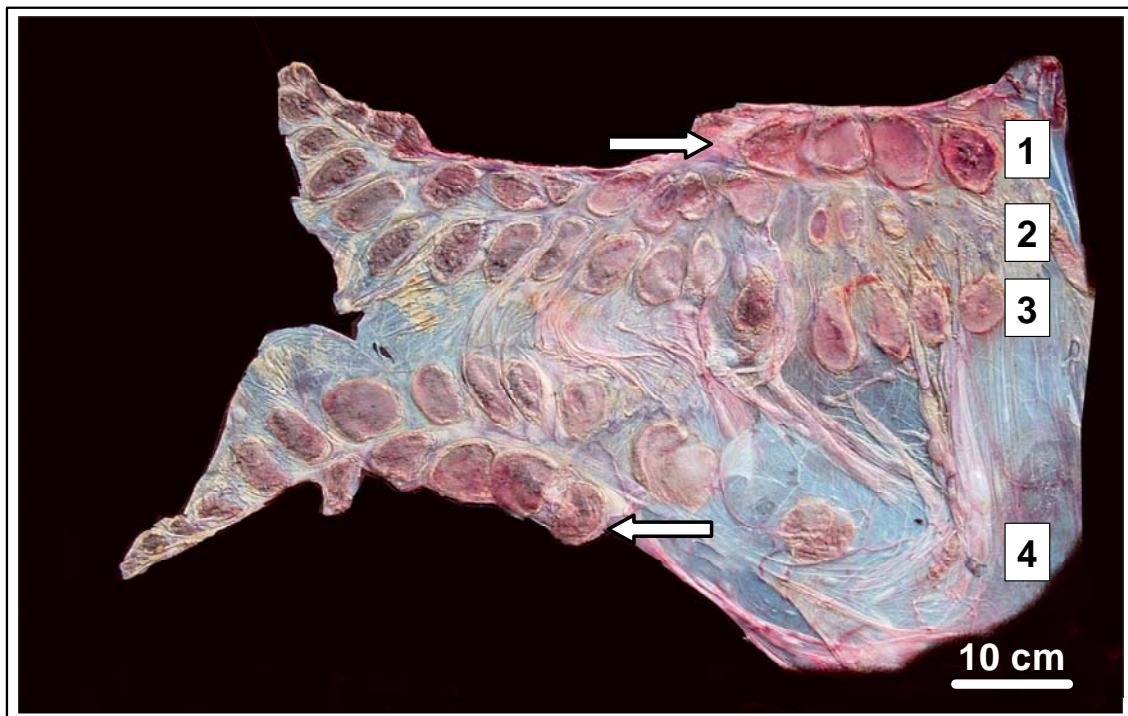


Figure 4.22. Photograph of the pregnant horn of an expelled buffalo foetal membrane. Four rows of cotyledons (numbers 1 – 4) are apparent throughout the horn. The disruption of cotyledonary row number 1 is artificial (white arrows).



Figure 4.23. Photograph of the non-pregnant horn of an expelled buffalo foetal membrane. Four rows of cotyledons occupy the base (left) and the central part of the horn. Three indistinct rows are visible in the tip (right) of the horn. Numbers 1 – 4 indicate rows.

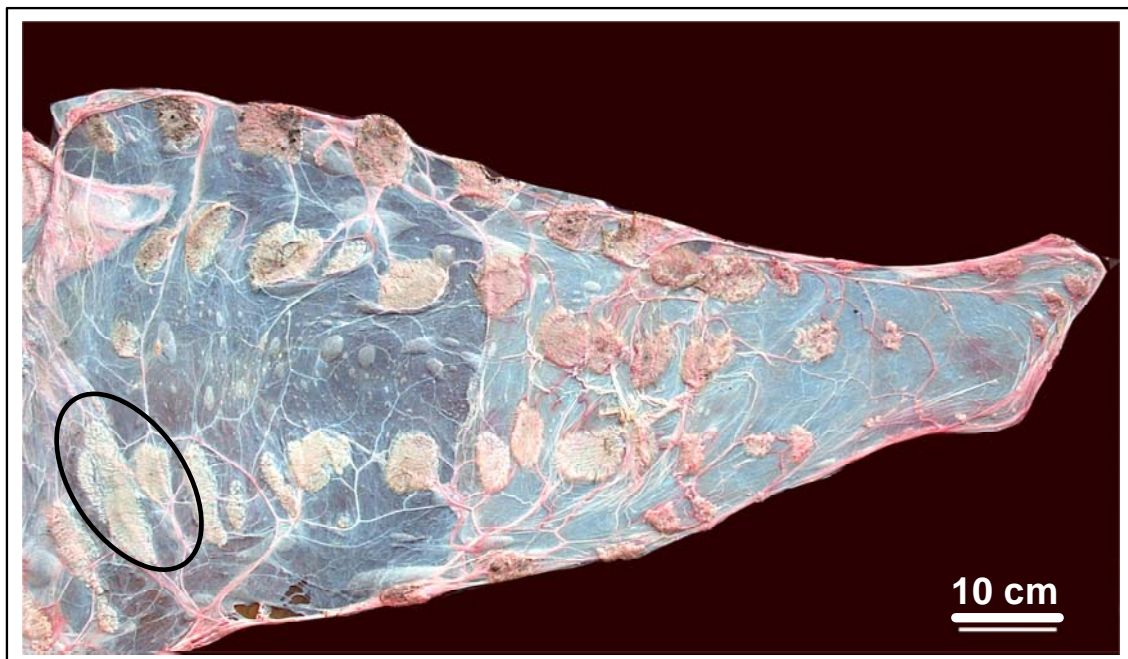
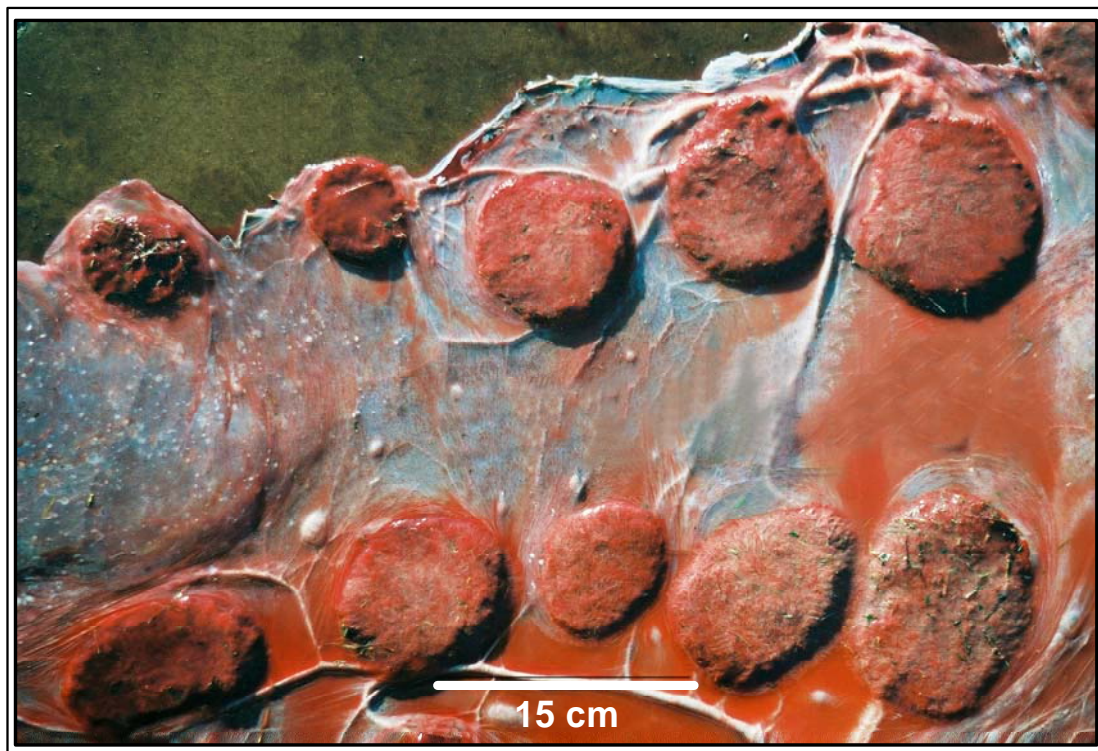


Figure 4.24. Photograph of the non-pregnant horn of an expelled buffalo foetal membrane. Variously shaped cotyledons arranged in an irregular manner are demonstrated. Four rows can be recognized along the centre of the horn but no distinct rows occur at the base (left) or the tip (right) of the horn. Enlargements of the elongated, pale cotyledons in the proximal horn (encircled) are illustrated in Figure 4.27.

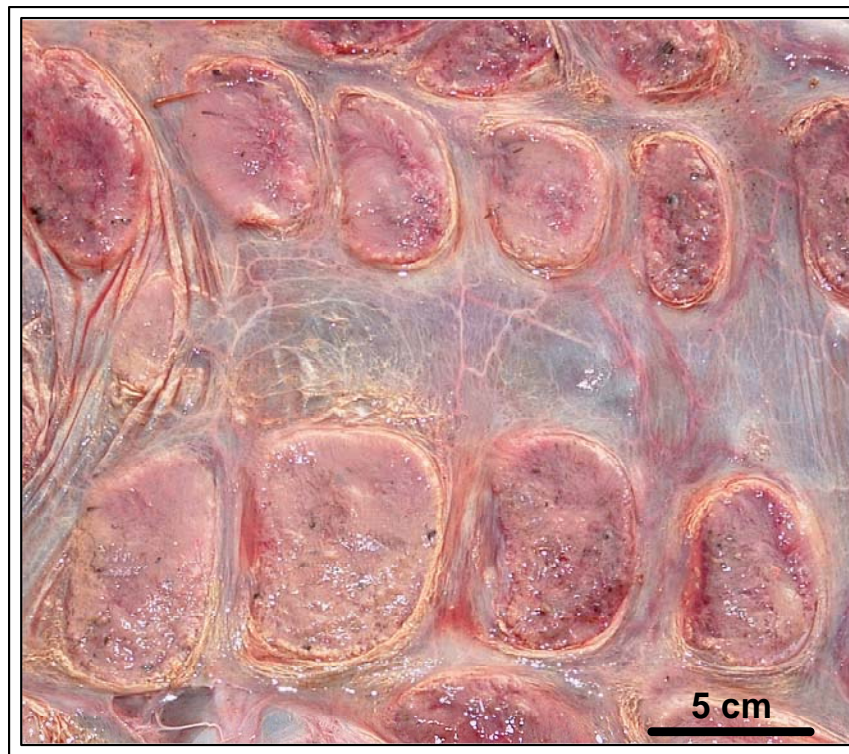


The most common shape of cotyledons was oval and elongated, but round and quadrilateral forms also occurred (Figure 4.25 and Figure 4.26). Fusion between two or more neighbouring cotyledons was observed, resulting in bizarrely-shaped cotyledons. The latter type is demonstrated in Figure 4.29, where a figure eight-shaped cotyledon resulted from the fusion of two neighbouring oval cotyledons. In Figure 4.28, two adjacent cotyledons appear to have fused. However, a small strip of smooth allantochorion was encountered between the two structures when the smaller cotyledon was carefully removed. Orientation of oval and elongated cotyledons was perpendicular to the longitudinal axis of the uterine horns in all foetal membranes studied (Figure 4.22, Figure 4.23 and Figure 4.24)

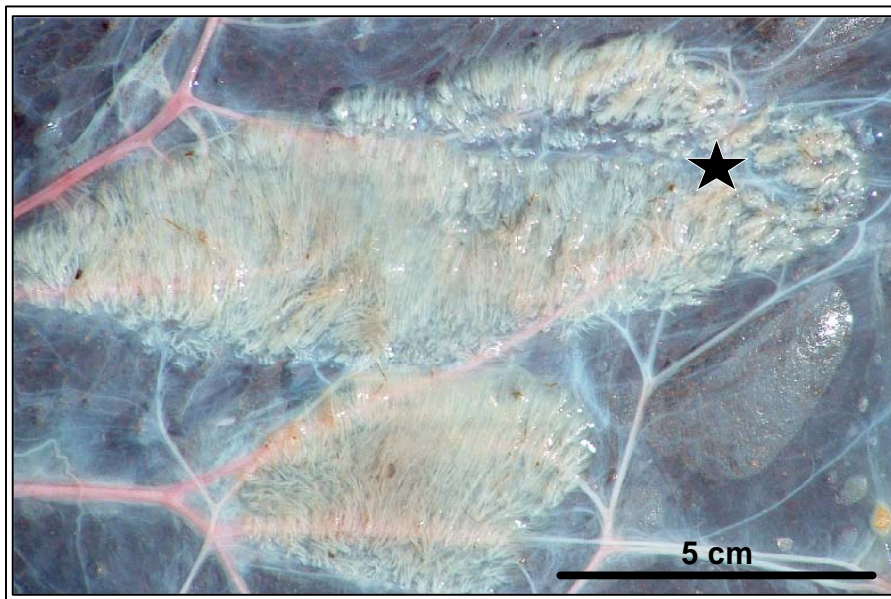
Most cotyledons were well demarcated from the surrounding smooth intercotyledonary membrane, measured approximately 0.5-1 cm in thickness and lay flat on the membrane surface. The majority of cotyledons had a fleshy appearance, as seen in Figure 4.25 and Figure 4.28. Some cotyledons, however, appeared thin and discontinuous as illustrated in Figure 4.24 and Figure 4.27, where chorionic villi are partly lacking on one cotyledon. In contrast, in four of six membranes chorionic villi occurred in a scattered fashion over variably sized areas without forming distinct, demarcated cotyledons. Very small, round to oval accumulations of chorionic villi also occasionally occurred in some of the membranes examined. Such areas were not included as data on cotyledonary size and distribution.



**Figure 4.25. Photograph of round and oval cotyledons in the non-pregnant horn of an expelled buffalo foetal membrane.**



**Figure 4.26.** Photograph of round and oval cotyledons in the pregnant horn of an expelled buffalo foetal membrane.



**Figure 4.27.** Photograph of incompletely formed cotyledons of a buffalo foetal membrane (enlargement of marked cotyledon of Figure 4.24). The elongated cotyledon on top demonstrates areas with incomplete or no chorionic villi (star). Both cotyledons occurred within a cotyledonary row.



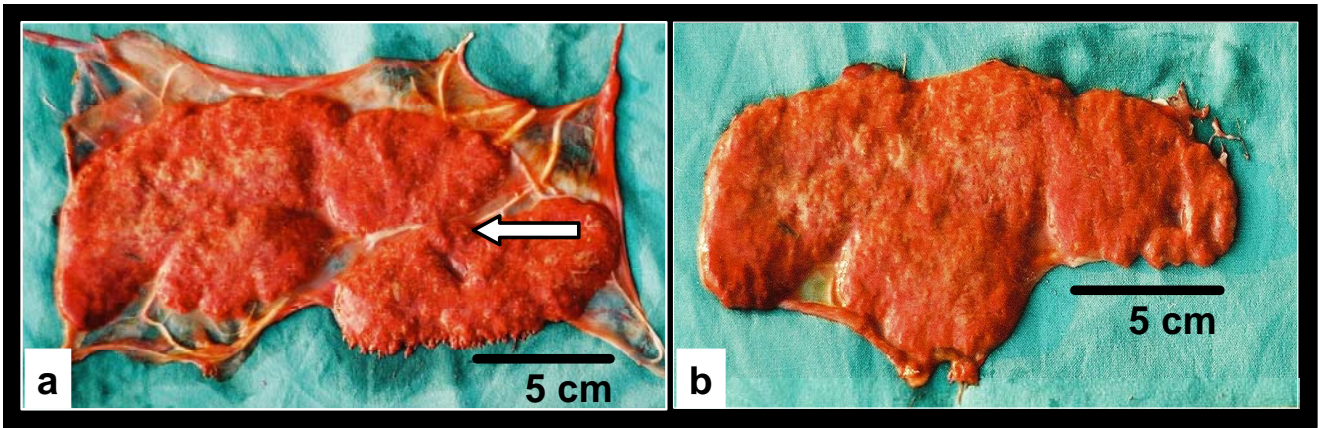


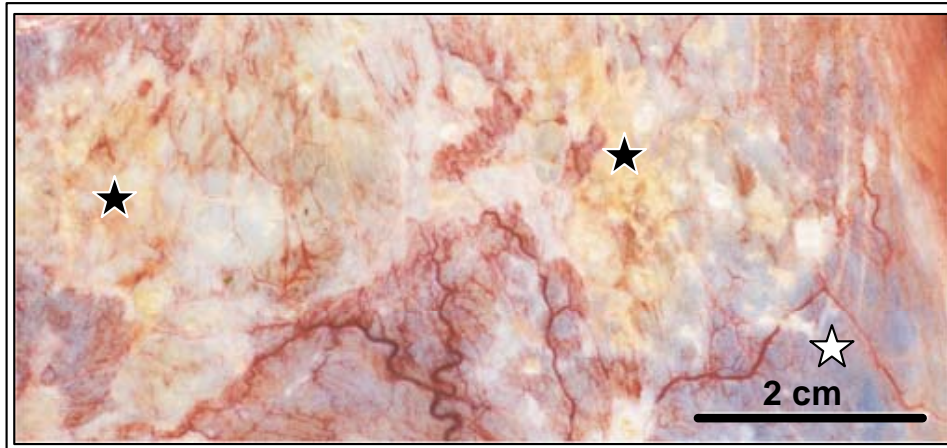
Figure 4.28. Photograph of cotyledons of an expelled buffalo foetal membrane. A misshapen cotyledon [a] demonstrating “pseudofusion” with an adjacent ovoid cotyledon. The arrow points to the possible area of fusion, but a small strip of smooth chorioallantois could be detected between the two structures. The same misshapen cotyledon after removal of the neighbouring structure is shown in [b].



Figure 4.29. Photograph of a figure eight-shaped cotyledon of an expelled buffalo foetal membrane. The particular shape is the result of fusion (arrow) between two adjacent cotyledons.

#### 4.3.5 Intercotyledonary areas and specific structures

The intercotyledonary chorioallantois had a smooth, transparent appearance over large parts of the foetal membranes. Five of the six samples displayed white to yellow proliferations on the smooth chorionic surface. The plaques were sometimes extensive in nature (Figure 4.30) or occurred as small units creating a grainy appearance on the surface of the membrane.



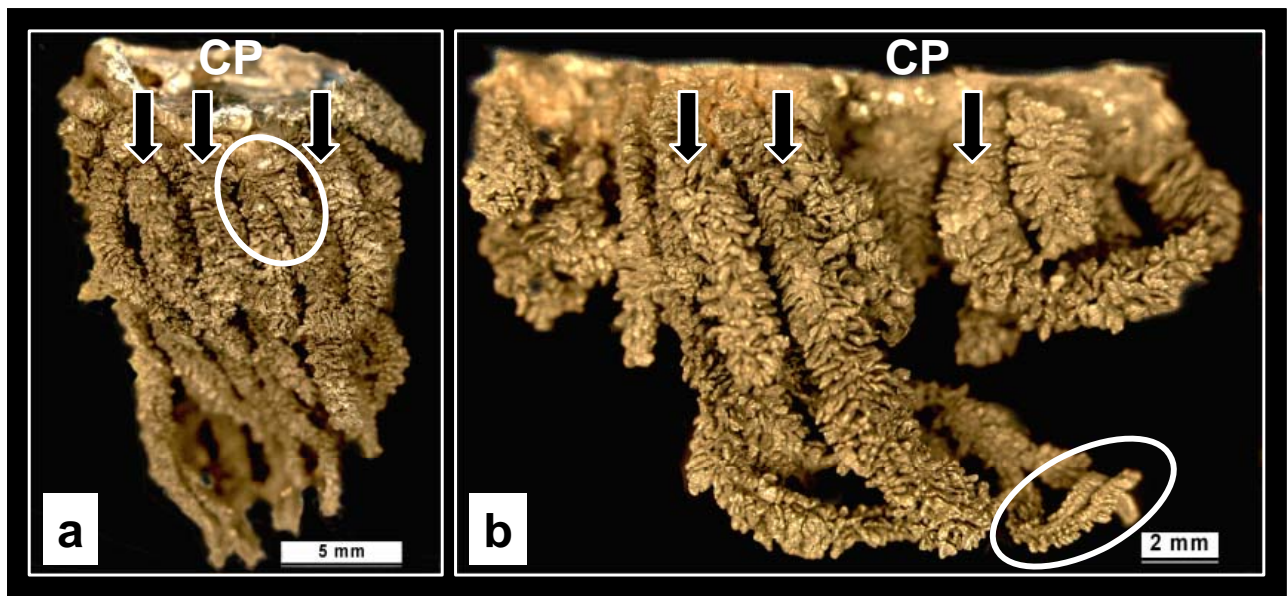
**Figure 4.30. Photograph of extensive white plaques (black stars) on the smooth, intercotyledonary chorioallantois (white star) of an expelled buffalo foetal membrane.**

In three of the six foetal membranes one or two hippomanes were found within the allantoic cavity. Hippomanes were oval measuring approximately 3 cm in diameter and 1 cm in thickness.

#### 4.4 Microscopic observations of the buffalo afterbirth

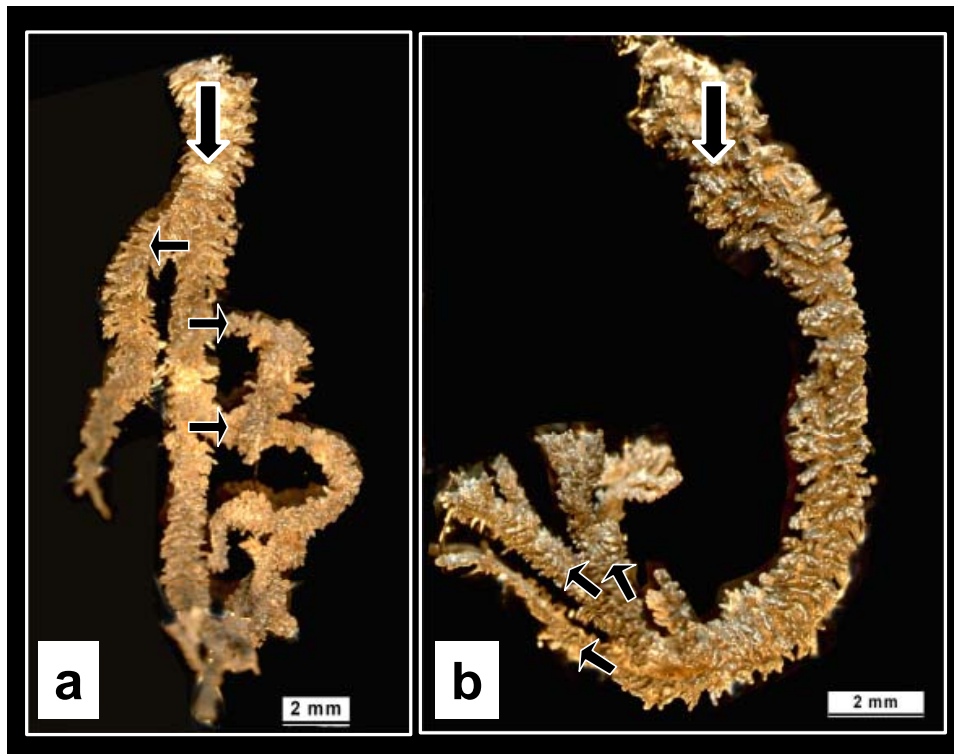
##### 4.4.1 Chorionic villous architecture

The 3-dimensional arrangement of the chorionic villi could be clearly visualised by stereomicroscopy and scanning electron microscopy. Each villous tree originated from the chorionic plate separately but in close proximity and parallel to neighbouring villous trees. The diameter of each villous tree remained constant over much of the tree's length. A gradual decrease in diameter only occurred towards the villous tip, which conferred a very slight conical shape to the villous tree. Many stem villi divided at an acute angle into further stem branches, which were generally slightly thinner in diameter than the villus of origin. The latter division occurred at various levels along the primary villus, that is, close to the chorionic plate, along the central part and / or towards the tip of the stem villus (Figure 4.31, Figure 4.32 and Figure 4.33).



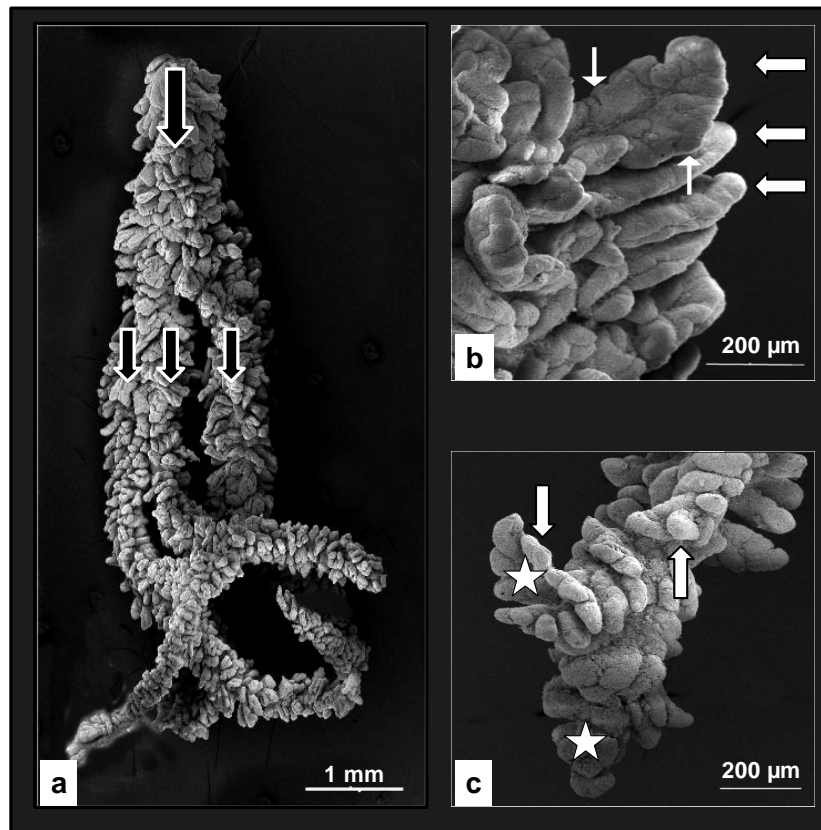
**Figure 4.31. Micrographs of chorionic villous trees projecting from the chorionic plate of an expelled buffalo foetal membrane ([a] and [b]). Villous trees (arrows) project separately and parallel from the chorionic plate (CP). Note the constant thickness along most parts of the villous trees prior to their thinning out towards the tips. Dividing stem villi are marked (ovals). (stereo microscopy)**



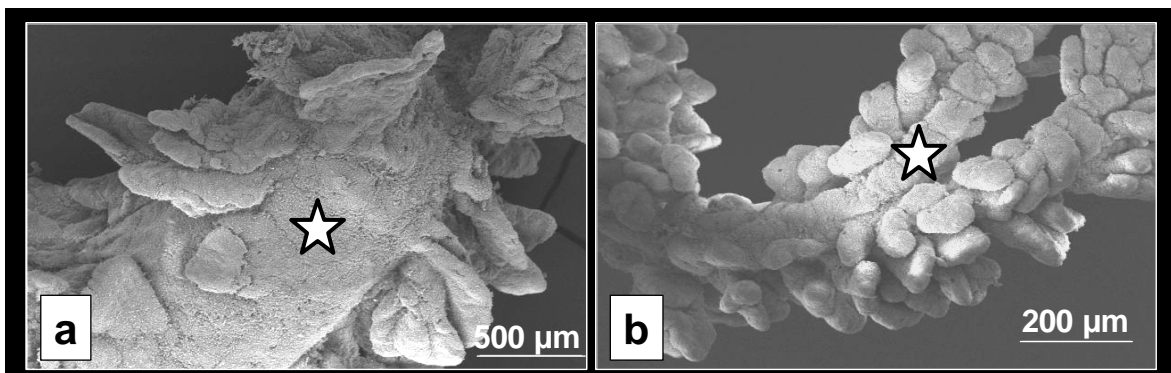


**Figure 4.32. Micrographs of single villous trees of an expelled buffalo foetal membrane. Note the branching of the stem villus (large arrows) into several stem villi (small arrows) at various levels [a] and towards the villous tip [b]. (stereo microscopy)**

Each stem villus demonstrated secondary (intermediate) and tertiary (terminal) villi. Secondary and tertiary villi varied in length and shape along each stem villus as well as between different stem villi of the same animal. However, secondary villi generally projected at approximately right angles or slightly acutely from each primary villus, closely resembling the surface of a dried pinecone. Most secondary villi were leaf-like, broad, dorso-ventrally flattened, and displayed an uneven, wavy border which represented tertiary villi (Figure 4.33). Most secondary villi stacked upon one another. Towards the tip of the stem villus, secondary villi became simpler, adopted a bulbous, finger-like shape and often lacked any further, terminal branching. Some parts of the stem villi lacked any branches of higher order and were referred to as “smooth areas” (Figure 4.34). SEM revealed hexagonal cell profiles over the entire surface of the chorionic villi.



**Figure 4.33. SEM micrographs of a single villous tree of an expelled buffalo foetal membrane. [a] A single villous tree (large black arrow) dividing into three stem villi (small black arrows). [b]: Enlargement of the proximal part of the villus shown in [a]. Leaf-like secondary villi branch at approximately right angles from the stem and are arranged in layers (white arrows). Note the undulating surface representing tertiary villi (small white arrows). [c]: Enlargement of the villous tip shown in [a]. Note the bulbous appearance of simple secondary villi (white large arrows) and the division of the primary villus into two final branches (stars).**



**Figure 4.34. SEM micrographs of proximal [a] and distal [b] villous parts of a chorionic villus of an expelled buffalo foetal membrane. Both regions reveal smooth areas (stars) devoid of secondary / tertiary villi.**

## 4.5 Comparison of villous architecture between African buffalo and cattle

### 4.5.1 Chorionic villous shape

Chorionic villi in cattle differed greatly in appearance from those observed in buffalo. They characteristically displayed a wide base at the chorionic plate, thinned out remarkably along their course and ended in a slender tip, demonstrating a conical shape reminiscent of a Christmas tree. Comparison between the two different villous shapes is illustrated in Figure 4.35.

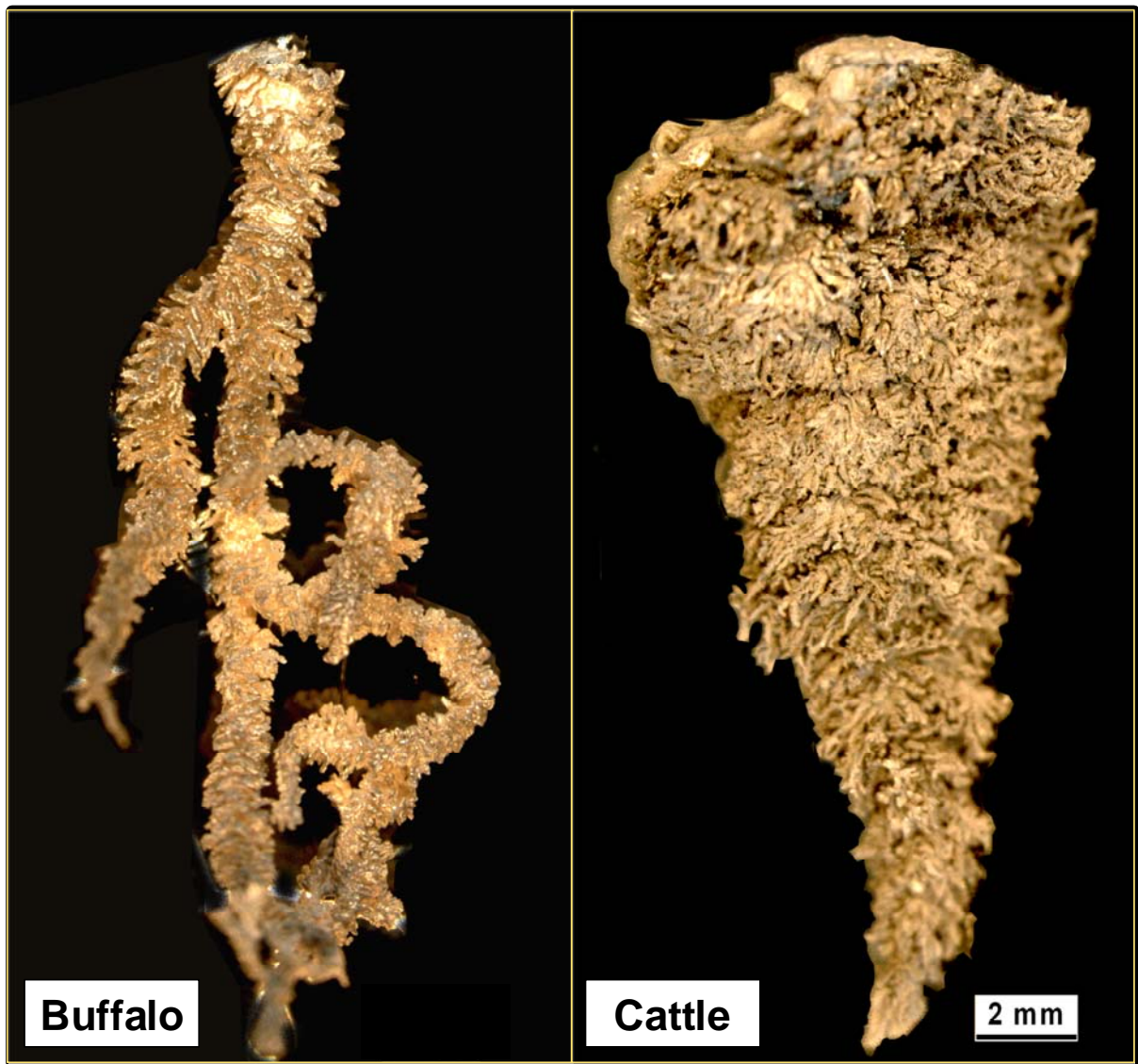
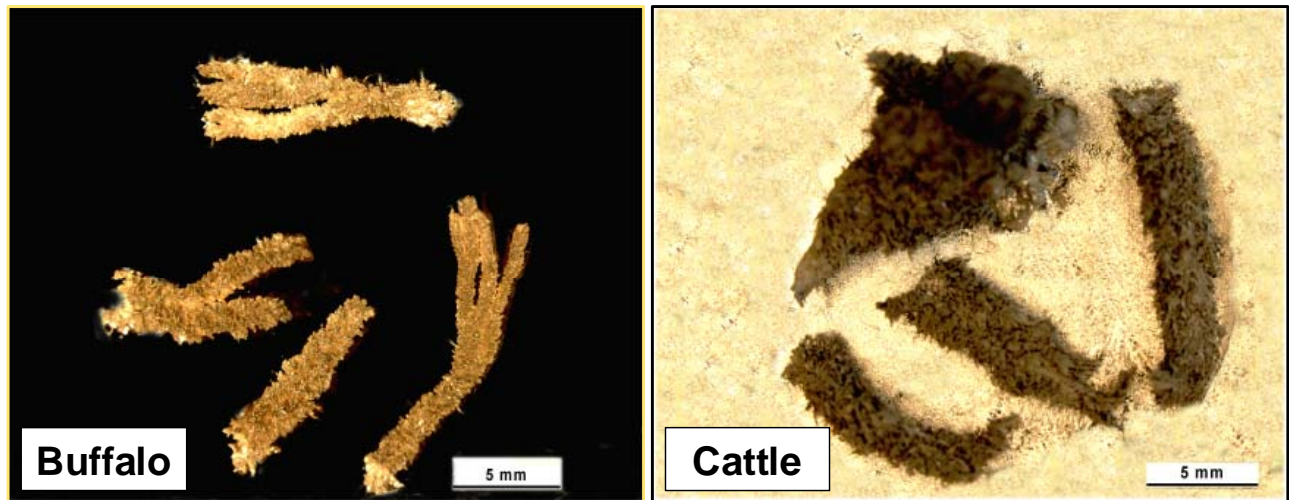


Figure 4.35. Micrographs (same magnification) of a single villous tree from buffalo and cattle. Note the slender villous tree branching into several primary villi in the buffalo compared to the conical, Christmas tree appearance in cattle. (stereo microscopy)



Additionally, at any level of the villous trees, villous diameter in buffalo is smaller than that of cattle. The division of the villous tip into several terminal branches occurs frequently in buffalo but seldom in cattle (Figure 4.36).



**Figure 4.36. Micrographs of distal parts of villous trees of buffalo and cattle. Note the differences in villous diameter and shape between buffalo and cattle as well as the frequent division into several terminal branches in the buffalo. (stereo microscopy)**

#### 4.5.2 Chorionic villous branching pattern

As in buffalo, chorionic villi branch up to the third order in cattle. In contrast to the buffalo, secondary villi appear long and slender and are equipped with distinct, pointed tertiary villi along the entire length of the stem villus (Figure 4.37 and Figure 4.38). The rather slender stem villus therefore projects long secondary villi, which, together with their terminal branches are responsible for the wider diameter of the entire villous tree in cattle when compared to buffalo.

Another major difference in villous appearance between the two genera was the lack of covering trophoblast epithelium over large parts of the villous trees in cattle, which revealed the presence of numerous convoluted capillaries. In cattle, only some terminal villi were partly covered by bulging trophoblast cells (Figure 4.39) and isolated ciliated cells were occasionally observed (Figure 4.40).



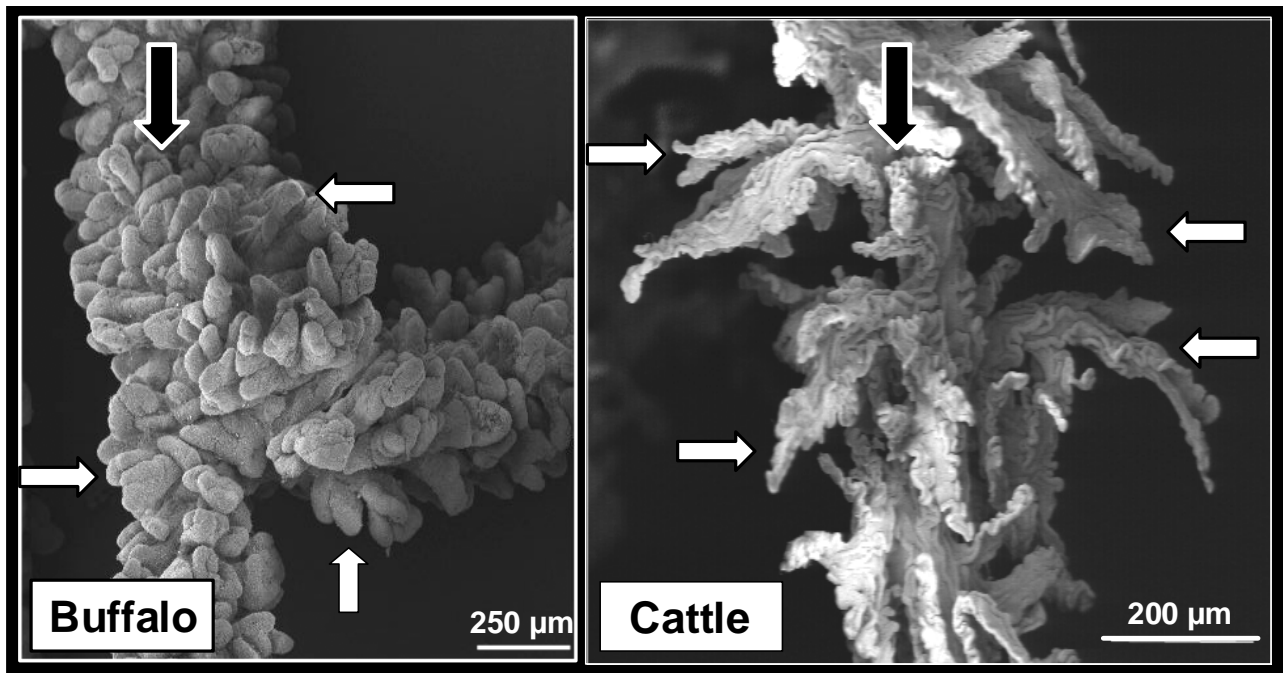


Figure 4.37. SEM micrographs of the central part of villous trees in buffalo and cattle. Stem villi (black arrows) of both genera project villi of higher order at approximately right angles. Note the simple (bulbous) appearance of secondary and tertiary villi (white arrows) in buffalo compared to their long and pointed appearance in cattle. The loss of trophoblast epithelium over the villous trees in cattle reveals sight of the rich capillary network supplying the entire villus. The epithelial loss is most probably an artefact.

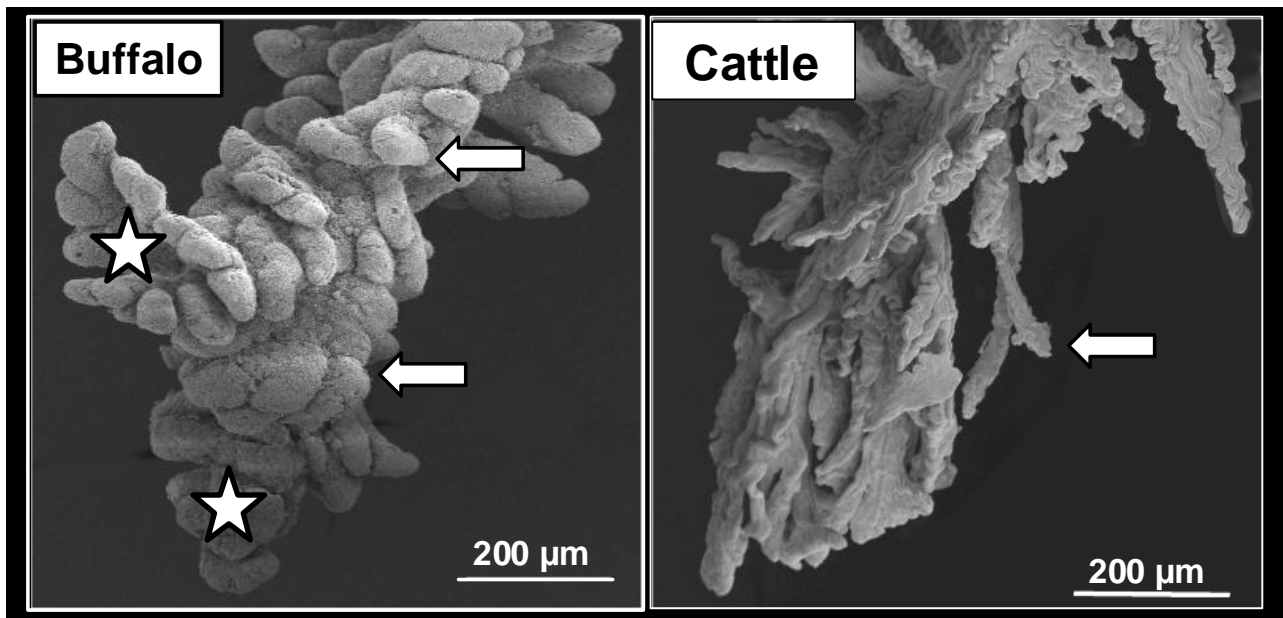
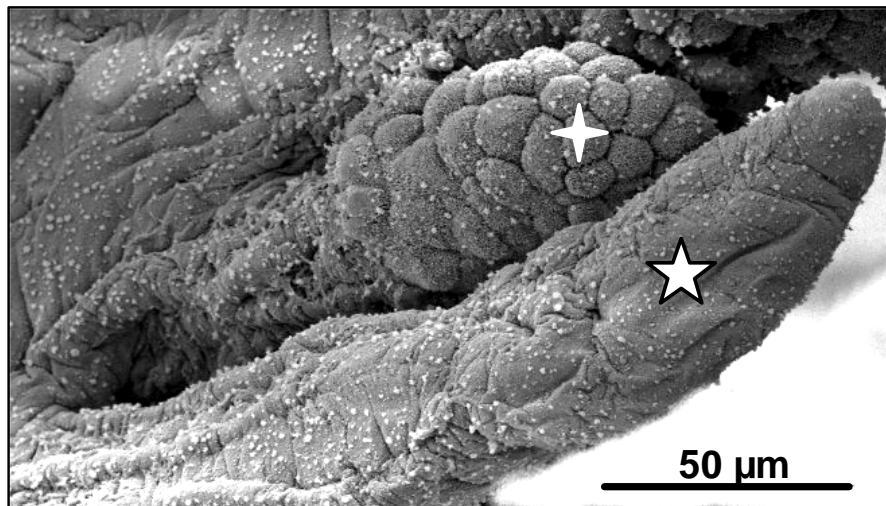
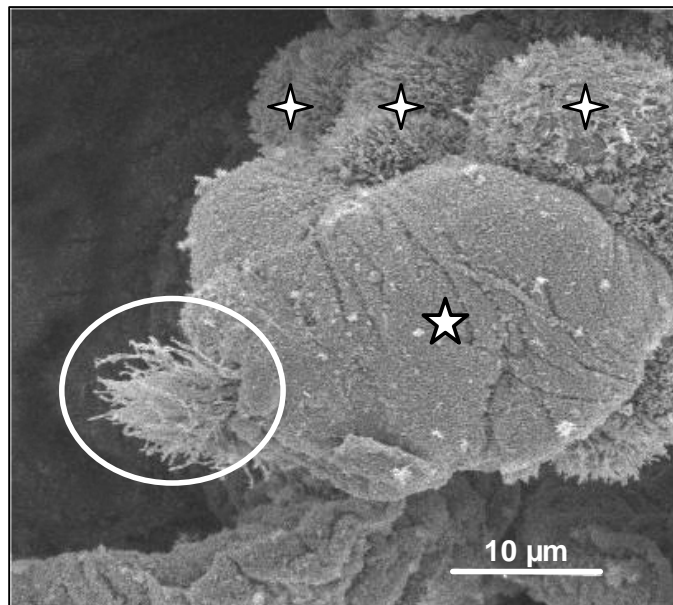


Figure 4.38. SEM micrographs of the distal part of the villous tree of buffalo and cattle. Villi of higher order (white arrows) are simple and bulbous in buffalo but long and slender in cattle.



**Figure 4.39. SEM micrograph of terminal branches of a villous tree in cattle. One terminal villus is covered with trophoblast cells (cross) whereas the other has apparently lost the epithelium revealing the exposed basement membrane (star). Note the pointed end of the terminal villus.**



**Figure 4.40. SEM micrograph of a tertiary villus in cattle. Note microvillous cells (crosses), a single ciliated cell (circle) and the intervening exposed basement membrane (star).**

## **5 Discussion**

### **5.1 Placentation in the African buffalo – current state of knowledge**

Considering the large number of mammalian species involved in comparative placentation studies in the past (for review, see Benirschke 2005), the neglect of the African buffalo placenta is rather surprising. Benirschke (2005) offers the only existing description of delivered foetal membranes of African buffalo on his web-page and mentions the importance and urgent need for studies on implanted placentas in this species.

To explain this lack of information on the African buffalo placenta, it is necessary to realize that many placental samples of “wild” mammals originate from animals kept in captivity, mainly in zoos. Pohle (2002) claims that the aggressive nature of African buffalo in captivity is the reason why only a few zoos presently keep this species. Only three zoos in Germany, for example, currently house one or other subspecies of African buffalo.

In the past, large numbers of African buffalo have been culled in various African National Parks and many reproductive studies have been based on samples obtained from both female and male buffalo (Grimsdell 1973; Sinclair 1974; Bartels *et al.* 1996; Herold 2003). However, no detailed examination of the reproductive organs has ever been reported. A possible reason for the previous lack of research on the reproductive organs may simply have been disinterest in buffalo reproduction (Bertschinger, personal communication) at the time when culling operations took place (Whyte 1996). The increasing focus on buffalo reproduction during the last decade is based on attempts at breeding so called “disease free buffalo” (buffalo free from Foot and Mouth disease, Tuberculosis and Corridor disease (Ebedes 1996)). Since buffalo are successfully bred in captivity, the resulting possibility of applying artificial reproductive technologies (i.e. intergeneric embryo transfer between African buffalo and cattle) in this species demands baseline knowledge of the reproductive system.

### **5.2 Placentome morphology**

#### **5.2.1 Placentome collection**

The first indication of differing placentomal morphology between African buffalo and cattle was the necessity to use fetotomy wire to separate placentomal tissue proper from the underlying endometrium. While blunt, manual placentome extirpation can be achieved in *Bos* (Gross *et al.* 1991; Gerber, personal communication), the latter was impossible in peri-partal *Syncerus*. In cattle, placentomes were twisted and separated from the underlying endometrium (Gross *et al.* 1991; Gerber, personal

communication). In African buffalo, twisting of placentomes was not possible due to their broad connection to the underlying endometrium.

### 5.2.2 Shape

The present placentome classification in African buffalo is based on palpation as well as macroscopic and microscopic observations of placentomes and corresponding cotyledonary tissue of released foetal membranes (see below).

Placentomes of African buffalo can be classified as being convex and non-pedunculated, and differ markedly from the convex, pedunculated placentomes described in cattle (Andresen 1927; Laven & Peters 2001). However, the term “convex” should not be used according to Mossman’s definition (Mossman 1987) of “a rounded, foetal surface and a definitely constricted pedicle”, as the buffalo placentome connects to the underlying endometrium via a broad base. A caruncular stalk is therefore lacking (see Figure 4.1).

Cross sections as apposed to longitudinal sections of placentomes were used for LM observations in the present study in order to prevent distortion of the results obtained, as advised by Mossman (1987) and Andresen (1927). Both authors report on the possibility of a single placentome appearing different (flat versus pedunculated) depending on the orientation (longitudinal or transverse) of the section.

Considering the dimensions of a medium sized cattle placentome (e.g. 4 x 4 cm), the material prepared by Boos *et al.* (2003a), and used in this study, did not generally demonstrate the caruncular stalk. However, the distinctly convex maternal plate and the curved arrangements of the villous tips demonstrated proof for its existence. Buffalo placentomes, in contrast, always displayed a straight maternal plate and a straight orientation of villous tips, thus demonstrating the lack of a caruncular stalk. Schmidt *et al.* (2005) report divergent placentomal development between African buffalo and cattle during early gestational stages (CRL up to 17 cm) and suggest a possible lack of stalk formation until term in the buffalo. When comparing endometrial tissue beneath early (CRL up to 17 cm), non-pedunculated placentomes in both genera it was observed that glandular tissue was present as a continuous layer in *Syncerus*, but missing beneath the central region of the placentome in *Bos*. The latter pattern of glandular distribution possibly serves as an indicator of subsequent stalk formation in cattle, as connective tissue constituting the pedicle generally lacks glandular profiles (Priedkalns & Leiser 1998). In contrast, the continuous presence of glands in the buffalo endometrium may indicate the lack of stalk formation throughout the remainder of pregnancy.



Descriptions of *Bubalus* placentomes are similar to these in *Syncerus*; a convex surface and a broad, inconspicuous (Yap 1974; Ram & Chandra 1984; Abd-Elnaeim *et al.* 2003) or absent (Abdel-Raouf & Bawadi 1966) caruncular stalk. *Bubalus* placentomes thus resemble *Syncerus* placentomes more than *Bos* placentomes.

However, Abd-Elnaeim *et al.* (2003) recently described the *Bubalus* placentome as “mushroom-shaped”, a term used rather inappropriately when viewing the placentome section illustrated in the paper. Although the photograph of a *Bubalus* placentome (longitudinal section) clearly presents features similar to those described in this study, namely, a convex chorionic and flat maternal plate, the naming of the latter structures as well as the schematic illustration shown below the photograph are rather misleading and contradictory.

However, the slight basal constriction (bending of the peripheral placentomal tissue) observed in one *Syncerus* placentome included in the present study might indicate that a few placentomes of the African buffalo placenta do have a very broad, inconspicuous stalk. This structure may be responsible for the slight deviation of peripheral caruncular crypts from their usually strictly vertical course within the placentome centre.

### 5.3 Placentome histology

Histological features of tissues constituting full term placentomes of *Syncerus*, *Bos* (Björkman 1954; Hager 1983; Hradecky *et al.* 1988a) and *Bubalus* (Sharma *et al.* 1983; Yap 1974) are very similar. In all three bovid genera, caruncular connective tissue septae compartmentalize placentomes into main crypts containing villous trees of various shapes, depending on the genus (Yap 1974; Hradecky *et al.* 1988a). Comparing horizontal cross-sections of *Syncerus* placentomes to similar sections of full term *Bos* placentomes illustrated in a paper by Hradecky *et al.* (1988a), the intergeneric differences in villous and crypt architecture, as shown in this study, are supported. The thick mesenchymal core of the broad villous stem in *Syncerus* projects rather short villi of higher order, thus the primary villus occupies the majority of space within the main caruncular crypts. In contrast, a complex network of long secondary and tertiary villi occupies most of the caruncular space in cattle and the villous stem itself is very slender (Hradecky *et al.* 1988a).

Although detailed studies on foetal and maternal vasculature in *Syncerus* placentomes are so far lacking, LM observations in the present study reveal evidence for a similar vascular pattern in placentomes of all three bovids (Ebert 1993; Leiser *et al.* 1997a; Pfarrer *et al.* 2001; Abd-Elnaeim *et al.* 2003). Primary structures (villous stems and primary crypt walls) contain large arteries and veins

whereas arterioles and venules found within secondary structures (intermediate villi/crypt walls) divide further into a rich capillary network supplying tertiary villi and the corresponding crypt wall.

In the epitheliochorial type of placenta, reduction in the distance between foetal and maternal vasculature is achieved via subepithelial capillaries which indent the basement membrane and closely approach the MVJ, and via the reduction of connective tissue elements within secondary and tertiary villous cores (Wooding & Flint 1994; Enders & Carter 2004). Present results indicate similar mechanisms at play in the African buffalo placenta (compare Figure 4.10, Figure 4.12 and Figure 4.19).

The occurring cell types, maternal crypt (CEC) and multinucleate (MNC) epithelial cells as well as trophoblast epithelial (TEC) and binucleate cells (BNC), including their basic histological and ultrastructural features are equally described in *Syncerus*, *Bos* and *Bubalus* placentomes (Björkman 1954; Yap 1974; Wooding & Wathes 1980; Hradecky *et al.* 1988a; Leiser 1990; Priedkalns & Leiser 1998; Schlafer *et al.* 2000).

One consistent feature is the BNC population in the chorionic trophoblast in all ruminant placentas so far examined. BNC have been studied extensively in the past (Björkman 1968; Wooding 1982a; Wooding 1983; Byatt *et al.* 1986; Wooding *et al.* 1994; Wooding *et al.* 1996; Wooding *et al.* 1997). but have only been described in the African buffalo in the trophoblast epithelium during early gestation (Schmidt *et al.* 2005) and in foetal membranes after their delivery (Benirschke 2005). In the present study, histological and ultrastructural features of BNC reveal the similarity between *Bos* and *Syncerus* genera regarding BNC distribution, life span and function. These observations support the reported involvement of BNC in maternally directed transport of mediator substances (e.g. placental lactogens) across the placental barrier and their release into the maternal compartment (Wooding & Flint 1994). Evidence for multinucleate trophoblast cells, as demonstrated by Klisch *et al.* (1999) in cattle, could not be provided in this study, as serial sections were not performed on samples collected. It is possible, however, that cells containing more than two nuclei remained undetected. The “double lamellar bodies” (DLB), mentioned to be characteristic for ruminant BNC (Wooding & Flint 1994) were never observed in buffalo BNC. This should, however, be interpreted cautiously, since DLB occur predominantly in young, developing BNC, and are therefore unlikely to be found in tissues of post-partal placentomes. In contrast to early gestational stages (CRL 2-17 cm, Schmidt *et al.* 2005), maternal MNC containing more than three nuclei are lacking within the crypt epithelium of fully developed placentomes as revealed in the present study. A transitional formation of tri-nucleate fetomaternal hybrid cells (MNC) as described for cattle during the entire pregnancy (Wooding & Flint

1994) can therefore be assumed to occur likewise in the buffalo placenta. Bovine trinucleate cells degenerate after releasing their granular content and their remnants are phagozytised by trophoblast epithelial cells. Cell remnants occurring within small feto-maternal gaps in the buffalo specimens (see Figure 4.16) might indicate a similar fate for these cells in the buffalo.

### 5.3.1 Placental maturation

In the present study, interpretation of epithelial structure and arrangement and the description of possible alterations caused by placental maturation were complicated by the following factors: no descriptions exist on “normal” histo-morphology of full term *Syncerus* placentomes prior to the possible occurrence of maturation processes; results from the H&E-stained slides differed markedly from those obtained from semi-thin TB-stained sections, although tissues originated from the same placentome. Differences between H&E and TB-stained sections included a disrupted (H&E) versus an intact (TB) MVJ as well as a squamous to low cuboidal epithelium (average height 12  $\mu\text{m}$ ) lacking lateral cell borders (H&E) versus an undisturbed, simple cuboidal epithelium (average height 15  $\mu\text{m}$ ) displaying distinct lateral cell borders (TB). Most probable reasons for these differing results are the use of different fixatives (formalin versus glutaraldehyde) and different processing techniques (wax versus resin embedding) applied. The superior resolution obtained from thinner resin sections would also impact on structural detail. Samples collected for TEM purposes were fixed more rapidly and more efficiently due to their smaller size (2 mm<sup>3</sup>) when compared to large LM samples (4 mm thickness), even though formalin generally penetrates tissue faster than glutaraldehyde (Hayat 2000). The disruption of the MVJ seen in H&E-stained sections could therefore be interpreted as an artefact, considering the sensitivity of delicate structures such as microvilli (Björkman and Sollen 1960), and the considerable tissue shrinkage caused by certain fixatives (Hayat 2000). Resin embedding (TEM samples) is reported to cause less tissue/cell shrinkage when compared to wax embedding (LM samples) (Bancroft & Stevens 2004). The lower epithelial cell height in H&E compared to TB-stained sections might therefore be caused, at least partially, by different degrees of cell shrinkage during fixation rather than by the staining itself.

In *Bos*, reduction of epithelial height and a partial/complete (Björkman & Sollen 1960; Holm *et al.* 1964; Woicke *et al.* 1986; Schoon 1989) loss of the maternal epithelium lead to the formation of a syndesmochorial-type placenta, as reported by some authors (Schulz & Merkt 1956; Schoon 1989) and represents the occurrence of major epithelial changes during placental maturation. The higher and largely continuous crypt epithelium observed in both H&E and TB-stained sections of *Syncerus* placentomes can therefore be distinctly distinguished from descriptions in *Bos*. An intact, cuboidal epithelium lining the crypts of *Bos* placentomes has been uniquely described in animals with retained

foetal membranes, thus in animals demonstrating insufficient placental maturation (Woicke *et al.* 1986; Sobiray 1992; Hayat 2000; Boos, personal communication). Consequently, considering epithelial height and continuity, the crypt epithelium of peri-partal *Syncerus* placentomes most closely resembles the crypt epithelium of insufficiently matured *Bos* placentomes.

Besides the epithelial dissimilarities mentioned above, numerous common indicators of cellular degeneration indicative for maturation exist between *Bos* and *Syncerus* epithelia. In the maternal epithelium (*Syncerus*) there are numerous pycnotic nuclei (H&E), swollen CEC with pale cytoplasm (TB), as well as myelin figures and degenerated mitochondria (TEM) (compare with *Bos* in Sobiray, 1992). In the trophoblast epithelium, numerous lipid droplets as well as lipofuscin granules with similar morphological properties occur in both *Syncerus* and *Bos* (Björkman & Sollen 1960) specimens. The lipofuscin granules, also known as "age pigment" may be considered as a conglomerate of undigested residues of mostly endogenous material (Young & Heath 2000).

Comparison of further maturation-related histological changes between *Bos* and *Syncerus*, such as the decline in BNC numbers (Gross *et al.* 1991; Wooding & Flint 1994) and fibrotic alterations (Willms 1986) are difficult due to the lack of information on pre-maturation samples in the African buffalo. The occurrence of moderate numbers of BNC in post-partal *Syncerus* placentomes indicates their continuing activity (migration and substance release) until term. The relatively thick maternal connective tissue septae (Figure 4.3) observed in this study indicate an increased fibrous as apposed to a decreased cellular tissue component during placental maturation. The involvement of leukocytes in maturation and separation of foetal membranes has not yet been proven in *Bos* (Woicke *et al.* 1986; Schoon 1989). However, the large numbers of leucocytes observed in some of the large vessels of *Syncerus* placentomes might indicate their involvement in inflammatory processes and therefore in separation and release of foetal membranes as suggested by Gunnik (cited by Davies *et al.* 2004) in cattle.

As early as about 8 months of gestation, the bovine crypt epithelium reduces in size to shorten the distance between foetal and maternal tissue for substance exchange by diffusion (Woicke *et al.* 1986), thus meeting the increasing demand for substances by the rapidly growing foetus towards the end of gestation. Present results reveal a less distinct decrease in epithelial height in *Syncerus*, possibly indicating a less efficient mechanism of feto-maternal exchange. This, combined with other factors (see 5.5) might lead to poorer foetal nutrient supply consequently requiring a prolonged gestational period in African buffalo as compared to cattle.



The continuous, mainly cuboidal crypt epithelium in peri-partal *Syncerus* placentomes does not appear to be involved in retained foetal membranes, as reported for cattle (Woicke *et al.* 1986; Sobiray 1992; Hayat 2000).

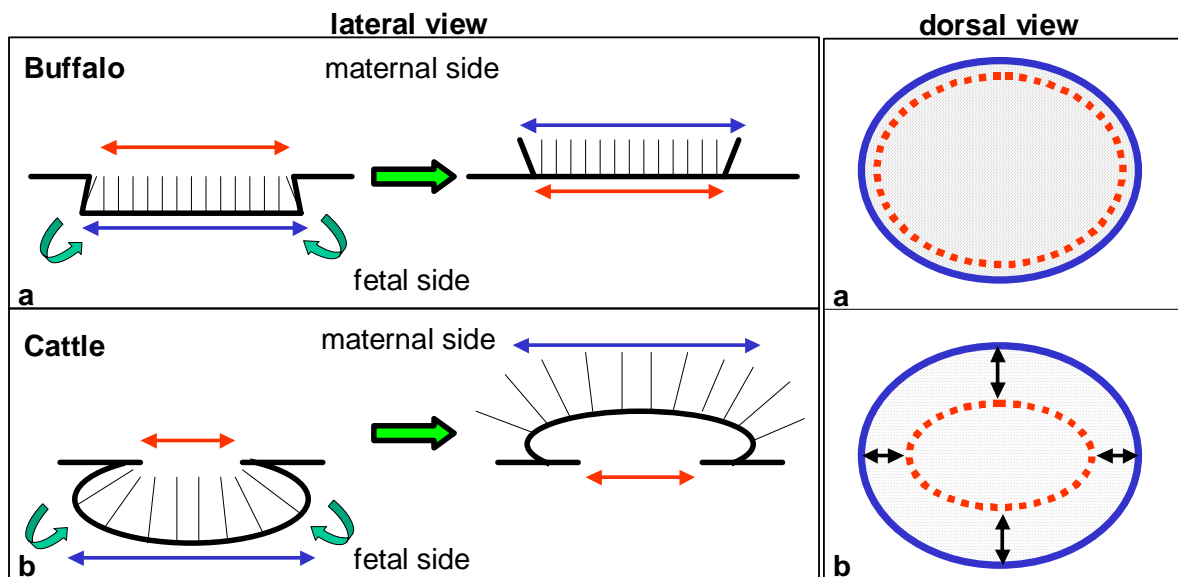
## 5.4 Morphological features of foetal membranes following their normal delivery

### 5.4.1 General aspects

The 24-hour survey of African buffalo kept in bomas allowed a close observation of the sequence of events before, during and after parturition. A time period of 2-3.5 hours from delivery of the buffalo calf to the expulsion of the foetal membranes is physiologic when compared to the 6–12 hour time lapse reported as normal in cattle (Arthur *et al.* 1996). When dealing with wild animals it was not always possible to collect the delivered afterbirth quickly enough to prevent trampling by other animals.

### 5.4.2 Macroscopic observations

Although afterbirths collected from cattle were not macroscopically examined in detail, differences in cotyledonary shape between *Syncerus* and *Bos* were noticed while handling *Bos* afterbirths during sample collection. Whereas *Syncerus* cotyledons could be turned inside out with ease, moderate force was necessary to do so in *Bos* cotyledons. These differences are illustrated in Figure 5.1 and demonstrate an obvious and easily recognizable difference in cotyledonary (and placentomal, see 5.4.2.1) morphology between the two genera.



**Figure 5.1. Schematic illustrations of buffalo [a] and cattle [b] cotyledons with adjacent smooth chorioallantois in lateral (left) and dorsal (right) view. In lateral view, cotyledons are turned towards the foetal side, thus “inside out”, when compared to their situation “in situ”. The red arrows (lateral views) and the red discontinuous lines (dorsal views) demonstrate the circumference of the round-ovoid area formed by bending of the foetal membranes (the marginal folds) at the site of transition between the smooth intercotyledonary and cotyledonary areas. The blue arrows (lateral views) and the blue lines (dorsal views) demonstrate the largest diameter of the cotyledon. Note the small size difference in structures marked red and blue in buffalo compared to the great difference in cattle, indicated by black arrows (dorsal view in b). This explains the difficulty experienced while pushing “large” bovine cotyledonary tissue (blue)**

**through a small (red) circular area and shows the differences in size of the marginal folds. Variation in cotyledonary height from rather flat [a] to high-convex [b] are also demonstrated.**

The membranous structure formed by the curved foetal membrane at the transition from the smooth to the cotyledonary parts of the foetal membranes represents the so-called “marginal fold” in bovine placentomes (Mossman 1987). This fold reaches into the “undercutting” endometrial tissue space, which develops around the placentome due to stalk formation. The comparably small dimensions of marginal folds in African buffalo, as represented in Figure 5.1 result from the lack of a caruncular stalk.

Amniotic plaques described on expelled foetal membranes in buffalo are reported to disappear after six months of gestation in domestic cows (Arthur *et al.* 1996). As all buffalo calves delivered were healthy, any pathological cause for the persistence of amniotic plaques on the foetal membranes is unlikely. “Hippomanes”, observed in buffalo and cattle, seem to be of no functional significance (Arthur *et al.* 1996). Necrotic tips of foetal membranes, as reported for *Bos* (Schlafer *et al.* 2000), were not observed in the placenta of African buffalo.

#### **5.4.2.1 Correlation between cotyledonary and placentome shape**

When Andresen (1927) examined expelled goat afterbirths, he reported that it was impossible to distinguish between a concave and a convex placentome shape by simply examining the cotyledonary shape on the afterbirth. Present results reveal that the cotyledonary shape on expelled foetal membranes serves as an indicator for the placentome shape regarding the existence or lack of a caruncular stalk (pedunculated versus non-pedunculated placentomes). Benirschke (2005) described a very flat cotyledonary shape on foetal membranes of African buffalo and Père David's deer (*Elaphurus davidianus*). The Père David's deer is known for its rather flat placentome shape (Benirschke 2005) and drawing a conclusion from a flat cotyledonary to a non-stalked placentome shape in African buffalo seems justified. Investigations of delivered foetal membranes therefore allow insight into all cotyledons/placentomes present on the entire placenta.

#### **5.4.2.2 Cotyledonary number, size, shape and distribution**

The present study revealed evidence for a multicotyledonary-type placenta in the African buffalo, as previously reported (Mossman 1987; Benirschke 2005). The number of placentomes ranging from 50-175 (Mossman 1987) typical for this placental type corresponds closely to cotyledonary numbers counted on *Syncerus* afterbirths in this study. However, cotyledonary numbers differed greatly between present observations and reports by Benirschke (2005) (n=3) and Durrant and Benirschke (1981) (n=1) performed on afterbirths of the African forest buffalo (*Syncerus caffer nanus*), which is considered as a subspecies of the African buffalo. The total number of 30 (Durrant & Benirschke

1981), 34, 52 and 54 (Benirschke 2005) cotyledons per afterbirth counted in the forest buffalo corresponds to cotyledonary numbers found in a single uterine horn in the present study (42, 57, 59 and 90). The total number of cotyledons counted over the entire foetal membrane was approximately 3-5 times higher (89, 109, 115, 156, 168 and 178 cotyledons, respectively) in *Syncerus* in this study than that of the forest buffalo (Durrant & Benirschke 1981, Benirschke 2005). The reasons for this discrepancy in cotyledonary numbers are unknown. A possible explanation may be that small cotyledons (e.g. under 2 cm in length) were, in contrast to the present study, not counted in previous reports (Durrant & Benirschke 1981; Benirschke 2005) or that parts of the afterbirths were damaged or eaten prior to collection. The total number of placentomes (or cotyledons of expelled foetal membranes) reported to range from 92 to 126 in *Bubalus* (Abdel-Raouf & Bawadi 1966; Ranjhan & Pathak 1993; Abd-Elnaeim *et al.* 2003) fit better with the results of the present study.

The uneven distribution of placentomes between pregnant and non-pregnant horn, as generally described in *Bos* and *Bubalus* placentas (Laven & Peters 2001; Ranjhan & Pathak 1993; Bertolini *et al.* 2002) could not be confirmed for the African buffalo in the present study. The observed difference (50.3 versus 49.7) was not significant (t-test,  $p < 0.05$ ).

*Syncerus* cotyledons demonstrated a great variability in length, although most ranged between 6.4 – 7.2 cm, similar to observations in cattle (Laven & Peters 2001), water buffalo (Abdel-Raouf & Bawadi 1966), and African buffalo (Benirschke 2005). Slight differences in cotyledonary length in respect of the total number of cotyledons per afterbirth can be noted. Whereas most cotyledons ranged in length between 7 and 8 cm on afterbirths with relatively low cotyledonary numbers (average of 104), most cotyledons measured 6 cm on foetal membranes with relatively large total cotyledonary numbers (average of 167). Interpreting this feature as a possible compensatory placental mechanism (fewer placentomes, larger size / more placentomes, smaller size) might be misleading, considering the low cotyledonary numbers combined with relatively small cotyledon size reported by Benirschke (2005) and the lack of correlation between these two parameters reported by Laven & Peters (2001) in *Bos*. However, in this study, the frequency of larger cotyledons was higher in the pregnant horn, a consistent characteristic typical for the *Bos* placenta (Laven & Peters 2001).

Similarities exist between *Syncerus* and *Bos* genera in the pattern of cotyledonary distribution according to their length (diameter) in the pregnant horn (compare Figure 5 in Bertolini *et al.* 2002 to Graph 4.2 in this study). As the most developed (largest) placentomes are usually located in the peri-embryonic region (Kingman 1948; Leiser 1975; Ram & Chandra 1984), it is not surprising to find a



higher number of larger cotyledons in this area. However, the largest cotyledon observed in this study (19.5 x 5cm) was found in the proximal part of the non-pregnant horn.

A moderate proportion of *Syncerus* cotyledons differed in shape from the common round to ovoid form usually described in cattle. Irregular, lobulated cotyledons observed in the present study were apparently due to fusion of neighbouring placentomes, a feature also described in water buffalo during advanced stages of gestation (Abdel-Raouf & Bawadi 1966; Abd-Elnaeim *et al.* 2003).

The pattern of cotyledonary distribution along expelled foetal membranes allows conclusions to be drawn regarding placentome distribution *in vivo*. However, a lack of one or more cotyledons, thus disrupting the generally described linear pattern of placentome distribution (Andresen 1927), does not necessarily imply the lack of a caruncle at this particular location. Laven and Peters (2001) report that during all gestational stages in cattle “all uteri had spare caruncles not covered by or attached to a cotyledon”. The occurrence of cotyledons in 2 mesometrial, and 2 anti-mesometrial rows along most parts of the uterine horns in African buffalo is in agreement with descriptions in *Bos* (Schlafer *et al.* 2000) and *Bubalus* (Abdel-Raouf & Bawadi 1966). An alternating arrangement of placentomes between the dorsal and ventral rows also occurs in the *Syncerus* placenta. Andresen (1927) has suggested that this arrangement facilitates a better blood supply. Adventitious and accessory placentation occurs likewise in *Syncerus* and *Bos* (Wild 1964). The function of accessory placentation in cattle is not clear. In earlier studies (cited by Wild, 1964), accessory placentomal development was often related to pathological or traumatic endometrial changes during previous pregnancies. Wild (1964) demonstrated that the formation of accessory cotyledons increased in placentas with a cotyledonary surface area below 4474 cm<sup>2</sup>. He concluded that accessory placentomes on the intercotyledonary chorion increased the functional surface area of small cotyledons, which contributed positively to the calf’s viability. In a more recent study on cattle (Laven and Peters 2001) no correlation could be found between placentome numbers in the pregnant and in the non-pregnant horn and increased formation of accessory placentomes did not occur. Therefore, no compensation for the lack of placentomal development in the one horn by increasing development in the other horn appears to take place.

The remarkable variability in placentome number, size, shape and distribution observed in *Syncerus*, *Bos* and *Bubalus*, suggests that the reserve functional capacity of the placenta is exceptionally large and able to cope with most demands. The report of the delivery of a viable cloned bovine calf with only 12 enlarged functional cotyledons at term is a remarkable example of the adaptive capacity of the placenta (Hill *et al.* 2001).

### 5.4.2.3 Foetal villousity

In an attempt to classify villi of cotyledonary placentas according to shape and pattern of branching, two types of villi have been recognized (Strahl 1911; Mossman 1987). One type is simple and slender with simple branches of higher order arising at an acute angle (e.g. deer). The other type demonstrates complex branching, with villi arising at a less acute angle from the stem (e.g. cow, sheep). Both types seem to be associated with particular placentome shapes, thus simple villi with a flat placentome and complex villi with a convex/concave placentome. This classification appears somewhat simplistic, considering the great variability in foetal villousity and placentome morphology within ruminants (Hradecky *et al.* 1988a) and the morphological changes which take place throughout gestation.

Morphology and dimensions of full term chorionic villi of cattle and horses are reported to be similar *in utero* and after delivery of foetal membranes (Björkman & Sollen 1960; Macdonald *et al.* 2000). The same phenomenon is assumed to be true for specimens collected from the African buffalo. The general characteristic of villous branching up to the third order is universal in *Bos*, *Bubalus* and *Syncerus*. Villous trees in *Bos* are reported to change from a broad-conical shape at mid-gestation to a tall-conical shape at term (Leiser *et al.* 1997a; Pfarrer *et al.* 2001). Changes in the outer shape of *Syncerus* villous trees during the course of gestation, as reported in cattle (Ebert 1993; Leiser *et al.* 1997a; Pfarrer *et al.* 2001), cannot be clarified, as *Syncerus* specimens from mid-gestation onward were not studied. However, the differences in villous shape and architecture between *Syncerus* and *Bos*, demonstrated during early gestation (CRL 17 cm, Schmidt *et al.* 2005), seem to continue until term when marked differences between the conical villous trees in *Bos* and the cylindrical villous trees in *Syncerus* are obvious (Figure 4.35). As with placentome shape, villous trees resemble each other more closely in the two buffalo genera, *Syncerus* and *Bubalus* than in *Bos*. Such similarities include the description of *Bubalus* villi as “cylindrical or conical” (Abd-Elnaeim *et al.* 2003) or “slender” (Yap 1974; Sharma *et al.* 1983), the ramification of stem arteries into two stem villi (compare Figure 4.32) and the occurrence of two villous types, rough and smooth (Abd-Elnaeim *et al.* 2003).

Leiser *et al.* (1997a) propose that the modified arrangement of villous trees (tall-conical in shape) in the full term *Bos* placentome allows for their denser arrangement, which results in a higher number of tree units per placentome. The almost complete indentation between foetal and maternal tissue is achieved via villous branching at right angles from the stem. This results in the exclusion of blind corners of foeto-maternal contact, thus achieving the maximal functional surface area of villous trees in a given space (Leiser *et al.* 1997a). In contrast to the conical structures in *Bos*, the more cylindrical, slender shaped villous trees in *Syncerus* and *Bubalus* promote an even denser arrangement of parallel-

orientated villi within each placentome. This particularly close proximity of villous trees within buffalo placentomes may be responsible for the occurrence of smooth areas along some stem villi due to the lack of space for developing branches of higher order as observed in the present study and reported by Abd-Elnaeim *et al.* (2003). As far as could be ascertained, this phenomenon has not been described in *Bos*.

A direct morphological comparison of branches of higher order between *Bos* and *Syncerus* was difficult due to the artificial loss of trophoblast epithelium from all *Bos* samples. However, three-dimensional corrosion cast studies on foetal villosity in *Bos* (Ebert 1993; Leiser *et al.* 1997a; Pfarrer *et al.* 2001) and *Bubalus* (Abd-Elnaeim *et al.* 2003) offered detailed, comparable material.

A consistent feature of villous trees in all three genera was the decrease in villous complexity from the base to the tip (Leiser *et al.* 1997a). In *Bos*, long and slender secondary branches were predominately well equipped with pointed tertiary villi, thus resembling the “sling-type” described by Ebert (1993) and as a whole resembling a long-fingered glove, decreasing in length towards the villous tip. In contrast, corresponding structures in *Syncerus* appeared leaf-like in proximal parts of the villus and bulbous in the distal part. Villi of higher order in *Syncerus* hence resembled those described in *Bubalus* (Abd-Elnaeim *et al.* 2003) rather than *Bos*. It is therefore surprising that, according to Abd-Elnaeim *et al.* (2003), interdigitation between foetal and maternal tissues is reported to be more elaborated in *Bubalus* than in *Bos*.

## **5.5 Placentome morphology and its possible influence on gestational length and afterbirth retention in *Syncerus*, *Bubalus* and *Bos***

Differing mean gestational lengths of 343 days in *Syncerus* (Knechtel 1993), 315 days in *Bubalus* and 280 days in *Bos* (Hafez & Hafez 2000) are remarkable, especially considering the comparably similar calf birth weight of an approximate average of 38 - 40 kg in all three genera (Arthur *et al.* 1996; Benirschke 2005; Hufana-Duran *et al.* 2004). To explain this phenomenon, an insight into foetal villosity and the degree of feto-maternal interdigitation (villous crypt architecture) in the three genera may offer an explanation although contradictory opinions are presented in recent literature.

As mentioned above (5.4.2.3), Leiser *et al.* (1997a) describe the placentomal rearrangement of villous trees and their morphological modification in vascular architecture (wide-conical villous trees become more slender and are more tightly arranged) in *Bos* as an adaptation to the ever-increasing need for transplacental substance exchange with progressive gestation. During the second half of gestation the total volume of capillary networks within terminal villi, the “working part”, of *Bos* placentomes,

increases with respect to vessel-volume within stem and intermediate villi, the “supplying part” (Leiser *et al.* 1997a). This increasing complexity of feto-maternal interlocking via well-developed tertiary villi thus enhances substance transfer via diffusion at the end of gestation as well as promoting the villous anchoring effect (Allen *et al.* 2003).

Allen *et al.* (2003) conclude that long pregnancy duration will give more time to form a complex ramification of villous trees. They argue e.g. that the microcotyledons are less ramified in horses than in donkeys, because the pregnancy duration of mares is one month shorter (11) than in donkeys (12). In bovids, Abd-Elnaeim *et al.* (2003) describe the development of a more elaborate feto-maternal interdigitation during the additional months of gestation in *Bubalus* (10 months) when compared to *Bos* (9 months). This morphological difference is suggested to result in a higher incidence of foetal membrane retention of 4.55 % in *Bubalus* compared to 1.25 % in *Bos* (Navarro 2002 cited in Abd-Elnaeim *et al.* 2003). The latter percentages contradict those given in other publications, including a rather recent workshop report (Allen, *et al.* 2003) where a higher afterbirth retention rate is stated to occur in cattle rather than in water buffalo.

According to this study, foetal villi and interdigitation patterns in African buffalo are less complex than in *Bos* and more closely resemble features of the water buffalo placenta. In contrast to other studies (Abd-Elnaeim *et al.* 2003; Allen *et al.* 2003) it is hypothesised that placental complexity may influence the duration of pregnancy rather than the other way round, thus short when complex and long when simple. A decreasing placental complexity (from *Bos* to *Bubalus* and finally to *Syncerus*) may correspond with decreasing placental efficiency, leading to the necessity of a longer gestation period in the two buffalo genera.

The above hypothesis is supported by the fact that retained foetal membranes are rarely encountered in African buffalo (Bertschinger, Cooper, Nel, Rogers, Tindall, personal communications). Retained foetal membranes have occasionally been observed in old buffalo cows or following difficult parturition and dystocias.

In the *Syncerus* placentome, various morphological features might support an easy and uncomplicated release of foetal membranes, namely the parallel arrangement of foetal villi, their slender shape and the rather simple, leaf-like features of tertiary villi. In *Bos*, thick edges (connective tissue tongues) of caruncular crypts containing collagen types I and III fix the broad-based chorionic villi within placentomal crypts (Boos *et al.* 2003b). Detachment of broad villi through a narrow opening (*Bos*) would certainly appear more difficult than detaching slender villi through a similar sized opening (*Syncerus*).

## 5.6 Histotrophic nutrition in the *Syncerus* placenta

Histotrophic nutrition by endometrial glands is very important in epitheliochorial placenta types (Vogel 2005). In cattle, areolae (dome-shaped spaces between gland openings and the overlying trophoblast) and marginal folds (folds of chorioallantois at the transition from cotyledonary to intercotyledonary chorion) are central structures for histotrophic nutrition of the foetus (Mossman 1987). Columnar, phagocytic trophoblast epithelial cells over marginal folds actively take up the secretions of endometrial glands around the base of the caruncular stalk of *Bos* placentomes (Mossman 1987).

Lack of stalk formation in *Syncerus* placentomes involves the reduction of marginal folds from early gestation (Schmidt *et al.* 2005) until term (present results). In the early pregnant African buffalo a particularly high endometrial gland activity is reported as a possible compensation for the reduced absorptive surface area of marginal folds (Schmidt *et al.* 2005). In the full term *Syncerus* placenta, the degree of glandular activity is unknown. However, numerous glandular profiles occur in the endometrial tissue beneath placentomes and a moderate, continuous glandular secretory activity can be suggested throughout gestation. Considering the greater number of placentomes observed in *Syncerus* compared to *Bos* in the present study, it appears feasible that the absorptive surface area of marginal folds might, in total, be similar in placentae of both genera. The role of histotrophic nutrition might therefore be comparable in the *Syncerus* and *Bos* placentas.

## 5.7 Placental morphology and phylogenetic relationships between *Syncerus*, *Bubalus* and *Bos*

Torpin (1971) emphasizes that similarity of placentation must indicate evolutionary relationships between mammals. Mossman (1987) notes that evolution of reproductive organs and foetal membranes have diverged far less, and followed a straighter line, than body evolution when compared in any single genus. In other words, dissimilar placentation of two given genera would exclude their close phylogenetic relation.

The multicotyledonary, synepitheliochorial placenta type described in all three bovid genera considered in this study does therefore follow the general rule that all genera within a family have similar placental types (Enders & Carter 2004). However, the closer resemblance in placentome morphology between *Bubalus* and *Syncerus* when compared to that described in cattle, might possibly reflect the closer relationship between the two buffalo genera than that between buffalo and *Bos*, as



previously suggested by craniological (Groves 1981) and genetic studies (Gallagher *et al.* 1999; Buntjer *et al.* 2002; Groves 1981; Janecek *et al.* 1996; Hassanin & Douzery 1999).

## **5.8 Implications of placental morphology for interspecies and intergeneric embryo transfer**

Currently, no reliable rules exist for predicting success in embryo transfer (ET) between two different species or genera. Systematic studies of uterine and placental structure in different species might provide helpful information in evaluating possible choices for interspecies transfer (Hradecky *et al.* 1987b; Hradecky *et al.* 1988a). It should also be noted that the relatedness of one species to another according to current zoological systems might not necessarily reflect the true degree of the relationship.

Factors that determine the degree of compatibility between embryos and uteri of various species and genera are not clearly understood. The importance of similarities in the general anatomy, reproductive organs, reproductive physiology and endocrinology between donor and recipient seems obvious, but the influence of variable factors such as placental structure and gestation length have not yet been defined. Studies done by Hradecky *et al.* (1987b, 1988b), where species compatibility for ET was based on observed similarities in placental structure, seem to underline the importance of structural homogeneity for the application of this technique.

Past attempts at intergeneric embryo transfer (ET) between *Bos* (donor) and *Bubalus* (recipient) (Drost *et al.* 1986) as well as *Syncerus* (donor) and *Bos* (recipient) (<http://www.embryoplus.com/index.html>) resulted in pregnancies but animals aborted in all cases during the second and third month of gestation. The time period during which abortion occurred in these trials corresponds time-wise with the differing stages of placentomal development between *Syncerus* and *Bos* (Schmidt *et al.* 2005). A foetal CRL of about 8 cm corresponds to approximately 65 days (~2 months) of gestation in cattle (Björkman 1954). At this stage, *Bos* placentomes develop a caruncular stalk whereas *Syncerus* placentomes remain non-pedunculated (Schmidt *et al.* 2005). In addition, villous trees are broad-conical with distinct secondary branches in *Bos* compared to slender, barely branched villous trees in *Syncerus*. Subsequent insufficient penetration of “*Syncerus*-specific villi” into “*Bos*-specific crypts” might be the result of structural incompatibility between tissues of the transferred foetus and the surrogate mother. Structural incompatibility has been reported in placentomes following interspecies and intergeneric ET between other ruminant species such as gaur and cattle (Hradecky *et al.* 1988b) as well as between domestic sheep and domestic goat (Dent *et al.* 1971, Hradecky *et al.* 1988b). For example, the delivery of an undersized, dead gaur calf after a prolonged gestation period of 9.5 months of the bovine mother

(Hradecky *et al.*1988b) supports our suggestion of incomplete villous-crypt interlocking, leading to insufficient nutrient supply and reduced foetal development. Prolongation of the gestation period can thus be considered as a compensatory mechanism, as has been suggested by Hradecky *et al.* (1988). Besides possible endocrinological, immunological and biochemical factors influencing the success of intergeneric ET, incompatibility between the mother and the transferred foetus may result from structural inconsistencies, such as differences in placentome shape, villous size and villous branching pattern.

Considering the closer similarity in gestational length and placentomal morphology between water buffalo and African buffalo, when compared to cattle, intergeneric ET between the two buffalo genera may be more likely to succeed and should be seriously considered in the future.

## 6 Conclusion

The present study provides the first histo-morphological description of the full term African buffalo placenta. Based on morphological and histological examinations of full term placentomes and of expelled foetal membranes, significant differences between African buffalo and cattle placentation are revealed.

The morphological similarities discovered between African buffalo and water buffalo placentae support the closer phylogenetic relationship between the two buffalo genera, *Syncerus* and *Bubalus*, than between buffalo and domestic cow. The results presented offer possible explanations for the one and two months longer gestation period in the African buffalo when compared to the water buffalo and cattle, respectively.

Histo-morphological features of reproductive organs are important factors that have to be considered in planning future research in reproductive physiology. Based on present results, the water buffalo, rather than cattle, can be expected to be more compatible as recipient for intergeneric transfer of African buffalo embryos.

## 7 References

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