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1 **The ovine placenta and placentitis – a review**

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11

12 **Abstract**

13 An appreciation of the complexities of placental structure and function is essential to
14 understanding the pathogenesis of infectious placentitis and abortion. This review
15 aims to illustrate aspects of ovine pregnancy and placentation that will assist both the
16 research worker and the diagnostic pathologist. Morphologically, the ovine placenta is
17 classified as being chorioallantoic, villous, cotyledonary and synepitheliochorial.
18 Apposition of foetal and maternal tissues in early pregnancy eventually leads to the
19 formation of the definitive placenta. Physiological features of placentation that are
20 essential to normal pregnancy and foetal development include modulation of immune
21 responses at the placental interface, increasing placental bloodflow to allow for
22 increasing foetal demand and the secretion of hormones for the recognition and
23 maintenance of pregnancy. Descriptions of the morphology of the near-term placenta
24 in a normal pregnancy and of the foetal membranes that are voided during normal
25 parturition provide the proper context for understanding the morphological changes

26 associated with placentitis and how these changes are likely to affect placental
27 function.

28

29 *Keywords:* placenta; placentitis; sheep; *Chlamydophila abortus*; *Toxoplasma*
30 *gondii*

31

32 **Introduction**

33 The placenta may best be defined as a functional apposition of maternal and foetal
34 tissues (Steven, 1975; Kaufman and Burton, 1994; Wooding and Flint, 1993). Its role
35 is not only to provide the foetus with nutrients and allow gaseous exchange but also to
36 permit the mother to tolerate the presence of what is essentially a “foreign body” or
37 semi-allogeneic foetus carrying paternal antigens. Assheton (1906) gave the earliest
38 detailed account of ovine placentation in the modern veterinary literature. His
39 description of sequential development and morphology was supplemented by
40 Wimsatt’s observations (1950) and by many subsequent studies (Lawn *et al.*, 1969;
41 Burton *et al.*, 1976; Wooding *et al.*, 1980; Wooding 1984). The objective of this brief
42 review is to illustrate some features of ovine placentation essential to understanding
43 the pathogenesis of abortion in sheep.

44

45 **Classification of placentae**

46 By definition, a placenta develops during pregnancy in all Eutherian (placental)
47 mammals. However, the final or definitive form of placentation differs in different
48 species. Placental classification is based on these differing anatomical features. In
49 sheep, the foetal component of the placenta is formed from the fusion of the avascular
50 chorion and the vascular allantois. This increases in surface area by developing

51 villous projections, which are clustered in discrete areas, known as cotyledons. Hence
52 the ovine placenta is classified as being chorioallantoic, villous and cotyledonary.
53 Alternatively, placentae of different species may be classified according to Grosser's
54 method (Wimsatt, 1950; Wooding and Flint, 1993; Kaufman and Burton, 1994). This
55 is based on the observation that the number of layers interposed between the maternal
56 and foetal circulation differs in different species. In species with epitheliochorial
57 placentation, all layers (endothelium, connective tissue and epithelium) are intact on
58 both sides of the placenta such that the chorion (foetal epithelial layer) is apposed to
59 the maternal epithelium. In other species, the maternal layers are effaced such that the
60 chorion is apposed to the endothelium of maternal blood vessels (endotheliochorial
61 placentation) or is bathed in maternal blood (haemochorial placentation). In the ovine
62 placenta, the chorion is apposed to a foeto-maternal syncytial layer, which replaces
63 the maternal epithelial layer within placentomes, prompting the designation
64 **synepitheliochorial** placentation (Wooding, 1992).

65

66 **Placental development**

67 In ruminants, the last stage of embryonic development prior to implantation involves
68 elongation of the blastocyst from a sphere of 1 mm diameter to a filamentous structure
69 of more than 10 cm in length (Rowson and Moor, 1966). Attachment is initiated at
70 around the 17th day of gestation over raised projections of the uterine wall, the
71 caruncles. The chorionic epithelium overlying uterine caruncles becomes focally
72 thickened and develops parallel folds, which fit into corresponding grooves on the
73 caruncular surface (Assheton, 1906). Formation of cylindrical villous projections
74 from these folds disrupts the orderly parallel arrangement. Villi gradually elongate
75 from 1 mm at 32 days gestation (dg) to 10 mm at 74 dg, becoming attenuated and

76 frond-like (Wimsatt, 1950). Primary villi extend towards the caruncular wall,
77 branching to form lower order villi. The septa that interdigitate with these villi are
78 branching projections of the caruncular wall, which extend in the opposite direction.
79 Placentomes reach a maximum size and weight at about 90 dg due to expansion of
80 foetal villi and their associated capillary network. The myxomatous connective tissue
81 core of villi progressively diminishes thereafter to allow for the expanding villous
82 surface (Assheton, 1906; Barcroft and Barron, 1946).

83

84 **Placental immunology and immune responsiveness in pregnancy**

85 Successful pregnancy requires immunological compromise by the dam to allow
86 acceptance of a “foetal allograft” (Medawar, 1953). Medawar proposed three possible
87 mechanisms to explain foetal survival: the existence of an anatomical barrier to
88 physically separate the dam from the foetus; the possibility that the foetus might not
89 be sufficiently antigenic to provoke a maternal immune response and/or
90 immunological tolerance of the dam towards the foetus.

91 In addition to constituting a physical barrier between mother and foetus, the
92 trophoblast layer of the placenta in humans, mice and ruminants does not express
93 classical MHC class I antigens (Entrican, 2002). Whilst neither ovine trophoblasts
94 nor foeto-maternal syncytia express MHC class I molecules, stromal cells in the
95 caruncular septa and endometrial epithelial cells in interplacentomal areas of the
96 placenta do (Gogolin-Ewens *et al.*, 1989).

97 Lymphocytic activation may be suppressed at the foeto-maternal interface. Factors
98 known to suppress *in vitro* activation of lymphocytes are secreted *in utero* (Hansen
99 and Liu, 1996). The trophoblast secretes interferon tau (IFN- τ), while both
100 trophoblast and endometrium secrete prostaglandin E₂ and the endometrial glands

101 secrete serpins (uterine milk proteins), all of which inhibit lymphocyte activation.
102 Furthermore, non-classical, class I MHC antigens expressed by trophoblasts prevent
103 maternal NK-mediated cytotoxic responses (Entrican and Wheelhouse, 2006).
104 Altered cytokine secretion profiles by maternal lymphocytes during pregnancy may
105 also be required to ensure pregnancy success. CD4⁺ T helper cells (Th cells) in mice
106 have been classified according to their cytokine secretion profiles as either Th1 or
107 Th2 (Mosmann *et al.*, 1986). Th1-type cytokines include interleukin 2 (IL-2) and
108 interferon gamma (IFN γ) which are pro-inflammatory and promote cell-mediated
109 immune responses. Th2-type cytokines, particularly interleukin 4 (IL-4), are
110 antagonistic to Th1-type cytokines and promote humoral responses. Secretion of
111 Th2-type cytokines by the normal human and murine conceptus led to the idea that
112 there might be a bias towards Th2-type immunity during normal pregnancy (Hauguel-
113 de Mouzon and Guerre-Millo, 2006). Consequently, it may be that Th1-type maternal
114 immune responses, as elicited by intracellular bacterial infection, are incompatible
115 with successful pregnancy (Raghupathy, 1997). Also the regulation and maintenance
116 of ovine pregnancy may involve other cells, such as T regulatory cells, recently
117 recognised as having an important role in human and murine pregnancy (Aluvihare
118 and Betz, 2006).
119 There is no published evidence to date of a similar bias in cytokine secretion profiles
120 during pregnancy in ruminants (Waldvogel *et al.*, 2000; Entrican, 2002), although
121 Innes *et al* (2001) did show that in cattle there appears to be a down regulation of
122 cellular response to the T-cell mitogen concanavlin A, at mid-gestation. Whether this
123 is a reflection of an alteration in the secretion of cytokines is not known. Pregnant
124 cattle, seropositive for bovine viral diarrhoea virus (BVDV), responded in the same
125 manner as non-pregnant seropositive cattle to experimental challenge with a non-

126 cytopathic strain of BVDV (Waldvogel *et al.*, 2000). Messenger RNA expression for
127 IFN γ and IL-4 by peripheral blood mononuclear cells (PBMCs) was similar in
128 pregnant and non-pregnant cattle. When stimulated *in vitro*, the capacity of ovine
129 PBMCs to produce IFN γ is not affected by pregnancy (Entrican, 2002) and the
130 secretion profiles of both IL-4 and IL-10 are not appreciably different in pregnant and
131 non-pregnant sheep (Wattegedera *et al.*, 2007).

132 Although the foetal side of the placenta is devoid of lymphocytes in normal
133 circumstances, the ovine foetus becomes increasingly immunocompetent as
134 pregnancy advances. In the foetal lamb the onset of specific immune responsiveness
135 correlates with increasing numbers of circulating lymphocytes and the increasing
136 mass of lymphoid tissue (Fahey and Morris, 1974). The ovine foetus usually
137 develops without exposure to foreign antigenic material and the normal ovine
138 placenta does not permit transfer of maternal immunoglobulin to the foetus such that
139 neonatal lambs are usually agammaglobulinaemic prior to ingesting colostrum
140 (Griebel, 1998). However, following antigen challenge *in utero*, the ovine foetus can
141 elaborate specific antibody as early as 64-66 dg. In addition, rejection of allografts by
142 the foetal lamb has been demonstrated (Schnickel and Ferguson, 1953; Silverstein *et*
143 *al.*, 1964; Niederhuber *et al.*, 1971). The capacity to reject allografts which develops
144 between 67 and 77 dg, is cell-mediated and independent of antibody (Silverstein *et*
145 *al.*, 1964). Immunocompetence of late gestational fetuses has been confirmed by oral
146 vaccination of fetuses and demonstration of subsequent protective immunity in
147 neonates (Gerds *et al.*, 2002).

148

149 **Placental bloodflow and foeto-maternal exchange**

150 Foetal de-oxygenated blood is carried to the placenta in the arterial system, where it
151 becomes oxygenated in the villous capillary network before it is transported back to
152 the foetus in the venous system. Although the juxtaposition of foetal and maternal
153 capillary networks, which are for the most part in parallel array with bloodflow in
154 opposite directions, suggested a counter-current arrangement (Barcroft and Barron,
155 1946), it is now thought that the net effect of various anastomoses is to create cross-
156 current exchange (Wooding and Flint, 1993). The efficiency of transfer of nutrients
157 and gases across the placenta does not appear to be affected by the number of layers
158 interposed between foetal and maternal blood as morphological features such as
159 indentation of surface epithelia by capillaries, so-called “intraepithelial capillaries”,
160 greatly reduce the diffusion distance. In addition, active transport mechanisms and
161 carrier molecules exist to facilitate transmembrane transfer (Wooding and Flint,
162 1993).

163 Pregnant sheep have been extensively used as a model system for the study of
164 placental angiogenesis and physiological control of placental bloodflow to provide a
165 better understanding of the pathogenesis of compromised pregnancies in humans
166 (Reynolds *et al.*, 2005; Reynolds *et al.*, 2006). Hence a great deal of information has
167 been generated about the magnitude of placental bloodflows in the ovine placenta and
168 how these flow rates are able to keep pace with the increasing requirements of the
169 rapidly growing foetus. Placental weight is maximal in sheep at about day 90 of
170 gestation. Thereafter, the increased demands of the foetus during the exponential
171 growth phase are met by increasing uterine and umbilical bloodflows (Makowski,
172 1968). This is achieved on the maternal side by increased size of the capillary bed and
173 on the foetal side by increased branching and surface area of the microvasculature
174 (Reynolds *et al.*, 2005).

175

176 **Endocrine control of pregnancy and parturition**

177 Progesterone is necessary for the establishment and maintenance of pregnancy,
178 ensuring that the endometrium is receptive to the conceptus and that the myometrium
179 remains quiescent. Prior to the maternal recognition of pregnancy, the cyclical *corpus*
180 *luteum* (CL) in the ovary is the source of this progesterone. The CL of pregnancy
181 continues to be the predominant source between 13 and 55 dg whereas placental
182 production of progesterone is sufficient to maintain pregnancy in ovariectomised ewes
183 from 55 days gestation (Allen, 1975). Prostaglandin $F_{2\alpha}$ ($PgF_{2\alpha}$) from the
184 endometrium initiates luteolysis in the cycling ewe (McCracken, 1971) but this
185 uterine luteolytic activity is abrogated by the presence of a conceptus *in utero* (Moor
186 and Rowson, 1966). Production of $IFN-\tau$ by trophoblasts between 8 and 21 days post
187 conception, exerts a local action on the endometrium thus blocking the pulsatile
188 secretion of $PgF_{2\alpha}$ and prolonging the lifespan of the CL (Bazer *et al.*, 1997).
189 Activation of the foetal hypothalamic-pituitary-adrenal axis in late gestation and
190 secretion of foetal cortisol induces the production of the enzymes 17α hydroxylase
191 and aromatase in the placenta which favour oestrogen synthesis at the expense of
192 progesterone synthesis. Maternal plasma levels of oestradiol 17β increase suddenly
193 whilst plasma levels of progesterone decline sharply immediately prior to parturition.
194 Spontaneous myometrial contractility is augmented by autocrine and paracrine release
195 of $PgF_{2\alpha}$ and parturition ensues. The precise nature of the cue for foetal cortisol
196 release is still somewhat uncertain. It is unclear if foetal maturation alone accounts
197 for cortisol secretion in late pregnancy or if an external stimulus such as foetal stress
198 is required (Wood, 1999).

199

200 Morphology of the late stage placenta – 120 days gestation to term

201 The combination of a foetal cotyledon and a maternal caruncle is referred to as a
202 placentome. Placentomes vary considerably in both size and number, ranging in size
203 from less than 10mm to more than 50mm in diameter and numbering from 20 to 70
204 per foetus. Although usually concave in shape the ovine placentome may also
205 occasionally occur in convex form similar to placentomes of cattle. In their more
206 usual form, each placentome when viewed from the foetal aspect appears as a round,
207 firm protuberance from the uterine wall, which glistens because of the overlying
208 chorioallantois (Fig. 1a). Chorioallantoic blood vessels enter each placentome at its
209 hilus, a centrally located depression demarcated by brown pigment. On cross section
210 of a placentome, the full extent of the brown-pigmented, haemophagous areas (or
211 arcade haematomata) is evident, confined as they are within a zone surrounding the
212 hilus (Fig. 1b).

213 Histologically, this hilar zone consists of primary chorioallantoic villi surrounded by
214 pools of blood and the hyalinised distal ends of caruncular septa (Fig. 1c).
215 Erythrocytes and aggregates of golden-brown granular pigment in the cytoplasm of
216 the tall columnar trophoblasts (chorionic epithelial cells) that line the primary villi
217 indicate that these cells are actively engaged in erythrophagocytosis. Although
218 derived from haem the pigment is non-ferruginous (Wimsatt, 1950) and does not stain
219 positively for ferric iron using Perl's Prussian blue method. The blood is maternally
220 derived and these haemophagous areas, which are also described in the placentae of
221 other mammals, are thought to function as a means of transferring iron from dam to
222 foetus (Burton, 1982). Another prominent histological feature within the hilar zone is
223 the presence of thick-walled chorioallantoic blood vessels, ranging in diameter from

224 100 to 800 microns. These vessels surrounded by the sparsely cellular connective
225 tissue matrix of the chorioallantois can be seen branching along the primary villi.
226 Deeper within placentomes, chorioallantoic villi of progressively lower order extend
227 in the direction of the caruncular wall, interdigitating with branching caruncular septa.
228 The trophoblast layer lining these villi consists for the most part of uninucleate
229 cuboidal cells but also features occasional binucleate cells (BNCs) whereas septa are
230 lined by flattened syncytial cells (Fig. 1d). Following implantation, BNCs migrate
231 from the trophoblast layer across to the maternal side of the placenta, fusing with
232 maternal epithelial cells to form foeto-maternal hybrid cells (Wooding, 1984).
233 Ongoing migration of BNCs gives rise to syncytial plaques lining the caruncular
234 septa, which persist within the placentome throughout pregnancy. BNCs produce
235 placental lactogen and pregnancy-associated glycoproteins, which are delivered to the
236 maternal side of the placenta by continued migration of BNCs across the placental
237 interface (Wooding, 1992; Ward *et al.*, 2002).

238 In interplacentomal areas, the chorioallantoic membrane, which is composed of two
239 epithelial surfaces that sandwich a core of loose connective tissue, is simply apposed
240 to the uterine mucosa or endometrium. The endometrium extends as far as the hilus of
241 each placentome. Endometrial epithelium is of simple columnar type, the underlying
242 stroma is densely cellular and deep to this compact layer a less cellular connective
243 tissue matrix surrounds the tubular endometrial glands. Occasional lymphocytes and
244 histiocytes are normally present within the endometrial stroma. However, more
245 noticeable is the presence of lymphocytes within the endometrial epithelium.
246 Although numerous in interplacentomal areas of the endometrium, these
247 intraepithelial lymphocytes (IELs) are never seen within the placentome (Gogolin-
248 Ewens *et al.*, 1989). As pregnancy advances, endometrial IELs increase in number,

249 size and granularity (Lee *et al.*, 1992). Approximately 25 *per cent* of IELs in the non-
250 pregnant uterus express the $\gamma\delta$ T cell receptor ($\gamma\delta$ TCR). The increase in the number
251 of endometrial IELs which occurs during pregnancy in the sheep is largely due to
252 expansion of the $\gamma\delta$ TCR⁺ subpopulation (Meeusen *et al.*, 1993). The function of these
253 cells is not clear. It has been suggested that in pregnant sheep, endometrial $\gamma\delta$ TCR⁺
254 IELs might suppress maternal anti-foetal immune responsiveness (Hansen and Liu,
255 1996). Alternatively, endometrial $\gamma\delta$ TCR⁺ IELs may have a role in maintaining the
256 integrity of the endometrium through lysis of the transient trinucleate cells which are
257 formed by fusion of invasive trophoblast BNCs with endometrial epithelial cells (Lee
258 *et al.*, 1997). A dramatic decline in the number of IELs in the ovine endometrium
259 occurs at parturition because of degranulation, apoptosis and migration of these cells
260 into the uterine lumen (Nasar *et al.*, 2002).

261

262 **Morphology of the foetal membranes after expulsion during normal parturition**

263 Rapid degeneration of the trophoblast layer occurs immediately postpartum in the
264 normal lambing ewe leading to prompt separation of cotyledons from the caruncles
265 (Steven, 1975; Perry *et al.*, 1975). The absence of this layer on lower order villi and of
266 any maternal component in the placental tissue expelled by the ewe has resulted in the
267 ovine placenta being described as “contradeciduate” (Assheton, 1906). It also means
268 that the term “foetal membranes”, rather than placenta, more accurately describes
269 what is available to the diagnostic pathologist for examination post-abortion. Note
270 that the maternal tissue voided with the foetal membranes in humans is referred to as
271 “decidua”. After parturition, complete involution of the caruncles takes 28 days and
272 involves necrosis and separation of septa from underlying stroma and regrowth of
273 epithelium from the caruncular edges (vanWyck *et al.*, 1972).

274 Foetal membranes expelled after normal lambing consist of cotyledonary and
275 intercotyledonary areas. In cotyledonary areas, large diameter blood vessels are
276 prominent in what had been the hilar zone of placentomes (Fig. 2a). Surrounding
277 these vessels and extending throughout the cores of the primary villi, a sparsely
278 cellular, myxomatous stroma occasionally features small, circumscribed fragments of
279 bone. The trophoblast layer is still intact overlying the larger primary villi and many
280 of these cells are heavily laden with haem-derived, pigment (Fig. 2b). Smaller villi are
281 completely denuded of trophoblasts but the branching pattern of the villous tree is still
282 evident, due to congestion of the blood vessels contained therein. The
283 intercotyledonary areas consist simply of the chorioallantoic membrane.

284 As foetal membranes are usually recovered from the stable floor, they are frequently
285 heavily contaminated with bedding and faecal material. On occasions where foetal
286 membranes have been retained *in utero* after foetal death, autolysis occurs very
287 quickly. These autolytic changes resemble those seen with sterile autolysis of the
288 foetus (Dillman and Dennis, 1976 and 1979). Macroscopically, the foetal membranes
289 become grey and friable whereas microscopically the tissue becomes increasingly
290 amorphous and eosinophilic.

291

292 **Morphological changes associated with placentitis**

293 Two distinctly different distribution patterns of pathological changes in foetal
294 membranes reflect the pathogenesis and sequential progression of placentitis
295 associated with abortifacient infectious agents.

296 In ovine toxoplasmosis, a protozoal infection, multifocal necrosis is triggered in the
297 placentomes following a maternal parasitaemia. *Toxoplasma gondii* tachyzoites
298 traverse the walls of the septal blood vessels to invade the contiguous foetal villous

299 tissue causing foci of coagulative necrosis that involve both maternal and foetal
300 elements (Fig. 3). These foci are associated with both foetal and maternal
301 mononuclear inflammation, which is relatively mild (Buxton, 1999). Typically once
302 established the foci become progressively larger as pregnancy proceeds, sometimes
303 becoming mineralised. The foetus becomes infected at the same time and whether it
304 survives or not is greatly influenced by the stage of gestation at which this is initiated.
305 Early in gestation foetal immune responses are too immature to respond effectively
306 resulting in early foetal death, but infection established later on, when the foetal
307 immune system is more developed and able to exert a progressively greater amount of
308 control over the parasite, can allow longer survival (Buxton, 1999).

309 An alternative pattern of pathological response is seen when suppurative or mixed-
310 cell inflammatory lesions initially develop in the hilar zone of the placentome. The
311 cause is usually bacterial and it is presumed that infection is blood borne and gains
312 access to the hilar trophoblasts via the arcade haematomata that develop in the hilus
313 after 58 days. This explains why the lesions are predominantly localised within these
314 haemophagous areas before extending peripherally to involve the chorioallantoic
315 membrane. This particular pattern is typified by, but not unique to, chlamydial
316 placentitis in sheep.

317 When pregnant sheep were inoculated with either *Campylobacter foetus*, sub-species
318 *foetus* [*Vibrio foetus*], *Brucella ovis*, *B. mellitensis*, *B. abortus* or *Listeria*
319 *monocytogenes* each bacterium initially localised within the haemophagous zones in
320 the placentomal hilus before spreading peripherally to contiguous areas of the
321 trophoblast (Jensen *et al.*, 1961; Mollalo *et al.*, 1963a, 1963b and 1963c; Mollalo and
322 Jensen, 1964). The *Brucella* species in particular exhibited a tropism for the ovine
323 trophoblast (Mollalo *et al.*, 1963a; 1963b and 1963c). Preferential growth of *B.*

324 *abortus* in bovine foetal tissues has been ascribed to the intracellular presence of
325 erythritol (Smith *et al.*, 1962). Furthermore, only host species in which erythritol was
326 found in placentae were susceptible to acute placentitis when challenged with
327 *Brucella* spp. (Keppie *et al.*, 1965). While both *Chlamydophila abortus* and *Coxiella*
328 *burnetii* also appear to grow preferentially in the foetal trophoblast (Zeman *et al.*,
329 1989; Sammin *et al.*, 2006), whether this is due to the presence of a similar trophic
330 factor(s) or other cause is not known.

331 Chlamydial placentitis does not develop until after 110 days gestation, whereupon the
332 severity of lesions progressively develops (Buxton *et al.*, 1990; Sammin *et al.*, 2006;
333 Maley *et al.*, 2008). In advanced stages, extensive loss of the trophoblast layer
334 throughout the hilar zone of placentomes is accompanied by a severe suppurative
335 inflammatory response at the exposed chorionic surface (Fig. 4). Inflammatory
336 changes are particularly severe at placentomal margins with marked chorioallantoic
337 arteritis and thrombosis occasionally accompanied by full-thickness necrosis of the
338 chorioallantoic membrane. Although placental pathology primarily involves the
339 foetal chorioallantois, focal endometritis may also be seen at the edge of placentomes.
340 Different patterns of leucocytic infiltration are apparent on foetal and maternal sides
341 of the placenta. Chorioallantoic infiltrates predominantly consist of neutrophils and
342 macrophages and it is suggested that a significant proportion of the latter express the
343 major histocompatibility complex class II molecule, produce tumour necrosis alpha
344 and are important in the pathogenesis of the infection (Buxton *et al.*, 2002), and as
345 such probably represent a non-specific, inflammatory response. On the other hand the
346 number of cells labelling positive for CD4, CD8, $\gamma\delta$ TCR or immunoglobulin are
347 relatively few (Buxton *et al.*, 2002; Sammin *et al.*, 2006). In contrast, intralesional
348 lymphocytes are very numerous on the maternal side of the placenta and consistently

349 feature more CD8⁺ than CD4⁺ T-cells, suggesting an antigen-specific response and an
350 important role for CD8⁺ T-lymphocytes in local immunity to *C. abortus* infection in
351 sheep (Sammin *et al.*, 2006).

352

353 **Pathogenesis of abortion**

354 Placentitis may result in placental failure and abortion for one of a number of reasons
355 and is most probably the result of a combination of these different factors. Reduced
356 efficiency of foeto-maternal exchange is an inevitable consequence of an
357 inflammatory process at the placental interface. In addition, chorioallantoic arteritis is
358 a distinct feature of the placentitis caused by fungi and some specific bacteria, most
359 notably *C. abortus* and such vascular damage is likely to impinge on placental
360 bloodflow. Therefore even when inflammatory changes are largely restricted to the
361 hilar zone of placentomes, the functional capacity and viability of extra-hilar areas
362 may also be affected.

363 Disruption of placental endocrine function is another likely sequel to placentitis.
364 Chlamydial placentitis in sheep has been shown to be accompanied by a reduction in
365 maternal plasma progesterone that occurs two weeks earlier than in normal
366 pregnancy, an increase in plasma oestradiol 17 β and elevated levels of prostaglandin
367 E₂ in foetal fluids (Leaver *et al.*, 1989). In addition, the systemic response of the
368 foetus to infection can be very marked and features premature activation and
369 hyperplasia of the adrenal cortex associated with elevated foetal plasma levels of
370 cortisol, as has been demonstrated in *B. abortus* infected foetal calves (Enright *et al.*,
371 1984).

372 Placentitis may also endanger the foetus by upsetting the precise immunological
373 balance that exists between it and the dam. For pregnancy to be successful, maternal

374 immunity is modulated locally in the placenta to enable the dam to accommodate the
375 foetus and not reject it. In bovine neosporosis it has been suggested that infection with
376 *Neospora caninum* in some circumstances may reach a certain undefined threshold in
377 the placenta so that the production of Th1-type cytokines is not adequately suppressed
378 and pregnancy fails and the foetus is aborted, so-called immune expulsion (Dubey *et*
379 *al.*, 2006). Subsequently the possibility that this might also occur in some cases of
380 ovine toxoplasmosis has been mooted (Buxton *et al.*, 2007).

381

382 **Conclusions**

383 Although much has been learned from experimental studies about the pathogenesis of
384 infectious abortion in sheep, this review illustrates that there are still significant gaps
385 in our understanding of the immunological basis for successful pregnancy in
386 ruminants and of how this is perturbed by infectious disease. A considerable
387 advantage in addressing this deficit is that the physiology of normal pregnancy,
388 including foetal development, has been so intensively studied in sheep. To date,
389 experimental studies of ovine toxoplasmosis and ovine chlamydial abortion have
390 primarily focussed on the immunological responses of sheep to both pathogens and on
391 the sequential pathological changes that occur in foetuses and placentae of infected
392 pregnant sheep. Characterisation of patterns of cytokine expression at the placental
393 interface and the identification of any changes in protein expression by the pathogens
394 during latent and active stages of infection will further our understanding of these two
395 infectious diseases of sheep, giving a clearer insight into the delicate balance that
396 exists between the pathogen and the infected host animal. Such studies on pregnant
397 animals must take cognisance of the fact that there are two distinct “hosts”, the foetus
398 and the dam. Although each is capable of responding to infection in different ways,

399 the foetus becomes increasingly immunocompetent as pregnancy advances such that
400 its response to infection will be greatly influenced by the timing of that threat relative
401 to the stage of gestation.

402

403 **Conflict of Interest Statement**

404 None of the authors (D. Sammin, B. Markey, H. Bassett, D. Buxton) has a financial or
405 personal relationship with other people or organisations that could inappropriately
406 influence or bias the paper entitled “The ovine placenta and placentitis – a review”.

407

408

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413

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644

645 **Figure Captions**

646 Fig. 1. Ovine placentome, normal pregnancy, 120 days gestation. (a) Chorioallantoic
647 blood vessels (BV) entering the hilus. (b) Cross section along the plane indicated by
648 dashed line in (a); hilar zone (HZ) demarcated by the dashed line contains
649 haemophagous areas. (c) Haemophagous area, location indicated by the larger box in
650 (b); a pool of maternal blood (*) lies between the distal end of a septum (S) and a
651 primary villus (V). (d) Interdigitation of villi (V) and a septum (S) within the area
652 indicated by the smaller box in (b); binucleate cells (bnc) occur in the trophoblast
653 layer lining a villus and foeto-maternal syncytia (fms) line the adjacent septum.
654 Magnifications: x40 (c), x200 (d).

655

656 Fig. 2. Cotyledon obtained from a normal lambing ewe. (a) Branching villi (V) extend
657 away from the hilus (H); a fragment of bone (arrowhead) is present in the villous
658 stroma; note the absence of septa. (b) Enlarged view of boxed area in (a); a villus
659 lined by pigment-laden trophoblasts (arrowheads) at left of picture is denuded of
660 trophoblasts at right of picture; note the sparsely cellular villous stroma (*).
661 Magnifications: x20 (a), x400 (b)

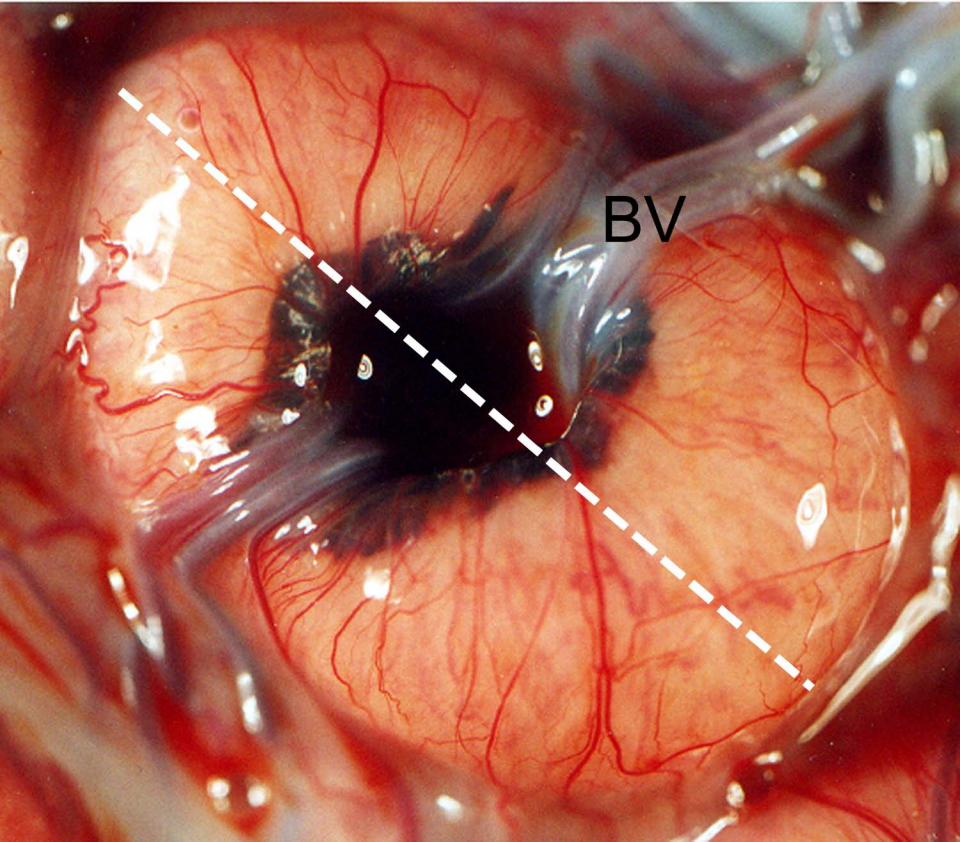
662

663 Fig. 3. Placentome from a ewe infected with *Toxoplasma gondii*. Foci of coagulative
664 necrosis (*) involve septa and adjacent villi. Magnification: x100.

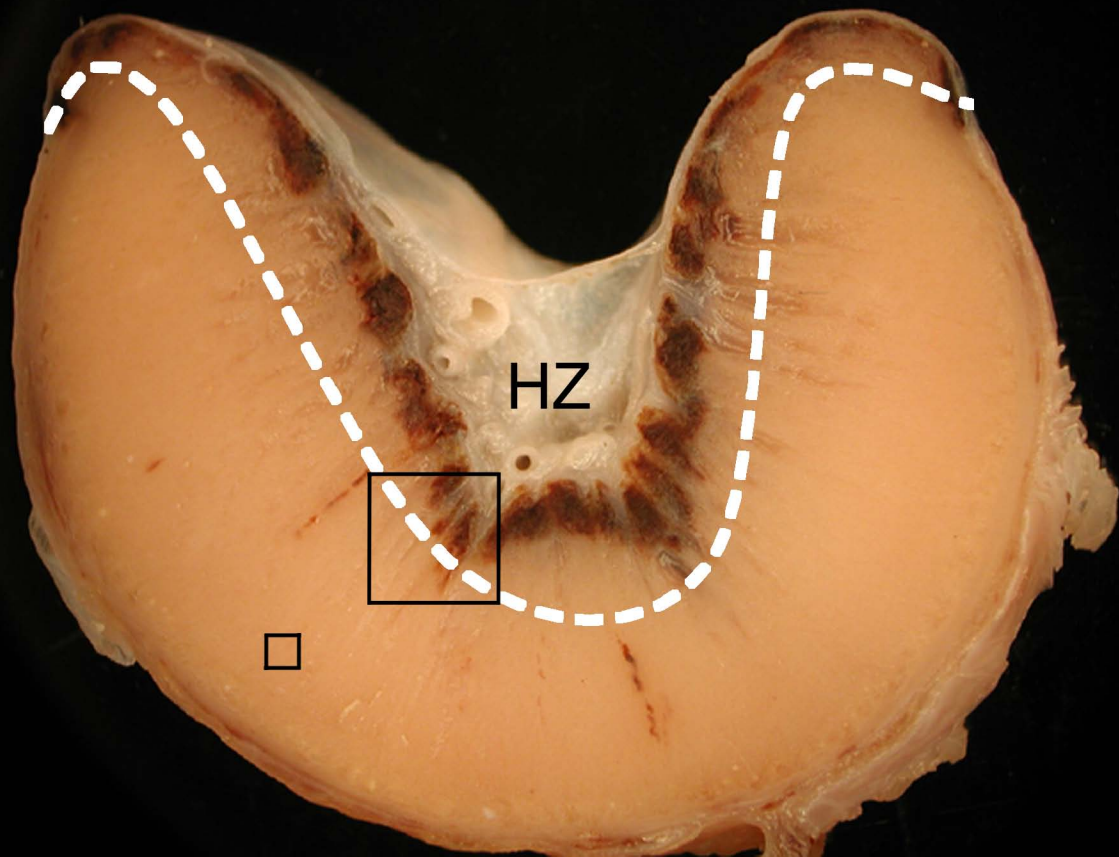
665

666 Fig. 4. Cotyledon from a ewe infected with *Chlamydophila abortus*. Severe
667 inflammation extending along the primary villi (V); fibrinopurulent exudate (*);
668 chorioallantoic vasculitis (arrowheads) at the hilus (H). Magnification x20.

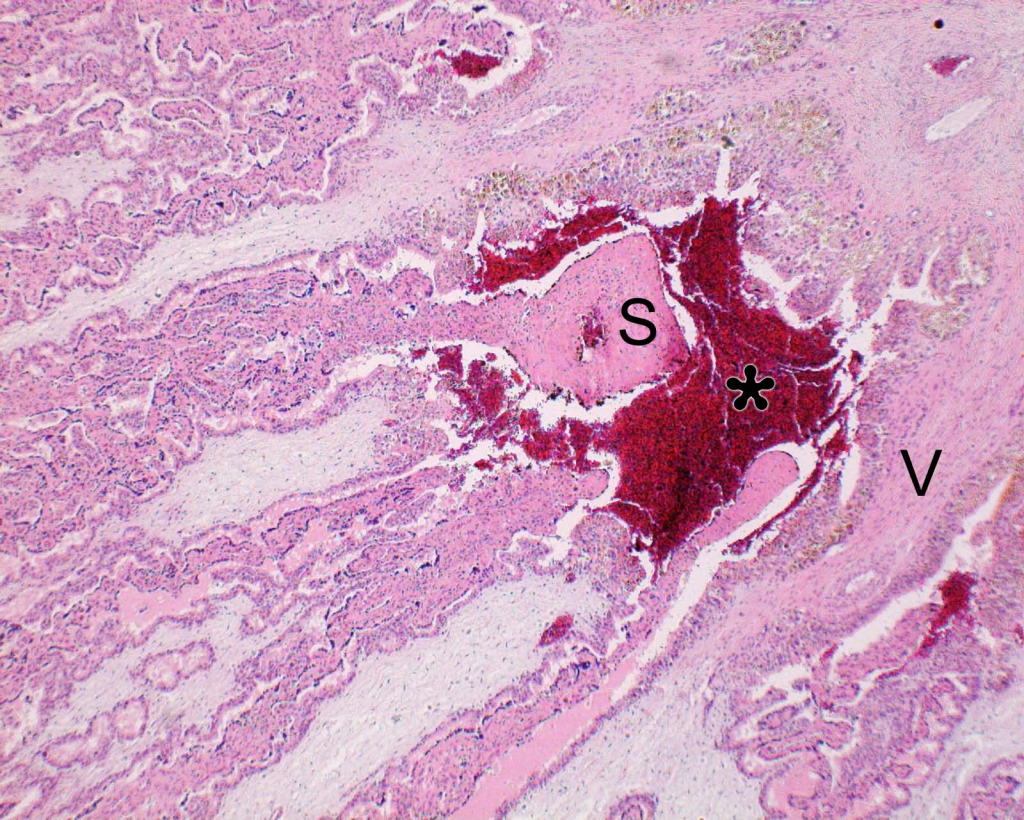
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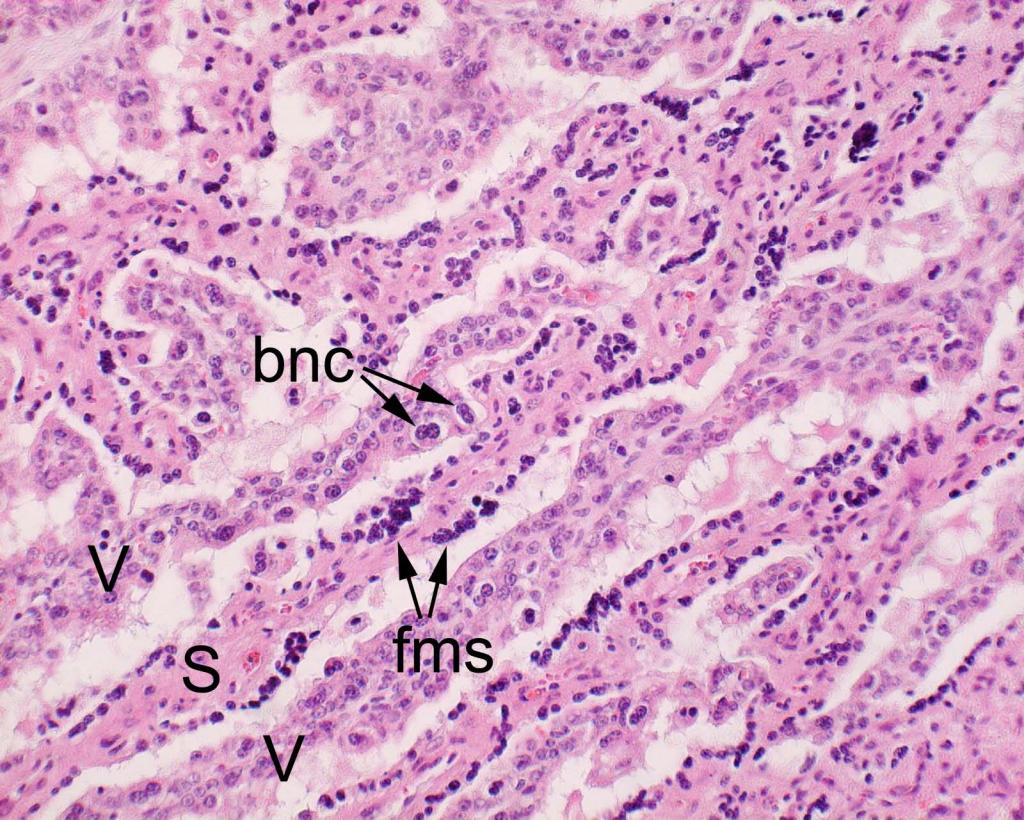
HZ



S

*

V



bnc



v

s

v



fms

