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Review

The bovine placenta *in vivo* and *in vitro*

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ABSTRACT

The gross anatomic features (cotyledonary type) and histologic classification (synepitheliochorial) of the bovine placenta have been known for many years. Thorough ultrastructural analysis as well as a variety of descriptive studies dealing with the localization of cytoskeletal filaments, extracellular matrix, growth factor systems, steroid hormone receptors, and major histocompatibility complex have contributed further significant knowledge. However, this knowledge was not sufficient to solve clinical placenta-based problems, such as retained fetal membranes. Owing to the complexity of the fetomaternal interface *in vitro*, culture systems have been developed. As trophoblast giant cells (TGC) are thought to be key players in the cattle placenta, most cell culture models attempt to overcome the pitfall of losing the entire TGC population *in vitro*. Nevertheless, distinct cell line–based *in vitro* systems such as cell monolayers or 3-dimensional (co-culture) spheroids were generated for the fetal (trophoblast) and maternal (uterine epithelium) placental compartments. Monolayers have been used to study for example, growth factor or hormonal signaling and TGC formation, whereas spheroids served as models for, for example, trophoblast attachment, uterine epithelium depolarization, and also TGC formation. In the future, the use of more improved culture models might lead to better treatments of retained fetal membranes and increased prevention of embryonic loss. In addition, the *in vitro* models could shed more light on the mechanisms of the differentiation of uninucleate trophoblast into TGC.

1. Classification and morphology

The gross anatomic appearance of the bovine placenta has been described extensively, and several terms were introduced. The term cotyledonary placenta, based on the presence of focal villous aggregations (cotyledons), is the one still and most frequently used [1]. In cattle, 100 to 140 cotyledons (polycotyledontophora) develop and attach to maternal preformed uterine caruncles to form placentomes, where fetal villous trees interdigitate with maternal crypts. With progressing gestational age, the placentomal size increases due to growth and elaboration of both components [2,3]. The fetomaternal interface within these placentomes represents the main exchange area for oxygen, nutrients as well as CO_2 and fetal metabolic products. Initially, the cattle placenta was termed syndesmochorial because the uterine epithelium (UE) seemed to have disappeared leaving the trophoblast in direct association with the endometrial connective tissue [4]. Later, it was shown that this was indeed not the case, assigning the bovine to the epitheliochorial placental types [5]. The fact that bovine trophoblast giant cells (TGC, or formerly binucleate cells) migrate and fuse with singular uterine epithelial cells to form trinucleate fetomaternal hybrid cells finally resulted in the classification as synepitheliochorial placental type [6].

Histologically, the fetal villous trees consist of an epithelial surface layer, the chorionic epithelium or trophoblast, and a mesenchymal core carrying branches of allantochorionic blood vessels. The outer layer of the

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maternal crypts, the UE, or more specifically caruncle epithelium (CE) is connected to the trophoblast to the extent that manual separation of both automatically leads to damage of either side. Oxygen and nutrients are delivered via blood vessels of the underlying stromal compartment. The vascular architecture of these blood vessels corresponds to the villous architecture, where stem villi branch into intermediate and terminal villi [7–9]. Owing to the synepitheliochorial condition, fetal and maternal vascular systems remain completely separated from each other [6].

2. Morphology during implantation and placental development

A specific characteristic of the bovine conceptus is the elongation of the placental membranes before attachment to the uterus [10,11]. Impressive growth takes place between gestational day (GD) 16 and 27 (for review [12]). At this time, the first cotyledons appear in the vicinity of the embryo, which subsequentially spread across the entire allantochorionic sac and finally attach exclusively to uterine caruncles. The precursors of the caruncles are nodules (100–140) which already develop during fetal organogenesis, protrude into the uterine lumen, and lack endometrial glands [2]. Following the sequence of cotyledon formation, the most developed placentomes are located near to the embryo [13]. Placentomes macroscopically grow only until GD 170. Later, the increasing fetal demand is satisfied by a progressive elaboration of the fetal villous trees and the corresponding maternal crypts leading to an equally growing exchange surface [8]. The processes of implantation and placentation in the bovine are characterized by distinct ultrastructural [3,13,14] and gene expression changes [15] in bovine endometrium and trophoblast. By GD 22, the chorionic epithelium consists of 80% uninucleate trophoblast cells (UTC) which are polarized and 20% nonpolarized TGC. Uninucleate trophoblast cells and the epithelium of the caruncles are, thus, forming the major part of the fetomaternal exchange surface [6]. Because both cell types are equipped with apical microvilli, an intervillous space (former uterine lumen) of 15 to 20 nm remains where pregnancy-associated glycoproteins (PAGs) can be found [16]. In the closely related sheep, the gap of the intervillous space is bridged by osteopontin an extracellular matrix (ECM) protein secreted by uterine glands (UG) which can bind to integrin receptors on both sides and, thus, could be responsible for trophoblast attachment to the endometrium [17]. The interaction of trophoblast cells with the endometrium is well documented in sheep. Here, osteopontin is produced in a progesterone-dependent manner [18], whereas TGC products affect the system of the UG [19].

3. Specific characteristics: TGC

Trophoblast giant cells, the second population of chorionic epithelial cells, display a species-specific behavior in the bovine, as they migrate through chorionic tight junctions to fuse with single epithelial cells of the caruncle [20,21]. Therefore, it seemed feasible to classify the bovine trophoblast to be moderately invasive [22]. The resulting fetomaternal hybrid cells deliver TGC products to the maternal placenta by exocytosis. Owing to their distinct appearance and behavior, TGC have been the focus of many studies. It has been shown that TGC develop from UTC [23] by tripolar acytokinetic mitosis [20]. Trophoblast giant cells occur from GD 16 onward [3,13,14]. Throughout gestation, around 20% of trophoblast cells are TGC [6], requiring that at any given time point a certain percentage of UTC differentiate into TGC because hybrid cells degenerate after migration and fusion [24]. However, toward term, the number of TGC clearly decreases [25]. After implantation, bovine hybrid cells have three nuclei because one binucleate TGC fuses with one epithelial cell of the caruncle. This is in contrast to other ruminants, such as the sheep, where larger syncytia (up to 25 nuclei) are formed by fusion of additional TGC with hybrid cells [6]. This distinct difference could be responsible for the high incidence of retained fetal membranes (RFM) in cattle compared with sheep where the epithelium of the caruncle is replaced mostly by large fetomaternal syncytia. Up to date, the important question if there is a population of stem cells which differentiate into TGC or if all UTC could do so is still to be answered [14,23]. In contrast, lineage dependence of the various subtypes of TGC has been shown in murine and rodents [26]. In fact, the invasive potential of murine TGC is maintained even when these cells differentiate out of trophoblast stem cell lines [27]. An intriguing question is also the nature of the signal(s) that induce(s) TGC formation. On the basis of published *in vivo* results, growth factors [28], compartmentalization of steroid synthesis [29], collagen type I (COL1) [30], or endogenous retroviruses [31] have been named as such. By use of cell culture experiments, it seems likely that TGC formation encompasses multiple steps which are differentially regulated [30,32].

4. Specific characteristics: ECM

Extracellular matrix carries out a multitude of functions such as mediating trophoblast attachment to the UE [33], inducing cell differentiation [30], being a reservoir of growth factors [34], or serving as tracks on which cells can migrate along [22]. Owing to the impressive plasticity of the transient organ placenta, which grows in a very short time to be expelled after birth, the ECM appears to be of special importance for placentation and placental function. In fact, ECM remodeling already begins in early pregnancy as far as COL1 in the endometrial stroma is concerned [35]. Any tissue remodeling involves major changes/alterations of the ECM composition and relies on a delicate balance between matrix synthesis and degradation. Extracellular matrix molecules such as type I collagen and type IV collagen (COL4), fibronectin, and laminin are synthesized mainly by fibroblasts, which can also produce the respective degrading enzymes, such as matrix metalloproteinases (MMP) [36]. Growth of the bovine placenta comprises first actual growth of maternal septa and fetal villous trees and second, the establishment of secondary and tertiary (exchange) branches. Near term, a process of placental maturation occurs [37,38], which finally results in the expulsion of fetal membranes and