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Enzymes in Bovine Placenta: A review

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Schlüsselwörter: Nachgeburtsverhaltung, antioxidative Enzyme, steroidogene und Prostaglandinmetabolismus Enzyme, Matrix-Metalloproteinase, DNA Reparaturenzyme, Indikatorenzyme.

Summary

The placenta is indispensable for the maintenance of pregnancy. It is responsible for the metabolism of the foetus and the mother and their connection. The enzymes that catalyse the metabolic reactions involved not only in biochemical pathways but also in the synthesis and breakdown of biologically active substances are widely distributed in placental cells and tissues. Their presence assures the physiological course of pregnancy and changes in their activity may cause pregnancy disorders.

The present review summarizes the knowledge on the enzymes in the bovine placenta, shedding light on their multifaceted functions and importance. It focuses on enzymes with a key role in antioxidant defence mechanisms, protecting both the foetus and the mother from the harmful effects of free radicals, on enzymes involved in the synthesis of sex steroids, a key aspect of hormonal regulation during pregnancy, and on enzymes responsible for prostaglandin metabolism that affect various physiological processes. It also defines the enzymes involved in the metabolism of connective tissue, which contribute to the structural integrity of this important organ and ensure its continued functionality throughout pregnancy. Finally, it provides information on enzymes related to DNA damage and on indicatory enzymes.

By presenting placental enzymes, we hope to provide insights into the biochemical processes that underlie the maintenance of a healthy pregnancy and placental development and maturation and to describe the potential implications of problems with the enzymes, e.g. the retention of foetal membranes (RFM).

Zusammenfassung

Enzyme in der Rinderplazenta – eine Übersicht

Die Plazenta ist für die Aufrechterhaltung der Trächtigkeit unverzichtbar. Sie ist für den ordnungsgemäßen Stoffwechsel des Fötus und der Mutter und deren Verbindung verantwortlich. Enzyme, die für verschiedene Stoffwechselreaktionen verantwortlich sind, die nicht nur an biochemischen Stoffwechselwegen, sondern auch an der Synthese und dem Abbau biologisch aktiver Substanzen beteiligt sind, sind in Zellen und Geweben der Plazenta weit verbreitet. Ihre Präsenz sichert den physiologischen Verlauf der Trächtigkeit und Veränderungen in ihrer Aktivität können Störungen verursachen.

Ziel der Übersicht ist es, den aktuellen Wissensstand über die in der Rinderplazenta vorhandenen Enzyme zu beschreiben und deren vielfältige Funktionen und Bedeutung zu beleuchten. Der Schwerpunkt liegt auf Enzymen, die eine Schlüsselrolle bei antioxidativen Abwehrmechanismen spielen und sowohl den Fötus als auch die Mutter vor den schädlichen Auswirkungen freier Radikale schützen, Enzyme, die an der Synthese von Sexualsteroiden beteiligt sind - ein wichtiger Aspekt der Hormonregulierung während der Trächtigkeit - sowie Enzyme, die für den Prostaglandin-Stoffwechsel verantwortlich sind, der sich auf verschiedene physiologische Prozesse auswirkt. Darüber hinaus werden die Enzyme definiert, die am Stoffwechsel des Bindegewebes beteiligt sind und zur strukturellen Integrität dieses wichtigen Organs beitragen und so dessen weitere Funktionalität während der Trächtigkeit sicherstellen. Abschließend werden Informationen zu

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Abbreviations: 15-PGDH = 15-hydroxyprostaglandin dehydrogenase; 20α-HSD = 20α-hydroxysteroid dehydrogenase; 3β-HSD = 3β-hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase; 5'-NU = 5'-Nucleotidase; 9K-PGR = prostaglandin E_2-9-ketoreductase; AA = arachidonic acid; ACE = angiotensin-converting enzyme; ADAMTS-1 = thrombospondin motif metalloproteinase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CAT = catalase; CE = perivascular epithelium; CK = creatine kinase; CSCs = perivascular stromal cells; CV = chorionic villi; E1 = estrone; E1S = conjugated estrone; ECM = extracellular matrix; GGT = γ-glutamyltransferase; GSH = glutathione; GSH-Px = glutathione peroxidase; GSH-Tr = glutathione transferase; LOX = lipoxygenases; MHC-I = major histocompatibility complex; MMP = matrix metalloproteinases; MPO = myeloperoxidase; MT-MMP = membrane-type metalloproteinase; OR = oestrogen receptors; OS = oestrogen sulfatase; OST = oestrogen sulfotransferase; P4 = progesterone; P450arom = aromatase cytochrome P450; P450c17 = cytochrome P450 17-hydroxylase/17,20-lyase; P450scc = cytochrome P450scc; P5 = pregnenolone; PARG = poly(ADP-ribose) glycohydrolase; PARP = poly(ADP-ribose) polymerase; PGs = prostaglandins; PGD_2 = prostaglandin D_2; PGE_2 = prostaglandin E_2; PGES = prostaglandin E-synthase; $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$; PGFS = prostaglandin F-synthase; PGH_2 = prostaglandin H_2; PGHS/COX = prostaglandin-endoperoxide synthases/cyclooxygenases; PLA_2 = phospholipase A_2; PR = progesterone receptors; RACE = 5-rapid amplification of cDNA end; RFM = retained foetal membranes; ROS = reactive oxygen species; RT-PCR = reverse transcription-polymerase chain reaction; SOD = superoxide dismutase; StAR = steroidogenic acute regulatory protein; TGC/BNC = binucleated giant trophoblast cells; TIMPs = tissue inhibitors of metalloproteinases; TPA = 12-O-tetradecanoylphorbol-13-acetate; TrxR = thioredoxin reductase; UTC = mononuclear trophoblast cells

Indikatorenzymen und anderen Enzymen bereitgestellt, die mit DNA-Schäden in Zusammenhang stehen.

Durch diese Abhandlung über Plazentaenzyme soll ein wertvoller Einblick in die Mechanismen der biochemischen Prozesse, die der Aufrechterhaltung einer gesunden Trächtigkeit zugrunde liegen sowie der Entwicklung und Reifung der Plazenta und die möglichen Auswirkungen bei Störungen dieser Enzyme gegeben werden, wie z.B. Nachgeburtsverhaltung.

Introduction

The placenta is a temporal, very specific organ that is responsible for the development of the foetus and the course of pregnancy. In the cow, it develops in the placentation phase, around 25–50 days of pregnancy, which follows implantation. From a histological point of view, the bovine placenta is defined as cotyledonary type: the chorionic villi form patchy structures over the surface of the chorionic sac. As a result, the maternal part (the caruncle) joins the foetal part (the cotyledons) to form the placentome. Cotyledons have branched villi that penetrate the areas of the endometrium of the uterine caruncles, or more specifically their crypts. The anastomosing sites of cotyledon villi and caruncle crypts constitute a maternal-foetal unit (Schlafer et al. 2000). The placental connection between the foetal and maternal parts is formed by epithelial layers, as the epithelium of the villi of the foetal placenta-trophoblast is in direct contact with the uterine epithelium (Haeger et al. 2019). The uterine epithelium persists but is modified into a hybrid foetal-placental syncytium as pregnancy progresses (Wooding 1992). This type of placenta can be described as synepitheliochorial (Wooding 1992), emphasizing the changes in the uterine epithelium through the formation of hybrid cells. The placental structure consists of uninucleated trophoblast cells (UTCs) and binucleated giant trophoblast cells (TGCs or BNC). When TGCs reach full differentiation (as indicated by their granular appearance) they can initiate the formation of the ruminant-specific form of the placenta (Wooding 2022). They migrate through the chorionic junctions and fuse with uterine epithelial cells, UTCs, to form hybrid trinucleated cells of the foetal and maternal placenta throughout pregnancy. In the cow, these cells release TGC products into the maternal placenta by exocytosis (Haeger 2019; Wooding 2022). The process is reviewed in more detail (placental development, histology, cellular biology of trophoblast and foetal macrophages) by Schlafer et al. (2000).

The assessment of placental maturation involves histological analysis of the maternal crypt epithelium. This is important because immature placentas are associated with retention of foetal membranes and delay of the third stage of labour (Boos et al. 2003).

Placentomes increase in size during pregnancy. The foetal-maternal interface enlarges through the branching of villi trees and their corresponding crypts, which requires connective tissue remodelling. During the course of pregnancy, biologically active molecules necessary for the correct activity of the placenta and the development of the foetus are produced. Some of them are placenta-specific but the majority are common to all cells and tissues of the body. They are all produced by metabolic pathways that rely on the activity of enzymes. Enzymes in the placenta also have a

critical role in hormone regulation. They are involved in the synthesis, metabolism and degradation of various hormones, including oestrogens, progesterone and prostaglandins. These enzymatic processes contribute to the maintenance of hormonal balance required for successful pregnancy and foetal development (McNaughton & Murray 2009) as well as placental maturation and induction of parturition.

The periparturient period in dairy cows is marked by significant hormonal, metabolic and immunological changes. Within this critical time frame, one of the most frequent disturbances is the retention of the foetal membranes (RFM), i.e., the failure to expel foetal membranes within 8–12 hours after parturition. RFM is a complex disorder influenced by multiple factors (Amin et al. 2013). Various elements influence the normal course of placental maturation, including the attenuation of antioxidative defence mechanisms against reactive oxygen species; a decreased prostaglandin E_2 to prostaglandin $F_{2\alpha}$ ratio within the foeto-maternal compartments of the placentome; and an altered status of steroid hormone receptors (McNaughton & Murray 2009). Likewise, many factors influence the expulsion of the foetal membranes, such as the immunological condition of the animal before delivery; inflammatory responses related to the placenta; remodelling of the connective tissue; cellular changes such as the apoptosis of trophoblast cells and MHC-I compatibility between mother and foetus cells; and normal hormonal balance and the maturity of the placenta (Attupuram et al. 2016).

Enzymes are divided into 6 classes in accordance to their catalytic action. In many cases, however, it is more practical to describe their actions based on the pathways in which they participate. In this review, we will discuss the functions of enzymes in the bovine placenta that are restricted to special placental functions (see Fig. 1). We will not consider house-keeping enzymes. A comprehensive understanding of the enzymatic system in the bovine placenta is fundamental for unravelling the molecular mechanisms of placental function and for exploring therapeutic strategies in reproductive health.

The literature contains no comprehensive overview of placental enzymes and of their possible roles in the physiological course of pregnancy or in pathological conditions related to placenta. We hope that our review will fill this gap.

Placental Enzymes

Antioxidant enzymes

Pregnancy is a physiological state with increased energy requirements. The intensity of oxidative stress may increase due to the higher oxygen utilisation, so it is important to protect the placenta from the increased production of free radicals. The process of placental maturation requires a hormonal interplay in which free radicals play their part and the balance between the

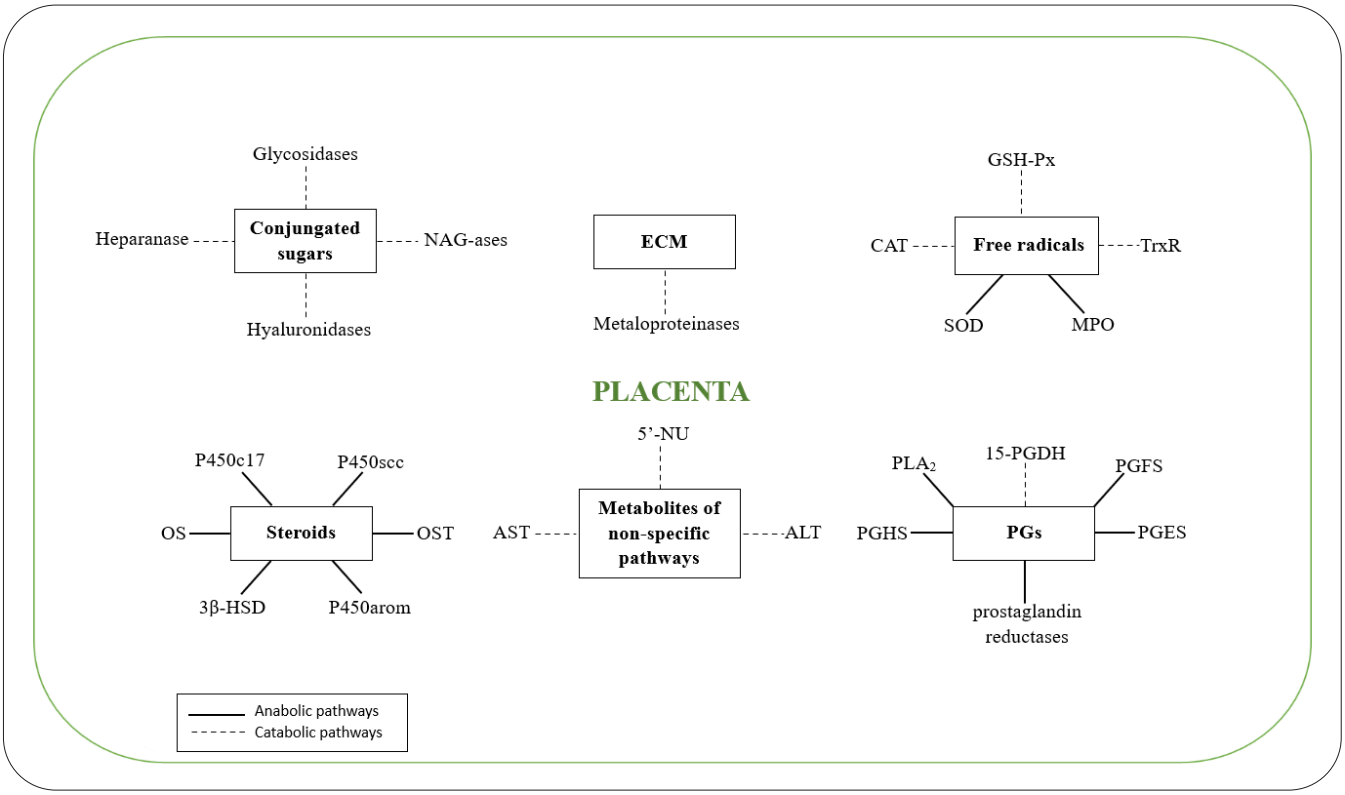

Fig. 1: Overview of placental enzymes and their substrates / Übersicht über Plazentaenzyme und deren Substrate

production and the neutralisation of free radicals may influence this hormonal interplay. Placental cells protect themselves from peroxidative damage by free radicals by enzymatic antioxidative defence (Gitto et al. 2002). The prevention of free radical excess in the placenta is important for appropriate metabolism and for maintaining redox balance during pregnancy and delivery (Weydert & Cullen 2010).

Free radicals are unavoidable products of aerobic metabolism. Small quantities of free radicals are important as signalling molecules and also have a role in the body's immune responses, cell differentiation and growth but excessive production or lack of regulation of free radicals can have serious biochemical consequences, such as changes in cell membrane permeability and damage to macromolecules such as fatty acids, proteins and nucleic acids (Dröge 2002). The disturbance in the balance between the production of free radicals and antioxidant defence leads to oxidative stress, which contributes to many diseases. In addition, free radicals are involved in ageing mechanisms (Lushchak 2014). Several enzymes are involved in antioxidative defence: intracellular glutathione peroxidase (EC 1.11.1.9; GSH-Px), glutathione transferase (EC 2.5.1.18; GSH-Tr), superoxide dismutase (EC 1.15.1.1; SOD) and catalase (EC 1.11.1.6; CAT). SOD quenches the free radicals, resulting in the formation of hydrogen peroxide (H_2O_2), which is broken down by GSH-Px and CAT. SOD activity can have an impact on CAT activity, because the presence of superoxide (O_2^-, the substrate of SOD) can inhibit CAT activity and, conversely, H_2O_2 (the substrate for CAT) can affect SOD activity (Kono & Fridovich 1982). Zinc (Zn) and copper (Cu) are cofactors of the SOD1 isoform and manganese (Mn) is a cofactor of SOD2. GSH-Px and GSH-Tr activities are related to glutathione (GSH); GSH levels are influenced by the activity of γ-glutamyl transpeptidase (GGT), which links γ-glutamyl residues to amino acids and small peptides (Kankofer & Maj 1997).

In the bovine placenta, GSH-Tr exists in an active reduced form and a less active oxidised form (Schäffer et al. 1988). Transformation from one to the other form is reversible. The enzyme has been characterized by electrophoresis and gel filtration chromatography and sedimentation coefficients have been measured by ultracentrifugation, indicating the size and shape of the protein. The enzyme belongs to the neutral PI class (Schäffer et al. 1988), suggesting that it is a member of a broader family of glutathione transferases that differ in properties and functions. This may indicate the existence of different subtypes of the enzyme.

According to available literature, activities of antioxidative enzymes are detected in the maternal and foetal parts of the placenta. Using Western blotting, Wawrzykowski et al. (2021) confirmed the presence of SOD and GSH-Px proteins in both tissues at 2 and 3 months of gestation and during parturition. The molecular weights were 35 kDa and 85 kDa. The authors usually applied a spectrophotometric method to determine enzymatic activity.

Antioxidant enzymes exist in different isoforms, depending on location or substrate specificity. Maternal and foetal bovine placental tissue express the CuZn-SOD (SOD1) and cGSH-Px isoenzymes. The specific roles and functions of SOD and GSH-Px isoenzymes in different conditions are still not fully understood. Administration of trace minerals (selenium (Se), Cu, Zn, Mn) at 230 and 260 days of gestation increases serum SOD activity (Machado et al. 2014), while administration of Se one month before calving increases GSH-Px activity (Ceballos-Marquez et al. 2010).

Research on antioxidant enzymes has mainly focused on the problem of RFM and on how reduced antioxidant capacity contributes to the condition. The activities of GSH-Px, GSH-Tr, SOD and CAT have been determined not only in physiologically released bovine placentas but also in retained placentas. GSH-Px and SOD activities were lower in the maternal part in cows with retained placenta than in released foetal membranes (Kankofer 1996a). Another study described that foetal tissue from healthy cows showed higher GSH-Px, CAT and SOD activity than in cows with retained placentas, while GSH-Px activity was high in maternal tissue in cows with and without RFM (Wischral et al. 2001). The enzyme activity was higher after caesarean sections, showing that the maternal body is exposed to higher oxidative stress than in spontaneous delivery. In the preterm group, GSH-Px, GSH-Tr and CAT showed the lowest activity, while SOD activity was the highest. In all groups, GSH-Px and CAT activities were higher in the foetal part than in the maternal part, while GSH-Tr activity showed the opposite relationship (Kankofer 2001a). There is a correlation between SOD and CAT activity (Kankofer et al. 1996a; Kankofer 2001a). In summary, the activity of all of these enzymes is affected by the mode of delivery and the time of parturition.

The activities of antioxidative enzymes are altered in blood of cows around parturition.

Also, changes on the tissue level can be seen in the placenta which confirms that the periparturient time is related to oxidative stress and, most probably, to the retention of foetal membranes as a result (Kankofer 2001b, 2002; Bernabucci et al. 2002; Yazlık et al. 2019; Khudhair et al. 2021; Sayiner et al. 2021). The changes in the activity of antioxidative enzymes were confirmed by the determination of non-enzymatic antioxidants as well as the products of peroxidative damage to macromolecules in bovine placenta (Kankofer 2001b; Kankofer et al. 2010).

Thioredoxin reductase (EC 1.6.4.5; TrxR) may be an important antioxidant defence mechanism in peripheral blood mononuclear cells (PBMCs) that is impaired during the perinatal period in cows (Sordillo et al. 2007). TrxR activity decreases during the first 21 days after calving, while elevated GSH-Px activity

is observed during this time. The cotyledon tissue of cows with RFM shows reduced myeloperoxidase (EC 1.11.1.7; MPO) activity (Gupta et al. 2005): the antimicrobial efficiency of neutrophils depends on this enzyme. Leukocytes, armed with a granular apparatus, have a key role in the body's innate defence mechanisms. When activated, they undergo degranulation, releasing MPO (Prokopenko & Aleshina 2006). Healthy cows have higher levels of MPO in cotyledons, indicating a greater influx of neutrophils at the foetal-maternal junction, facilitating the release of foetal membranes (Gupta et al. 2005).

During the synthesis of steroid hormones, free radicals are generated as by-products but oestrogen can nevertheless have antioxidant effects as it stimulates SOD activity, at least in mice (Strehlow et al. 2003). 17-beta-estradiol has antioxidant properties, preventing the ROS production resulting from angiotensin-II activity. It also reduces the production of free radicals in smooth muscle cells by elevating the levels of MnSOD (manganese) and ecSOD (extracellular superoxide dismutase) while not affecting CuZn-SOD, GSH-Px, or CAT activity (Strehlow et al. 2003).

Steroidogenic enzymes

Steroid hormones such as progesterone (P4) and oestrogens are essential for sustaining pregnancy and proper foetal development. P4 is mainly produced by the *corpus luteum* in the ovary but as pregnancy progresses, particularly between 180 and 240 days, the placenta contributes significantly to the production of this hormone (Schuler et al. 2008). The main regions of steroid production within the bovine syncytial placenta are the foetal cotyledons (Hoffmann et al. 1979; Conley & Ford 1987), which have this ability at both early and late stages of pregnancy. In contrast, significant P4 synthesis in the maternal caruncle is not observed until 100–120 days of gestation (Shemesh 1990). Cows experiencing retained foetal membranes show elevated P4 levels on the day of parturition, suggesting incomplete lysis of the *corpus luteum* (Matton et al. 1987) but P4 is gradually reduced at parturition in cows with typical calving (Matton et al. 1979). The decrease in P4 before delivery allows the enzymatic activity necessary for the placenta to separate (Maj & Kankofer 1997). P4 biosynthesis in the bovine placenta is stimulated by a mechanism that is independent of cyclic nucleotides but mediated by Ca^{2+} (Shemesh et al. 1984).

The placenta also produces a significant amount of oestrogens and their concentrations increase significantly during pregnancy. The first increase in the mother's peripheral blood is observed between days 110 and 120 and the concentration increases further around day 230–250. Nevertheless, oestrogen production is already detectable at day 33 of gestation (Hoffmann et al. 1997). In the placenta, oestrogens are mainly in the form of estrone (E1) but there is more conjugated estrone (E1S) than free estrone, especially in allantoic fluid, suggesting conjugation of free estrone by the foetus (Eley et al. 1979). The role of oestrogens in the placenta, from the beginning to late gestation, has not yet been fully elucidated. In the period preceding parturition, the functions include stimulation of the uterine myometrium, maturation of the placenta and preparation of the birth canal (Schuler et al. 2008).

The localization of hormonal receptors gives indications on the sites of hormonal action. Immunohistochemical analysis has shown that P4 receptors (PR) are present in the caruncle at each stage of pregnancy, specifically in the stromal cells and capillary pericytes, while it was found in the arterial walls detected only during delivery. Oestrogen receptors (OR) are also present in perivascular stromal cells, the perivascular epithelium and the pericytes of perivascular capillaries. As for PR, most of it is localized to the nuclei of fibrocyte-like cells (Hoffmann & Schuler 2002). ORα-specific mRNA can be detected in bovine placentas using RT-PCR (Schuler et al. 2002), confirming OR expression. The presence of ORα in caruncular stromal and epithelial cells implies that these cells are potential targets for oestrogens produced in the placenta and could potentially regulate caruncular growth. The results are consistent with earlier findings on the proliferative activity of caruncular stromal cells expressing Orα (Schuler et al. 2000). They suggest that placental steroids may have a role in regulating proliferation, particularly in the maternal part. In addition, there is a temporary decrease in proliferation in caruncular stromal cells, in particular between days 150 and 270, consistent with local oestrogen concentrations in the tissue (Hoffmann & Schuler 2002). Immunohistochemistry shows that the concentrations of ORα and ORβ in bovine placentas increase differently, indicating that the two isoforms of the receptor have different physiological roles in the tissue (Schuler et al. 2005). In contrast to ORα, which is expressed only in the caruncle, ORβ is also expressed in the cotyledons and this may be related to the control of TGC differentiation and placental steroidogenesis. Placental oestrogens and P4 appear to be important in controlling the growth, differentiation and function of the placenta (Schuler et al. 2002). Treatment of cows prior to parturition with the oestrogen receptor antagonist tamoxifen caused no changes in length of gestation, calving or the rate of retained placenta. Oestrogen concentrations were typical for cows in late pregnancy, while progesterone concentrations were significantly lower in treated animals (Janowski et al. 1996). Oestrogens may exert effects that are not mediated by classical oestrogen receptors. In the final weeks of pregnancy, the oestrogen concentrations are so high that the receptors are almost completely saturated (Janowski et al. 1996). An increase immediately before delivery may not significantly affect receptor saturation, suggesting that a late

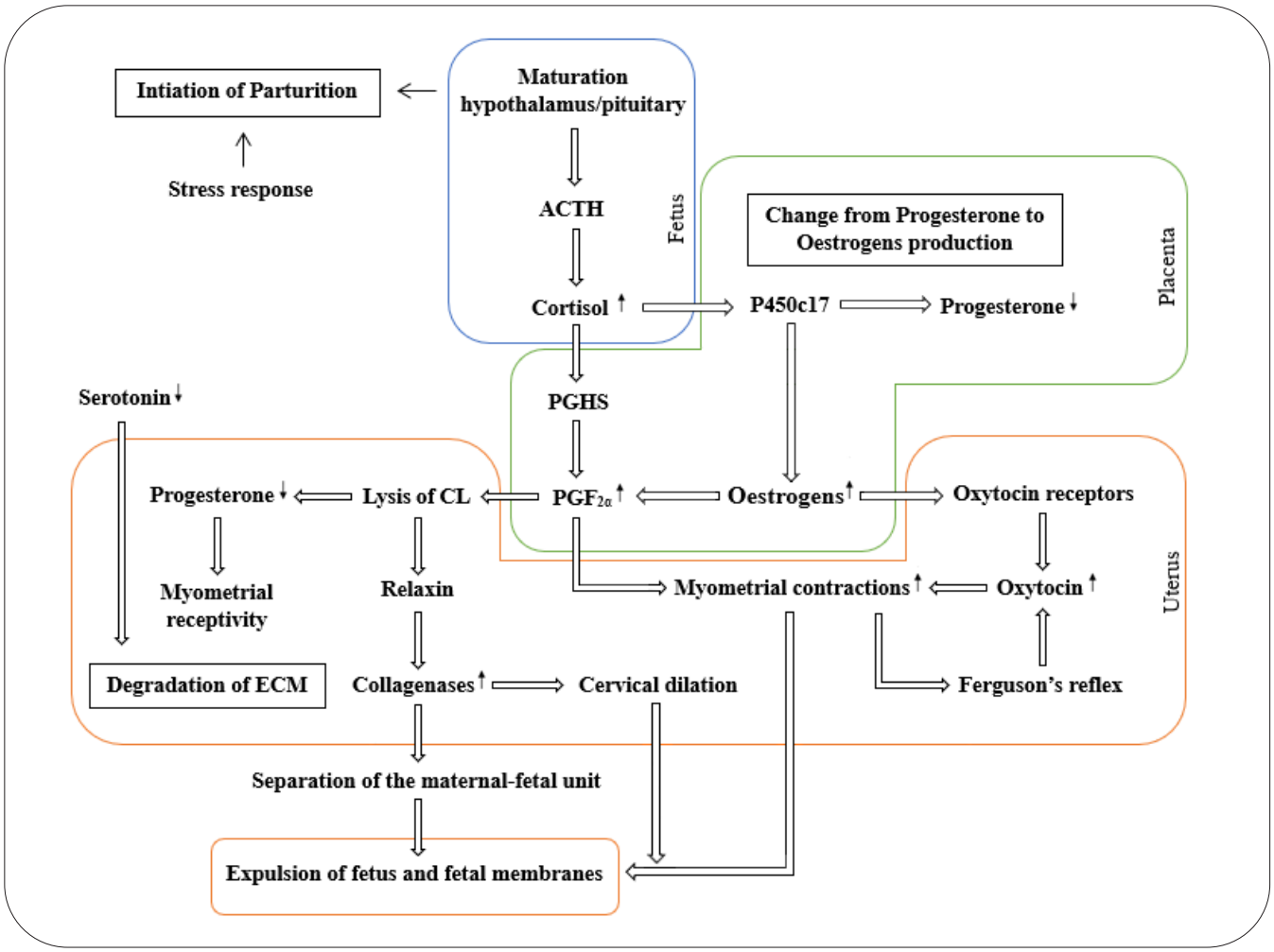

Fig. 2: Hormonal cascade of the regulation of parturition. ACTH = Adrenocorticotropic hormone, P450c17 = Cytochrome P450 17-hydroxylase/17,20-lyase, PGHS = prostaglandin-endoperoxide synthases (cyclooxygenases), $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$, CL = *corpus luteum*, ECM = extracellular matrix / Hormonelle Kaskade der Geburtsregulation. ACTH = Adrenocorticotropes Hormon, P450c17 = Cytochrom P450 17-Hydroxylase/17,20-Lyase, PGHS = Prostaglandin-Endoperoxid-Synthasen (Cyclooxygenasen), $PGF_{2\alpha}$ = Prostaglandin $F_{2\alpha}$, CL = *corpus luteum*, ECM = extrazelluläre Matrix

rise in oestrogen may not affect placental maturation (Sauerwein et al. 1989). The role of this oestrogen excess requires elucidation and it is unclear whether the excess oestrogen is used for further steroid conversion or for a non-genomic action.

Specific distribution patterns of steroid hormone receptors in bovine placentas indicate precise hormonal regulation based on cell type, gestational stage and tissue location. Receptor immunoreactivity reflects hormonal changes during pregnancy and delivery, with altered receptor activity indicating RFM (Boos et al. 2000). ORα in the *tunica media* of uterine blood vessels may also mediate uterine blood flow (Boos et al. 2006). Except for progesterone and oestrogens, androgens may also be active products of placental steroidogenesis in cattle. Khatri and Bhutto (2014) confirmed the expression of the androgen receptor (AR) in the placenta with the highest expression at day 50 and the lowest at day 150.

In ruminants, the initiation of parturition can be attributed to foetal maturation of the hypothalamic-pituitary-adrenal axis (Thorburn & Challis 1979). During this time (7 days before delivery to 3 hours before delivery), foetal plasma cortisol levels gradually increase (Comline et al. 1974). The increase in cortisol can be imitated by the administration of exogenous glucocorticoids to the maternal compartment of the pregnant cow (Adams & Wagner 1970; Jöchle 1973). Dexamethasone may initiate labour in cattle (270 days of gestation), causing a decrease in progesterone by inducing luteal regression rather than by affecting placental progesterone metabolism (Conley et al. 2019). At the same time, maternal plasma oestrogen concentration may increase (Comline et al. 1974) and remain elevated as long as the synthetic glucocorticoid flumethasone is present. After removal of the drug, steroid levels return to the control group's values (Möstl et al. 1985). Changes in placental steroid metabolism from progesterone to oestrogen production are

a result of elevated concentrations of cortisol, leading to increased activity of enzymes such as cytochrome P450 17-hydroxylase/17,20-lyase (P450c17) and potentially aromatase. The activity of these enzymes in placental microsomes (of ovine placental cotyledons) during late gestation and dexamethasone-induced delivery has been investigated. In animals undergoing induced labour, dexamethasone elevates both P450c17 mRNA expression and placental protein content. Glucocorticoids stimulate the transcription of the gene encoding this enzyme (France et al. 1988). In other words, corticoids appear to reduce the source of progesterone in the myometrium, increasing its sensitivity due to oxytocin to rising oestrogen concentrations. This seems to create conditions that precede normal parturition (Jöchle 1973).

Steroid biosynthesis begins with the conversion of cholesterol to pregnenolone (P5), a C21 steroid that is an intermediate in the synthesis of P4 and also serves as a precursor for other steroid hormones. This conversion is catalysed by the cholesterol side-chain cleavage system of cytochrome P450scc, which is located in the mitochondrial inner membrane. The first step is hydroxylation at C22 leading to the formation of 22-hydroxycholesterol, followed by the formation of 20,22-dihydroxycholesterol and cleavage of the C20-C22 side chain to form pregnenolone. The entire chain is oxygen-dependent and NADPH+ is an essential cofactor (Lieberman & Lin 2001). Free cholesterol must be supplied from cellular stores across the mitochondrial membrane to the P450scc to sustain steroidogenesis. Substrate transfer requires a protein factor - steroidogenic acute regulatory protein (StAR) (Stocco & Clark 1996). Using Northern blot, expression of the StAR gene has been demonstrated in cotyledons and caruncles tissues (Pilon et al. 1997). Immunohistochemistry has located the StAR protein mainly in foetal-maternal internodes and Western blotting shows a single band of 30 kDa in foetal and maternal tissue at all stages of gestation (from day 50 to day 120). Posttranscriptional regulation may play a significant part in controlling the abundance of the protein (Verduzco et al. 2012).

The P450scc cytochrome is present in placental tissue, demonstrating that the placenta can directly produce P4 from cholesterol (Shalem et al. 1988). The enzyme is in the mitochondria of mononuclear cells, which are present in both the cotyledon and caruncle during the mid-gestational period. However, no expression of P450scc is seen in binucleate cells (Ben-David & Shemesh 1990). Although P450scc is present in UTCs but not in TGCs, both tissues are involved in P4 synthesis. UTC is the site of pregnenolone production, which is further converted to P4 in TGC. Foetal cotyledons can secrete P4 in both early and late stages of pregnancy (Shemesh et al. 1992). P450scc is expressed in foetal cotyledons throughout pregnancy (Conley et al. 1992), although P450scc mRNA expression is higher in the second trimester than in the first (Takagi et al. 2007), so P450scc tends to increase in cotyledons during pregnancy, possibly contributing to the placenta's greater ability to synthesise hormones. However, significant P4 synthesis by the maternal

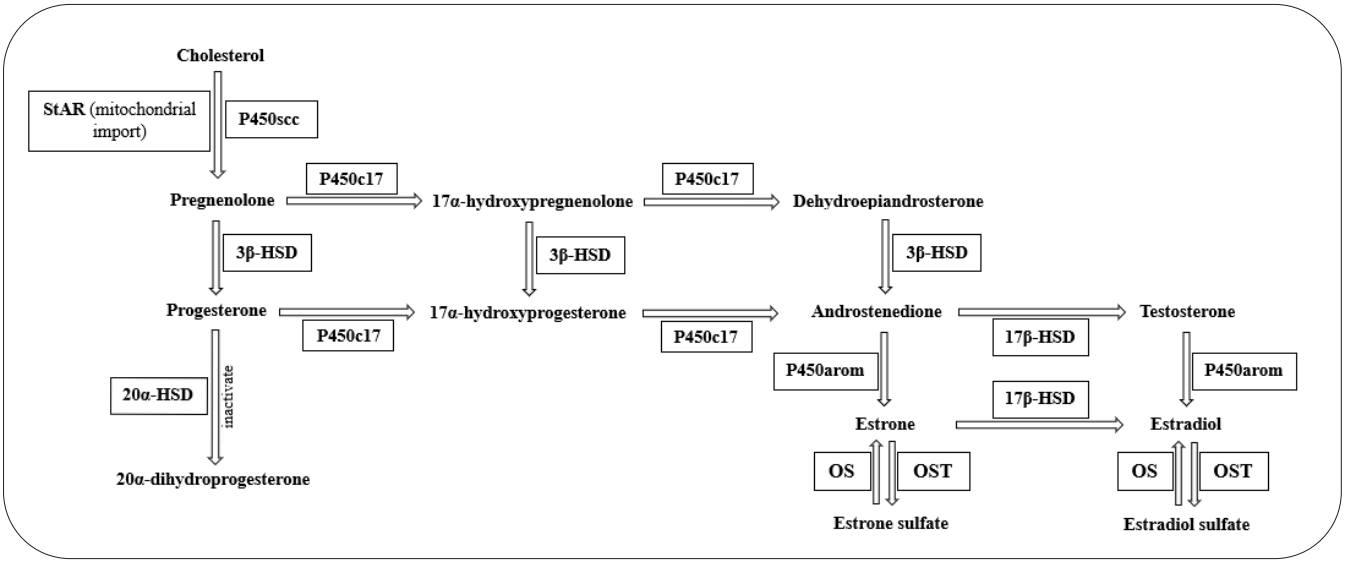

Fig. 3: The cascade of steroid hormone metabolism and the enzymes that catalyse the reactions: cytochrome P40scc, P450c17/CYP17 = cytochrome P450 17-hydroxylase/17,20-lyase, 3β-HSD = 3β-hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase, P450arom = aromatase cytochrome P450, 17β-HSD = 17β-hydroxysteroid dehydrogenase, OS = Oestrogen sulfatase, OST = Oestrogen sulfotransferase, 20α-HSD = 20α-hydroxysteroid dehydrogenase; StAR = steroidogenic acute regulatory protein; enzyme abbreviations are enclosed in boxes. / Die Kaskade des Steroidhormonstoffwechsels und die Enzyme, die die Reaktionen katalysieren: Cytochrom P40scc, P450c17/CYP17 = Cytochrom P450 17-Hydroxylase/17,20-Lyase, 3β-HSD = 3β-Hydroxysteroid-Dehydrogenase/Δ5-Δ4-Isomerase, P450arom = Aromatase Cytochrom P450, 17β-HSD = 17β-Hydroxysteroid-Dehydrogenase, OS = Östrogensulfatase, OST = Östrogen-Sulfotransferase, 20α-HSD = 20α-Hydroxysteroid-Dehydrogenase; StAR = steroidogenes akutes regulatorisches Protein; Enzymabkürzungen sind eingerahmt.

caruncles is observed only after 100–120 days of gestation, while P450scc is expressed early in pregnancy. Therefore, increased P4 production is not associated with a change in P450scc levels (Shemesh et al. 1992). The expression of P450scc and adrenodoxin (an iron-sulphur protein necessary for electron transfer) in caruncle cells remains constant in the first and second trimester but in the first trimester the maternal tissue produces low levels of P4, even though the substrate is supplied. The second trimester sees an increase in the production of P5 and thus P4 (Izhar et al. 1992).

Further transformation of P5 can follow the Δ5 and/or the Δ4 pathway. In the Δ5 pathway, P5 undergoes 17α-hydroxylation to 17-hydroxypregnenolone, while the Δ4 pathway leads to its conversion to P4 (Miller 1988; Tsumagari et al. 1994). The membrane-bound 3β-hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase (3β-HSD) is NAD^+-dependent. The enzyme has two distinct enzymatic activities and is responsible for catalysing the synthesis of P4 from P5 (Morel et al. 1997) as well as having a key role in converting various Δ5-3β-hydroxysteroids into their corresponding Δ4-3-ketosteroids. As a result, 3β-HSD is essential for the biosynthesis of a wide range of steroid hormones, including P4, androgens, oestrogens, glucocorticoids and mineralocorticoids. 3β-HSD occurs in a number of isoforms, each with tissue-specific expression and substrate selectivity (Mason et al. 1997). The 3β-HSD isoenzymes have been studied in various species, including cattle, and the cDNAs that encode them have been identified. A notable discovery is that a single protein unit contains the activity of 3β-HSD and Δ5-Δ4 isomerase (Morel et al. 1997). 3β-HSD mRNA can be detected in the bovine trophoblast in differentiating TGCs using *in situ* hybridization (Özalp 2005). 3β-HSD activity is higher in bovine cotyledons than in caruncles, although activity in cotyledons is low until the 4th month of gestation. Between 4 and 7 months, it increases significantly and reaches a peak at 8 months, persisting until delivery and decreasing at parturition. Cotyledon P4 levels mirror the fluctuations in 3ß-HSD activity. P4 is metabolised in the cotyledons before it is transferred into the foetal blood (Tsumagari et al. 1994). Cotyledon tissue homogenates show high activity of 3β-HSD on days 220–270 (Schuler et al. 1994).

Cytochrome P450 17-hydroxylase/17,20-lyase (P450c17; CYP17) has two distinct catalytic functions. The 17-hydroxylase catalyses the hydroxylation of P5 or P4, resulting in the formation of 17-OH P5 or 17-OH P4. The 17,20-lyase cleaves the C17,20 bond, resulting in the formation of dehydroepiandrosterone (DHEA) or androstenedione (Liu et al. 2005). High amounts of androgens are produced by the placenta (Möstl et al. 2023) and androgens may have a role in parturition (cervical ripening; Mahendoo et al. 1999). However, P450c17 does more than merely produce steroid sex hormones. When only 17-hydroxylase activity is present, glucocorticoids such as cortisol are produced (Miller 2002). P450c17 is exclusively found in the UTCs, where it seems to be rapidly suppressed once UTC enters the TGCs differentiation pathway. High abundance is found throughout the cotyledonary trophoblast between 80 and 160 days but thereafter staining is limited to primary chorionic villi (CV) and secondary CV branching sites, until day 270. During this period, P450c17 is up-regulated until parturition but there is no observable expression of P450c17 in the chorionic plates and intercotyledonary trophoblast. This suggests the involvement of a maternal factor in regulating the expression of P450c17. The findings show the contrasting steroidogenic capabilities of bovine UTCs and TGCs, highlighting their distinct roles in oestrogen synthesis (Schuler et al. 2006a). P450c17 expression is significantly lower in placental tissues than in foetal adrenal glands. In addition, there is a temporal correlation between foetal adrenal activation and P45017a expression in the placenta, which occurs in the early stages of pregnancy (Conley et al. 1992).

Aromatase cytochrome P450 (P450arom) converts androgens (androstenedione or testosterone) into oestrogens, such as estrone and estradiol. P450arom is expressed in the ovary and the placenta but oestrogen production is tissue-specific (Hinshelwood et al. 1993). P450arom activity in caruncular tissue is much lower than in cotyledons and is unchanged at 4–6 months of pregnancy. Foetal cotyledons show high P450arom activity in the 5th month and immediately after parturition: estrone concentrations in cotyledons and caruncles change in parallel throughout pregnancy. Estrone concentrations are about 4.6-times higher after delivery than in the 5th month. Estrone concentration is also correlated with estradiol concentration (Tsumagari et al. 1993). Immunohistochemistry shows P450arom in TGCs, with more intense staining in mature cells. P450arom abundance is higher, while P450c17 is down-regulated when UTCs enter the TGC differentiation pathway (Schuler et al. 2006a).

The expression of genes encoding steroidogenic enzymes such as P450scc, 3β-HSD, P450c17 and P450arom has been studied using real-time PCR. The transcripts Hsd3b, Cyp11A, Cyp19 and Cyp17 can be detected in the placenta and *corpus luteum* of cattle during pregnancy, although in varying concentrations. Cyp11A1W and Hsd3b are predominant in the *corpus luteum* and a high concentration of Cyp19 is found in the placenta, while its concentration is very low in the *corpus luteum*. The Cyp17 concentration is low in both tissues but highest during parturition (Vanselow et al. 2004). In cattle, the expression of the Cyp19 gene encoding P450arom is regulated by different tissue-specific regions. The main placental promoter is UTR (5′-untranslated region) 1.1 and the methylation pattern and chromatin structure of this region are correlated with the transcriptional activity of Cyp19. The 1.1 promoter is more active in foetal cotyledons, being hypomethylated compared to methylated maternal

caruncles. DNase I hypersensitive sites have also been identified in the promoter region, suggesting that chromatin accessibility has a role in regulating Cyp19 expression (Vanselow et al. 2001).

20α-hydroxysteroid dehydrogenase (20α-HSD) converts P4 to the inactive steroid 20α-dihydroprogesterone (20α-OHP) (Seong et al. 2003). The enzyme reduces the effect of P4 in the placenta and is involved in foetal development and the onset of labour (Sudeshna et al. 2013). Its roles in various aspects of bovine reproduction have attracted attention, such as *corpus luteum* function and the maintenance of pregnancy. Recombinant 20α-HSD has a molecular weight of approximately 37 kDa (Naidansuren & Min 2012). The mRNA transcripts of 20α-HSD in the placenta have been examined and the activity of the enzyme has been determined by the rate of conversion of NADP to NADPH. The gene is expressed at the end of gestation, at day 283, with strong expression on the cotyledon side and in the *corpus luteum* at the late oestrous cycle in ovaries. The observation is consistent with the requirement for P4 regulation during luteolysis and at the end of pregnancy (Naidansuren et al. 2011). UTCs show positive staining in all sections of the cotyledonary trophoblast, with a noticeable trend toward stronger staining in the chorionic plate and apical tertiary villi (Schuler et al. 2006b). In early pregnancy, at days 30, 60 and 90, 20α-HSD is present in the villi of the cytotrophoblast (Kim et al. 2014). The results suggest that 20α-HSD is involved in the maintenance of early pregnancy. During a specific timeframe, the *corpus luteum* employs 20α-HSD to regulate the production of P4 and initiate the process of self-reconstruction through the Casp-3 apoptosis signalling mechanism (Lee et al. 2023). The expression of 20α-HSD/Casp-3 signals in the chorionic villi section increases gradually from day 30 to day 90 and apoptosis of trophoblast cells increases after 90 days (Lee et al. 2022).

Oestrogen sulfatase (OS; StS) and oestrogen sulfotransferase are involved in the regulation of oestrogen levels in the bovine placenta. Oestrogen sulfotransferase (EC 2.8.2.4; OST; SULT1E1) catalyses sulfoconjugation, the attachment of a sulfate group to the 3-OH group of oestrogens (Strott 1996). Sulfoconjugated oestrogens are generally considered biologically inactive as they have a reduced binding affinity for oestrogen receptors (Hoffmann & Schuler 2002) but they can be used locally to produce biologically active free oestrogens because the placenta can deconjugate them thanks to the presence of oestrogen sulfatase (Dwyer & Robertson, 1980; Greven et al. 2007). The action of steroid hormones is based on the interaction of sulfation and desulfation processes, as well as the transport of sulfated steroids across the membrane (reviewed by Mueller et al. 2015).

The bovine placenta contains two forms of oestrogen sulfotransferase. The active form is a dimer with an approximate mass of 72 kDa, that consists of two similar subunits (Moore et al. 1988). The cDNA of oestrogen sulfotransferase (SULT1E1) corresponds to a protein with a maximum molecular weight of 34.6 kDa (Nash et al. 1988). The SULT1E1 protein was localized to foetal chorionic villi giant cells using a monoclonal antibody against purified bovine oestrogen sulfotransferase (Brown et al. 1987). *in situ* hybridization has shown that SULT1E1 mRNA is found largely in TGC (Ushizawa et al. 2007). Although transcripts and proteins corresponding to oestrogen-specific sulfotransferase have been found in both UTC and TGC, there are indications of higher concentrations of transcripts in TGCs and higher abundance of protein in UTCs. These findings resolved the discrepancies over the localization of the enzyme and suggest that post-transcriptional mechanisms play a significant part in controlling protein levels during TGC differentiation (Polei et al. 2014). Studies of the bovine trophoblast have revealed a biosynthetic pathway for the production of oestrogens (Schuler et al. 1994). The activity of OST and OS in the cotyledons and caruncle of cattle can be followed by measuring the conversion of E1 to E1S in tissue homogenates and subcellular fractions. During the latter half of pregnancy and at birth, OST activity is mainly associated with cytosol and does not change significantly, while OS activity decreases during birth. This finding is puzzling as there is an increase in free oestrogens at this time. OS activity can be found in the microsomal and mitochondrial fractions of cotyledon tissue; OST activity is higher in the cotyledon and OS activity in the caruncular tissues (Hoffmann et al. 2001). Oestrogen sulfatase activity can be found in the endometrium, allanto-chorionic membrane and placenta, while the allanto-amnion has no activity. Importantly, placental tissue shows a significantly higher conversion rate than the endometrium. OS activity in the placenta of parturient animals is significantly higher than in placentas obtained on day 262. The results indicate that in pregnant cows not only the placenta but also the endometrium and the allanto-chorion can deconjugate oestrogens. The increased OS activity in the placenta during parturition suggests that OS contributes to the increase of free oestrogens in the maternal blood toward parturition (Janszen et al. 1995). Alternatively, the increase in free oestrogens prior to parturition may be a result of the synthesis of new E1 rather than increased hydrolysis of conjugated oestrogens accumulated in the foetal-placental compartment (Hoffmann & Schuler 2002). Plasma oestrogen concentrations increase before calving (Henricks et al. 1972; Hoffmann et al. 1976), with OS largely produced in the maternal part (Greven et al. 2007). Immunohistochemistry detects OS mainly in caruncular epithelial cells, with the pattern dependent on the gestational period. On days 100–240, staining is restricted to the chorionic plate and the basal primary and secondary chorionic villi. A significant increase in staining intensity is seen on days 273–282

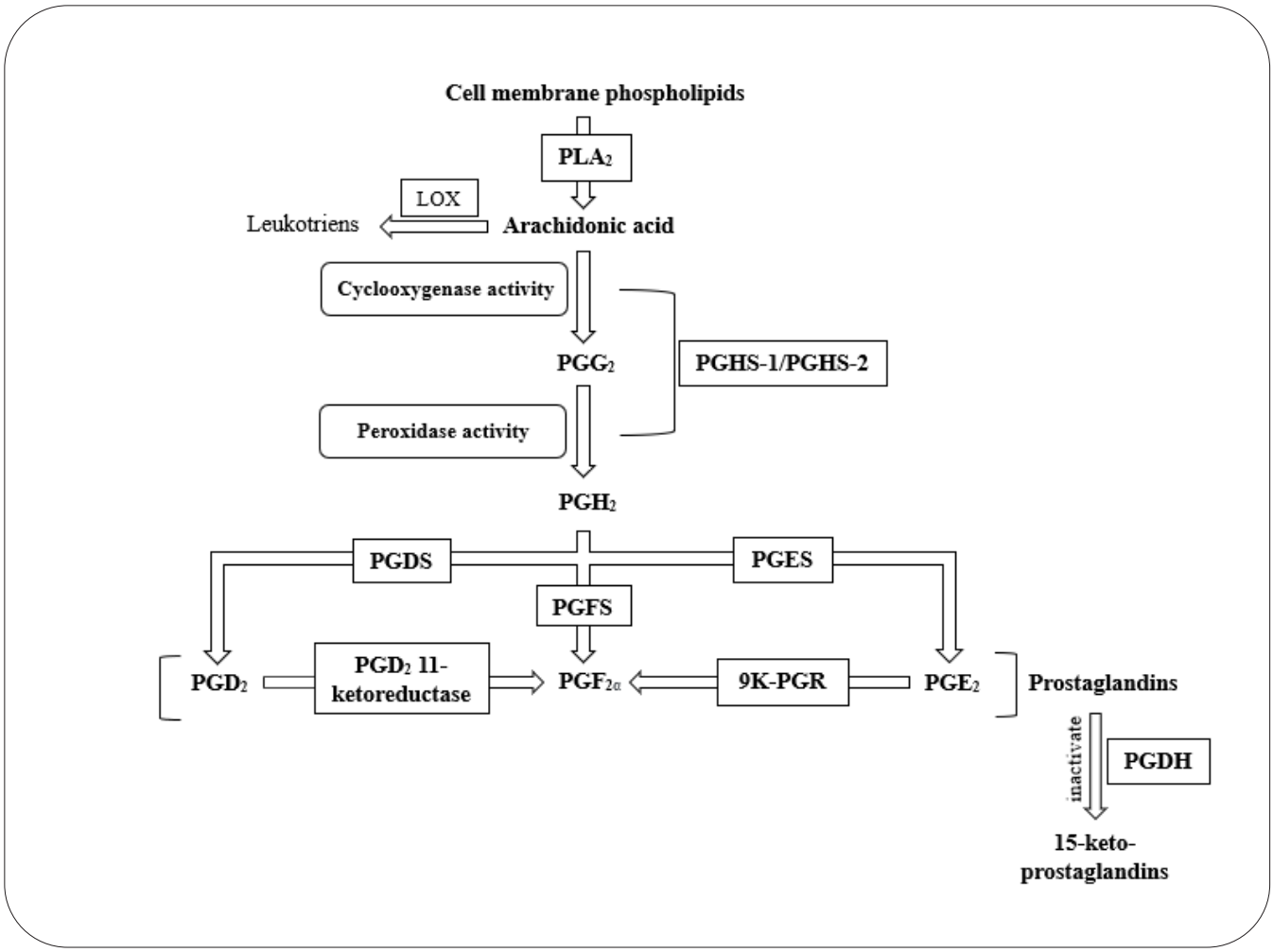

Fig. 4: The cascade of prostaglandin metabolism, and enzymes that catalyse the reactions: PLA_2 = phospholipase A2, LOX = lipoxygenases, PGHS-1/PGHS-2 = prostaglandin-endoperoxide synthases (cyclooxygenases), PGDS = prostaglandin D-synthase, PGFS = prostaglandin F-synthase, PGES = prostaglandin E-synthase, 9K-PGR = 9-keto prostaglandin reductase, PGDH = 15-hydroxyprostaglandin dehydrogenase; enzyme abbreviations are enclosed in boxes. / Die Kaskade des Prostaglandinstoffwechsels und die Enzyme, die Reaktionen katalysieren: PLA_2 = Phospholipase A2, LOX = Lipoxygenasen, PGHS-1/PGHS-2 = Prostaglandin-Endoperoxid-Synthasen (Cyclooxygenasen), PGDS = Prostaglandin-D-Synthase, PGFS = Prostaglandin-F-Synthase, PGES = Prostaglandin E-Synthase, 9K-PGR = 9-Keto-Prostaglandin-Reduktase, PGDH=15-Hydroxyprostaglandin-Dehydrogenase; Enzyme sind umrahmt.

and during delivery and includes all parts of the caruncular epithelium. The OS activity in the caruncle may be related to the utilization of sulfoconjugated cholesterol or P5, taken from maternal blood and delivered in free form to the trophoblast. The finding of low levels of OS in a small fraction of TGCs is consistent with previous results (Hoffmann et al. 2001). Reactivity was confirmed using Western blot, showing a single band of 64 kDa, and the molecular weight was the same as that obtained of OS in human placental homogenates. Evaluation of OS mRNA by RT-PCR showed a gradual 2.5-fold increase from mid-pregnancy to around luteolysis. The simultaneous presence of OST and OS suggests a local system that regulates the availability of active oestrogens in the placenta (Greven et al. 2007). During the final 21 days of pregnancy, there is a reduced correlation between the concentrations of free and conjugated estrone in the plasma. This suggests alterations in the regulation of placental oestrogen synthesis before parturition. The changes may indicate the activation of cytochrome P450c17 after day 270 of pregnancy (Hoffmann et al. 1997).

Enzymes involved in prostaglandin metabolism

Prostaglandins (PGs) are important tissue hormones for the course of pregnancy and parturition. They affect uterine motility, facilitate the expulsion of the placenta (Gross et al. 1991) and modulate leukocyte function. PGs are metabolites of the arachidonic acid (AA) cascade and their catabolism requires a series of enzymes (Rocca & FitzGerald 2002). Steroids, such as cortisol and P4, can modulate the synthesis and activity of prostaglandin enzymes in the placenta, affecting prostaglandin synthesis and metabolism. Disruptions in the metabolism of PGs are linked to RFM (Gross

et al. 1987, Slama et al. 1994; Kankofer 1999, 2002; Wischral et al. 2001).

Phospholipase A_2 (PLA_2; EC.3.1.1.4.1) catalyses the hydrolysis of the fatty acid ester bond in phospholipids, releasing free fatty acids including AA (Burke & Dennis 2009), which is metabolised by enzymes such as prostaglandin-endoperoxide synthases and lipoxygenases (LOX) to produce various prostaglandins and leukotrienes (Funk 2001). PLA_2 is active in placental tissues around parturition and the activity of enzyme in bovine placenta correlates with the fatty acid content. The enzyme is more active in the maternal part, where AA and linoleic acid concentrations are higher, than in the foetal part (Kankofer et al. 1996b). Foetal tissue is the main source of AA metabolites and metabolism is significantly increased in the last month of pregnancy (Hoedemaker et al. 1991). Maternal tissues from cows with RFM contain more arachidonic and linoleic acid. The accumulation of fatty acids is a consequence of the inhibition of PGF2a production due to a decrease in plasma oestrogen concentration (Wischral et al. 2001).

Prostaglandin-endoperoxide H_2 synthase (PGHS), also known as cyclooxygenase (COX), is responsible for the biosynthesis of prostaglandin H2 (PGH_2) from AA. PGH_2 is the precursor of other prostaglandins (Smith et al. 1996). PGHS occurs in two main isoforms, PGHS-1 and PGHS-2, and a notable expression of the PGHS-2 isoform occurs in the placenta (Fuchs et al. 1999). The level of PGHS-2 transcripts in foetal cotyledons and placentomes increases as gestation progresses, with strong expression observed at term. There is no significant difference in PGHS-2 expression before and after the onset of labour (Fuchs et al. 1999). Immunohistochemical techniques confirm the increase in PGHS-2 expression in the prepartum period (Schuler et al. 2006b). PGHS-2 can be detected in UTC from 100 days until parturition. Between 100 and 235 days, the protein is largely localized to the chorionic plate and the adjacent basal areas of the chorionic villi. At 270 and 284 days of gestation, PGHS-2 staining increases significantly and extends to the secondary and tertiary villi of the chorionic villi. Before and after parturition, the entire villi structure stain for UTC and prenatal placental PGHS-2 up-regulation starts before luteolysis. The increase in expression during the prepartum period has been confirmed by RT-PCR (Schuler et al. 2006b). These findings are consistent with general trends in PGHS-2 mRNA patterns (Takagi et al. 2008), with a significant difference in the overall expression of PGHS-2 between pregnancy and postpartum in caruncles and cotyledons. Expression remained relatively stable during the postnatal period, at least until 6 hours after parturition.

PGHS-2 expression has also been studied in the endometrium (Arosh et al. 2002; Rantala et al. 2014). Interestingly, there is higher prostaglandin production in the non-pregnant period than in the pregnant state. In addition to the presence of prostaglandin synthase, both in non-pregnant and pregnant endometrial tissues, an inhibitory effect on the conversion of arachidonic acid to prostaglandins was noted. Inhibition is high on days 16–20, 25 and 31 of pregnancy (Basu & Kindahl 1987).

Prostaglandin E-synthase (PGES) and prostaglandin F-synthase (PGFS) catalyse the conversion of PGH_2 to other prostanoids. Under the influence of PGES, prostaglandin E_2 (PGE_2) is formed, while PGFS leads to the formation of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Kankofer 1999). During the perinatal period, prostaglandin synthesis in the foetal placenta changes, shifting from PGE to PGF production before the expulsion of foetal membranes. Any disruption of the balance may be associated with placental retention (Gross et al. 1987). Cows with retained placentas tend to produce lower amounts of $PGF_{2\alpha}$ and higher amounts of PGE_2 (Gross et al. 1987; Slama et al. 1993). $PGF_{2\alpha}$ is mainly synthesized during normal foetal membrane expulsion and PGE_2 when the foetal membranes are retained (Gross & Williams 1988). Cows with RFM show reduced PGF_{2a} catabolism (Kankofer et al. 1994). The PGF2α concentration is higher in foetal cotyledons than in the maternal part but tissues from cows with retained foetal membranes show significantly lower values (Leidl et al. 1980). Similarly, $PGF_{2\alpha}$ concentrations in cows with RFM are significantly lower than in cows without RFM, in both maternal and foetal tissues (Takagi et al. 2002). As for PGE_2 concentrations, there are no significant differences between the groups at the initial time point (0 hours). However, six hours after delivery, PGE_2 content in the RFM group increases significantly (Takagi et al. 2002). The alterations in PGs concentrations relate to the changes in the activity of the enzymes involved in their metabolism.

Throughout the gestational period, the mRNA expression of PGES remains lower than that of PGFS but the same cannot be said for the postpartum period, when there is more mRNA for PGES. Cotyledons have higher expression of both PGFS and PGES mRNA. There are no significant differences in PGFS expression between the early and late detachment groups of animals (Takagi et al. 2008). Consistently, there is a 2.6-fold increase in PGFS mRNA transcript between mid-pregnancy and the prepartal period, with slightly lower levels observed at birth (Schuler et al. 2006b). All regions of the cotyledonary trophoblast stain positive for PGFS at UTC, with a trend toward higher staining intensity in the chorionic plate and apical tertiary villi than in other sections of the chorionic tree. Other placental cell types, and mature TGCs, show no signals. Similar to PGHS-2, PGFS is down-regulated in UTC as they differentiate into TGCs (Schuler et al. 2006b). Generally, PGES expression patterns during gestation and postpartum mirror those of PGHS-2 but are reversed compared to the patterns of PGFS expression (Takagi et al. 2008). UTCs are the main source of foetal placental

prostaglandins, consisting mainly of PGF. TGCs do not produce significant amounts of prostaglandins from arachidonic acid (Gross & Williams 1988). There are no significant differences in the transcription patterns of PGHS-2 and PGES genes in perinatal blood from cows with normal puerperium and uterine inflammation, so these genes cannot serve as markers of predisposition or the presence of puerperal uterine infections. The lowest expression of both genes during labour coincided with maximum cortisol levels (Silva et al. 2008). Glucocorticoids have an inhibitory effect on PGHS-2 expression (Lindstrom & Bennett 2004). PGF synthesis also takes place in the bovine endometrium (Madore et al. 2003). Vasoactivity-related systems, i.e. angiotensin-converting enzyme (ACE), endothelin-1, ET-A receptor and ET-B receptor, may be associated in bovine placentas with arachidonic acid cascade enzymes (PGHS-2, PGES and PGFS), which may have a role in placental development and foetal membrane detachment (Takagi et al. 2008).

In the oxidative metabolism of prostaglandins, 15-hydroxyprostaglandin dehydrogenase (EC 1.1.1.141; 15-PGDH) catalyses the oxidation of the 15-hydroxyl group, leading to the formation of inactive 15-keto-prostaglandins (Tai et al. 2007). In bovine placenta, PGDH is primarily localised in uninucleated trophoblast cells (Von Hof et al. 2017). The Michaelis constant (Km) of 15-PGDH is 5.7 x 10^{-6} mol/dm^3 and the maximum rate (Vm) is 0.825 nmol/s. 15-PGDH can be completely inhibited by furosemide, a loop diuretic medication, at a concentration of 90 μM/dm^3 (Kankofer et al. 1995). The enzyme activity can be measured spectrophotometrically through the reduction of NAD^+ to NADH. Foetal placental tissue has higher activity than maternal placental tissue and the presence of retained foetal membranes has no effect on 15-PGDH, except in cows that undergo caesarean sections in term. There is a negative correlation between maternal placental enzyme activity and P4 concentration, as well as between P4 concentration and total oestrogens. It appears that the stage of pregnancy as well as the hormonal environment affect 15-PGDH activity (Kankofer et al. 1994). $PGF_{2\alpha}$ concentrations in the foetal compartment are about ten times higher than in the maternal compartment (Erwich et al. 1988) and the level of $PGF_{2\alpha}$'s main metabolite, 13,14-dihydro-15-keto-prostaglandin F2α, is higher in peripartum plasma several days before parturition in cows with RFM than in cows without RFM (Bosu et al. 1984). Electrophoretic separation reveals five fractions with 15-PGDH activity (Kankofer et al. 1995). 15-PGDH expression assessed by semi-quantitative PCR is higher at 3 months of gestation than at 6 months but the highest expression was recorded after parturition, i.e. when involution occurs. In addition, the expression is P4-dependent and does not differ significantly between retained and released bovine placenta (Von Hof et al. 2017), which is consistent with previous findings (Kankofer et al. 1994). The expression of 15-PGDH in the bovine endometrium changes during the oestrous cycle but persists at the beginning of pregnancy. 15-PGDH is located near the site of prostaglandin production, which allows it to regulate the activity. It is mainly found in glandular epithelial cells and stroma cells (Parent et al. 2006).

Prostaglandin reductases reduce the ketone group of prostaglandins, converting $PGF_{2\alpha}$ to PGE and vice versa. The production of $PGF_{2\alpha}$ involves three steps: reduction of PGH_2 by 9,11-endoperoxide reductase, reduction of PGD_2 by PGD_2 11-ketoreductase and the reduction of PGE_2 by 9-keto prostaglandin reductase, also known as 9K-PGR. This particular enzyme may have dual functionality, with both 9K-PGR and 20α-hydroxysteroid dehydrogenase (20α-HSD) activity potentially undertaken by one and the same enzyme (Madore et al. 2003). 9K-PGR may contribute to $PGF_{2\alpha}$ production in cattle. It regulates prostaglandin concentrations and activities in various physiological processes, including the perinatal period (Kankofer 1999). The Michaelis constant for prostaglandin E2-9-ketoreductase (9K-PGR) from the placenta after delivery is 117 μM and the maximum velocity 183 pmol/min. Spectrophotometric measurements of enzyme activity show an increase in activity towards parturition and significantly higher values are recorded in retained placental tissues than non-retained samples (Kankofer & Wiercinski 1999). The data may indicate a link between alterations in 9K-PGR activity and a retained placenta (Kankofer et al. 2002). Together with PGFS, 9K-PGR could be pivotal in the regulation of specific prostaglandins within the endometrium during the peri-implantation period (Asselin & Fortier 2000).

PGF2α accumulation and PGHS expression are significantly suppressed by dexamethasone treatment, although foetal placental cells are not sensitive to dexamethasone treatment during the first and second trimester (Izhar 1992). Aglepristone (a steroid with antiprogesterone activity) and dexamethasone cause a reduction in the mRNA expression of PGDH (Von Hof et al. 2017).

Placental hydrolases (enzymes of connective tissue)

Connective tissue is very important for placental formation. Remodelling of extracellular matrix (ECM) in the connective tissue allows the uterus to enlarge and the placenta to increase in size and form the placentomes (Boos et al. 2003). The ECM consists of structural macromolecules, along with specialised adhesive proteins such as fibrillins, fibronectins, decorin, dermatopontin and laminin, as well as proteoglycans (Kim et al. 2011) The composition of connective tissue is regulated by the enzymes involved in its metabolism such as among others collagenases, hyaluronidase or N-acetylglucosaminidase (NAGase).

Collagens are among the most important structural proteins of connective tissue. Collagens are degraded by substrate-specific collagenases, also known as matrix metalloproteinases (MMPs) (Jabłońska-Trypuć et al. 2016). The activity of bovine placental MMPs depends on the availability of metal ions such as Zn^{2+}, Ca^{2+} and Mg^{2+}. The highest activity is achieved in the presence of $CaCl_2$ (Alderton et al. 2004). Metalloproteinases can exist as free or membrane-anchored forms. They are synthesized in the inactive form (proMMP) and activated in the extracellular space (Murphy et al. 1999). Activation requires cleavage, which involves the hydrolysis of a peptide fragment at the N-terminus, characterized by a high proportion of cysteine (Wang et al. 2000).

MMP activity is tightly regulated at the transcription level by hormones and by molecules, such as endogenous tissue inhibitors of metalloproteinases (TIMPs). Maintaining a balance between MMP and TIMP activity is an important part of physiological processes during pregnancy, such as vascular remodelling and angiogenesis (Raffetto & Khalil 2008). MMPs are also involved in trophoblast invasion and implantation (Hirata et al. 2003) and they are important in changes in the mammary gland during lactation (Rabot et al. 2007). Excessive MMP activity can promote tissue destruction and tumour invasion (Amălinei et al. 2010).

MMP gene expression is influenced by steroid hormones, including glucocorticoids and P4. P4 suppresses proMMP-1, proMMP-3 and proMMP-7, while stimulating the production of TIMP-1 and TIMP-2 (Salamonsen 1996). 12-O-tetradecanoylphorbol-13-acetate (TPA) stimulates the production of MMP-2 and -9 in cultured bovine endometrial stromal cells, while P4 inhibits the expression of both MMPs. TPA and transforming growth factor β (TGFβ) cause a significant increase in proMMP-9 production. TPA also increases the expression of MT1-MMP, proMMP-1 and MMP-3 (Hashizume et al. 1997). IL-1α, TNFα and HGF increase TIMP-1 protein levels in a bovine trophoblast (BT-1) cell line without changing the mRNA levels, while TPA increases both protein and mRNA expression of TIMP-1 in BT-1 cells (Hirata et al. 2003).

The gelatinases MMP-2 and MMP-9 can degrade IV collagen (Nagase et al. 2006). MMP-2 can also digest fibrillar collagen in a manner similar to collagenases (Patterson et al. 2001). It is noteworthy that the collagenolytic activity of MMP-2 is much weaker than that of MMP-1, while their cooperation is believed to be essential for the efficient removal of collagen from connective tissue during inflammatory and invasive processes (Aimes & Quigley 1995).

MMPs and TIMPs can be important in regulating processes in the bovine placenta during both physiological and pathological pregnancy. This applies in particular to MMP-2 and MMP-9, as well as to membrane-type metalloproteinase (MT-MMP) MMP-14. MMP-14 can act as an activator of MMP-2 by binding TIMP-2 on the cell surface (Strongin et al. 1995). The activity of both 72 kDa (proMMP-2) and 92-kDa (proMMP-9) gelatinases was demonstrated by zymography in bovine term placenta. ProMMP-2 is active in the foetal and maternal parts of the bovine placenta, while proMMP-9 is active only in the maternal part. proMMP-2 activity is higher in cows with foetal membrane retention than without it, while proMMP-9 activity shows the opposite relationship. The foetal form of proMMP-9 thus has no function in the release or retention processes. The retained placenta contains an active form of MMP-2 with a molecular weight of 68 kDa, while healthy cows have active forms of 68, 64 and 60 kDa. Deficiency of 68 kDa MMP-degrading enzymes in the maternal and foetal parts may affect collagen hydrolysis and thus the proper release of foetal membranes (Maj & Kankofer 1997). Less of both forms of the enzyme, inactive and active, are produced in the middle and late stages of pregnancy in both maternal and foetal tissue, while the inactive form is predominant from day 150 to day 250 (Kizaki et al. 2008). Gelatinase activity is important in the formation and growth of the bovine placenta. Immunohistochemistry has shown the presence of MMP-2 mainly in the caruncular stroma in early and late pregnancy. The epithelium adjacent to the chorionic villi cells stains positive from the 2nd month of pregnancy. MMP-9 is concentrated in the same area and in the maternal epithelium, which is negative for MMP-2. During pregnancy, the endothelium and the *tunica media* cells of the maternal and foetal blood vessels express both MMP-2 and MMP-9 (Walter & Boos 2001). The localization of MMP-2 and MMP-14 in bovine placentomes has also been investigated during induced and term parturition. In early pregnancy, MMP-2 is confined to the chorionic plate and foetal villi, while the maternal stroma shows faint staining in late pregnancy. In UTCs, MMP-2 immunoreactivity increases significantly from the 4th until the 9th month of gestation. On days 60–150, MMP-14 is localized in the foetal mesenchyme and as pregnancy progresses the immunoreactivity increases in the foetal villi and in UTCs (Dilly et al. 2011). The TIMP-2 protein is located exclusively in binucleated giant cells during the entire gestation (Walter & Boos 2001; Dilly et al. 2011).

The expression patterns of MMP-2 and MMP-9 differ in the pre- and postnatal periods. In maternal tissues, MMP-9 expression during pregnancy remains low compared to MMP-2. Despite the lack of significant differences in MMP-2 expression at this time, there is an increase in expression postpartum in animals with earlier and later membrane release. Foetal tissues show reduced expression of MMP-2 throughout pregnancy and after delivery. This suggests that the caruncle tissue is the main source of MMP-2 in the bovine placenta. There is no expression of MMP-9 in cotyledons during pregnancy but MMP-9 mRNA is found after parturition, increasing significantly in the late release group after 6 hours. Expression of TIMP-2 increases

as pregnancy progressed, with markedly higher expression in the 3rd trimester in both tissue types. After delivery, there is a decrease in expression, which remains low for 12 hours. The decrease in MMP-2 and TIMP-2 expression correlates with a reduced ability to produce steroids in the placenta (Takagi et al. 2007). Early pregnancy and the peri-implantation period are characterized by a high expression of MMP-2 mRNA (Kizaki et al. 2008). However, the highest expressions are found during the oestrous cycle. In the middle period of pregnancy, MMP-2 abundance decrease and decline to low levels as pregnancy progressed, before increasing again in late pregnancy. Higher expressions in the peripartum period may be necessary for placental release. The mRNA expression of MMP-9 remain stable throughout pregnancy and is clearly lower than that of MMP-2. The patterns are consistent with the results of protein zymographic analysis. There are significant differences in gene expression between animals experiencing RFM after induction of birth with PGF2 and glucocorticoids or premature section and those releasing foetal membranes (Dilly et al. 2011). The mRNA expression of TIMP-2 and MMP-2 are notably higher in animals with RFM. The changes in transcription and the localization of proteins point to mechanisms that determine placental retention in cattle. The genes MMP-2, MMP-9, MMP-1, MMP-3, TIMP-2 and MT1-MMP, as well as EMMPRIN (extracellular matrix metalloproteinase inducer), disintegrin and ADAMTS-1 (thrombospondin motif metalloproteinase), are expressed at higher levels in late pregnancy, more so in the intercaruncular area than in the caruncular area. MMP-2 is dominant throughout pregnancy and its action during implantation and before birth is associated with the regulation of TIMP-2, disintegrin, EMMPRIN, ADAMTS-1 and MT1-MMP (Kizaki et al. 2008). mRNAs of MMP-1, MMP-3, MMP-9, MMP-13 and MMP-16, as well as of TIMP-2 and TIMP-3, can be found in bovine placenta during calving (Streyl et al. 2012). The expression of these MMPs is higher in placental endometrial samples. MMP-3 activates proMMP-9 and indirectly activates MMP-13, which in turn cleaves proMMP-9. MMP-16 is an activator of proMMP-2 and its activation is enhanced by TIMP-3 and TIMP-2. The expression of extracellular matrix collagenases (MMP-1 and MMP-13) is higher in caruncle tissue but there are no significant differences between RFM and non-RFM cows (Wakamiya et al. 2009).

Gelatinases persist in the second half of pregnancy and there is an increased MMP-2 activity in the perinatal period (Hiebel et al. 2019). This seems to be a key element for placental release after delivery. Acupuncture treatment can increase MMP-2 immunoreactivity, tissue concentration and enzymatic activity in dairy cows, leading to cotyledon and caruncle separation (Hiebel et al. 2019). However, it is worth noting that a major role in placental separation is attributed to collagenases. Collagen connections between the mother and foetus are thought to be important in the cotyledon-caruncle bond. The foetal cortisol signal causes changes in the P4 : relaxin ratio, which increases collagenase activity prior to parturition (Beagley et al. 2010). Collagen breakdown is important in placental release, as confirmed by the successful use of bacterial collagenase by injection into the umbilical arteries, with the expected results in the treatment of placental retention (Eiler & Hopkins 1993). Administering collagenase into the uterine artery during caesarean section also proved effective in reducing the incidence of RFM (Guérin et al. 2004). There is a substantial presence of type III collagen in cotyledons acquired from cows experiencing retained foetal membranes, as opposed to type I collagen. The observation is linked to the irregular synthesis or breakdown of collagen polymers, which may affect the mechanical properties of the placenta. It is possible that dexamethasone has a direct effect on this mechanism as a result of its ability to decrease collagenase production in *in vitro* cultures of connective tissue cells (Sharpe et al. 1990). Administration of dexamethasone to cows 5 days before the expected calving time, followed by $PGF_{2\alpha}$ (to 8 hours) postpartum, led to a reduction in the incidence and duration of membrane retention compared to the control groups (cows treated with saline). Discrepancies in the timing of dexamethasone and $PGF_{2\alpha}$ administration, both before and after parturition, may explain the observed differences in membrane retention and release (Gross et al. 1986). However, the results are not consistent with a subsequent study of the effect of prostaglandin treatment (cloprostenol or dinoprost) on the incidence of RFM. Cows induced with cloprostenol and dexamethasone experienced earlier calving and demonstrated less variability than those induced with dexamethasone alone but prostaglandins administered within one hour of induced labour proved ineffective at reducing the incidence of RFM (Garcia et al. 1992). The exact relationship between $PGF_{2\alpha}$ and collagen metabolism remains unclear.

Along with the activity of collagenases, the action of other placental enzymes such as hyaluronidase and N-acetylglucosaminidase is associated with retention or release of foetal membranes. Attachment between the maternal and foetal parts of the placenta is based on proteoglycan molecules within the extracellular matrix. Any changes in the activity of enzymes involved in proteoglycan metabolism can result in placental retention (Kankofer et al. 2000). Changes have been seen in the patterns of proteins linked to bound sugar molecules in animals experiencing foetal membrane retention (Wawrzykowski et al. 2019). Sugar molecules in conjugated proteins change during pregnancy, reflected in changes in placental glycosidases activity. Glycosidases have a significant impact on ECM regulation (Jamioł et al. 2020).

Hyaluronidase (a proteoglycan-degrading enzyme) degrades hyaluronic acid, one of the conjugated sugars

in connective tissue that assures the appropriate thickness of connective tissue and may facilitate the adhesion of cells. When decomposed, hyaluronic acid loses its properties. Its breakdown may be the result of enzymatic activity or of peroxidative damage to sugar molecules. Hyaluronidase activity can be detected in bovine term placenta (Kankofer et al. 1998). The enzyme, which is most probably of lysosomal origin, works optimally at pH 5.0 and its activity is revealed by two bands in zymographic analysis. Spectrophotometric determination has confirmed the results of zymographic analysis. Hyaluronidase activity may be regulated by substrate availability, which may be dependent on linoleic acid (18:2) and on PGE_2 (Hennig et al. 1995). The substrate is available in bovine placenta, as confirmed by electrophoresis and sugar-specific staining (Wawrzykowski et al. 2019). The enzyme has similar activities in the maternal and the foetal parts of the term placenta and the activities in retained placenta tissues are higher than those in physiologically released placenta (Kankofer et al. 1998). Collagen fibres may be surrounded by mucopolysaccharides (Nimni & Harkness 2018). Proteoglycan-degrading enzymes expose collagens and make them available for MMPs. This provides a good rational for treating the retention of foetal membranes by collagenase and hyaluronidase. The latter had no influence, even though it was tested at different concentrations and incubation times: unlike collagenase, it did not facilitate collagenolysis or cotyledon-caruncle separation. However, the enzyme was isolated from the testis and had different properties than that of placental origin (Eiler & Hopkins 1992).

N-acetylglucosaminidase (EC.3.2.1.30) is mainly known as a marker of inflammation in the mammary gland but the enzyme is involved in adhesive reactions of cells thanks to its participation in the metabolism of hyaluronate metabolites (Linker et al. 1955). It breaks β-glycosidic bonds of terminal N-acetylglucosamine or N-acetylgalactosamine residues. The reactions may modify the cell surface and influence not only cell recognition but also cell adhesion. N-acetylglucosaminidase may cooperate with MMPs and hyaluronidases in the remodelling and cell adhesion of connective tissue. For this reason, it was interesting to determine the activity of the enzyme in bovine placenta. Using spectrofluorimetry, the activity was found to be higher in the maternal and foetal part of the term placenta and lower in cases of retained placenta (Kankofer et al. 2000). The enzyme remains under the control of the sex steroids progesterone and oestrogens (Hussain et al. 1992).

Other galactosidases in the bovine placenta include β-galactosidase, α-1-fucosidase, β-N-acetylhexosaminidase and sialidase. These enzymes are present in the contact layers between the maternal and foetal parts. The β-galactosidase and sialidase activities exhibit similar upward trends as parturition approaches but are lower in retained foetal membranes than in non-retained foetal membranes. Conversely, the α-l-fucosidase activity tends to decrease leading up to parturition and is also reduced in retained foetal membranes. The activity of β-N-acetyl hexosaminidase varies between placental parts, with a decreasing trend in the maternal tissue and an increasing trend in the foetal tissue. Changes in enzyme activities coincide with alterations in the pattern of sugar moieties detected in electrophoretic gels (Jamioł et al. 2020).

Heparanase is an endoglycosidase able to cleave heparan sulfate in proteoglycans. Along with fibrillar proteins (collagens), proteoglycans are essential components of the ECM that surrounds cells and provides structural support (Nasser 2008). By breaking down components of the ECM, heparanase regulates various cellular processes, enabling trophoblasts to migrate and invade more efficiently. The role of heparanase is not well characterized. It appears to be important during pregnancy, where it contributes to the development and function of the bovine placenta. Northern blot analysis and RT-PCR show that heparanase mRNA is virtually undetectable in the conceptus before implantation (day 17). After implantation, heparanase is expressed in the foetal membrane containing the cotyledon (days 27–34) and continues to be expressed in the cotyledon, in the intercotyledonary foetal membrane and the caruncle on days 60, 120 and 260. There is no expression in the intercaruncular endometrium during days 30 to 120. The caruncle shows increased expression as gestation progresses and the time of cotyledon formation in the bovine placenta correlates with the expression. On day 240 of gestation, heparanase mRNA is found within the binucleate cell-rich fraction extracted from the cotyledon, although other cells produce heparanase (Kizaki et al. 2001). Western blot analysis (day 60) reveals two immunoreactive proteins with approximate molecular weights of 55 kDa and 65 kDa. The heparanase cDNA has been cloned by RT-PCR and RACE. *In situ* hybridization shows that heparanase mRNA is localized in placental binucleated cells at days 60 and 210 of gestation (Kizaki et al. 2003). RT-PCR analysis shows the expression of heparanase and its substrate - syndecans (SDC1, SDC2 and SDC4) – in the bovine placenta from day 35 to the due date, with a significant increase in heparanase expression at the end of gestation (Hambruch et al. 2017). Western blotting shows higher protein concentrations only in samples at late gestation, when immunohistochemistry shows heparanase to be localized in non-nucleated trophoblast cells, some TGCs and maternal epithelial cells. During pregnancy, the most intense signal for heparanase is in trophoblast cells, gradually shifting toward the maternal compartment as the delivery date approaches and reaching the highest expression at the end of pregnancy. The expression and localization of the enzyme differs in animals with and without RFM. The ability of heparanase to degrade the matrix may facilitate a timely reduction

in foetal-maternal adhesion, thereby promoting postpartum membrane separation (Hambruch et al. 2017). Abnormal expression or activity of heparanase has been linked to foetal growth restriction in humans and this may also be the case in cattle. While the role of heparanase in the human placenta has been extensively studied due to its importance in pregnancy-related disorders such as preeclampsia and preterm labour, there has been more limited research on its functions in the bovine placenta (Haimov-Kochman et al. 2002; Ginath et al. 2015; Naeh et al. 2022).

Placental transferases/DNA repair enzymes

Poly(ADP-ribose) polymerase (PARP; EC 2.4.2.30), a 113 kDa enzyme, participates in poly(ADP-ribose) metabolism, e.g. after oxidative damage to molecules such as DNA. It is activated when DNA strand breaks occur. It uses NAD^+ as a substrate, which is also necessary for various metabolic reactions of oxidoreductases, including prostaglandin metabolism (Kankofer & Guz 2003a; Hegedűs & Virág 2014). PARP overactivation may lead to acidification (Affar et al. 2002) and to a decrease in cellular NAD^+ and ATP. This is one of the biochemical signs of oxidative stress (Pieper et al. 1999). The interplay of poly(ADP-ribose), prostaglandin metabolism and oxidative damage can contribute to proper or improper placental release. The level of 8-hydroxy-2'-deoxyguanosine (8-OH-dG) gives an indication of the extent of oxidative DNA damage. The group of cows subjected to term caesarean sections with RFM had elevated levels of this metabolite in both the maternal and the foetal parts. However, maternal placental tissues from the spontaneous delivery groups without RFM also showed elevated levels of 8-OH-dG relative to tissues with RFM (Kankofer & Schmerold 2002). Western blotting detects PARP in bovine term placenta, suggesting that the enzyme is important in placental development and function. A semiquantitative analysis showed less enzymatic protein in the foetal than in the maternal part of the placenta (Kankofer & Guz 2003b). PARP1 is the most widely studied member of the PARP family. However, recent studies have highlighted the emergence of other DNA-dependent PARPs, such as PARP2 and PARP3 (De Vos et al. 2012). In addition to promoting efficient repair and thus prolonging cell life, PARP affects metabolic processes and its overactivation has a role in ageing and cell death (Murata et al. 2019). Proper regulation of these processes is important for gene expression and the maintenance of genome integrity (De Vos et al. 2012). Poly(ADP-ribose) glycohydrolase (PARG), a 110 kDa protein, has a closely related activity; it degrades the products of PARP catalytic action. Western blotting has confirmed the presence of PARG in bovine term placenta. In contrast to PARP, the amount of PARG enzymatic protein is higher in the foetal than in the maternal part of the placenta. There are significant differences in PARP enzymatic protein content between cases of retained and spontaneously released placentas (Kankofer et al. 2004). Further research is needed to unravel the complex molecular mechanisms by which PARP affects placental function and how its dysregulation contributes to complications in pregnancy.

Indicatory enzymes

Many intracellular enzymes are usually restricted to particular cellular organelles, including the cytoplasm and mitochondria. However, under certain conditions, such as injury, infection or cellular stress, cells can experience breakdown of or damage to their membranes. When this happens, intracellular enzymes may be released into the extracellular space, where they are not normally found. The release of intracellular enzymes into the extracellular environment can serve as a diagnostic marker for various diseases (Elazab 2015).

Significant metabolic changes in cows occur mainly during the transition period, i.e. 3 weeks before parturition to 2–3 weeks after parturition, and during lactation. Intense physiological changes can lead to the emergence of factors that induce negative energy balance and trigger abnormal metabolic events, such as increased fat mobilization and synthesis of ketogenic bodies (Djokovic et al. 2019), as well as impaired immune function (Ingvartsen & Moyes 2015). These periods have a significant effect on the values of certain blood parameters. Metabolic disorders account for more than half of the documented ailments in cattle (Mordak & Nicpoń 2006). They can include RFM, which occurs during the first 8 hours after parturition (Goff & Horst 1997). RFM may be associated with increased activity of the enzymes: aspartate aminotransferase alanine aminotransferase and creatine kinase in serum (Elazab 2015). Aspartate aminotransferase (EC 2.6.1.1; AST) and alanine aminotransferase (EC 2.6.1.2; ALT) are involved in amino acid metabolism, while γ-glutamyltransferase (EC 2.3.2.2; GGT) transports the amino acid and is involved in glutathione metabolism. 5'-Nucleotidase (EC 3.1.3.5; 5'-NU) plays a significant part in nucleotide metabolism and nucleotide distribution in cells, while creatine kinase (EC 2.7.3.2; CK) is involved in cellular energy metabolism, especially in tissues with high and variable energy requirements, such as muscle. The activities of AST, ALT, GGT, 5'-NU and CK in the placental tissues of cows with and without retained foetal membranes have been compared (Kankofer & Maj 1997) to study the intensity of metabolism in placental cells in physiological conditions and *in situ*ations altered by the preservation of foetal membranes. AST activity is lower in the maternal part, while GGT is higher in the maternal part in cows with RFM than in a group of healthy cows. No significant differences were observed in ALT activity in cows with and without RFM. In both groups,

the maternal part of the placenta showed significantly higher 5'-NU activity than the foetal part. The only noticeable difference in CK activity was a significantly lower activity in the foetal part of the placenta in cows with RFM. The findings suggest changes in amino acid metabolism in cases of retained placenta (Kankofer & Maj 1997).

The literature also presents evidence that changes in AST, ALT, GGT or CK activity can be detected in the general circulation of cows. These enzymes are not specific for the placenta but an increase in their activity in blood may indicate that septic processes of RFM affect the liver. Cows with RFM show elevated plasma AST activity, both in the prepartum period and around the time of calving. CK activity is also higher in cows with RFM. The increase in CK could be attributed to degradation of muscle tissue resulting from increased energy demand and insufficient lipid mobilization, which elevates AST activity. GGT concentrations are higher in cows with RFM but they remain within physiological ranges (Yazlık et al. 2019). Similarly, AST, ALT and GGT are elevated in the serum of cows with RFM (Enculescu & Škrbić 2021). During the first two weeks after calving, the activity of these enzymes is higher than in late pregnancy (Djokovic et al. 2019). Significant fluctuations in antioxidant enzymes in the blood coincide with changes in metabolic parameters, suggesting a correlation between antioxidant enzymes and metabolic factors. There is an increase in GSH-Px values simultaneously with an increase in AST and GGT values (3 and 8 days postpartum) (Sayiner et al. (2021). The literature lacks comprehensive data on the metabolic profile enzymes ALT, AST, GGT, 5'-NU and CK in the bovine placenta.

Conclusions

We have attempted to summarize the available information on bovine placental enzymes. The importance of these enzymes in the biochemical activity of the placenta during pregnancy and their significance for the successful progress of pregnancy are underestimated. Enzymes are responsible for all reactions occurring in the placenta, contributing to its proper functioning. Enzymes influence the concentrations of many biologically active proteins and are regulated on hormonal and allosteric levels. While the determination of placental enzymes in blood has not been discussed in detail, it is essential to recognize their catalytic activity. The study of enzyme activity in serum, in combination with other laboratory data, is crucial for understanding the progression of disease.

Although we did not focus on the disorder RFM, it is hard not to mention it when describing placental enzymes during pregnancy and during labour. The many factors that can act long before delivery and only reveal themselves during delivery or that act during labour itself make it difficult to prevent problems with placental separation. But it is crucial to understand the physiological processes that regulate the attachment, separation and expulsion of foetal membranes. Separation of the placenta occurs when the foetal-maternal unit is mature and this is influenced, for example, by decreasing numbers of TGC chorionic cells and by changes in endocrine and collagenic content. The metabolism of prostaglandins also changes and metabolic disorders can occur.

The knowledge gained from enzyme studies may pave the way for the development of new targeted treatments in cases where placental lesions require therapeutic interventions. However, the complex interactions in the bovine placenta, along with the mechanisms regulating its structural and functional maturation, require further study. In particular, we lack understanding of the interactions in the foetal-maternal unit that lead to its death or ageing and we have insufficient knowledge of the regulatory role of oestrogens in the placenta, which remains an intriguing area.

Fazit für die Praxis:
Die enzymatischen Regulationsvorgänge in der Plazenta des Rindes sind essentiell für die Entwicklung des Fötus und einen komplikationslosen Ablauf der Geburt, indem sie die Konzentrationen vieler biologisch aktiver Proteine unter hormonaler Steuerung beeinflussen. Verschiedene Faktoren können allerdings schon lange vor der Geburt eine Auswirkung auf Geburtsstörungen, wie z.B. Nachgeburtsverhaltung haben. Die Kenntnis der Wirkungsmechanismen und Aktivität plazentarer Enzymproteine kann zu einem besseren Verständnis des Gesundheitszustands und der Fortpflanzungsprobleme von Tieren führen. Darüber hinaus kann die weitere Erforschung der molekularen Ursachen von Fehlfunktionen in Zukunft Ansatzpunkte für die Prävention dieser Erkrankungen ergeben.

Conflict of interest
The authors declare no conflict of interest.

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