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Uterine and placental expression of HPGD in cows during pregnancy and release of fetal membranes

Jessica von Hof[a], Nele Sprekeler[a], Gerhard Schuler[b], Alois Boos[a,1], Mariusz P. Kowalewski[a,*,1]

[a] Institute of Veterinary Anatomy, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, 8057 Zurich, Switzerland
[b] Clinic for Obstetrics, Gynecology and Andrology of Large and Small Animals, Faculty of Veterinary Medicine, Justus-Liebig-University Giessen, 35392 Giessen, Germany

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ABSTRACT

15-Hydroxyprostaglandin dehydrogenase (HPGD) plays a key role in prostaglandins (PGs) catabolism. Its expression and activity appear to be regulated by progesterone (P4). We investigated the HPGD mRNA-expression and protein localization in placentomes and interplacental uterine sites throughout gestation (Study I), and after fetal membranes retention (RFM) compared with normally delivered fetal membranes (DFM) (Study II). Furthermore, we analyzed the influence of aglepristone (AP), dexamethasone (GC) or cloprostenol (CP), on HPGD expression in bovine placentomes (Study III). Tissues from late gestation (D272) and at normal term (NT) served as controls. HPGD was highest in all sites at the beginning of pregnancy and at (NT). Following induced parturition HPGD was lower after (AP) and (GC) compared with (NT), and was similar in RFM and DFM. Placentomes stained primarily in fetal compartments; interplacentomal signals were observed in endometrial glandular and luminal epithelium. Results indicate that HPGD may play a role during establishment and termination of gestation.

1. Background

In ruminants, prostaglandin (PG) E_2 (PGE_2) and PGF2α are key factors involved in important processes in the female reproductive system, including ovulation and luteolysis [1–3], implantation and maintenance of pregnancy [4], induction of parturition, ripening and softening of the cervix, involution of the uterus [5,6], and likely also in the release of fetal membranes [7]. In cattle, cortisol, which is synthesized and secreted by the fetus, induces parturition by increasing placental estrogen production, which leads to intensified production of PGF2α and thus decreasing progesterone (P4) secretion [3,8]. Increased myometrial contractions and softening of the birth canal parallel these events. As reviewed elsewhere in detail [9,10], the prepartum luteolytic cascade is largely controlled by the activity of cyclooxygenases (COX or PTGS), in particular by its inducible form, *i.e.,* COX2 (PTGS2). Prostaglandins are synthesized from membrane-bound phospholipids *via* utilization of phospholipase A_2-derived arachidonic acid (AA) as a substrate for COX [9]. PGH_2 is the first product in this pathway, which is subsequently converted to either PGD2, PGE2 or PGF2α due to the activity of PGD-synthase (PGDS), PGE2-synthase (PTGES) or PGF2α-synthase (PGFS), respectively [9]. Additionally, PGF2α can result from the activity of 9-ketoprostaglandin reductase (9K-PGR) utilizing PGE_2 for its synthesis, or from the reduction of PGD_2 by 11-ketoreductase [10].

Type 1 NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase (HPGD) is the key enzyme regulating the bioavailability of PGs. It controls their activities by catalyzing the reversible oxidation of PGs into the biologically inactive forms 13,14-dihydro-15-keto-PGF2α (PGFM) and 13,14-dihydro-15-keto PGE2 (PGEM) [11,12]. There is strong evidence that HPGD activity is maintained by P4 [13,14] and inhibited by progesterone receptor blockers [15,16] and glucocorticoids [17,18]. The complex regulatory mechanisms underlying these changes remain, however, not fully understood.

Although there are several studies on HPGD expression [19–21], regulation [22,23] and function [12,24], its expression and cellular localization have never been investigated comprehensively in

* Corresponding author at: Institute of Veterinary Anatomy Vetsuisse Faculty University of Zurich Winterthurerstrasse 260, CH-8057 Zurich, Switzerland.
E-mail addresses: jessica.vonhof@uzh.ch (J. von Hof), nelesprekeler@gmx.de (N. Sprekeler), gerhard.schuler@vetmed.uni-giessen.de (G. Schuler), boosa@vetanat.uzh.ch (A. Boos), kowalewskipl@yahoo.de, kowalewski@vetanat.uzh.ch (M.P. Kowalewski).
[1] These authors contributed equally to this work.

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uterine and placentomal tissues during different stages of bovine pregnancy. Furthermore, the potential role of HPGD in controlling the maintenance of pregnancy and the induction of parturition in cattle is poorly understood. In several previous studies, hormones such as the glucocorticoid dexamethasone and the PGF2α analog cloprostenol, as well as compounds interfering with P4 function, *e.g.*, the selective progesterone-receptor (PGR) blocker aglepristone, have been widely tested on the induction of parturition in ruminants [25–28] and other species like dogs [29–31] and humans [14,18]. Their effects on diminishing plasma P4 concentrations and, thereby, their luteolytic potential, were investigated [28,32–35]. Additional effects of using aglepristone in cows, besides shortening the gestation length, include lowered myometrial activity and reduced softening of the birth canal compared to naturally calving cows [36]. The probability of retention of fetal membranes is higher in cows in which parturition is induced with hormonal medication [37,38]. Dexamethasone is proven to upregulate COX2 and CYP17A1 (17alpha-hydroxylase-C17, 20-lyase) in the cotyledonary trophoblast [36], which results in activation of the delta-5 pathway of placental steroid biosynthesis [39]. This leads to decreased P4 synthesis in favor of estrogens. However, the roles of aglepristone, cloprostenol and dexamethasone in regulating HPGD and, thereby, availability of PG in the complex preterm signal cascade in cattle are still not fully understood. Therefore, in order to characterize their effects on maternal HPGD expression levels, and thereby the potential involvement of HPGD in the prepartum endocrine cascade in cows, preterm animals were treated with the P4 receptor blocker aglepristone (withdrawal of both placental and luteal P4 effects), the glucocorticoid dexamethasone (mimicking the prepartal increase in fetal cortisol), or the PGF2α analog cloprostenol (induction of luteolysis). Furthermore, bovine uteri and placentomes were collected from different stages of pregnancy and mRNA expression and immunohistochemical localization of HPGD were examined to shed new light on PG metabolism and the coordination of pathways leading to parturition in cattle.

2. Methods

2.1. Animals

All animal experiments were conducted in accordance with animal welfare legislation. Details about the experimental groups and studies, and the animal experiment permissions are shown in Table 1.

Study I included interplacentomal specimens and placentomes of 35 Holstein-Friesian cows at different stages of pregnancy (3rd to 9th months), which were collected at a slaughterhouse within 30 min after slaughtering [40,41] ($n \geq 3$ animals/month of pregnancy). The gestational age was estimated according to fetal crown-rump length as described elsewhere [40,41]. In this study tissue material was also included from spontaneously calving cows of the university dairy herd at the Hannover School of Veterinary Medicine Foundation, Hannover, Germany, which released the *secundinae* within 12 h after expulsion of the fetus (discharged fetal membranes; DFM; n = 5).

In Study II, DFM placentomal specimens were used and the mRNA levels of HPDG were compared with those expressed in patient cows undergoing routine cesarean section at the clinic of the Hannover School of Veterinary Medicine Foundation, but retaining the fetal membranes for more than 12 h (RFM; n = 5).

In DFM cows, placentomes were collected *per vaginam* within one h after expulsion of the fetus using an elongated effeminator according to Richter, as modified by Reisinger (Hauptner, Solingen, Germany) [40]. In RFM patient cows, surgeries were performed under local anesthesia with Procaine (Isocaine, 2% solution, Selectavet, Weyarn, DE), 40 ml paravertebral and 80 ml at the site of the incision, and a single placentome was collected per animal [41].

For Study III, placentomal specimens were collected from animals following preterm cesarean section at day 272, and at induced parturition and normal parturition. All these cows were of the Holstein-Friesian breed with a physiological singleton pregnancy.

More specifically, as described by Shenavai et al. [32,36], elective cesarian sections were performed in untreated cows pre-term on day 272 (D272; n = 3) after insemination and placentomes were collected to examine the clinical situation directly before the final labor-inducing signals occur, but on the same day when the treated cows were expected to deliver their calves [32,36]. Furthermore, pregnant cows were treated on day 270 after artificial insemination with 3 g (~5 mg/kg of body weight) of the PGR blocker aglepristone (AP; Alizine Virbac Bad Oldesloe, DE; n = 3; withdrawal of placental and luteal P4 effects) in 100 ml solvent s.c. at four different sites of the lower thoracic wall. The same procedure was repeated 24 h later on the contralateral side of the thorax. The cows treated with glucocorticoid (GC; n = 4; mimicking the prepartal increase in fetal cortisol) received 25 mg dexamethasone-21-undecanoate (Devamed®; Topkim, Istanbul, TR), and the cows treated with prostaglandin (CP; n = 3; induction of luteolysis) received 150 μg D-cloprostenol (Dalmazin®; Selectavet Dr. Otto Fischer GmbH, Weyarn-Holzolling, DE) on day 270 after artificial insemination [36]. All cows were observed every 2 h for signs of impending labor. Immediately after expulsion of calves, placentomes were collected *per vaginam*. Additionally, placentomes from spontaneously calving (d280.5 ± 1.3) cows (normal term = NT; n = 5) were removed *per vaginam* immediately after the expulsion of healthy calves. *Secundinae* were released in a timely manner in these animals [36].

2.2. Tissue sampling and processing

As described by Boos et al. [41], for Study I at least two randomly selected and comparatively large placentomes and two segments of the interplacentomal uterine wall including adherent allantochorion were collected at the slaughterhouse from randomly selected cows at different stages of pregnancy. Macroscopically visible caruncles were excised with the adherent allantochorion and the surrounding uterine wall. In DFM and RFM groups, only a single placentome was collected from each experimental or inpatient cow. For Study III, three to five placentomes – but no uterine wall segments – were collected *per vaginam* immediately after parturition from each cow.

As described by Sprekeler et al. [42], for Study I and II the placentomes were then divided into pieces of 10 × 10 mm and three samples per placentome were selected for further processing as follows: (1) uterine wall oriented tissue cube, *i.e.*, placental tissue including maternal plate and material of the placentomal stalk; (2) allantoic sac oriented tissue cube, *i.e.*, placental tissue including fetal plate material, and finally (3) tissue cube collected from the equatorial periphery also including fetal plate and placental tissue. The samples were embedded in Tissue-Tek II (Miles, Elkhart, IN, USA) and attached onto cork lamellae, snap frozen in liquid nitrogen and stored at −80 °C [42]. For immunohistochemistry (IHC), besides collecting the interplacental uterine parts, the connective fetal tissues were dissected from the placentomes directly after collection and centrally located cross-sections of about 5 mm in thickness were prepared [42]. Tissue samples were then fixed in 4% neutral buffered formaldehyde for 24 h, washed in tap water, dehydrated in a graded ethanol series and acetic acid-*n*-butyl ester, and finally embedded in paraffin as previously described [41].

For Study III, placentomes were placed on ice and rinsed in 4 °C cold PBS buffer, divided into cross-sections of approximately 5 mm thickness and fixed in 4% neutral buffered formaldehyde and pro-

Table 1
Experimental groups.

Study	Description	Number of cows	Animal experiment permissions
I and II	3rd–9th month of gestation Uterine and placentomal tissues; collected at slaughterhouse	n ≥ 3 per month	Animal experiments were approved by the administration of the district Hannover, and performed according to the German Law for the Protection of Animals (TierschG) and the recommendations of the German Society of Laboratory Animal Science (GV-SOLAS).
	DFM: Uterine and placentomal tissues of spontaneously calving cows with **d**ischarged **f**etal **m**embranes <12 h after the expulsion of the calf; samples collected within one hour after expulsion of the calf	5	
	RFM: Placentomes of spontaneously calving cows with **r**etained **f**etal **m**embranes >12 h; samples collected within one hour after expulsion of the calf	5	
III	AP: Placentomes of cows, which were treated with aglepristone (AP) on day 270 after artificial insemination. Samples collected immediately after calving.	3	Animal experiments were approved by the local ethical committees on the use of animals for research purposes at the regional council (Regierungspräsidium Giessen, no. V54-19c-20-15(1) Gi 18/14-Nr.41/2007; LAVES, 33.0-42502-04-09/1634; Ethical Committee of Uludag University of Veterinary Medicine, No. 401/1510-26.03.2007).
	CP: Placentomes of cows treated with cloprostenol (PGF2α, Cl) on day 270 after artificial insemination. Samples collected immediately after parturition	3	
	GC: Placentomes of cows treated with dexamethasone (GC) on day 270 after artificial insemination. Samples collected immediately after parturition	4	
	NT: Placentomes of spontaneously calving cows without medical treatment; **n**ormal **t**erm (NT)	5	
	D272: Placentomes of untreated cows with Cesarian section (CS) on day 272 after insemination. Samples collected during CS	3	

cessed further as described above. No frozen material was available for this study.

2.3. RNA isolation and semi-quantitative real-time (TaqMan) PCR

Total RNA was isolated from collected placentomal and/or uterine tissues used for Studies I and II using TRIZOL-Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. For Study III, RNA-isolation was performed on formalin-fixed, paraffin-embedded (FFPE) tissue samples using the RNeasy FFPE-Kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocols and as previously described [43,44]. The RNA content and quality were measured by spectrophotometry (NanoDrop 2000C; Thermo Fisher Scientific AG, Reinach, CH). DNAse treatment was accomplished with RQ1 RNAse-free DNAse (Promega, Dübendorf, CH) following the manufacturer's instructions in order to remove genomic DNA contaminants using 100 ng (Studies I and II) or 400 ng (Study III) of total RNA for each sample. Reverse transcription was performed with the GOLD RNA PCR Core Kit (Gene Amp, Applied Biosystems, Foster City, CA, USA) and carried out in an Eppendorf Mastercycler (Vaudaux-Eppendorf AG, Basel, CH) using an established running protocol (8 min at 21 °C, 15 min at 45 °C followed by 5 min at 99 °C).

Semi-quantitative real-time (TaqMan) PCR of HPGD and the two housekeeping genes SDHA and GAPDH [45] (for accession numbers and primer sequences see Table 2) was performed using the automated fluorometer ABI PRISM 7500 Fast Real Time PCR system (Applied Biosystems, Rotkreuz, CH) as described previously [40,46]. The samples were quantified in duplicate using the Fast Start Universal Probe Master (ROX; Roche Diagnostics Schweiz AG) on 96-well optical plates (Applied Biosystems), with cDNA corresponding to 100 ng or 400 ng of total RNA per sample that was DNAse-treated and synthesized as described above. Negative controls consisted of autoclaved water (instead of cDNA) and the so-called RT minus controls (samples which were not reverse transcribed). The reaction mixture of 25 μl included 12.5 μl Fast-Start Universal Probe Master (Roche Diagnostics, Mannheim, DE), 150 nM of the forward and 150 nM of the reverse primers, 200 nM TaqMan probe and 5 μl cDNA. Primers and 6-carboxyfluorescein (6-FAM)- and 6-carboxytetramethyl-rhodamine (TAMRA)-labeled probes (Table 1) were designed with GenScript Inc. Software (Piscataway, NJ, USA) and ordered from Microsynth AG, Balgach, CH. The amplification reaction was conducted under the following conditions: denaturation at 95 °C for 10 min, 40 cycles at 95 °C for 15 s and 60 °C for 60 s. The two reference genes (SDHA and GAPDH) were used to calculate the relative gene expression of HPGD using the comparative CT method (ΔΔ CT method) in compliance with the manufacturer's instructions for the ABI 7500 Fast Real-Time PCR System as described by Kowalewski et al. [47,48]. Accordingly, the CT slope method was applied to check the efficiency of the PCR reactions that were set up to ensure approximately 100%. The specificity of the selected PCR products for each gene was confirmed by commercial sequencing (Microsynth AG).

2.4. Immunohistochemical (IHC) analysis

For IHC localization of HPGD protein in bovine tissues, sections of 3 μm thickness were cut from formalin-fixed and paraffin-embedded samples and mounted on SuperFrost Plus microscope slides (Menzel-Gläser, Braunschweig, DE). The sections were deparaffinized in xylol, rehydrated through a graded ethanol series and rinsed in tap water as previously described [42,49]. Antigen retrieval was performed in 10 mM citrate buffer pH 6.0 and endogenous peroxidase activity was quenched by 0.3% H_2O_2 in methanol. Subsequent to a wash in IHC buffer/0.3% Triton X, (0.8 mM Na_2HPO_4, 1.47 mM KH_2PO_4, 2.68 mM KCl, 137 mM NaCl) pH 7.2–7.4, the slides were treated with 10% normal goat serum to block non-specific binding sites. Afterwards, the sections were incubated with polyclonal, affinity purified, custom-made guinea pig anti-canine HPGD antibody (Eurogentec S.A., Seraing, BE; [49]) overnight at 4 °C at a dilution of 1:1000. This antibody was designed by using the canine peptide sequence HFQDYETTPFHAKTQ – C – terminal amino acids 252–266 of the HPGD sequence [GenBank:AFF60303] displaying 86% homology with the bovine sequence. For negative and isotype controls, the primary antibody was replaced by IHC buffer/0.3% Triton X, or by using pre-immune guinea pig serum matching the protein concentration of the antiserum, respectively. On the next day, the sections were washed with [IHC/0.3% Triton X] and incubated with a biotinylated secondary goat anti-guinea pig antibody (AI-7000;

Table 2
Sequences of primers and (TaqMan) probes.

Primer	Primer sequence	Accession ♯	Product length (bp)
HPGD forward	5′-GAA TCT CGA AGC AGG TGT CA-3′		
HPGD reverse	5′-CCA GCT TTC CAA AGT GGT CT-3′	NM_001034419.2	106
HPGD probe	5′-TGA TCG GCC ACA TCG CAC TG-3′		
SDHA forward	5′-ATG GAA GGT CTC TGC GCT AT-3′		
SDHA reverse	5′-ATG GAC CCG TTC TTC TAT GC-3′	NM_174178	79
SDHA probe	5′-ACA GAG CGA TCA CAC CGC GG-3′		
GAPDH forward	5′-GCG ATA CTC ACT CTT CTA CCT TCG A-3′		
GAPDH reverse	5′-TCG TAC CAG GAA ATG AGC TTG AC-3′	NM_001034034.1	92
GAPDH probe	5′-CTG GCA TTG CCC TCA ACG ACC ACT T-3′		

Vector Laboratories, Burlingame, USA) at 1:100 dilution at room temperature for 1 h. After repeated rinsing in IHC/0.3% Triton X, tissue slides were treated with the streptavidin-avidin-peroxidase Vectastain ABC kit (Vector Laboratories) to enhance the signals. For final detection of signals, the Liquid DAB+ substrate kit (Dako Schweiz AG, Baar, CH) was added and the reaction was stopped after approximately 10 min. The tissue sections were rinsed for 10 min in tap water, counterstained with hematoxylin, dehydrated through serial ethanol dilutions and coverslipped mechanically in Pertex® (RCM 2000®; Medite, Dietlikon, CH).

2.5. Statistics

For statistical tests the software program GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA) was used.

The datasets of semi-quantitative real-time PCR were tested for normality using the Kolmogorov-Smirnov Test. The results of HPGD mRNA expression in placentomes from Studies II and III were normally distributed and therefore the datasets were tested by a parametric one-way analysis of variance (ANOVA) followed by Dunn's multiple comparison test. The remaining datasets for the uterine and placentomal HPGD mRNA expression during different stages of gestation (Study I) did not show normality, so a non-parametric one-way analysis of variance (Kruskal-Wallis test) followed by Dunn's post-test was applied. The numerical data are presented as the mean ± SD. In all cases $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Expression of HPGD mRNA

Study I: stages of pregnancy

HPGD expression in uterine and placentomal tissues was investigated by semi-quantitative real-time (TaqMan) PCR. HPGD-specific mRNA was detected in all preparations between the 3rd and 9th month of gestation and post-partum. Despite high individual variations, in placentomes the highest mRNA expression of HPGD was observed immediately post-partum (DFM), compared with the 6th to 9th month of pregnancy ($p < 0.05$) (Fig. 1A).

HPGD mRNA expression in the bovine uterine wall was significantly higher in the 3rd compared with the 6th month of pregnancy ($p < 0.05$) (Fig. 1B).

Study II: DFM versus RFM.

The mRNA expression of HPGD did not differ significantly between the two groups ($p > 0.05$) (Fig. 1C).

Study III: NT, D272, CP, AP and GC

The treatments used in this study had different effects on placentomal HPGD mRNA expression. Thus, its levels were significantly lower in cows treated with aglepristone (AP) and dexamethasone (GC) compared with untreated cows at normal term (NT). Placentomes of cows during late gestation (D272)

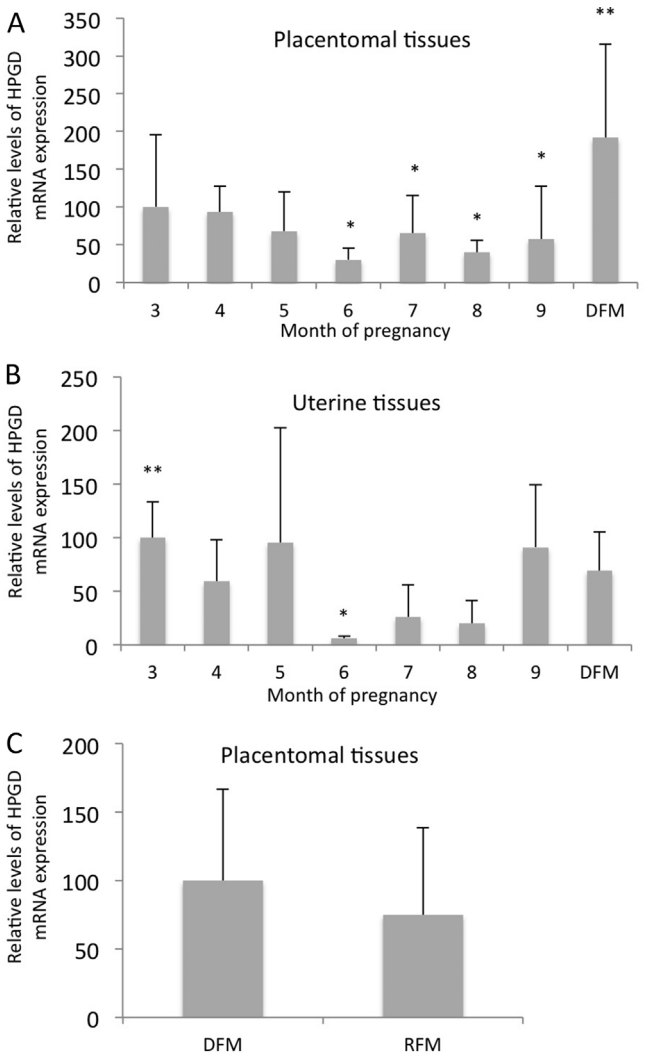

Fig. 1. Placentomal and uterine mRNA expression of HPGD at different stages of gestation and at term. In placentomes and uteri, HPGD was detected in all RNA preparations between the 3rd and 9th months of pregnancy and at term, with changes that were time-dependent. The highest mRNA expression was found during establishment and termination of gestation. Thus, (A) placental HPGD expression during pregnancy was significantly higher immediately post partum (DFM) compared to month 6 till 9 of pregnancy ($p < 0.05$). (B) uterine HPGD expression during gestation was in the 3rd month significantly higher than in the 6th month ($p < 0.05$). The datasets showed no normality, therefore a non-parametric one-way analysis of variance (Kruskal-Wallis) followed by Dunn's post-test was applied. (C) No significant differences were detected between cows with (DFM) or without (RFM) discharged fetal membranes ($p > 0.05$). The datasets of group DFM and RFM were normally distributed and one-way ANOVA was applied followed by Dunn's multiple comparison test. Numerical data are presented as means ± SD.

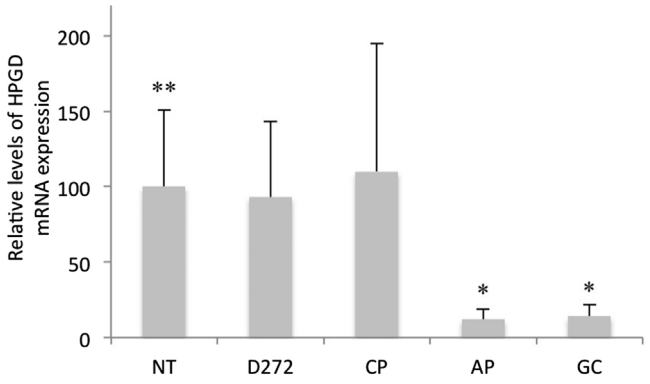

Fig. 2. Placentomal expression profile of bovine HPGD mRNA in groups NT, D272, CP, AP and GC. Detection of significantly lower placentomal HPGD mRNA levels in groups medicated with aglepristone (AP) or dexamethasone (GC) (*) compared with untreated cows at normal term (NT) (**). P-values: NT: AP = $p < 0.001$; NT: GC = $p < 0.01$; NT: D272 = $p > 0.05$; NT: CP = $p > 0.05$. Measured by real-time PCR (Taqman). Data were normally distributed and analyzed using one-way ANOVA followed by Dunn's multiple comparison test. Numerical data are presented as the mean ± SD.

and of cows treated with cloprostenol (CP) showed no significant differences in HPGD mRNA expression compared to controls (NT). In detail: NT *vs.* AP = $p < 0.001$; NT *vs.* GC = $p < 0.01$; NT *vs.* D272 = $p > 0.05$; NT *vs.* CP = $p > 0.05$, Fig. 2.

3.2. Localization of HPGD protein

Placentomal tissues

In all placentomes, staining was clearly detected in the trophoblast and the epithelium covering the maternal part of the placentomes (caruncle), with staining intensity appearing stronger in the trophoblast compared to the caruncular epithelium. No considerable differences of staining were found in relation to the localization of HPGD within the placentomal villous tree (PVT; fetal side) or the caruncular crypt system (Fig. 3A, C–H). In the trophoblast, immunostaining was present in uninucleate trophoblast cells (UNC), but not in binucleate trophoblast cells (BNC) (Fig. 3D–H). No staining was detected in maternal and fetal stroma (Fig. 3C–E). The staining intensity appeared strongest during post partum while it seemed weaker in the middle of pregnancy (Fig. 3A, D, H). In all groups, the localization pattern of HPGD remained constant (Fig. 3A–H). In some of the tissue samples, nuclear staining was detected, which was interpreted as non-specific background staining.

Uterine tissues

HPGD immunoreactivity was mainly localized in the endometrial glandular (GE) and luminal epithelium (Fig. 4A–D). The staining appeared weaker in the stroma and in the myometrial smooth muscle cells (MM = myometrium) (Fig. 4B). Immunostaining seemed to be more intense in the 3rd month of gestation (Fig. 4A and B) especially when compared to the 6th month of gestation (Fig. 4C and D) where it appeared weaker. The localization pattern remained constant throughout pregnancy and at post partum (Fig. 4A and D).

4. Discussion

The aim of the present study was to examine the expression, and thereby indirectly, the potential involvement of HPGD in the endocrine prepartum events in cattle, and in particular in the regulation of PGs release during bovine pregnancy and parturition. The three different treatments intervene in different regulatory mechanisms during the complex signal cascade leading to luteolysis and finally to parturition in cattle. Thus, dexamethasone imitates the birth-triggering factor cortisol, which is synthesized by the fetus after maturation of the hypothalamic-pituitary-axis in late pregnancy [50,51]. This, in turn, stimulates cotyledonary uninucleate trophoblast cells (UNC) to produce COX2, which results in upregulation of prostaglandin synthesis [52]. Glucocorticoids are thought to be co-responsible for induction of CYP17A1 activity in placental cotyledons, thereby participating in a prepartal switching mechanism redirecting steroid synthesis from delta-4 and delta-5 steroidogenic pathways in favor of estrogen instead of progesterone synthesis [39,53]. Concomitantly, sulfonated estrogens may be activated by increased expression of estrogen sulphatase [54,55]. Prepartal up-regulation of estrogens and the simultaneous decrease in progesterone lead to upregulation of PG synthesis, resulting in increased prepartum output of endometrial and myometrial PGF2α in the cow [56,57].

The competitive P4 receptor blocker aglepristone causes direct abolition of placental and luteal P4 effects. Cloprostenol, as an analog of PGF2α, simulates the effect of natural endogenous prostaglandin, which together with oxytocin, stimulates myometrial contractility and is directly involved in luteolysis as shown, *e.g.*, in cattle and sheep [58,59]. These hormonal changes finally lead to the onset of parturition.

Based on the results obtained in the present study, HPGD indeed seems to participate in these events. Thus, HPGD-specific mRNA was detected in all RNA preparations throughout the observation period and at normal and induced parturition, displaying time-dependent changes, and indicating its functional role in particular during the establishment and termination of pregnancy, when its uterine and placental expression levels, respectively, were the highest. By revealing the lowest placentomal and uterine expression levels during the mid-gestation, followed by significantly increased mRNA levels in placentomes at parturition, the expression pattern of HPGD appears to correlate negatively with the placentomal activity of 3β-hydroxysteroid dehydrogenase (3βHSD) and local placentomal concentrations of P4 showed before [60]. This further implies its functional role during the onset of parturition.

Similar effects were observed by others in the placenta of rats which exhibited a decline in placental HPGD activity until mid-gestation, followed by its increased activity at term [61]. Our findings showed the clearly detectable immunostaining of HPGD protein in the fetal compartments of placentomes. In agreement with other studies in cows and sheep, for example [20,62], immunostaining in placentomal trophoblast cells was generally restricted to the cytoplasm of UNCs, and no expression was detected in binucleate trophoblast cells (BNC). The exact role of HPGD in trophoblast cells of the placenta and fetal membranes is still not clear, however, the co-localization of HPGD with PG-synthesizing enzymes like COX2 and 20α-hydroxysteroid dehydrogenase (20α-HSD)/prostaglandin F-synthase (PGFS/AKR1B5) in the UNCs indicates that PG metabolism occurs mainly in the fetal components of the placenta [63,64]. With respect to uterine tissues, we detected immunostaining of HPGD protein mainly in epithelial cells of the glands and the luminal epithelium, whereas apparently weaker staining intensity was detected in cells of maternal stroma and smooth muscles. These results are equivalent to findings by other authors who examined HPGD expression in the endometrium of cows [65]. This also suggests that in the bovine uterus and placenta, synthesis and catabolism of PGs are located in the same cell compartments thus allowing for efficient regulation of PGs activity in an autocrine and paracrine manner. In addition, in uterine tissues of different species like human and cattle, PG-synthesizing-enzymes COX2 and PGFS/AKR1B5 were predominantly found colocalized with HPGD in glandular epithelium and maternal stroma [10,16,65,66].

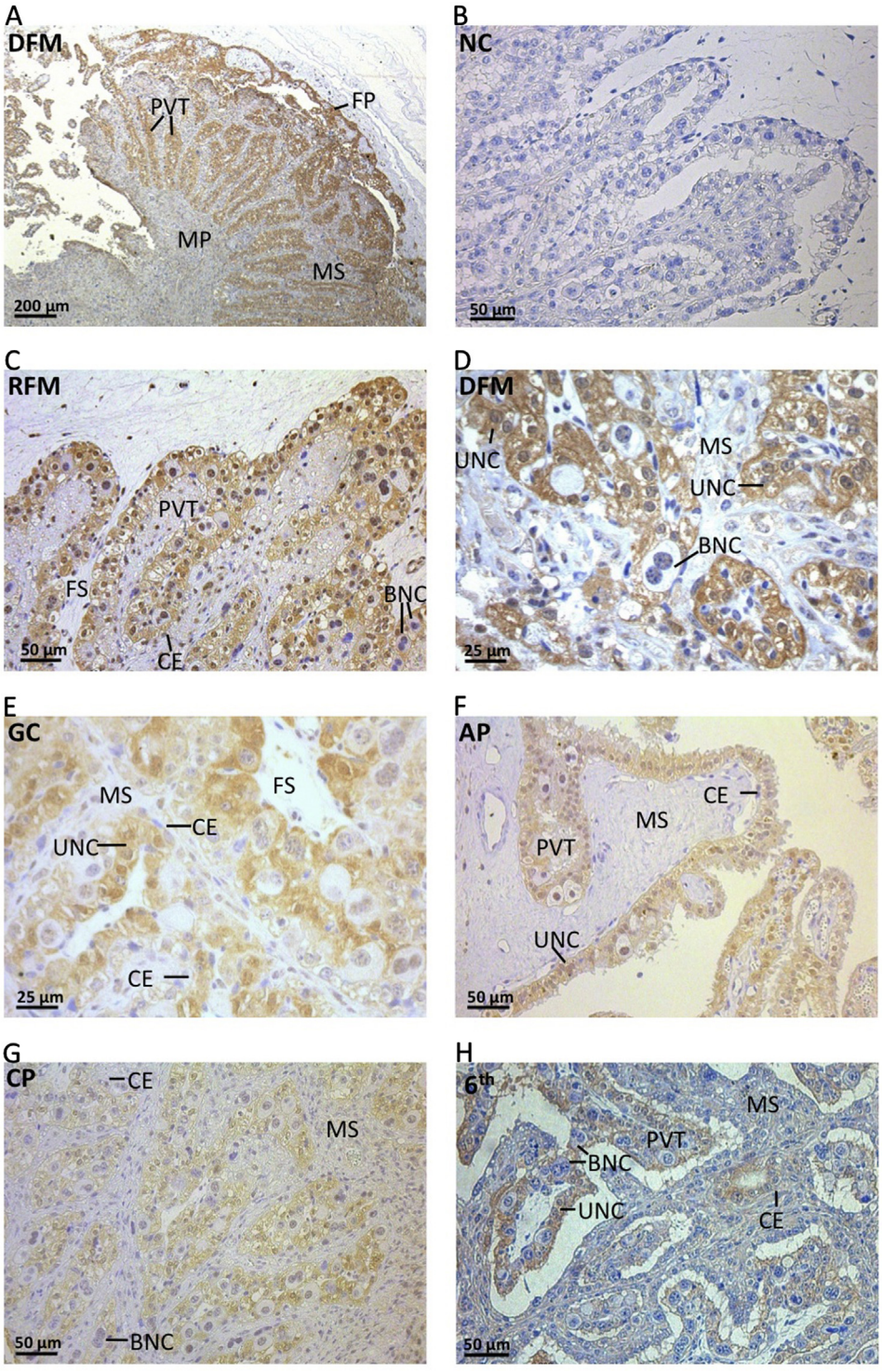

Fig. 3. Immunolabeling of HPGD protein in bovine placentomes. Representative tissue sections are shown. Clearly visible HPGD staining was found in uninucleate trophoblast cells (UNC) and the adherent fetal plate (FP in A). Clearly weaker staining was observed in the caruncular epithelium (CE). No cytoplasmic staining was detected in binucleate trophoblast cells (BNC), maternal septum (MS) and fetal stroma (FS). (A) and (D) Group of spontaneously calving cows with discharged fetal membranes (DFM); overview of the placentomal villi tree (PVT) is shown in (A). (B) Negative control (isotype control; NC). (C) Group of spontaneously calving cows with retained fetal membranes (RFM). (E) Group of cows treated with dexamethasone (GC). (F) Group of cows with aglepristone treatment (AP). (G) Group of cows treated with cloprostenol (CP). (H) Placental tissue collected in 6th month of gestation.

The increase of placental and uterine HPGD expression around term may have a local regulatory function. HPGD not only limits the levels of each of the major PGs (*i.e.*, PGE_2 and PGF2α), but also controls the equilibrium between these two hormones. During parturition, the ratio between PGF2α and PGE_2 changes [67] and HPGD could be involved in that mechanism. However, HPGD does not seem, to be involved in the etiology of fetal membrane retention in cows, as at least when it comes to its mRNA expression and protein distribution patterns, they did not differ significantly between retained and normally released placenta.

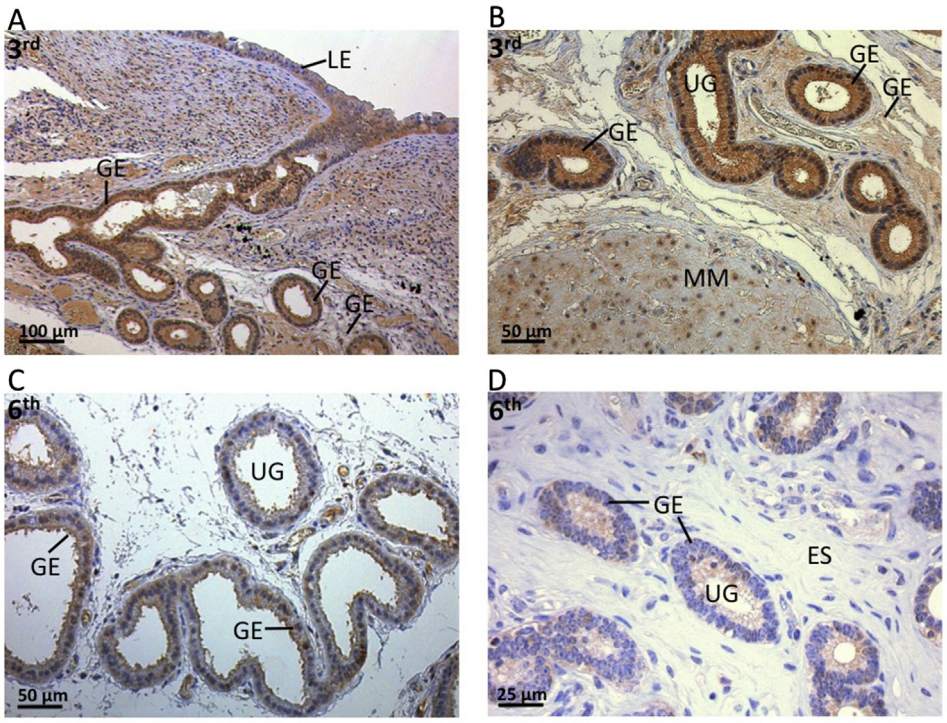

Fig. 4. Immunostaining of HPGD protein in bovine uterine tissue during pregnancy. Representative tissue sections are shown. HPGD was mainly detected in the epithelium (GE, glandular epithelium) of the uterine glands (UG) and the luminal epithelium (LE). Weaker labeling was observed in the smooth muscle cells and the endometrial stroma (ES). MM = myometrium. Immunostaining appeared stronger in the 3rd month (A, B) and weaker in the middle of gestation (C, D).

Furthermore, since uterine PGs are needed locally for the establishment of pregnancy in different species like cattle, sheep, pigs and horses [67–72], the elevated expression of HPGD at the beginning of gestation could be involved in protecting gestation from PGs entering the maternal blood stream. The myometrial expression during that time may indicate its contribution to maintaining myometrial quiescence, protecting it from the contractility-stimulating function of PGs.

The aglepristone and dexamethasone treatments resulted in a similar decrease of HPGD expression, most probably due to the withdrawal of P4, since the stimulating effect of progesterone on HPGD is removed [13,14,23]. Due to the stimulation of HPGD mRNA expression by progesterone, HPGD was shown to be down-regulated by PGR blockers like aglepristone and mifepristone in humans [15,16,73]. Similarly, in the placenta and uterus of guinea pigs, mifepristone invokes a significant decrease of HPGD activity [74]. Besides being a strong competitive antagonist of PGR, additionally, mifepristone reveals an affinity for glucocorticoid receptors [75,76]. The present results provide strong support that also in cattle the HPGD up-regulating effect of P4 is blocked by aglepristone.

Cortisol results in a significant down-regulation of HPGD and leads to a potent inhibition of the PGF2α to PGFM conversion in primary cultures of human placental and chorionic trophoblast cells [77]. Co-incubation with P4 was not able to reverse the cortisol-induced inhibition of HPGD activity [77]. However, there is evidence that regulation of HPGD expression and activity is species- and cell type- or organ- specific. Thus, for example, HPGD activity in fetal rat lung increases after treatment with dexamethasone from day 20–22 of pregnancy [78], but conversely, renal tissues of rats show a withdrawal of HPGD activity after dexamethasone treatment [79]. In cattle and humans [14,77], placental glucocorticoids appear able to block HPGD expression. Our results showed no significant differences in mRNA expression of HPGD between cows medicated with cloprostenol (CP) compared with animals of the control group (NT). There is evidence, however, that 17-β estradiol increases the activity of HPGD in human placentas [80] and rat uteri [81,82], and after PGF2α-induced luteolysis a switch in the estrogen:progesterone ratio in favor of estrogen is observed in cows [20]. This, together with the high prostaglandin concentration, could be the reason for the high HPGD mRNA expression in the PG group.

Clearly, the presented data obtained in its quantitative part at the mRNA level, are not definite. The exact involvement of HPGD in the endocrine prepartum events in cattle and the underlying mechanisms causing the withdrawal of HPGD by glucocorticoids and aglepristone remain to be further investigated in studies involving higher number of animals and presenting functional approaches, including protein expression levels and enzyme activity studies.

5. Conclusions

In bovine placentomes and uterus, HPGD is suggested to play a regulatory role during establishment and termination of pregnancy and in protection of the fetus against maternal prostaglandins and myometrial contractions. Its uterine and placental expression appears to be time-dependent and, within the placenta, it is localized predominantly in the fetal part. Thus, its expression is restricted mainly to the cytoplasm of UNCs, where it seems to be colocalized with the PG-providing 20α-HSD/AKR1B5. At term, HPGD withdrawal can be induced by aglepristone and the glucocorticoid dexamethasone. These data support the assumption that HPGD is a P4-dependent enzyme.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

JVH was involved in the coordination and performance of the experiments, in the evaluation and interpretation of the data and the drafting of the manuscript. NS designed a part of the project, was involved in the coordination and performance of the experiments, in the evaluation and interpretation of the data and supervision of the performance of the experiments. GS designed a part of the project (Study III) and was involved in sampling procedures. MPK and AB designed and supervised the project and performance of the experiments, were involved in the interpretation of the data, and drafting and revising the manuscript. Additionally, AB was involved in sampling procedures. All authors read and approved the final manuscript.

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