Placental vascular development, cellular proliferation and angiogenic factor expression in beef heifers throughout gestation

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The objective of this study was to characterize placental vascular development, cellular proliferation and angiogenic factor expression throughout gestation in beef heifers. Distinct expression patterns of VEGFR1 and VEGFR2 suggest complementary roles in maternal and fetal placental vascular development. These findings provide a baseline for future studies evaluating the effects of maternal nutrition and management on placental function and fetal growth.

Summary

Placental vascular development is essential for fetal growth, but the temporal regulation of angiogenic signaling throughout gestation in beef cattle is not well understood. Therefore, the objective of this study was to characterize placental vascular development, cellular proliferation and angiogenic factor expression throughout gestation in beef heifers. Placentomes were collected from 80 primiparous crossbred Angus beef heifers at 10 gestational stages (days 34, 50, 63, 83, 161, 181, 250, 260, 272 and parturition). Immunofluorescence was used to evaluate vascular development (CD31 and CD34), cellular proliferation (Ki67), and angiogenic and vasoactive factors (VEGF, VEGFR1, VEGFR2, endothelial nitric oxide synthase (eNOS) and soluble guanylyl cyclase [sGC]). Capillary area density (CAD), capillary number density (CND) and protein expression were quantified in the maternal caruncle (CAR) and fetal cotyledon (COT). Placental vascularity increased throughout gestation, with CAD increasing from 1.00 ± 0.77% to 12.47 ± 0.61% in the COT and from 3.76 ± 0.77% to 8.62 ± 0.61% in the CAR ($P < 0.01$). Cellular proliferation also increased during gestation and remained greater in the CAR than the COT during late pregnancy. Expression of eNOS increased during early to midgestation before declining during late gestation, whereas sGC expression was highest during early gestation and increased again near term. Distinct regional differences were observed in VEGF receptor expression, with VEGFR1 predominating in the maternal CAR and VEGFR2 in the fetal COT. Strong VEGF immunostaining was also observed in binucleate giant cells (BNGCs), suggesting a potential role in placental angiogenesis and maternal-fetal communication. Overall, placental vascular development, endothelial proliferation and angiogenic signaling changed dynamically throughout gestation, providing baseline information for future studies evaluating placental function, maternal nutrition and fetal growth in beef cattle. Observed in VEGF receptor expression. VEGFR1 was greater in the maternal CAR, particularly at days 63 and 181 ($P < 0.01$), whereas VEGFR2 was greater in the fetal COT, peaking at day 83 and remaining elevated during late gestation. In conclusion, placental vascular development, endothelial proliferation and angiogenic signaling changed dynamically throughout gestation. VEGFR1 predominated in maternal placental tissue, whereas VEGFR2 predominated in fetal placental tissue, and strong VEGF expression in binucleate giant cells (BNGCs) suggests these specialized trophoblasts cells may contribute to placental angiogenesis and maternal-fetal communication.

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Introduction

The placenta is a transient but highly specialized organ that supports fetal growth by facilitating the exchange of oxygen, nutrients and metabolic waste between the maternal and fetal circulations (Reynolds and Redmer, 2001). As gestation progresses, placental vascular development must increase to meet the growing metabolic demands of the fetus. In ruminants, the placentome, composed of the maternal caruncle (CAR) and fetal cotyledon (COT), is the primary site of maternal-fetal exchange; therefore, proper vascular development is essential for placental function, fetal growth and pregnancy success (Borowicz et al., 2007). Placental vascular development is regulated through coordinated processes of angiogenesis and cellular proliferation. Endothelial cell markers CD31 and CD34 are widely used to identify placental capillaries and quantify vascular development, whereas Ki67 is a reliable marker of cellular proliferation (Scholzen and Gerdes, 2000). Angiogenesis is primarily regulated by vascular endothelial growth factor (VEGF) and its receptors, VEGFR1 and VEGFR2, while placental vascular function is further controlled by the nitric oxide pathway through endothelial nitric oxide synthase (eNOS) and soluble guanylyl cyclase (sGC), which regulate placental blood flow (Reynolds and Redmer, 2001). Although placental vascular development has been extensively studied in sheep, relatively little information is available regarding placental vascularity, cellular proliferation and angiogenic factor expression throughout gestation in beef cattle. Therefore, the objective of this study was to characterize placental vascular development, cellular proliferation and the expression of key angiogenic and vasoactive factors throughout gestation in beef heifers, providing a baseline for future studies of placental function, fetal growth and maternal nutrition.

Procedures

The study included 80 pregnant crossbred Angus beef heifers managed at the North Dakota State University Beef Cattle Research Complex. Placental tissues were collected throughout gestation (approximately 280 days) on days 34 (n = 5), 50 (n = 5), 63 (n = 8), 83 (n = 8), 161 (n = 7), 181 (n = 10), 250 (n = 7), 260 (n = 9), 272 (n = 8) and at parturition (n = 13). At tissue collection, fetal and placental weights were recorded, and placentomes were dissected for histological evaluation. Placentomes were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 5 µm and mounted on microscope slides. Tissue sections were stained using immunofluorescence to evaluate placental vascular development, cellular proliferation and angiogenic factor expression. Endothelial cells were identified using CD31 and CD34. Cellular proliferation was assessed using the nuclear proliferation marker Ki67. Angiogenic and vasoactive proteins evaluated included VEGF red1, VEGFR1, VEGFR2, eNOS and sGC. Images were captured using a Mica Microhub fluorescence microscope (Leica Microsystems Inc., Buffalo Grove, Illinois), and quantitative image analysis was performed using Image-Pro Premier software (Version 9.0.1; Media Cybernetics, Inc., Silver Spring, MD). Capillary area density (CAD) and capillary number density (CND) were measured separately in the fetal cotyledon (COT) and maternal caruncle (CAR) of each placentome. Protein expression and Ki67 labeling index were quantified as the percentage of positively stained areas within each region. Data was analyzed using the PROC GLM and PROC REG procedures of SAS (Version 9.4; SAS Institute Inc., Cary, NC). Differences among days of gestational and placental regions were considered statistically significant at $P \leq 0.05$, with $P \leq 0.01$ indicating stronger statistical evidence,and regression analyses were performed to characterize changes in placental vascularity and angiogenic factor expression throughout gestation.

Results and Discussion

Placental and fetal weights increased throughout gestation, following a sigmoidal (S-shaped) growth pattern characterized by slow early growth, rapid mid-gestation growth, and leveling off near term, with excellent model fit (placental weight, $R^2 = 0.9388$; fetal weight, $R^2 = 0.9827$; Figure 1). Placental growth occurred earlier and increased gradually, whereas fetal growth accelerated after approximately day 160. This pattern indicates that placental development precedes rapid fetal growth, increasing the capacity for nutrient and oxygen exchange. Similar growth patterns have been reported in sheep and other ruminants (Reynolds and Redmer, 2001; Reynolds et al., 2005).

During early gestation (days 34-83), CAD was greater in the CAR than in the COT ($P < 0.05$), whereas by late gestation (days 250-272), both CAD and CND were greater in the COT ($P < 0.01$; Table 1). Capillary area density increased approximately 12-fold in the COT compared with 2.3-fold in the CAR, while CND increased more than 11-fold in the COT and 4-fold in the CAR. Cellular proliferation (Ki67) also increased throughout gestation and was greater in the CAR from days 63 to 272 ($P < 0.05$), indicating continued maternal placental remodeling. Together, these findings demonstrate a developmental shift from the maternal placenta during early gestation to rapid fetal placental vascular expansion during late gestation, supporting the increasing nutrient demands of fetal growth (Reynolds et al., 2023).

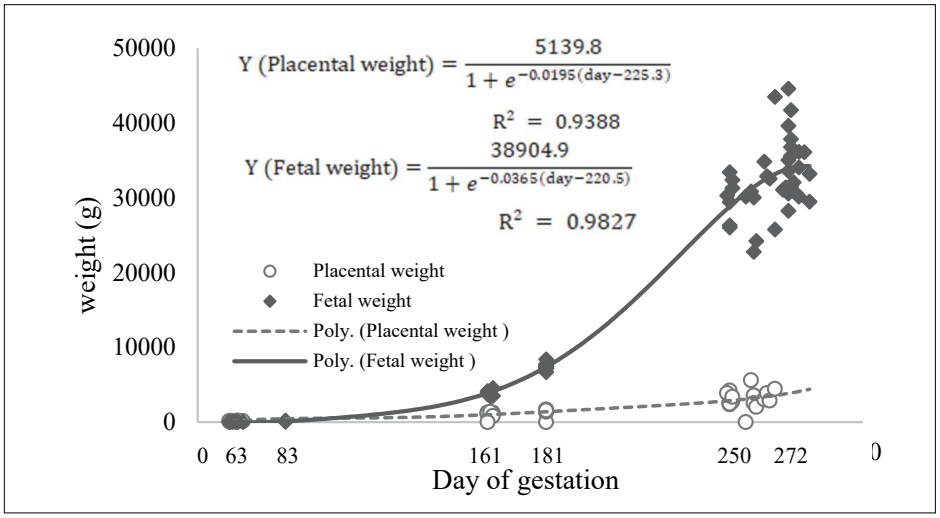

Figure 1. Relationship between placental weight and fetal weight in beef cattle.

Table 1. Changes in placental vascularity and cellular proliferation throughout gestation.

Measurement	Days of gestation	Region[1]		*P*-value[2] of region
		COT	CAR	
(CD31&CD34) Capillary Area Density, %	34	1.003 ± 0.768	3.757 ± 0.768	**0.0007**
	50	2.223 ± 0.768	5.244 ± 0.768	**0.0002**
	63	3.505 ± 0.701	6.438 ± 0.701	**<0.0001**
	83	5.546 ± 0.701	7.079 ± 0.701	**0.0352**
	161	7.955 ± 0.649	7.349 ± 0.649	0.3639
	181	8.521 ± 0.543	7.518 ± 0.543	0.0741
	250	11.528 ± 0.649	8.373 ± 0.649	**<0.0001**
	260	12.021 ± 0.551	8.541 ± 0.574	**<0.0001**
	272	12.466 ± 0.607	8.623 ± 0.607	**<0.0001**
	Parturition	18.275 ± 0.716	N/A	
(CD31&CD34) Capillary Number Density, %	34	0.055 ± 0.034	0.081 ± 0.034	0.4870
	50	0.068 ± 0.031	0.184 ± 0.031	**0.0022**
	63	0.097 ± 0.031	0.211 ± 0.031	**0.0010**
	83	0.123 ± 0.029	0.231 ± 0.029	**0.0017**
	161	0.231 ± 0.024	0.265 ± 0.024	0.2782
	181	0.260 ± 0.029	0.276 ± 0.029	0.5349
	250	0.512 ± 0.027	0.323 ± 0.027	**<0.0001**
	260	0.598 ± 0.049	0.328 ± 0.032	**<0.0001**
	272	0.618 ± 0.021	0.319 ± 0.021	**<0.0001**
	Parturition	0.419 ± 0.02	N/A	
Ki-67 (%Li)	34	3.302 ± 3.751	6.391 ± 3.751	0.5618
	50	14.999 ± 3.751	18.991 ± 3.751	0.4536
	63	16.434 ± 3.424	34.649 ± 3.424	**0.0003**
	83	21.646 ± 3.424	41.611 ± 3.424	**<0.0001**
	161	27.527 ± 3.424	50.220 ± 3.424	**<0.0001**
	181	29.222 ± 2.652	54.482 ± 2.652	**<0.0001**
	250	38.352 ± 3.170	59.265 ± 3.170	**<0.0001**
	260	32.487 ± 2.145	41.528 ± 2.458	**<0.0001**
	272	27.958 ± 2.965	36.847 ± 2.965	**0.0368**

[1]CAR = caruncle; COT = cotyledon;
[2]*P*-values in bold indicate significant differences between COT and CAR.

In the COT, eNOS expression increased from 14.08 ± 4.02% on day 34 to 22.31 ± 3.68% on day 181 before declining during late gestation ($P \leq 0.01$; Figure 2). In contrast, sGC expression was highest during early gestation (35.09 ± 10.34% on day 34), decreased during midgestational and increased again near term (15.60 ± 4.27% on day 272), indicating dynamic regulation of nitric oxide signaling. VEGF was expressed throughout gestation in both placental regions, with particularly strong immunostaining in binucleate giant cells (BNGCs). Because BNGCs are specialized trophoblast cells involved in placental development and maternal-fetal communication (Klisch and Leiser, 2003), their strong VEGF expression suggests a role in placental angiogenesis and vascular remodeling, consistent with the established roles of VEGF and nitric oxide signaling in placental vascular function (Reynolds and Redmer, 2001).

As shown in Figure 3, VEGFR1 and VEGFR2 exhibited distinct regional expression patterns. VEGFR1 expression was greater in the maternal CAR than in the fetal COT, particularly at days 63 and 181 ($P < 0.01$). In contrast, VEGFR2 predominated in the fetal COT, peaking at day 83 (35.50 ± 8.17%) and remaining elevated during late gestation ($P < 0.01$). Because VEGFR2 is the primary receptor mediating VEGF-induced angiogenesis, whereas VEGFR1 regulates VEGF signaling and vascular organization, these expression patterns suggest active angiogenesis in the fetal placenta and vascular stabilization in the maternal placenta, consistent with previous reports in ruminants (Reynolds and Redmer, 2001).

Overall, placental vascular development, endothelial proliferation and angiogenic signaling changed dynamically throughout gestation. A developmental shift from the maternal caruncle during early gestation to the fetal cotyledon during mid- and late gestation was accompanied by

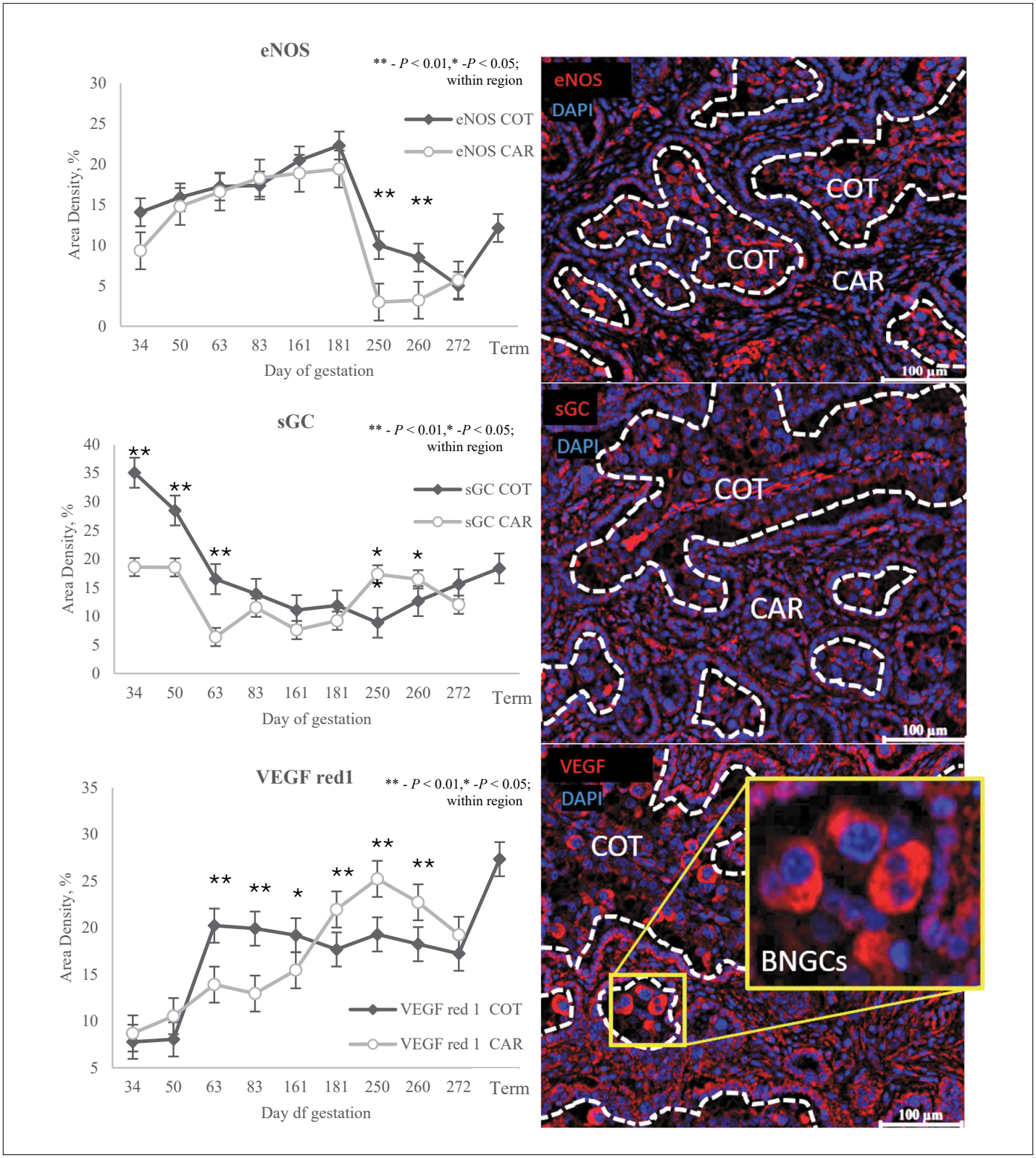

Figure 2. Gestational regulation of eNOS, sGC, and VEGF expression in COT and CAR of beef heifer placentomes.

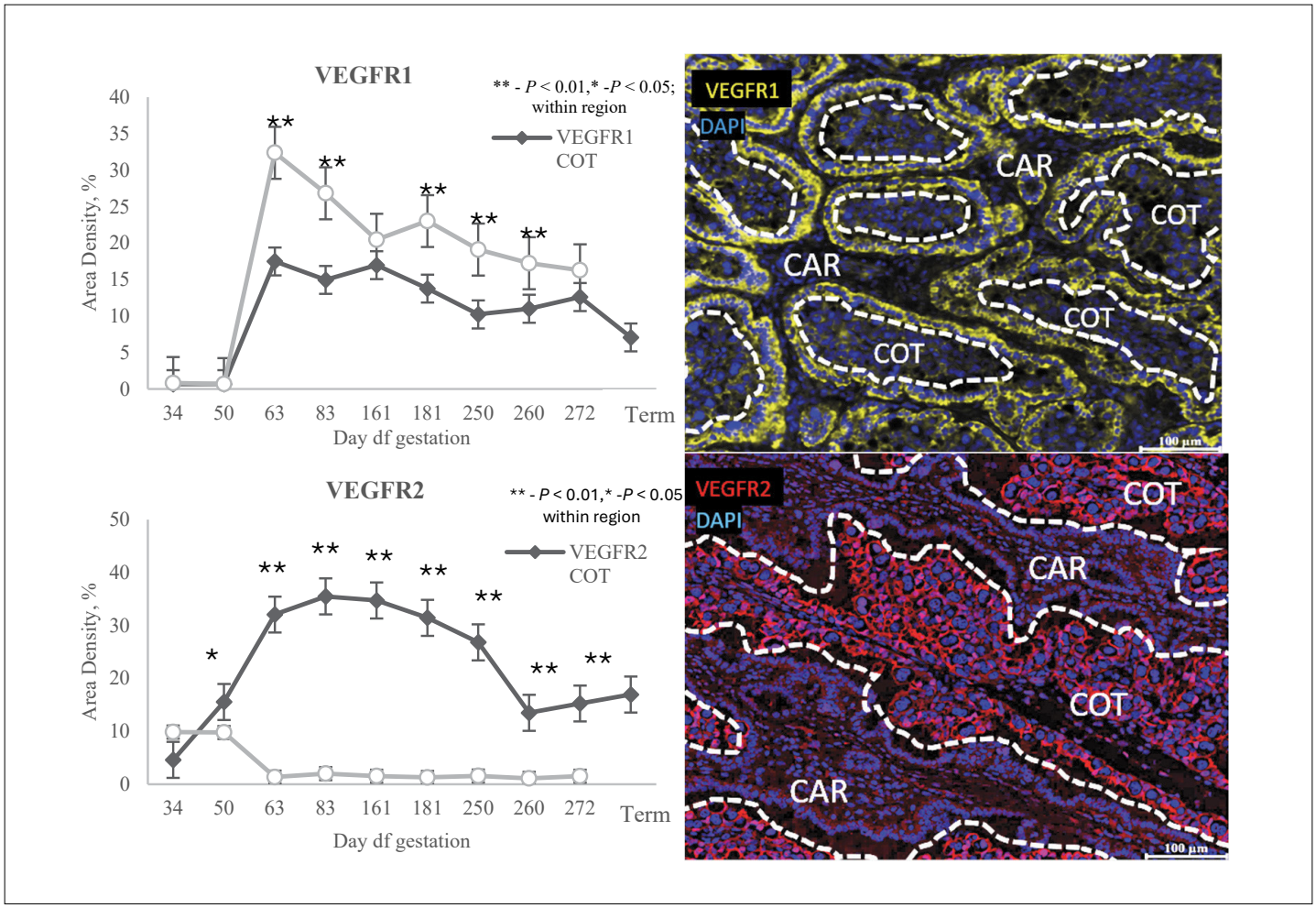

Figure 3. Gestational expression of VEGFR1 and VEGFR2 in CAR and COT of beef heifer placentomes.

predominant VEGFR1 expression in the maternal placenta and VEGFR2 expression in the fetal placenta. Strong VEGF expression in binucleate giant cells further suggests that these specialized trophoblast cells may contribute to placental angiogenesis and maternal-fetal communication.

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