Hypoxia increases glucose transporter 1 expression in bovine corpus luteum at the early luteal stage

Ryo NISHIMURA1,2)*, Hiroki HASEGAWA2), Masamichi YAMASHITA3), Norihiko ITO3), Yoshiharu OKAMOTO3), Takashi TAKEUCHI3), Tomoaki KUBO4), Kosuke IGA5), Koji KIMURA6), Mitsugu HISHINUMA1) and Kiyoshi OKUDA6,7)

1)Laboratory of Theriogenology, Joint Department of Veterinary Medicine, Faculty of Agriculture, Tottori University, 4-101 Koyama-cho Minami, Tottori 680-8553, Japan
2)Laboratory of Reproductive Physiology, Graduate School of Agriculture, Tottori University, 4-101 Koyama-cho Minami, Tottori 680-8553, Japan
3)Joint Department of Veterinary Clinical Medicine, Faculty of Agriculture, Tottori University, 4-101 Koyama-cho Minami, Tottori 680-8553, Japan
4)United Graduate School of Veterinary Science, Gifu University, Gifu 501-1193, Japan
5)Division of Livestock and Forage Research, Beef Cattle Production Group, Tohoku Agricultural Research Center, NARO, Iwate 020-0198, Japan
6)Laboratory of Reproductive Physiology, Graduate School of Environmental and Life Science, Okayama University, Okayama 700-8530, Japan
7)Obihiro University of Agriculture and Veterinary Medicine, Hokkaido 080-8555, Japan

ABSTRACT. A major role of the corpus luteum (CL) is to produce progesterone (P4). The CL has immature vasculature shortly after ovulation, suggesting it exists under hypoxic conditions. Hypoxia-inducible factor-1 (HIF1) induces the expression of glucose transporter 1 (GLUT1). To clarify the physiological roles of GLUT1 in bovine CL, we examined GLUT1 mRNA expression in the CL under hypoxic conditions by quantitative RT-PCR. We also measured the effects of glucose (0–25 mM) and GLUT1 inhibitors (cytochalasin B, STF-31) on P4 production in bovine luteal cells. GLUT1 mRNA expression in bovine CL was higher at the early luteal stage compared to the other later stages. Hypoxia (3% O2) increased GLUT1 mRNA expression in early luteal cells, but not in mid luteal cells. Glucose (0–25 mM) increased P4 production in early luteal cells, but not in mid luteal cells. Both GLUT1 inhibitors decreased P4 production in early and mid luteal cells. Overall, the results suggest that GLUT1 (possibly induced by hypoxic conditions in the early CL) plays a role in the establishment and development of bovine CL, especially in supporting luteal P4 synthesis at the early luteal stage.

KEY WORDS: corpus luteum, glucose transporter-1, HIF1, hypoxia, progesterone

The corpus luteum (CL) is an organ that is temporarily formed during the female reproductive cycle. The CL is formed from a ruptured follicle after ovulation and is coupled with rapid angiogenesis [29, 30, 32, 33], which is stimulated by a variety of growth factors [30, 31, 33]. The strongest growth factor is vascular endothelial growth factor (VEGF) [8]. VEGF is strongly induced by hypoxia-inducible factor-1 (HIF1) [9], and plays a role in the angiogenesis of newly formed CLs in cattle [2, 11, 23]. Follicle rupture shortly after ovulation has been suggested to be induced under hypoxic conditions as a result of bleeding and due to its immature vasculature [1, 23]. In addition to the angiogenic action of VEGF, several systems are thought to be induced as a result of HIF1 activity during CL formation.

HIF1 is an obligatory heterodimeric protein composed of HIF1A and the aryl hydrocarbon receptor nuclear translocator (ARNT), both of which are members of the basic-helix-loop-helix (bHLH)-containing PER-ARNT-SIM (PAS) domain family [39]. ARNT expression is not affected by oxygen concentration, whereas HIF1A is rapidly ubiquitinated under hypoxia, which targets the protein for degradation by the proteasome [13, 15, 34]. In bovine CL, HIF1A expression is highest during luteal development and has a similar expression pattern to that of VEGF [23]. This suggests HIF1 is important for angiogenesis during bovine CL development. HIF1 induces the transcription of genes related to several physiological systems, such as angiogenesis, erythropoiesis, glycolysis, and apoptosis [40]. Glucose transporter 1 (GLUT1) is one of 13 facilitative sugar transporters that...
has tissue-specific distribution and is induced by HIF1 [40]. GLUT1 is expressed in the ovaries of rat [17, 18], mouse [44], dog [29], and sheep [42]. GLUT1 mRNA is also expressed in bovine CL [22]. However, the roles of GLUT1 and its relationship with hypoxia in bovine CL remain unclear.

In the present study, we investigated the physiological roles of GLUT1 in bovine CL by examining GLUT1 mRNA expression in the CL during the estrous cycle. The effects of hypoxia on GLUT1 mRNA expression in cultured bovine luteal cells was also examined. Furthermore, to examine whether glucose and GLUT1 regulate luteal progesterone (P4) synthesis, we evaluated the effects of glucose and GLUT1 inhibitors on P4 production in cultured luteal cells.

MATERIALS AND METHODS

Collection of CLs

Ovaries with CLs from Holstein cows were collected at a local abattoir 10–20 min after exsanguination. Luteal stages were classified as early, developing, mid, late, or regressed by macroscopic observation of the ovary and uterus, as previously described [21]. After stage determination, CLs (n=4/stage) were immediately separated from the ovaries, rapidly frozen in liquid nitrogen, and stored at −80°C until being processed for RNA isolation. For cell culture experiments, ovaries with CLs (1–3 ovaries per experiment) were submerged in ice-cold physiological saline and transported to the laboratory.

Cell isolation

Early and mid luteal tissues were enzymatically dissociated, and luteal cells were cultured as previously described [25, 27]. The luteal cells were suspended in a culture medium, consisting of DMEM and Ham’s F-12 medium (Life Technologies Corp., Grand Island, NY, U.S.A.; No. 12634-010), supplemented with 5% calf serum (Life Technologies Corporation; No. 16170-078) and 20 µg/ml gentamicin (Wako Pure Chemical Industries, Osaka, Japan; No. 078-06061). Cell viability was determined to be greater than 85% by trypan blue exclusion. Cells in the cell suspension consisted of approximately 70% small luteal cells, 20% large luteal cells, 10% endothelial cells or fibrocytes, and no erythrocytes [25].

Cell culture and experiments

For the determination of mRNA expression, dispersed luteal cells were seeded at 2.0 × 10⁵ viable cells per ml in 24-well cluster dishes (Greiner Bio-One, Frickenhausen, Germany; No. 662160), while 48-well cluster dishes (Thermo Fischer Scientific, Rochester, NY, U.S.A.; No. 130187) were used for the determination of P4 production. In both instances cells were cultured in a N₂-O₂-CO₂-regulated incubator with a humidified atmosphere of 5% CO₂ at 37.5°C (ESPEC Corp., Osaka, Japan; No. BNP-110). After 12 hr of culture, the medium was replaced with fresh medium containing 0.1% BSA and 5 ng/ml sodium selenite. Thereafter the experiments described below were carried out. Glucose-free medium (Nakalai Tesque, Kyoto, Japan; No. 09893-05) was specifically used for the experiment examining the effects of glucose (0.25, 2.5 and 25 mM) on P4 production. Cell culture under conditions examining different levels of O₂ (2% or 20%) was performed using N₂-O₂-CO₂-regulated incubators (ASTEC, Fukuoka, Japan; No. APM30D), and cells were cultured for 24 hr. Following incubation, the cell culture supernatant was used for the determination of P4 concentrations, and total cellular RNA was extracted for the determination of GLUT1 mRNA. For GLUT1 inhibition, cytochalasin B (a non-specific GLUT inhibitor; Sigma-Aldrich, St. Louis, MO, U.S.A.; No. C6762; 10 µM) and STF-31 (a specific GLUT inhibitor; Sigma-Aldrich; No. SML1108; 10 µM) were added with or without human chorionic gonadotropin (hCG; ASKA Pharmaceutical Co., Ltd., Tokyo, Japan; 1 U/ml).

RNA isolation and cDNA synthesis

Total RNA was prepared from CL tissue and cultured luteal cells using RNeasy, according to the manufacturer’s directions (Qiagen GmbH, Hilden, Germany; No. 74106). Total RNA (1 µg) was reverse transcribed using a PrimeScript 1st strand cDNA synthesis kit (Takara Bio Inc., Otsu, Japan; No. 6110A).

Real-time polymerase chain reaction (PCR)

Gene expression was measured by real-time PCR using a 7500 FAST thermal cycler (Applied Biosystems, Tokyo, Japan) and the KAPA FAST ABI prism qPCR kit (KAPA, Boston, MA, U.S.A.; No. KK4604) starting with 1 ng of reverse-transcribed total RNA. The expression of 18S ribosomal RNA (18SrRNA) was used as an internal control. Specific primers with 50–60% GC-contents for GLUT1 and 18SrRNA were synthesized. Briefly, the primers for GLUT1 were 5′-AGACACCTGAGGAGCTGTTC-3′ (5′primer, 20 mer) and 5′-GACATCACTGGCTGGCTGAAG-3′ (3′primer, 20 mer); and for 18SrRNA were 5′-TCGCGGAAGGATTTAAAGTG-3′ (5′primer, 20 mer) and 5′-AAACGGCTACCACATCCAAG-3′ (3′primer, 20 mer). The PCR conditions were 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 60 sec. Use of the KAPA FAST ABI prism qPCR mixture at elevated temperatures resulted in reliable and sensitive quantification of the RT-PCR products with high linearity (Pearson correlation coefficient r>0.99). To analyze the relative level of expression of each mRNA, the 2⁻ΔΔCT method was used [20].

P4 concentration determination

Concentrations of P4 were determined directly from the cell culture medium using an enzyme immunoassay (EIA), as previously described [24]. The standard curve ranged from 0.391 to 100 ng/ml, and the effective dose of the assay for 50% inhibition (ED50) was 4.5 ng/ml. The intra- and inter-assay coefficients of variation were 5.3% and 8.4%, respectively.

doi: 10.1292/jvms.17-0284
Statistical analysis

All experimental data are shown as the mean ± SEM. Data on the effects of glucose and GLUT1 inhibitors on P4 production are shown as a percentage of the control. The statistical significance of differences between the amounts of GLUT1 mRNA (Fig. 1) during the estrous cycle and on the effects of glucose and GLUT1 inhibitors on P4 production (Figs. 3 and 4) were assessed by analysis of variance (ANOVA) followed by a multiple comparison with Bonferroni correction. The statistical significance of the difference between various oxygen conditions on the amounts of GLUT1 mRNA (Fig. 2) were assessed by Student’s t-test.

RESULTS

GLUT1 mRNA expression

GLUT1 mRNA was expressed in bovine CL throughout the estrous cycle and was highest at the early luteal stage (Fig. 1; P<0.05). GLUT1 mRNA expression in early luteal cells increased significantly after 24 hr of hypoxia (Fig. 2A; P<0.05). Mid luteal cells did not show the same response (Fig. 2B).

Effects of glucose and GLUT1 inhibitors on P4 production in early and mid luteal cells

Glucose increased P4 production in early luteal cells in a dose-dependent manner (Fig. 3A; none vs. 0.25, 2.5 mM glucose: P<0.05; none vs. 25 mM glucose: P<0.01), whereas it did not affect P4 production by mid luteal cells (Fig. 3B). The nonselective GLUT inhibitor cytochalasin B only decreased P4 production in early luteal cells stimulated by hCG (Fig. 4A; P<0.05), while the GLUT1-specific inhibitor STF-31 decreased P4 production in early luteal cells without and with hCG stimulation (Fig. 4A; P<0.05). Both GLUT1 inhibitors decreased P4 production in mid luteal cells stimulated by hCG (Fig. 4B; P<0.05). Minor inhibitory effects were also observed in mid luteal cells without hCG stimulation (Fig. 4B).

DISCUSSION

The present study reconfirmed the previous finding that GLUT1 mRNA was expressed throughout the estrous cycle in bovine CL [22], and revealed that GLUT1 mRNA expression levels in the CL were greatest at the early luteal stage. Compared to normal culture conditions (20% O2), GLUT1 mRNA expression increased in bovine early luteal cells, but not in mid luteal cells, under hypoxic conditions (3% O2). Development of the bovine early CL is suggested to occur under hypoxic conditions, since HIF1A protein expression is highest at early luteal stage CL than at the other CL stages [23]. The present study also found that glucose is necessary for luteal P4 production, since GLUT1 inhibition resulted in decreased P4 production. These findings suggest that, in early CL tissue, hypoxic conditions induce HIF1A expression, which induces GLUT1 expression [40], which in turn increases P4 production, the primary role of the CL. GLUT1 is also suggested to function in the bovine mid CL, since GLUT1 mRNA is expressed in bovine CL throughout the estrous cycle. However, GLUT1 mRNA expression did not increase under hypoxic conditions.
EFFECT OF HYPOXIA ON GLUT1 EXPRESSION IN CL

Consistent with the present results, Nishimoto et al. [22] reported high GLUT1 mRNA expression in bovine CL stage I (Days 1–4 after ovulation). However, they also reported high expression in stage II (Days 5–10) and stage III (Days 11–17). The discrepancies may be due to differences in stage classification between the two studies. We classified Days 2–3 as early stage CL, which could result in excessive expression in the early CL. Our finding of high GLUT1 expression in the early CL is also consistent with the dynamics of HIF1, whose expression is highest at the early luteal stage in bovine CL [23]. However, further analyses of the timings of expression of GLUT1, HIF1 and other proteins are needed to clarify the sequence of these events.

The dynamics of GLUT1 mRNA expression in CL during the estrous cycle observed in the present study are similar to those of HIF1 as well as VEGF in bovine CL [23]. These results are in agreement with the recent report for canine CL [29]. Similar expression level dynamics of SCL2A1 (GLUT1 gene), HIF1A, and VEGF were demonstrated in canine CL during diestrus, and suggest a functional inter-relationship that results in the formation of the GLUT1 protein [29]. Rather than bovine luteal cells, GLUT1 mRNA has been demonstrated to be induced by hypoxic conditions in several types of cells in several species, including bovine retinal endothelial cells [37], rat fibroblasts [4], mouse mammary epithelial cells [36], rat and ovine placental cells [5],

Fig. 3. Dose-dependent effects of glucose on P4 production by cultured bovine early luteal cells (A) and mid luteal cells (B). The cells were cultured in glucose-free medium without or with glucose (0.25, 2.5 and 25 mM) for 24 hr (early: n=3, mid: n=3). All values are represented as a percentage of the control (none) value (mean ± SEM). The concentration of P4 in the early and mid luteal control cells was 193 ± 25.7 ng/ml and 613 ± 36.7 ng/ml, respectively. Asterisks indicate significant differences compared with none (*P<0.05, **P<0.01), as determined by ANOVA followed by a multiple comparison with Bonferroni correction.

Fig. 4. Effect of GLUT1 inhibitors (cytochalasin B [CytoB], STF-31 [STF]) on P4 production by cultured bovine early luteal cells (A) and mid luteal cells (B). Cells were cultured in medium containing 5% calf serum without or with GLUT1 inhibitor (cytochalasin B; 10 µM, STF-31; 10 µM) in combination without or with hCG stimulation (1 U/ml) for 24 hr (early: n=6, mid: n=5). All values are represented as a percentage of the control (none without hCG) value (mean ± SEM). The concentration of P4 in the controls for early and mid luteal cells was 238 ± 19.5 ng/ml and 674 ± 58.0 ng/ml, respectively. Asterisks indicate significant differences (P<0.05), as determined by ANOVA followed by a multiple comparison with Bonferroni correction.
porcine proximal tubule cells [43], and human glioma cells [19]. These results, together with the present findings, suggest that hypoxic conditions in the CL induce HIF1, resulting in the transcription of GLUT1 and VEGF to support the physiological function of CL across species.

Cyclic mono-phosphate (cAMP) was found to stimulate GLUT1 transcription in 3T3-L1 adipocytes following 2–16 hr of culture [14]. This suggests cAMP directly modulates GLUT1 levels in 3T3-L1 adipocytes. cAMP was also found to stimulate the transcriptional activity of the GLUT1 promoter in rat myoblasts and a 33 bp sequence lying 5′ upstream of the transcriptional start site was important for the effects of 8-bromo-cAMP [38]. Ogura et al. [26] also demonstrated by Northern and immunoblot analyses that 8-bromo-cAMP stimulates GLUT1 mRNA and protein expression in a human choriocarcinoma cell line. In the present study, GLUT1 inhibitors, cytochalasin B and STF-31, significantly inhibited P4 production in hCG-stimulated cells, whereas their inhibitory effects were weak in cells without hCG stimulation. hCG binds to the LH/CG receptor which connects adenylyl cyclase inside the cells to induce cAMP accumulation for downstream signal transduction [12, 41]. Results from the present study therefore suggest that following LH/CG receptor stimulation GLUT1 expression increases and cAMP accumulates, thereby resulting in increased P4 production by bovine luteal cells.

In bovine CL, P4 is thought to be a lutetotropic factor since it exerts a positive feedback loop on its receptor [35] and protects luteal cells from apoptosis [28]. In endometrial cells, P4 has been suggested to directly regulate GLUT1 expression via its own receptor [10, 16], thereby enhancing GLUT1 expression and translocation to the plasma membrane [16]. In the bovine ovary, hypoxic conditions have been suggested to support P4 synthesis during luteinization [6, 7]. Our finding that hypoxic conditions increased GLUT1 mRNA expression suggests that GLUT1 supports P4 synthesis during luteinization in the bovine ovary. It is also possible that P4 enhances GLUT1 mRNA expression via its own receptor, thereby increasing luteal glucose uptake [3] and corresponding with higher steroidogenic outputs during CL establishment.

In conclusion, our results suggest that the hypoxic conditions during early bovine CL development enhance GLUT1 expression via the transcriptional activity of HIF1. GLUT1, in turn, contributes to CL function by supporting P4 production.

ACKNOWLEDGMENTS. This research was supported by Grant-in-Aid for Young Scientists (B) (Grant Number JP16K18803) of the Japan Society for the Promotion of Science (JSPS). We are grateful to Dr. Tsuyoshi Yamaguchi and Dr. Tatsufumi Usui of Tottori University for the technical assistance in measuring P4 concentrations. We are also grateful to Dr. Naoki Kitamura of Tottori University for the technical assistance in isolating bovine luteal cells.

REFERENCES

1. Amselgruber, W. M., Schäfer, M. and Sinowatz, F. 1999. Angiogenesis in the bovine corpus luteum: an immunocytochemical and ultrastructural study. *Anat. Histol. Embryol.* **28**: 157–166. [Medline] [CrossRef]

2. Berisha, B., Schams, D., Kosmann, M., Amselgruber, W. and Einspanier, R. 2000. Expression and tissue concentration of vascular endothelial growth factor, its receptors, and localization in the bovine corpus luteum during estrous cycle and pregnancy. *Biol. Reprod.* **63**: 1106–1114. [Medline] [CrossRef]

3. Chase, C. C., Jr., Del Vecchio, R. P., Smith, S. B. and Randel, R. D. 1992. In vitro metabolism of glucose by bovine reproductive tissues obtained during the estrous cycle and after calving. *J. Anim. Sci.* **70**: 1496–1508. [Medline] [CrossRef]

4. Chen, C., Pore, N., Behrooz, A., Ismail-Beigi, F. and Maity, A. 2001. Regulation of glut1 mRNA by hypoxia-inducible factor-1. Interaction between H-ras and hypoxia. *J. Biol. Chem.* **276**: 9519–9525. [Medline] [CrossRef]

5. Das, U. G., Sadig, H. F., Soares, M. J., Hay, W. W. Jr. and Devaskar, S. U. 1998. Time-dependent physiological regulation of rodent and ovine placental glucose transporter (GLUT1-protein). *Am. J. Physiol.* **274**: R339–R347. [Medline] [CrossRef]

6. Fadhillah, Y., Yoshioka, S., Nishimura, R. and Okuda, K. 2014. Hypoxia promotes progesterone synthesis during luteinization in bovine granulosa cells. *J. Reprod. Dev.* **60**: 194–201. [Medline] [CrossRef]

7. Fadhillah, Y., Yoshioka, S., Nishimura, R., Yamamoto, Y., Kimura, K. and Okuda, K. 2017. Hypoxia-inducible factor 1 mediates hypoxia-enhanced synthesis of progesterone during luteinization of granulosa cells. *J. Reprod. Dev.* **63**: 75–85. [Medline] [CrossRef]

8. Ferrara, N. and Davis-Smyth, T. 1997. The biology of vascular endothelial growth factor. *Endocr. Rev.* **18**: 4–25. [Medline] [CrossRef]

9. Forsythe, J. A., Jiang, B. H., Iyer, N. V., Agani, F., Leung, S. W., Koos, R. D. and Semenza, G. L. 1996. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. *Mol. Cell. Biol.* **16**: 4604–4613. [Medline] [CrossRef]

10. Frolova, A., Flesser, L., Chi, M., Kim, S. T., Foyouzi-Yousifi, N. and Moley, K. H. 2009. Facilitative glucose transporter type 1 is differentially regulated by progesterone and estrogen in murine and human endometrial stromal cells. *Endocrinology* **150**: 1512–1520. [Medline] [CrossRef]

11. Gabler, C., Plath-Gabler, A., Killian, G. J., Berisha, B. and Schams, D. 2004. Expression pattern of fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) system members in bovine corpus luteum endothelial cells during treatment with FGF-2, VEGF or oestradiol. *Reprod. Domest. Anim.* **39**: 321–327. [Medline] [CrossRef]

12. Garverick, H. A., Smith, M. F., Elmore, R. G., Morehouse, G. L., Agudo, L. S. and Zahler, W. L. 1985. Changes and interrelationships among luteal LH receptors, adenylyl cyclase activity and phosphodiesterase activity during the bovine estrous cycle. *J. Anim. Sci.* **61**: 216–223. [Medline] [CrossRef]

13. Huang, L. E., Gu, J., Schau, M. and Bunn, H. F. 1998. Regulation of hypoxia-inducible factor 1α is mediated by an O2-dependent degradation domain via the ubiquitin-proteasome pathway. *Proc. Natl. Acad. Sci. U.S.A.* **95**: 7987–7992. [Medline] [CrossRef]

14. Kaestner, K. H., Flores-Riveros, J. R., McLenithan, J. C., Janicot, M. and Lane, M. D. 1991. Transcriptional repression of the mouse insulin-responsive glucose transporter (GLUT4) gene by cAMP. *Proc. Natl. Acad. Sci. U.S.A.* **88**: 1933–1937. [Medline] [CrossRef]

15. Kallio, P. J., Wilson, W. J., O’Brien, S., Makino, Y. and Poellinger, L. 1999. Regulation of the hypoxia-inducible transcription factor 1α by the ubiquitin-proteasome pathway. *J. Biol. Chem.* **274**: 6519–6525. [Medline] [CrossRef]

16. Kim, S. T. and Moley, K. H. 2009. Regulation of facilitative glucose transporters and AKT/MAPK/PRKAA signaling via estradiol and progesterone in the mouse uterine epithelium. *Biol. Reprod.* **81**: 188–198. [Medline] [CrossRef]
17. Kodaman, P. H. and Behrman, H. R. 1999. Hormone-regulated and glucose-sensitive transport of dehydroascorbic acid in immature rat granulosa cells. *Endocrinology* **140**: 3659–3665. [Medline] [CrossRef]

18. Kol, S., Ben-Shlomo, I., Ruutuinen, K., Ando, M., Davies-Hill, T. M., Rohan, R. M., Simpson, I. A. and Adashi, E. Y. 1997. The midcycle increase in ovarian glucose uptake is associated with enhanced expression of glucose transporter 3. Possible role for interleukin-1, a putative intermediary in the ovulatory process. *J. Clin. Invest.* **99**: 2274–2283. [Medline] [CrossRef]

19. Li, S., Wang, J., Wei, Y., Liu, Y., Ding, X., Dong, B., Xu, Y. and Wang, Y. 2015. Crucial role of TRPC6 in maintaining the stability of HIF-1α in glioma cells under hypoxia. *J. Cell Sci.* **128**: 3317–3329. [Medline] [CrossRef]

20. Livak, K. J. and Schmittgen, T. D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2^(-ΔΔC(T)) Method. *Methods* **25**: 402–408. [Medline] [CrossRef]

21. Miyamoto, Y., Skarzynski, D. J. and Okuda, K. 2000. Is tumor necrosis factor a trigger for the initiation of endometrial prostaglandin F(2a) release at luteolysis in cattle? *Biol. Reprod.* **62**: 1109–1115. [Medline] [CrossRef]

22. Nishimoto, H., Matsutani, R., Yamamoto, S., Takahashi, T., Hayashi, K. G., Miyamoto, A., Hamano, S. and Tetsuka, M. 2006. Gene expression of glucose transporter (GLUT) 1, 3 and 4 in bovine follicle and corpus luteum. *J. Endocrinol.* **188**: 111–119. [Medline] [CrossRef]

23. Nishimura, R. and Okuda, K. 2010. Hypoxia is important for establishing vascularization during corpus luteum formation in cattle. *J. Reprod. Dev.* **56**: 110–116. [Medline] [CrossRef]

24. Nishimura, R., Sakumoto, R., Tatsukawa, Y., Acosta, T. J. and Okuda, K. 2006. Oxygen concentration is an important factor for modulating progesterone synthesis in bovine corpus luteum. *Endocrinology* **147**: 4273–4280. [Medline] [CrossRef]

25. Okuda, K., Korzekwa, A., Shibuya, M., Murakami, S., Nishimura, R., Tsubouchi, M., Woclawek-Potocka, I. and Skarzynski, D. J. 2004. Possible role of interleukin-1β in the regulation of bovine corpus luteum function. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **286**: R2065–R2071. [Medline] [CrossRef]

26. Ogura, K., Sakata, M., Okamoto, Y., Yasui, Y., Takadokoro, C., Yoshimoto, Y., Yamaguchi, M., Kurachi, H., Maeda, T. and Murata, Y. 2000. 8-bromo-cyclicAMP stimulates glucagon transporter 1 expression in a human choriocarcinoma cell line. *J. Endocrinol.* **164**: 171–178. [Medline] [CrossRef]

27. Okuda, K., Miyamoto, A., Sauerwein, H., Schweigert, F. J. and Schams, D. 1992. Evidence for oxytocin receptors in cultured bovine luteal cells. *Biol. Reprod.* **46**: 1001–1006. [Medline] [CrossRef]

28. Okuda, K., Korzekwa, A., Shihbaya, M., Murakami, S., Nishimura, R., Tsubouchi, M., Woclawek-Potocka, I. and Skarzynski, D. J. 2004. Progesterone is a suppressor of apoptosis in bovine luteal cells. *Biol. Reprod.* **71**: 159–175. [Medline] [CrossRef]

29. Papa, P. C., Sousa, I. M., Silva, R. S., de Fátima, L. A., da Fonseca, V. U., do Amaral, V. C., Hoffmann, B., Alves-Wagner, A. B., Machado, U. F. and Kowalewski, M. P. 2013. Glucose transporter 1 expression accompanies hypoxia sensing in the cyclic canine corpus luteum. *Reproduction* **147**: 81–89. [Medline] [CrossRef]

30. Redmer, D. A. and Reynolds, L. P. 1996. Angiogenesis in the ovary. *Rev. Reprod. 1*: 182–192. [Medline] [CrossRef]

31. Reynolds, L. P., Killilea, S. D. and Redmer, D. A. 1992. Angiogenesis in the female reproductive system. *FASEB J.* **6**: 886–892. [Medline]

32. Reynolds, L. P., Grazul-Bilska, A. T. and Redmer, D. A. 2000. Angiogenesis in the corpus luteum. *Endocrinology* **141**: 1–9. [Medline] [CrossRef]

33. Reynolds, L. P., Grazul-Bilska, A. T., Killilea, S. D. and Redmer, D. A. 1994. Mitogenic factors of corpora lutea. *Prog. Growth Factor Res.* **5**: 159–175. [Medline] [CrossRef]

34. Salceda, S. and Caro, J. 1997. Hypoxia-inducible factor 1α (HIF-1α) protein is rapidly degraded by the ubiquitin-proteasome system under normoxic conditions. Its stabilization by hypoxia depends on redox-induced changes. *J. Biol. Chem.* **272**: 22642–22647. [Medline] [CrossRef]

35. Schams, D. and Berisha, B. 2004. Regulation of corpus luteum function in cattle—an overview. *Reprod. Domest. Anim.* **39**: 241–251. [Medline] [CrossRef]

36. Schams, D. and Kowalewski, M. P. 2013. Glucose transporter 1 expression accompanies hypoxia sensing in the cyclic canine corpus luteum. *Reproduction* **147**: 81–89. [Medline] [CrossRef]

37. Takagi, H., Gun, G. L. and Ciello, L. P. 1998. Hypoxia upregulates glucose transport activity through an adenosine-mediated increase of GLUT1 expression in retinal capillary endothelial cells. *Diabetes* **47**: 1480–1488. [Medline] [CrossRef]

38. Vihals, F., Ferré, I., Fandos, C., Santalucia, T., Testar, X., Palacin, M. and Zorzano, A. 1997. Cyclic adenosine 3′,5′-monophosphate regulates GLUT4 and GLUT1 glucose transporter expression and stimulates transcriptional activity of the GLUT1 promoter in muscle cells. *Endocrinology* **138**: 2521–2529. [Medline] [CrossRef]

39. Wang, G. L., Jiang, B. H., Rue, E. A. and Semenza, G. L. 1995. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. *Proc. Natl. Acad. Sci. U.S.A.* **92**: 5510–5514. [Medline] [CrossRef]

40. Wenger, R. H. 2002. Cellular adaptation to hypoxia: O2-sensing protein hydroxylases, hypoxia-inducible transcription factors, and O2-regulated gene expression. *FASEB J.* **16**: 1151–1162. [Medline] [CrossRef]

41. Williams, M. T., Clark, M. R., Ling, W. Y., LeMaire, W. J., Caron, M. G. and Marsh, J. M. 1978. Role of cyclic AMP in the actions of luteinizing hormone on steroidogenesis in the corpus luteum. *Adv. Cyclic Nucleotide Res.* **9**: 573–582. [Medline] [CrossRef]

42. Williams, S. A., Blache, D., Martin, G. B., Foot, R., Blackberry, M. A. and Scaramuzzi, R. J. 2001. Effect of nutritional supplementation on quantities of glucose transporters 1 and 4 in sheep granulosa and theca cells. *Reproduction* **122**: 947–956. [Medline] [CrossRef]

43. Zapata-Morales, J. R., Galicia-Cruz, O. G., Franco, M. and Martinez Y Morales, F. 2014. Hypoxia-inducible factor-1α (HIF-1α) protein diminishes glucose transport expression in renal epithelial tubular cells (LLC-PK1) under hypoxia. *J. Biol. Chem.* **289**: 346–357. [Medline] [CrossRef]

44. Zhou, J., Bievre, M. and Bondy, C. A. 2000. Reduced GLUT1 expression in Igf1−/− null oocytes and follicles. *Growth Horm. IGF Res.* **10**: 111–117. [Medline] [CrossRef]